ASSESSING THE NEUROTOXICOLOGICAL RISK OF METHYLMERCURY EXPOSURE FOR BELUGA WHALES (DELPHINAPTERUS LEUCAS) HARVESTED IN THE MACKENZIE DELTA ESTUARY

by

Sonja Kerstin Ostertag

B.Sc., McGill University, 2004
M.Sc., McGill University, 2008

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTORATE OF PHILOSOPHY
IN
NATURAL RESOURCES AND ENVIRONMENTAL STUDIES

UNIVERSITY OF NORTHERN BRITISH COLUMBIA

February 2014

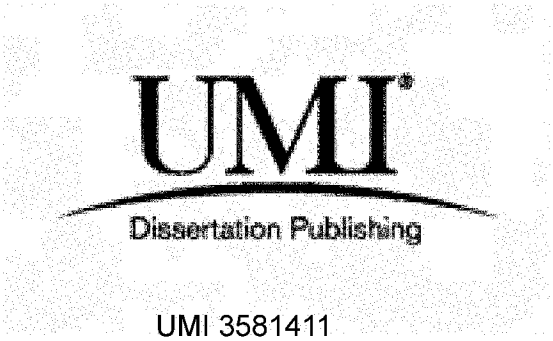
© Sonja Ostertag, 2014

UMI Number: 3581411

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.



UMI 3581411
Published by ProQuest LLC 2014. Copyright in the Dissertation held by the Author.
Microform Edition © ProQuest LLC.
All rights reserved. This work is protected against
unauthorized copying under Title 17, United States Code.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Approval Page

Abstract

Arctic marine mammals are exposed to numerous environmental contaminants and some of these compounds are known to damage mammalian nervous systems. Three methods were used to assess neurotoxicological risk of methylmercury (MeHg) exposure for beluga whales (*Delphinapterus leucas*) in the eastern Beaufort Sea population: characterization of mercury (Hg) accumulation and speciation in brain tissue, neurochemical and molecular biomarkers, and behavioural observations. To conduct this research, I worked closely with the communities of Tuktoyaktuk, NT and Inuvik, NT to conduct three field-sampling seasons on Hendrickson Island, NT, which is a traditional beluga-harvesting site used by Inuvialuit. Community members participated in this project as mentoring students, mentors, interviewees, and research assistants.

Total Hg concentrations (median; mg kg^{-1} wet weight, ww) were 2.34 (0.06 to 22.6, 81) (range, n) in temporal lobe, 1.84 (0.12 to 21.9, 77) in frontal lobe 1.84 (0.05 to 16.9, 83) in cerebellum, 1.25 (0.02 to 11.1, 77) in spinal cord and 1.32 (0.13 to 15.2, 39) in brain stem. The concentrations of MeHg ranged from 0.03 to 1.05 mg kg^{-1} ww and labile inorganic Hg (iHg) ranged from below detection limit to 1.59 mg kg^{-1} ww. Molar concentrations of selenium (Se_T) consistently exceeded Hg_T in the five brain regions analyzed. Harvesters (n=11) observed differences in evasive strategies used by beluga whales during the hunt, which varied with the concentration of Hg_T analyzed in brain tissue. At molecular and/or neurochemical levels, components of the dopaminergic, cholinergic, GABAergic and glutamatergic signaling pathways appeared to be sensitive to MeHg exposure. Furthermore, monoamine oxidase activity and muscarinic acetylcholine receptor binding were negatively associated with Hg_T to Se_T molar ratios (*p*

< 0.05), and mRNA expression for mAChr m1 was positively associated with Hg_T to Se_T ratio ($p < 0.05$).

The weight of evidence based on the outcomes from these studies suggests that MeHg exposure may be of toxicological concern for beluga whales from the Eastern Beaufort Sea population. The implications of MeHg-exposure for beluga whales from the eastern Beaufort Sea population at both physiological and population levels are still unclear.

Table of Contents

List of Tables

Chapter 5

Chapter 6

Chapter 7

List of Figures

Glossary

AMAP: Arctic Monitoring and Assessment Program
CHL: chlordane;
CBz: chlorobenzene
DDT: dichlorodiphenyltrichloroethane
dw: dry weight
EBS: eastern Beaufort Sea
FJMC: Fisheries Joint Management Committee
FNP: flunitrazepam
GABA: γ-aminobutyric acid
HBCD: hexabromocyclododecane
HCH: hexachlorocyclohexane
Hg: mercury
iHg: inorganic Hg
ISR: Inuvialuit Settlement Region
OC: organic contaminant
mAChR: muscarinic acetylcholine receptor
MAO: monoamine oxidase
MeHg: methylmercury
MK-801: Dizocilpinehydrogen maleate
NCP: Northern Contaminants Program
nss: Not statistically significant
NMDA: N-methyl-D-aspartate
PAH: polycyclic aromatic hydrocarbon
PBDE: polybrominated diphenyl ether
PCB: polychlorinated biphenyl
PCDD: polychlorinated dibenzo-p-dioxin
PFC: perfluorinated compounds
PFCA: perfluorinated carboxylic acid
PFSA: perfluorinated sulfonic acid
POP: persistent organic pollutant
ww: wet weight
QNB: quinuclidinyl benzilate
Se: selenium
TEK: Traditional Ecological Knowledge
TSK: Tradtional Scientific Knowledge

Acknowledgements

A heartfelt thank you to the community of Tuktoyaktuk for sharing your knowledge about beluga whales with me. This project would not have been possible without the kindness, teachings and support provided by Frank and Nellie Pokiak and their family. Thank you Robin Felix, Ronald Felix, Eric Loring, Lisa Loseto, Eddie Lucas, Marie Noel, Jocelyn Noksana, Kayla Nuyaviak, Dale Panaktolok, Mikkel Panaktolok, Charles Pokiak, James Pokiak, Maureen Pokiak, Myrna Pokiak, Rebecca Pokiak, Verna Pokiak, Kate Snow, Brandon Voudrach and Ryan Walker for making the sampling program and community visits enjoyable and successful.

I have greatly appreciated the opportunity to study with Dr. Laurie Chan and his UNBC research team. Laurie gave me the opportunity of a lifetime to study beluga whales in the Beaufort Sea with an amazing group of researchers and community members. I am grateful for Laurie's guidance and support on this research journey. I am also very thankful for the support, friendship and guidance offered by Dr. Nil Basu and his students at the University of Michigan. I appreciated the opportunity to collaborate with Drs. Feiyue Wang, Gary Stern and Marcos Lemes. I have benefitted from the feedback and support from my committee members: Drs. Andrea Gorrell, Stephen Raverty, Mark Shrimpton, and Gary Wilson.

My family has been incredibly supportive during my entire PhD journey– from the move to Prince George, BC from Montreal, QC, to many field seasons in the Inuvialuit Settlement Region, and the ups and downs that life has to offer. Thank you Julia and Colleen for many inspiring conversations about this project, Sebastian and Dave for your support and understanding, Joachim and Matthias for your encouragement and positivity, and my grandparents for your curiosity, love and prayers.

Living and learning in Prince George, British Columbia would not have been as fulfilling without the energy and dedication of the PGSO, PGCM, CNSC and the Sea to Sands Conservation Alliance. Thank you Maria for teaching me about balance and mindfulness through our many sessions together. Finally, thank you to all of you who became my Prince George family and are working towards making this world a better place. I have appreciated your friendship and company on the adventures that have been plentiful in the last seven years.

Finally, this research would not have been possible without the generous financial support from Aboriginal Affairs and Northern Development Canada, BC Leadership Chair in Environmental and Aboriginal Health, Fisheries Joint Management Committee, Nasivvik Centre for Inuit Health and Changing Environments, the Natural Sciences and Engineering Research Council and University of Northern British Columbia.

Dedication

This thesis is dedicated to

Alyssa, for your knowledge and friendship;

Anthony, for your youthful energy; and

My grandmothers, for your love, prayers and chocolate.

Chapter 1. Introduction

Contaminants are transported from southern latitudes to the Arctic via atmospheric and oceanic circulation, and river discharges (Braune et al., 2005). Heavy metals, organic contaminants (OCs), and radionuclides bioaccumulate in Arctic aquatic ecosystems (Atwell et al., 1998; Dietz et al., 2000; Loseto et al., 2008b; Mackey et al., 1996; Stern et al., 2005; Tomy et al., 2004). The far-reaching impacts of pollution were only recognized in the 1970s, when it was discovered that the Arctic was contaminated with organic pollutants (Barrie et al., 1992). Unlike most OCs, mercury (Hg) occurs naturally; however, anthropogenic activities are responsible for the release of the majority of Hg into the environment (Nriagu and Pacyna, 1988). Marine mammals and humans are susceptible to accumulating contaminants such as persistent organic pollutants (POPs) and Hg due to their long life-spans and high trophic position (Chan et al., 1995; Dewailly et al., 1993; Hoekstra et al., 2003; Loseto et al., 2008b). Inuit continue to harvest marine mammal species including beluga whales (*Delphinapterus leucas*), narwhal (*Monodon monoceros*), polar bears (*Ursus maritimus)* and ringed seals (*Pusa hispida*), for food, employment, or both (Hovelsrud et al., 2008). Therefore, the accumulation of persistent organic pollutants and heavy metals in the Canadian Arctic is an issue of concern for both humans (Van Oostdam et al., 2005) and wildlife (Fisk et al., 2005).

Past research has shown that beluga whales accumulate higher levels of OCs and heavy metals than terrestrial mammals due to their trophic position and long life span (Dietz et al., 2000; Lockhart et al., 2005; Stern et al., 2005). Animal feeding trials have demonstrated that a number

of these contaminants are also capable of disrupting components of mammalian systems integral to animal health (Birnbaum and Tuomisto, 2000; Clarkson, 1997; Costa et al., 2007; Fisk et al., 2005; Fournier et al., 2000; Lehmler et al., 2005; Mathieu et al., 1997). Contaminants in wildlife lead to increased exposure to toxins for consumers of traditional foods (Van Oostdam et al., 2005), but may also cause adverse impacts to animal health. The effects of contaminants on marine mammal health are poorly understood in part due to the challenges associated with correlating contaminant exposure to various adverse health outcomes in wild populations (Fisk et al., 2005). Recent studies detected levels of Hg in the brains of belugas in the western Arctic that exceed thresholds of effect in other animal species (Lockhart et al., 2005), which suggests that mercury exposure could lead to adverse effects in beluga whales at current levels.

Mercury is neurotoxic and may affect brain function in highly-exposed animals (Clarkson, 1997). Mercury neurotoxicity could lead to loss of critical components of animal function required for thriving and surviving in the wild. For example, acute MeHg exposure in humans has been associated with negative impacts to the visual system and neuro-motor function, peripheral neuropathy, dysarthria, tremor, cerebellar ataxia, gait disturbance and audiological impairment (Council, 2000). Although lab-based studies are useful for addressing specific biochemical or physiological effects, field studies are essential for understanding the real-world effects of chronic exposure to multiple contaminants (Rhind, 2009). Bringing together the results from diverse studies is necessary for the action of pollutants on multiple species and ecosystem function to be elucidated (Rhind, 2009). Neurochemical changes may represent early and reversible indicators of neurological harm because they occur prior to the onset of overt

functional or structural damage (Manzo et al., 1996; Manzo et al., 2001). Neurochemical biomarkers from diverse signaling pathways have been used to assess potential neurotoxicological risk of MeHg exposure in terrestrial mammals, avian species and marine mammals; results have suggested that environmental exposure to MeHg was associated with neurochemical variation in diverse species (Basu et al., 2005a; Basu et al., 2006a; Basu et al., 2005c; Basu et al., 2007b; Basu et al., 2007c; Basu et al., 2009; Hamilton et al., 2011; Rutkiewicz et al., 2010; Scheuhammer et al., 2008). Therefore, a neurochemical biomarker approach may provide valuable information about potential neurotoxicity in wildlife exposed to MeHg and other neurotoxins.

Inuvialuit harvest beluga whales for food in the western Canadian Arctic, and through collaboration with northern organizations and harvesters, researchers have collected high quality samples for monitoring and research. Inuvialuit have a strong interest in conservation of the environment; as Inuit Elder, Billy Day noted:

> "The land, the animals, the waters, the whales, and the fish were very important to our ancestors and still are to us. Even during negotiations for our land claim-settlement, our elders told us that the land and waters had looked after them for centuries and would look after us for many more if we looked after our environment" (Day, 2002).

Therefore, the Fisheries Joint Management Committee (FJMC) was established under the Inuvialuit Final Agreement to provide advice on the administration of the rights and obligations related to fish and marine mammals (Inuvialuit Final Agreement, 1987). Beluga whale research fits within one of the main objectives of the FJMC's beluga management plan "to provide for a

harvest that generates the greatest net benefit to the Inuvialuit while ensuring the long-term sustainability of beluga in the Canadian Beaufort Sea" (FJMC, 2001). Aboriginal knowledge has been increasingly recognized for its contribution to co-management and environmental impact assessments (Usher, 2000). Furthermore, it is likely that scientific and local observations of environmental change could be brought together to identify new avenues for further exploration, compare observations from different scales and discuss potential mechanisms that explain both sets of observations (Huntington et al., 2004). Inuit knowledge about beluga whales is gained through observations made during harvesting activities and travel (Byers and Roberts, 1995; Mymrin et al., 1999). Recent studies have attempted to bridge traditional ecological knowledge (TEK) and traditional scientific knowledge (TSK) in Arctic ecological research (Gagnon and Berteaux, 2009; Gilchrist et al., 2005; Huntington et al., 2004); finding ways to bridge TEK and TSK could potentially further our understanding of beluga whale health in a changing environment.

My thesis research was conducted over six years, which included three sampling seasons, three community visits and one large community workshop. My thesis reflects a collaborative approach to beluga research; I was a member of a comprehensive beluga-sampling team for the Hendrickson Island Beluga Study (HIBS) from 2008 to 2012. The HIBS was a multi-year research program, aimed at studying the risk of contaminants and assessing the health of harvested beluga whales. Samples were collected on Hendrickson Island from hunter-harvested beluga whales and the process of collecting samples from harvested whales gave me the opportunity to learn about a different culture and way of life, and taught me first-hand about the

value placed on beluga whales by Inuvialuit. Although this thesis is focused on the assessment of neurotoxicologicla risk of MeHg exposure for beluga whales, this work would not have been possible without learning extensively about the relationship between people and their environment in the Arctic, and the linkages between environmental and human health research. Navigating the social dimension of Arctic research is extremely important because poor communication strategies regarding contaminants have resulted in fear, confusion and health impacts in the communities involved (Furgal et al., 2005). Given that researchers conducting studies in the Arctic are predominantly non-Inuit, in part due to the low high school completion rates among Inuit residing in the Arctic (Richards, 2008), they must learn to communicate effectively to ensure that research studies taking place in the Arctic are addressing community needs and responding to community concerns.

Research Aim and Objectives

The central nervous system is particularly sensitive to MeHg toxicity (Clarkson and Magos, 2006) and preliminary analyses of Hg in beluga brains suggested that recent exposure to MeHg might reach levels associated with toxicity in other animals (Lockhart et al., 2005). However, brain tissues samples are not routinely collected and analyzed in beluga whale biomonitoring programs to study Hg accumulation and potential toxicity. Therefore, the principle objective of this thesis was to investigate the potential risk of neurotoxicity associated with Hg exposure in beluga whales harvested in the Mackenzie Delta Estuary of the Inuvialuit Settlement Region.

Consistent with the aim of this research, four objectives were developed:

1. to assess toxicological risk of Hg exposure in harvested beluga by comparing Hg concentrations and speciation to threshold levels of toxicity observed in mammals;
2. to determine the relationship between Hg concentration, speciation and stoichiometric relationship with selenium, to neurochemical and molecular biomarkers of neurosignaling pathways;
3. to investigate the relationship between harvesters' observations of beluga whale behaviour during harvesting and Hg exposure; and,
4. to assess the toxicological significance of Hg accumulation in beluga whales from the eastern Beaufort Sea population.

Thesis structure and contributions

Structure

This thesis is made up of four separate manuscripts that have been published or are in review for publication in peer-reviewed journals. The manuscripts were prepared with co-authors, whose contributions are outlined in the written statement (Appendix 4). Collaboration with co-authors has been particularly important for sample analysis, due to the diversity of methods used and tissue samples analyzed for the studies included in this thesis. Overall, collaboration with co-authors provided the opportunity for the scope and depth of analysis to be expanded for the research presented in this thesis.

Contribution of Chapters

Chapter 1 provides the context for this research, outlines the goals and objectives, and presents the structure and contributions of the following five chapters.

Chapter 2 provides a review of the literature related to beluga whales, beluga whale research, Hg in the Arctic, and Hg toxicity. This chapter does not attempt to be an exhaustive review of the literature, and instead strives to provide the context for the research studies presented in this thesis.

Chapter 3 presents the first manuscript, "Mercury distribution and speciation in different brain regions of beluga whales (*Delphinapterus leucas*)", which is published in the journal *Science of the Total Environment* (Ostertag et al., 2013). The article provides analytical data on the distribution and speciation of Hg in five brain regions sampled from hunter-harvested beluga whales. Furthermore, the stoichiometric relationship between Hg and selenium (Se) was explored, and the predictability of mercury concentration in brain tissue based on Hg concentrations measured in more frequently sampled tissue (e.g. kidneys, liver, muktuk, muscle and blood).

Chapter 4 presents the second manuscript, "Mercury and selenium exposure is associated with molecular and neurochemical biomarkers in two brains regions of Arctic beluga whales (*Delphinapterus leucas*)". In this manuscript, I assessed the relationship between Hg concentration, speciation and co-accumulation with Se, with variation of neurochemical and

molecular components of the GABAergic and glutamatergic signaling pathways in beluga whales.

Chapter 5 presents the third manuscript, "Methylmercury and selenium exposure were associated with biomarkers of the cholinergic and dopaminergic signaling pathways in Arctic beluga whales (*Delphinapterus leucas*)". In this manuscript, I assessed the relationship between Hg concentration, speciation and co-accumulation with Se, with variation of neurochemical and molecular components of the cholinergic and dopaminergic signaling pathways.

Chapter 6 presents the fourth manuscript "Inuvialuit observations during harvesting activities linked mercury exposure to differences in beluga whale (*Delphinapterus leucas*) behaviour". In this manuscript, I documented local observations of beluga whale behaviour during harvesting activities, to assess whether differences in beluga whale behaviour were associated with mercury concentration.

Chapter 7 provides a summary of key findings from the four research papers, presents conclusions regarding the toxicological risk of Hg exposure for beluga whales from the eastern Beaufort Sea population, and discusses potential next steps for community-based monitoring in the ISR.

Chapter 2. Literature Review

1. Beluga Whales

1.a Background

Beluga whales (*Delpinapterus leucas*) have a semi-circumpolar distribution with significant populations inhabiting the northern coasts of Alaska, Canada, Greenland and Norway (Jefferson, 2008). The main diet of beluga whales in the western Canadian Arctic is fish, squid and invertebrates (Loseto et al., 2009; Loseto et al., 2008a). Worldwide, the population of beluga whales exceeds 150 000, with summering populations concentrated in western Hudson Bay and eastern Beaufort Sea (Jefferson, 2008). The eastern Beaufort Sea (EBS) beluga stock migrates seasonally to the southeastern Beaufort Sea and Amundsen Gulf; they are larger and older than animals harvested from eastern Arctic beluga populations (Luque and Ferguson, 2010).

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recognizes seven distinct populations of beluga whales in Canadian waters based on their summer distributions and genetic differences (COSEWIC, 2004). Population estimates of whales are based on aerial surveys followed by corrections for whales missed due to diving behaviour (O'Hammill et al., 2004). In the east, the estimated population of the St. Lawrence population is 900-1000, the Ungava Bay population is too small to estimate and the eastern Hudson Bay population numbers around 2000 individuals but is declining rapidly. The western Hudson Bay population is a minimum of approximately 23 000 animals, the eastern High Arctic – Baffin Bay population is estimated to be 20 000 animals, but it may be potentially two distinct populations: the west Greenland population numbering around 5000 belugas and the north Water population, which

numbers approximately 15 000 belugas. The Cumberland Sound population is made up of approximately 1500 animals (may have increased since the 1980s), and a conservative estimate of the EBS population is 39 000 animals. Genetic techniques have been used to distinguish stocks of belugas based on mitochondrial DNA; however, there is evidence that stocks mingle during their seasonal migrations and the summer distribution of whales in Hudson Bay do not reflect distinct stocks (DFO, 2001). The ranges of some beluga populations are known to overlap and the distinction between eastern and western Hudson Bay stocks is disputed by Inuit in Nunavik (O'Hammill et al., 2004; Tyrrell, 2007).

1.b Harvesting

Beluga whales are harvested for subsistence throughout their circumpolar range, excluding Svalbard. Commercial hunting of belugas in eastern Canada reduced their numbers and populations in the St. Lawrence Estuary and eastern Canadian Arctic, and many of these stocks have not fully recovered (DFO, 2001; DFO, 2007). Hunting of the High Arctic population in Greenland may be causing a significant decline in their population (Alvarez-Flores and Heide-Jorgensen, 2004). Commercial hunting is permitted in Greenland and the harvest rate has not declined, although the harvest has been regulated in recent years (Sejersen, 2001).

Based on the archeological record, beluga whales made up approximately half of the diet of pre-contact Inuit of the Mackenzie Delta (Friesen and Arnold, 1995). Beluga hunting typically occurs in the month of July, when beluga whales migrate through the warm waters of the Mackenzie Delta Estuary (Harwood and Smith, 2002). Inuvialuit beluga hunters commonly hunt

from 4.6 m long aluminum boats and harpoon the whale before killing it, to make retrieval easier (Harwood and Smith, 2002). Between 1990 and 1999, the total annual number of landed beluga whales on the shores of the Beaufort Sea and Amundsen Coast was 111 (Harwood and Smith, 2002). Beluga whales from this population are also harvested by residents of some coastal villages in Alaska (average 64 per year between 1995 and 2000) and possibly by residents of Chukotka, Russia (Harwood and Smith, 2002). Beluga whales travel through Kugmallit Bay in the Mackenzie River Estuary during their summer migrations (COSEWIC, 2004). Hunters from Tuktoyaktuk butcher beluga whales on Hendrickson Island following the hunt and generally return to Tuktoyaktuk immediately after butchering the whale to process the muktuk (skin and blubber) and mipku (dry meat).

1.c Beluga management in Canada

Local, regional and federal organizations manage the marine mammal resources in the three Canadian Inuit land claim regions in which belugas are harvested (table 2.1). In the Inuvialuit Settlement Region, the Fisheries Joint Management Committee (FJMC) was established under the Inuvialuit Final Agreement, in which the DFO and Hunters and Trappers Committees co-manage the fisheries and beluga populations (FJMC, 2001). The beluga management plan was developed by the FJMC to ensure that Inuvialuit can continue to harvest beluga whales from the Canadian Beaufort Sea, while ensuring the long-term sustainability of beluga in the Canadian Beaufort Sea" (FJMC, 2001). The St. Lawrence Estuary Beluga population is currently listed as *threatened* under Canada's Species at Risk Act; Fisheries and Oceans Canada has led the recovery strategy, and responsibility for the recovery of this population is shared between the

DFO, and other jurisdictions and agencies (DFO, 2012).

Table 2.1 Overview of management structure for beluga whales (*Delphinapterus leucas*) in Canada.

Region	Local	Regional	Federal	International
Inuvialuit Settlement Region	Hunters and Trappers Committees	Fisheries Joint Management Committee	Department of Fisheries and Oceans	Alaska and Inuvialuit Beluga Whale Committee
Nunavut	Hunters and Trappers Associations	Nunavut Wildlife Management Board		Canada/Greenland Joint Commission on the Conservation and Management of Narwhal and Beluga
Nunavik	Hunting Trapping and Fishing Association	Nunavik Marine Region Wildlife Board		N/A
Nunatsiavut	Hunters and Trappers Organization	Torngat Joint Fisheries Board		

1.d Traditional ecological knowledge

In recent decades, there has been increasing recognition that Indigenous knowledge could contribute to governance processes such as co-management and environmental impact assessments (Usher, 2000). This recognition was one of the outcomes of sustained advocacy, the negotiation of comprehensive land claims across the north, and the development of formal Environmental Impact Assessments and review processes, in addition to legal developments within the Supreme Court of Canada and lower court rulings (Usher, 2000). In northern Canada, Indigenous knowledge is recognized in the Northwest Territories as "a valid and essential source of information about the natural environment and its resources" (Territories, 2005). Internationally, specific recommendations to establish marine and Arctic programmes that

include the use of traditional ecological knowledge (TEK) for the conservation of biodiversity were presented during the workshop on traditional knowledge and biological diversity (Programme, 1997). More recently, efforts have been made in to integrate or bridge TEK with traditional scientific knowledge in Arctic ecological research (Gagnon and Berteaux, 2009; Gilchrist et al., 2005; Huntington et al., 2004).

There are many definitions of TEK, and one broad definition of TEK is "knowledge gathered and maintained by groups of people, based on intimate experience with their environment" (Huntington et al., 2004). Traditional ecological knowledge has provided valuable information about marine mammals in the Arctic (Carter and Nielsen, 2011; Ferguson et al., 2012). Inuit knowledge and wisdom about beluga whales is associated with decades of observations, and includes hunters' and elders' knowledge of beluga whale behaviour and predation (Byers and Roberts, 1995). Observations of beluga whale diving behaviour, feeding, migration, communication and response to disturbance were made by Indigenous hunters and elders of Chukotka, Russia while hunting beluga whales or pursuing walrus (*Odobenus rosmarus divergens*) and seals (*Phoca* spp. and *Erignathus barbatus)* (Mymrin et al., 1999).

1.e Beluga whale research in the ISR

Beluga whales have been sampled periodically since the 1970s in the Mackenzie Delta Estuary (Lockhart et al., 2005) in conjunction with beluga harvesting activities. The Pokiak family has sampled beluga whales for the DFO beluga-monitoring program since 2000 and whale monitors were hired annually by the FJMC to document information about harvested whales. The

Hendrickson Island Beluga Study (HIBS) began in 2008, to inform scientists, policy makers and Inuvialuit about the effects of contaminants and climate change on beluga health. The HIBS is one of several community-based monitoring programs in the Inuvialuit Settlement Region, NWT. The main goals of this study were to increase our understanding of beluga health, and to determine a baseline for beluga health as their summering habitat (Eastern Beaufort Sea) is undergoing changes.

The HIBS research team was composed of four researchers from government and university institutions: L. Loseto (Victoria, BC; Winnipeg, MB), M. Noel (Victoria, BC), S. Raverty (Abbotsford, BC) and S. Ostertag (Prince George, BC). During fieldwork, the research team worked alongside N. Pokiak and F. Pokiak (Tuktoyaktuk, NT), mentoring students and the local whale monitors. Students from Tuktoyaktuk and Inuvik, NT were hired in 2008, 2009, 2010 and 2011 to assist or lead with sampling and lab analyses. The research team collaborated with an extensive network of researchers including Gary Stern (Winnipeg, MB), Feiyue Wang (Winnipeg, MB), Marcos Lemes (Winnipeg, MB), Brian Laird (Ottawa, ON), Laurie Chan (Ottawa, ON), Lois Harwood (Yellowknife, NT), Ole Nielsen (Winnpeg, MB), Gregg Tomy (Winnipeg, MB), and Peter Ross (Victoria, BC).

2. Arctic Contaminants

2.a Background

Mercury is released into the environment from natural and anthropogenic sources; gaseous elemental Hg(0) is degassed from soils and surface waters, and released during the combustion of fossil fuels and incineration of waste (Council, 2000). Elemental Hg(0) is easily volatilized and may be transported for a year or more on wind currents, before it is oxidized to its divalent state (Hg(II)) and deposited in the environment (Martin et al., 2011). Although the Arctic lacks point sources of Hg emissions, up to 300 tonnes of Hg are transported annually to the Arctic from southern latitudes (AMAP, 2003; Outridge et al., 2008; Skov et al., 2004). Following deposition, methylating bacteria can convert bioavailable inorganic Hg(II) to the highly toxic monomethylmercury (MeHg) (Kirk et al., 2008; Lindberg et al., 2007). Methylmercury is readily absorbed by organisms, with approximately 95% of MeHg in fish being absorbed into the bloodstream of humans (Clarkson and Magos, 2006). Methylmercury biomagnifies in aquatic ecosystems, and there is a millionfold increase in MeHg concentration from seawater to top predators (Clarkson and Magos, 2006).

Mercury is transported to the Arctic from southern latitudes at a rate of 200 to 300 tonnes per year, from both anthropogenic and natural sources (Dietz et al., 2009). Mercury levels in Arctic biota are an order of magnitude higher today than in the pre-industrial period and approximately 74 to 94% of Hg in biota is estimated to originate from anthropogenic Hg emissions (Dietz et al., 2009). Mercury levels are consistently higher in biota from the western Canadian Arctic than the European Arctic (Riget et al., 2005). Concentrations of cadmium, lead and arsenic have also

increased in the Arctic due to anthropogenic activities including agriculture and the combustion of coal (Barrie et al., 1992).

Organic contaminants of concern in the Arctic include 'legacy' contaminants that were used in high volumes in the past and are now strictly regulated or banned (e.g. polychlorinated biphenyls, PCBs; dichlorodiphenyltrichloroethane, DDT), and current-use chemicals such as perfluorinated compounds (PFCs) and endosulfan (Barrie et al., 1992). Organic contaminants found in Arctic biota can be classified as industrial and commercial organic compounds (e.g. PCBs), chlorobenzenes, dioxins, PFCs and brominated flame retardants), organic pesticides (e.g. DDT, toxaphene, hexachlorocyclohexanes and chlordane) and polycyclic aromatic hydrocarbons (e.g. Benzo(a)pyrene) (Barrie et al., 1992; Letcher et al., 2010). Many OCs in the Arctic are now included in the Stockholm Convention due to their persistence, long-range transport and toxicity. From the 1970s to the 1990s, decreasing trends of most 'legacy' contaminants have been observed; however, emerging contaminants of concern have also surfaced (e.g. PFCs, brominated flame retardants) (Braune et al., 2005). In general, the concentration of OCs in marine mammals were found in the following decreasing order: $\Sigma PCB > \Sigma CHL \approx \Sigma PFSA > \Sigma CBz \approx \Sigma HCH \approx \Sigma Toxaphene \approx PFCA > \Sigma PBDE > HBCD$ (Letcher et al., 2010).

2.b Beluga whale exposure to mercury and organic contaminants

Environmental contaminants of primary concern for beluga health in the Arctic have been identified as organic pesticides, polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Fisk et al., 2005). Elevated Hg concentrations have been observed in marine mammals,

including beluga whales in the western Canadian Arctic compared to the eastern Canadian Arctic (Wagemann et al., 1998). Furthermore, a spike in age-adjusted Hg concentrations was observed in belugas from the Beaufort Sea in the 1990s (Braune et al., 2005). Although Hg concentrations have decreased in recent years in the eastern Beaufort Sea beluga population, Hg concentrations in this population of whales are significantly higher than concentrations observed in the 1980s (Lockhart et al., 2005). Furthermore, modern beluga whales have Hg concentrations that are 4- to 17- times higher than pre-industrial levels, according to the analysis of Hg in beluga whale teeth collected from archeological sites and harvest camps (Outridge et al., 2002; Outridge et al., 2009). However, Hg concentrations were consistently higher in belugas from the St. Lawrence Estuary (1.42 – 756 μg/g (n=35)) than the Arctic (0.04 – 182 μg/g (n=94); Beland et al., 1993).

Concentrations of OCs in Arctic belugas were highest in the eastern Canadian Arctic, east Greenland and Svalbard. Arctic beluga whales were exposed to PCBs (geometric mean, range for males only; 3690 (1250 - 10900) ng g^{-1} lw), DDT (2521 (695 – 9150) ng g^{-1} lw), cyclodienes (473 (48 – 4690) ng g^{-1} lw), chlorobenzenes (377 (149 – 957) ng g^{-1} lw), cyclodienes (473 (48 – 4690) ng g^{-1} lw), hexachlorocyclohexane (lindanes, 119 (32 – 440) ng g^{-1} lw) and polybrominated diphenyl ethers (34 (13-96) ng g^{-1} lw) (Kelly et al., 2008). In previous studies, OCs were consistently found at lower concentrations in brain tissue than in other organs (i.e. blubber, liver, kidney and muscle) of east Pacific gray whales (*Eschrichtius robustus*, (Krahn et al., 2001)), Black Sea harbor porpoises (*Phocoena phocoena relicta*, (Weijs et al., 2010)) and harbor seals (*Phoca vitulina*) from the southern coast of Norway (Bernhoft and Skaare, 1994). My thesis focused on the potential neurotoxicity associated with MeHg exposure for beluga whales because

'legacy' OC concentrations appear to be decreasing in Arctic biota (Braune et al., 2005), the blood-brain-barrier may reduce the transfer of OCs to the brain (Bernhoft and Skaare, 1994), and OC concentrations are lower in biota from the western Canadian Arctic than other regions of the Arctic (Letcher et al., 2010). Although concentrations of OCs in brain tissue of beluga whales in the Beaufort Sea may be relatively low and decreasing, OC exposure could be of neurotoxicological concern given the neurotoxicity of certain PCB congeners associated in particular with *in utero* and lactational exposure (Kodavanti, 2005).

2.c Policies

The 'Minamata Convention on Mercury' is a global, legally binding treaty developed to reduce Hg emissions to the environment. On January 19, 2013, governments agreed to the text of the Minamata Convention and on October 10, 2013 the treaty was adopted and signed by 93 countries. The temporal trends from the past decades are inconsistent in biota; however, there is strong evidence that Hg levels have increased from pre-industrial times to the present in some Arctic biota (Braune et al., 2005; Dietz et al., 2009). Although Hg emissions have been reduced in many countries in recent years, one new coal-burning energy plant is coming on line every week in India and China (Berry and Ralston, 2008), therefore anthropogenic mercury releases will continue to be of global concern.

3. Mercury toxicokinetics and toxicity

3.a Background

The half-life of MeHg in the body has been estimated to be approximately 44 d in humans, although the half-life of the body burden of Hg was 98 d (Smith et al., 1994). In the brain, the half-life of MeHg likely varies between species, but it has been estimated to be on the order of days or weeks for MeHg and months to years for iHg (Aschner and Aschner, 1990). Methylmercury is removed from the body by demethylation followed by excretion of inorganic Hg (iHg) in feces and urine (Smith et al., 1994). In whale livers, it has been observed that iHg may form granules made up of Hg and selenium (Se), to become inert (Lockhart et al., 2005).

Mercury neurotoxicity varies with its speciation (i.e. inorganic or MeHg) (Clarkson and Magos, 2006; Michalke et al., 2009) and the complexes it forms with other molecules (e.g. MeHg-cysteine or tiemannite). Methylmercury and iHg are both neurotoxic; however, Se may bind to iHg to form HgSe, a non-toxic Hg complex (Bjorkman et al., 1995). Demethylation of MeHg followed by the formation of compounds similar to mercury selenide (HgSe) was linked to successful detoxification of Hg-exposed humans (Korbas et al., 2010). Marine mammals and perhaps also some bird species are able to demethylate MeHg and immobilize it in the liver as HgSe (Riget et al., 2007). Previous studies have suggested that Se may be involved in a bio-transformation process in the brain, which may be initiated by the demethylation of MeHg followed by the formation of inert HgSe granules (Nigro and Leonzio, 1996). Therefore, Se may also reduce the toxicity of Hg by reducing oxidative stress associated with Hg exposure, and by

forming inert compounds with Hg and forming bis(methylmercury) selenide (Khan and Wang, 2010).

Modern day MeHg-poisoning events in Iraq and Japan increased our understanding of the neurotoxicological risks of MeHg exposure for adults and the developing fetus (Mergler et al., 2007), and similar outcomes have been observed in mammalian wildlife (Basu and Head, 2010). Methylmercury exposure has been linked to neurochemical disruption with resulting sensory and motor deficits (Sirois and Atchison, 1996). At a cellular level, Hg poisoning was associated with the loss of neuronal cells in the granular layer of the cerebellum and visual cortex of adult humans (Hunter and Russell, 1954). Clinical symptoms of Hg toxicity include cerebellar ataxia, constriction of the visual field and damage to the auditory region of the temporal lobe (Clarkson, 1997; Ekino et al., 2007). Clinical symptoms of Hg intoxication include the loss of motor coordination (Bellum et al., 2012), abnormal movements and convulsions (Takeuchi et al., 1977) and loss of balance (Farina et al., 2005). Macaque monkeys had impaired high-frequency hearing and visual damage following postnatal MeHg exposure (Newland and Paletz, 2000). Prenatal exposure to MeHg caused mental retardation, severe neurological impairments, and diffuse damage to the brain (Clarkson, 1997; Mergler et al., 2007). Exposure to MeHg *in utero* has been linked to neurobehavioural deficits and decreases in fine motor function in the Faroe Islands and the Seychelles longitudinal studies, respectively (Mergler et al., 2007).

Exposure to MeHg has been associated with neurochemical disruption in environmentally-exposed wildlife (see Table 2.2). Biomarkers of neurochemical variation associated with Hg

exposure have been used in previous wildlife studies on piscivorous fish and mammals to relate MeHg exposure to neurochemical endpoints. Methylmercury exposure in loons, eagles, mink, otters and polar bears was associated with changes in receptors (density and/or binding affinity) and enzyme activity: NMDA receptor decreased with increasing total Hg (Hg_T, ΣMeHg and iHg) levels in polar bears (Basu et al., 2009), wild mink (Basu et al., 2007c), loons and eagles (Scheuhammer et al., 2008); muscarinic receptor levels was positively correlated to Hg concentrations in wild mink (Basu et al., 2005a), loons and eagles (Scheuhammer et al., 2008) but the opposite relationship was found in river otters (Basu et al., 2005d). A negative correlation between Hg_T and the dopamine receptor was also observed in wild river otters and wild mink (Basu et al., 2005a). A negative relationship between $GABA_A$ receptor binding levels and Hg was observed in captive mink (Basu 2010), while monoamine oxidase and acetylcholinesterase activities were negatively correlated to brain Hg in wild river otters (Basu et al., 2007b).

Table 2.2 Variation of monoamine oxidase (MAO) activity, muscarinic acetylcholine receptor (mAChR), *gamma*-aminobutyric acid receptor (GABA-R) and N-methyl-*ID*-aspartate receptor (NMDA-R) associated with MeHg exposure in select mammalian and avian species.

Animal	MAO	mAChR	GABA-R	NMDA-R	Reference
Mink (captive) (*Mustela vison*)	-	↗[a]	↘	↘	(Basu et al., 2008; Basu et al., 2010; Basu et al., 2007c)
Wild mink (*mustela vison*)	-	↗	-	↘	(Basu et al., 2005a; Basu et al., 2007c)
River otter (*Lontra cadeis*)	↘	↘	-	-	(Basu et al., 2005d; Basu et al., 2007b)
Polar bear (*Ursus maritimus*)	-	-	-	↘	(Basu et al., 2009)
Bald eagle (*Haliaeetus leucocephalus*)	-	↗	-	↘	(Rutkiewicz et al., 2011; Scheuhammer et al., 2008)
Common loon (*Gavia immer*)	-	↗ ns	-	↘ ns	(Hamilton et al., 2011; Scheuhammer et al., 2008)
Herring gulls (*Larus argentatus*)	-	ns	-	ns	(Rutkiewicz et al., 2010)
Little brown bats (*Myotis lucifugus*)	↘[1] ↗[2]	-	-	↘	(Nam et al., 2010)
Spotted seatrout (*Cynoscion nebulosus*)	-	-	-	-	(Adams et al., 2010)
Salmon (*Salmo salar L.*)	↘	-	-	-	(Berntssen et al., 2003)

1 = contaminated site; 2 = non contaminated site;
a = m1 and m2 subunits for mAChR

3.b Interaction of MeHg and iHg with neurochemical signaling pathways

Previous studies have suggested that the interaction of MeHg or iHg with cysteine residues may inhibit, stimulate or damage components of the dopaminergic (Gomes et al., 1976), cholinergic (Castoldi et al., 1996), γ-aminobutyric acid (GABA) (Huang and Narahashi, 1997; Narahashi et al., 1994), and glutamatergic (Albrecht and Matyja, 1996) signaling pathways. The following subsections provide a brief background about the cholinergic, dopaminergic, GABA-ergic, and glutamatergic signaling pathways, including an overview of potential interactions of MeHg and iHg with select components of these signaling pathways.

3.b.1 Cholinergic signaling pathway

Cholinergic signaling pathways have been linked to essential physiological processes including learning, memory, stress response and modulation of sensory information (Reis et al., 2009). The muscarinic acetylcholine receptor (mAChR) may play a critical role in physiological processes including thermoregulation, motor function and feeding (Bymaster et al., 2003; Wess, 2004). There are five mAChR subtypes (m1-m5) in mammals, and they each have distinct pharmacological and functional properties (Wess, 1996). The distribution of mAChR subtypes is heterogeneous in mammalian brains, with m1 abundance greater in the forebrain (i.e. cerebrum) and m2 more abundant in the hindbrain (i.e. cerebellum) (Wess, 1996). There are differences in signaling associated with the receptor subtypes. In particular, m1, m3 and m5 are coupled to G proteins of the $G_{q/11}$ family, which mediate the activation of phospholipase C and subsequent release of Ca^{2+}; the m2 and m4 mAChR subtypes are selectively linked to G-proteins of the $G_{i/o}$ class, which mediate the inhibition of adenylyl cyclase but are not linked to Ca^{2+} release (Wess, 1996).

Methylmercury has a high affinity for sulfhydryl groups and was found to inhibit agonist binding to m1 and m2 muscarinic receptors in rat brain cortical membranes (Castoldi et al., 1996). The m1 subtype may be more sensitive than m2 to Hg exposure based on *in vivo* and *in vitro* experiments (Basu et al., 2008; Castoldi et al., 1996). Mercuric chloride and MeHg may modify mAChR activity by binding to the binding site of the mAChR and competitively inhibiting ligand- binding (Abd-Elfattah and Shamoo, 1981). The binding of MeHg to mAChR has been linked to disruption of Ca^{2+} homeostasis in cerebellar granule cells, and has been suggested as a cause of cell-regulated death (apoptosis) or the downregulation of mAChR (Limke et al., 2004). Furthermore, inhibiting binding of the m3 mAChR reduced the effect of MeHg on the amplitude of Ca^{2+} elevations (Limke et al., 2004). Methylmercury and Hg^{2+} inhibited radioligand binding (*in vitro*) to the mAChR receptor in ringed seal brain homogenate (Basu et al., 2006a). An increase in mAChR density was observed in rat hippocampus and cerebellum following chronic exposure to low doses of MeHg, which may have been due to the inhibition of acetylcholine synthesis and the competitive antagonism of MeHg at the receptors (Castoldi et al., 2003).

3.b.2 Dopaminergic signaling pathway

Dopamine plays an important role in cognition, emotion, memory processes and learning (Dalley and Everitt, 2009). Monoamine oxidase (MAO) is present in two forms in the brain, and is responsible for the oxidative deamination of a number of biogenic amines including dopamine (Shih et al., 1999). Two forms of MAO exist, MAO-A has higher affinity for the substrates serotonin, norepinephrine, dopamine, and the inhibitor clorgyline, whereas MAO-B has higher affinity for phenylethylamine, benzylamine, and the inhibitor deprenyl (Shih et al., 1999). Both

MAO-A and B are located throughout the brain in the outer membrane of mitochondria (Green and Youdim, 1975) and are encoded by different genes (Bach et al., 1988; Grimsby et al., 1991). Methylmercury may exert an effect on MAO either by directly binding to thiol groups on the enzyme (Chakrabarti et al., 1998) or by altering mitochondrial function (Komulainen et al., 1995).

3.b.3 The GABA-ergic signaling pathway

The $GABA_A$ receptors form GABA-gated chloride ion channels that are responsible for inhibitory synaptic transmission (Penschuck et al., 1999). The major $GABA_A$ receptor subtypes are made up of a pentameric assembly of distinct subunits (α, β and $\gamma 2$) including six unique α subunits (α_1- α_6) that have been cloned to date (Vicini and Ortinski, 2004). The decarboxylation of glutamate gives rise to GABA, which is an inhibitory neurotransmitter. $GABA_A$ receptors are associated with sedation, sleep induction, and myorelaxation, as well as anxiety, seizures and amnesia (Vicini and Ortinski, 2004). Furthermore, the benzodiazepine binding site on the $GABA_A$ receptor modulates the activity of the $GABA_A$ receptor (Chebib and Johnston, 1999).

In cell cultures, MeHg chloride and mercury chloride ($HgCl_2$) inhibited the uptake of GABA by cultured astrocytes from newborn rat cerebral cortex (Brookes and Kristt, 1989) and $HgCl_2$ increased the affinity of $GABA_A$ receptor for GABA (Huang and Narahashi, 1996). Mercury was found to modulate GABA-induced inward currents: $HgCl_2$ induced a larger inward current and MeHg suppressed this inward current (Arakawa et al., 1991; Narahashi et al., 1994). In cerebellar granule cells in culture, inhibition of $GABA_A$ receptor-mediated currents was observed when cells were exposed to 0.1 uM MeHg (Yuan et al., 2005). Following MeHg

exposure, a decrease in $GABA_A$ receptor levels could occur to maintain homeostasis of GABAergic signaling following Hg exposure (Basu et al., 2010). Decreased $GABA_A$ receptor levels occur in neurons following exposure to GABA and various $GABA_A$ receptor agonists (Barnes, 1996). Inhibition of the $GABA_A$ receptor could potentially lead to an excitatory effect (Sunol et al., 2008); therefore, receptors and enzymes that mediate GABAergic signaling are tightly regulated to protect neurons from excitotoxicity (Reis et al., 2009). Regulation of $GABA_A$ receptors may include desensitization of the receptor, endocytosis and degradation of subunit polypeptides and the repression of subunit gene expression (Barnes, 1996).

3.b.4 Glutamatergic signaling pathway

The N-methyl-D-aspartate receptor (NMDA-R) is a separate class of glutamate-gated ion channels, and is activated by the artificial glutamate analogue N-methyl-D-aspartate. Glutamate is thought to be one of the major excitatory transmitters in the brain and may be used by up to 40% of synapses (Coyle and Puttfarcken, 1993). NMDA receptors are double-gated and open only when two conditions are met simultaneously; glutamate must be bound to the receptor and the membrane must be strongly depolarized (Johnson and Ascher, 1987). NMDA receptors are critical for long term potentiation (Harris et al., 1984); however, overstimulation of the NMDA-R can cause neuronal excitotoxicity, and has been linked to the loss of neurons, Alzheimer's disease, and other neurodegenerative diseases (Cull-Candy et al., 2001).

A glutamate-mediated excitotoxic mechanism of MeHg neurotoxicity is supported by several studies. Methylmercury induced an increase in extracellular glutamate (Juarez et al., 2002); the

activation of NMDA receptors was found to play a key role in glutamate-associated excitotoxicity of astrocyte cultures (Albrecht and Matyja, 1996). The NMDA receptor was linked to increased release of dopamine following MeHg and iHg exposure *in vivo,* and the inhibition of nitric oxide synthase (NOS) activity reduced the release of dopamine (Faro et al., 2002; Vidal et al., 2007). Astrocytes play a key role in regulating the transport and clearance of neurotransmitters in the synaptic space (Fitsanakis and Aschner, 2005).

Excitotoxicity can be caused by the overstimulation of the NMDA receptor (Coyle and Puttfarcken, 1993); therefore, MeHg-associated increases in extracellular glutamate could have implications for neurotoxicity. Furthermore, the NMDA receptor was linked to DNA damage with MeHg exposure *in vivo* (Juarez et al., 2005), and increased Ca^{2+} influx and apoptosis of cerebellar granule cells following Hg^{2+} exposure (Rossi et al., 1997).

4. Assessing potential toxicity

4.a Characterizing the risk of toxicity in wildlife

In general, two approaches have been taken to identify and characterize the risk of toxicity associated with contaminants in Arctic species (Dietz et al., 2013). The first approach used a comparison of the concentration of contaminants in Arctic species and toxicity thresholds or known levels of toxicity. The extrapolation of risk was based on threshold levels that are usually taken from laboratory studies or field studies. Challenges with extrapolation include the differences in exposure regimes between laboratory animals and wildlife (e.g. chronic vs acute, short term vs long-term, single contaminant vs mixtures of contaminants), inter-species

differences in sensitivity, and differences in additional stressors faced by free-ranging animals (Dietz et al., 2013). The second approach investigated potential toxic effects by studying the response of biomarkers (indicators of biological response) to contaminants (Skaare et al., 2002). Although biomarkers can involve any biological change from a molecular to ecosystem level, the term generally refers to changes at lower levels of biological organization that are associated with exposure to contaminants (Skaare et al., 2002).

To identify potential behavioural effects of MeHg exposure, contaminant exposure data must be linked to whole-animal observations. Inuit observe beluga whales during harvesting activities and travel, and thereby gain substantial knowledge about these animals (Byers and Roberts, 1995; Mymrin et al., 1999). Collecting information about beluga whales using both traditional scientific knowledge (TSK) and traditional ecological knowledge (TEK) could provide the opportunity to compare observations made at different scales (i.e. chemical and whole-animal) (Huntington et al., 2004).

4 b. Methods

Given the sensitivity of the central nervous system (CNS) to the neurotoxic effects of Hg, it is relevant to determine Hg concentrations, distribution, speciation and potential detoxification in the CNS to assess health risks due to MeHg exposure. Analysis of potential neurochemical disruption associated with MeHg exposure in beluga whales would provide much needed information about potential risk of current MeHg exposure levels for these cetaceans. Finally,

integrating TSK and TEK could provide evidence of a physiological effect of MeHg exposure on beluga whales. The methods used for the studies presented in this thesis are outlined below.

4.b.1 Radioligand binding assays

Radioligand binding assays use a ligand that is labeled with a radioactive isotope (e.g. ^{3}H, ^{125}I or ^{35}S) that binds to a receptor to make detection of the receptor possible (Leysen et al., 2010). Either homogenized or sliced tissue, or cells in culture can be used for these assays. The tissue preparation is first incubated with the labeled ligand, and the bound labeled ligand is collected and detected using various techniques (e.g. filtration techniques combined with radioactivity counting) (Leysen et al., 2010). For ^{3}H-labeled ligands, a liquid scintillation cocktail is added to the filters, the energy emitted by the radioisotopes first excites the aromatic solvent molecules in the cocktail, and the fluor molecules absorb and re-emit this energy, which is detected by a photomultiplier detector (Staff, 2004). To determine the maximum number of receptor binding sites in the tissue preparation (B_{max}), the specific binding is calculated by subtracting the non-specific binding from the total binding (Leysen et al., 2010). The k_d value is equal to half of the maximum binding, and is calculated by nonlinear regression using a curve fitting program (e.g. GraphPad Prism; GraphPad software, La Jolla, USA) (Leysen et al., 2010).

4.b.2 Monoamine oxidase assay

Monoamine oxidase activity may be quantified through a horseradish peroxidase-coupled reaction using Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine). Briefly, tyramine is a

substrate for both MAO-A and MAO-B, and the oxidation of tyramine by MAO produces hydrogen peroxide. Amplex Red combined with horseradish peroxidase reacts with hydrogen peroxide in a 1:1 stoichiometry, to produce the fluorescent compound resorufin (Held, 2003). Quantification of resorufin produced occurs via fluorescence spectroscopy, which records the excitation and emission spectra (Lakowicz, 2006). A microplate reader uses a xenon flash lamp as a light source, and the specific excitation wavelength is selected with a monochromator (Lakowicz, 2006). A mirror with a hole is used to transmit the excitation and also to direct the fluorescence toward the detector optics (Lakowicz, 2006).

4.b.3 Real Time Polymerase Chain Reaction

Reverse transcriptase is a ribonucleic acid (RNA)-dependent DNA polymerase that synthesizes DNA complementary to an RNA template, using a mixture of the four deoxyribonucleotides in the presence of a short oligonucleotide primer or random hexamers (Rapley, 2010). Real time Polymerase Chain Reaction (RT PCR) generates an exponential increase in copies of a DNA template, which can be quantified based on the relationship between the amount of starting target and the amount of PCR product (Arya et al., 2005). Specific detection of PCR product (amplicons) is carried out based on two available chemistries: double-stranded DNA (dsDNA) binding dyes and fluorescent probes (Arya et al., 2005). In the case of DNA-binding dyes, SYBR® Green 1 binds to dsDNA and emits a strong signal (Arya et al., 2005). The fluorescence signal increases during polymerization and decreases upon denaturing; therefore, measurement of fluorescence occurs at the end of the elongation step of each PCR cycle (Arya et al., 2005). SYBR® Green 1 binds all dsDNA; therefore, amplification of non-specific PCR products and

primer-dimers reduces the specificity of the assay (Arya et al., 2005). Fluorescent probes (e.g. TaqMan probes) can be used in combination with specific forward and reverse primers; fluorescence emission increases during PCR amplification when the probe anneals to the target and Taq polymerase cleaves the probe (Arya et al., 2005).

Absolute quantitation depends on quantifying the initial number of target copies through the use of a standard curve (Livak and Schmittgen, 2001). The Pfaffl method provides a model for relative quantification of a target gene transcript in comparison to a reference gene transcript (Pfaffl, 2001). With this method, PCR efficiency (E) for each target and reference gene is calculated based on the crossing point (CP) cycle number and the input of cDNA (ng), according to the equation: $E = 10^{(-1/slope)}$ (Pfaffl, 2001). The ratio is defined as the expression of a target gene in the sample versus the control, compared to the reference gene: ratio = $(E_{target})^{\Delta CP\ target\ (control - sample)}/(E_{reference})^{\Delta CP\ ref\ (control-sample)}$. The comparative threshold method is another commonly used method for calculating relative changes in gene expression, and does not require a standard curve (Arya et al., 2005). The $2^{-\Delta\Delta Ct}$ is calculated based on the relative expression of the target compared to the reference gene or calibrator ($-\Delta\Delta Ct = \Delta Ct$ (sample) - ΔCt (control)) (Arya et al., 2005). This method requires similar amplification efficiencies for the target and reference genes (Arya et al., 2005). An internal reference gene or calibrator is required to minimize the errors associated with the starting amount of RNA, the quality of RNA, differences in cDNA synthesis efficiency and PCR amplification (Arya et al., 2005). Reference genes must be selected to normalize the RNA values, and their expression should be consistent at all stages of development and throughout different experimental conditions (Arya et al., 2005). Typically, housekeeping

genes such as beta-actin, glyceraldehyde-3-phosphate dehydrogenase and ribosomal RNA are used for normalization (Arya et al., 2005).

4.b.4 Traditional ecological knowledge questionnaires

There are many definitions of TEK, and one broad definition of TEK is "knowledge gathered and maintained by groups of people, based on intimate experience with their environment" (Huntington et al., 2004). Questionnaires have been identified as one of many methods for documenting TEK (Huntington, 2000). The strength of questionnaires is that they provide consistency and allow comparisons to be made between respondents and over time; however, semi-directed interviews provide a greater depth and breadth of knowledge and may reveal unanticipated information (Huntington, 2000). Traditional ecological knowledge has provided valuable information about marine mammals in the Arctic (Carter and Nielsen, 2011; Ferguson et al., 2012). Inuit knowledge and wisdom about beluga whales is associated with decades of observations, and includes hunters' and elders' knowledge of beluga whale behaviour and predation (Byers and Roberts, 1995).

5. Responsibility and accountability in northern research

Research that takes place in the north must abide by a number of ethical principles (ACUNS, 2003; ITK and NRI, 2007) and regulatory requirements. In particular, Inuit communities expect meaningful consultation, inclusion and communication throughout the research process (ITK and NRI, 2007). Reporting of research results is of particular concern among Inuit communities,

especially the timing and formatting of researchers' reporting procedures (ITK and NRI, 2007). In fact, early and frequent communication, local training, and community participation in research activities were identified as being critical for building trusting research relationships in natural science research in Nunavut (Gearheard and Shirley, 2007). This may be of even more importance in beluga whale research, given the high cultural and nutritional value of beluga for many Inuit. The significance of beluga extends beyond the consumption of meat and muktuk, and includes the whale-hunting complex (Tyrrell, 2007), and food-sharing, which reinforces social relations (Condon et al., 1995). In general, whales play an important role in maintaining Inuit identities, culture and well-being (Freeman et al., 1998). Traditional harvesting activities are also linked to Inuit well-being: 'the land teaches us not only the technical skills of aiming a gun or harpoon or skinning a seal, but also what is required to survive, giving confidence to our people' (Watt-Cloutier, 2003).

Chapter 3. Mercury distribution and speciation in different brain regions of beluga whales (*Delphinapterus leucas*)

Names and Affiliations of Authors:

1. Sonja K. Ostertag
Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, British Columbia, Canada, V2N 4Z9
ostertag@unbc.ca

2. Gary A. Stern
Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, Manitoba, Canada, R3T 2N6
Centre for Earth Observation Science, Department of Environment and Geography, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2
Gary.Stern@dfo-mpo.gc.ca

3. Feiyue Wang
Centre for Earth Observation Science, Department of Environment and Geography and Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2
feiyue.wang@ad.umanitoba.ca

4. Marcos Lemes
Centre for Earth Observation Science, Department of Environment and Geography, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2
Marcos.lemes@ad.umanitoba.ca

5. Hing Man Chan
Center for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5
laurie.chan@uottawa.ca

Abstract

The toxicokinetics of mercury (Hg) in key species of Arctic ecosystem are poorly understood. We sampled five brain regions (frontal lobe, temporal lobe, cerebellum, brain stem and spinal cord) from beluga whales (*Delphinapterus leucas*) harvested in 2006, 2008, and 2010 from the eastern Beaufort Sea, Canada, and measured total mercury (HgT) and total selenium (SeT) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or Hg analyzer, and the chemical forms using a HPLC-ICP-MS. At least 14% of the beluga whales had HgT concentrations higher than levels of observable adverse effect (6.0 mg kg^{-1} wet weight (ww)) in primates. The concentrations of HgT differed between brain regions; median concentrations (mg kg^{-1} ww) were 2.34 (0.06 to 22.6, 81) (range, n) in temporal lobe, 1.84 (0.12 to 21.9, 77) in frontal lobe, 1.84 (0.05 to 16.9, 83) in cerebellum, 1.25 (0.02 to 11.1, 77) in spinal cord and 1.32 (0.13 to 15.2, 39) in brain stem. Total Hg concentrations in the cerebellum increased with age ($p < 0.05$). Between 35 and 45% of HgT was water-soluble, of which, 32 to 41% was methylmercury (MeHg) and 59 to 68% was labile inorganic Hg. The concentration of MeHg (range: 0.03 to 1.05 mg kg^{-1} ww) was positively correlated with HgT concentration, and the percent MeHg (4 to 109%) decreased exponentially with increasing HgT concentration in the spinal cord, cerebellum, frontal lobe and temporal lobe. There was a positive correlation between SeT and HgT in all brain regions ($p < 0.05$) suggesting that Se may play a role in the detoxification of Hg in the brain. The concentration of HgT in cerebellum was significantly correlated with HgT in other organs. Therefore, HgT concentrations in organs that are frequently sampled in bio-monitoring studies could be used to estimate HgT concentrations in the cerebellum, which is the target organ of MeHg toxicity.

Introduction

Beluga whales (*Delpinapterus leucas*) have a semi-circumpolar distribution with significant populations inhabiting the northern coasts of Alaska, Canada, Greenland and Norway (Jefferson, 2008). Worldwide, the population of beluga whales exceeds 150 000, with summering populations concentrated in western Hudson Bay and eastern Beaufort Sea (Jefferson, 2008). The eastern Beaufort Sea beluga stock is known to migrate seasonally to the southeastern Beaufort Sea and Amundsen Gulf (Figure 3.1); they are larger and older than animals harvested from eastern Arctic beluga populations (Luque and Ferguson, 2010). The main diet of beluga whale is fish, squid and invertebrates (Loseto et al., 2009; Loseto et al., 2008a). Beluga whales can accumulate high concentrations of organic and metal contaminants due to their trophic position (Dietz et al., 2000; Lockhart et al., 2005; Stern et al., 2005) and long lifespans (Luque and Ferguson, 2010).

Mercury (Hg) is a global pollutant and a potent neurotoxicant (Gladden et al., 1999). Elevated concentrations of Hg (> 1 mg kg^{-1} ww) were reported in brain tissue of beluga collected in the western Arctic from 1998 to 2002 (Lockhart et al., 2005). Mercury exposure has been associated with neurochemical disruption in environmentally-exposed wildlife (Basu et al., 2005b; Basu et al., 2005d; Basu et al., 2009; Scheuhammer et al., 2008). Therefore, beluga whales may be at risk of Hg-related neurochemical disruption.

Mercury concentrations in the Arctic have increased significantly due to anthropogenic activities, and current estimates suggest that 72 to 94 % of Hg in biota in the Arctic comes from

anthropogenic emissions (Dietz et al., 2009). A previous study of beluga teeth collected from recent harvests, and archeological sites in the Inuvialuit Settlement Region (ISR), NT, Canada, indicated that Hg concentrations have increased by an order of magnitude since the 16th century, with Hg concentrations in older animals harvested in 1993 estimated to be 16.7 times greater than expected in their pre-industrial counterparts (Outridge et al., 2002). The risk of increased Hg exposure in beluga whales is poorly understood. As beluga whale is an important part of the Inuit diet, the well-being of the beluga whale population has implications for food security and public health (Wesche and Chan, 2010).

To assess toxicokinetics associated with Hg exposure in beluga whales, it is necessary to determine the concentrations, speciation and distribution of Hg in the brains of exposed animals. Beluga whales are primarily exposed to methylmercury (MeHg) from the diet (Loseto et al., 2008b), which is the most neurotoxic Hg species (Yokel et al., 2006). More than 95% of ingested MeHg is absorbed (Aberg et al., 1969) and may cross the blood brain barrier via neutral amino acid transporters (Aschner and Aschner, 1990). Methylmercury may undergo demethylation in the brain (Shapiro and Chan, 2008; Yokel et al., 2006), and co-exposure to selenium (Se) and Hg may increase the rates of MeHg uptake, demethylation, and inorganic Hg (iHg) retention in the brain(Bjorkman et al., 1995; Magos and Webb, 1980; Newland et al., 2006).

In this study, we analyzed Hg concentration, distribution and speciation in beluga tissue samples from different brain regions in order to assess the toxicological significance of current Hg and MeHg concentrations in the Arctic beluga whale populations.

Material and methods

Sample Collection

Five brain regions from harvested beluga whales were sampled at Hendrickson Island between June 30 and July 25 in 2006, 2008 and 2010, and at East Whitefish station in 2006 (Figure 3.1). These locations are two traditional beluga-hunting camps in Kugmallit Bay (KB), Inuvialuit Settlement Region (ISR), NT. Prior to each sampling season, a research permit and scientific license to fish were obtained from the Aurora Research Institute and Department of Fisheries and Oceans. The sampling program was approved and supported by the Tuktoyaktuk and Inuvik Hunters and Trappers Committees for all sampling years.

Grey matter (cortex) from the cerebellum (C), frontal lobe (FL), and temporal lobe (TL), and white matter from the brain stem (BS; only in 2010) and spinal cord (SC) were dissected and placed into pre-weighed, acid-washed scintillation vials. Whole brains were collected and frozen in the field in 2006 ($n = 46$) and later dissected at UNBC; in 2008 ($n = 24$) and 2010 ($n = 15$), samples were dissected in the field and frozen immediately (approx -20 °C). Whole blood was collected into BD Vacutainer® blood collection tubes (with K_2EDTA; Becton Dickinson) from the neck of harvested whales in 2008. Kidney, muscle, muktuk (skin and fat), and liver samples were collected on Hendrickson Island and frozen on site in a portable freezer at -20 °C. Animal age was estimated from a thin section of a tooth by counting individual growth layer groups in the dentine at the Freshwater Institute, Winnipeg, Manitoba, Canada (Stewart, 2006).

Sample analysis

Brain samples were freeze-dried for 72 hours at -80 °C and homogenized individually with a glass rod. Blood samples were thawed at room temperature and vortexed thoroughly prior to analysis. Total Hg (Hg_T) in blood and 2008 brain samples were measured using a total mercury analyzer (MA-2000, Nippon Instruments Corp., Osaka, Japan) and the methods are described in more detail elsewhere (Basu et al., 2005d). In brief, ~10 mg freeze-dried sample or 100 µL blood (29 v/v % in RNA preservative solution; 70% ammonium sulfate (Omnipur), 25 mM sodium citrate (Omnipur), 10 mM EDTA disodium salt dihydrate (Omnipur) was embedded in carbonate powder (sodium carbonate (Sigma Aldrich), calcium hydroxide (Fisher) and activated alumina (Nippon Instruments Corp.), followed by thermal decomposition at 800 °C for six minutes. Mercury vapour was then trapped by gold amalgamation, thermally desorbed and then measured by cold-vapour atomic absorption spectrometry (wavelength = 253.7 nm). Recovery of Hg_T in the standard reference material (DOLT-4; Dogfish Liver Certified Reference Material for Trace Metals; National Research Council) was 98.8 ± 0.7% of the certified value and Hg_T in blanks samples were -0.02 ± 0.06 ng mg^{-1} for a 10 mg sample. Total Hg concentration data from the MA-2000 were correlated to values obtained following acid digestion ($r = 0.99$, $p < 0.05$), and measured with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as described below.

Total Hg and total selenium (Se_T) in all brain samples (2006, 2008 and 2010) were analyzed by ICP-MS (Agilent Technologies, 7500 CX) following a modified acid digestion (Armstrong and Uthe, 1971). In brief, ~10 mg dry weight (dw) homogenized sample was rinsed with acetone

(Omnisolv) to remove lipids, and digested using trace metal grades nitric acid (Fisher), hydrochloric acid (Sigma) and H_2O_2 (Fisher), and heating at 100 °C for five hours. Total Hg (average of mass 200, 201 and 202) and Se_T (mass 82) were quantified by ICP-MS under the following parameters: plasma power 1550 W, nebulizer gas flow rate 1.05 L/min, Micromist Nebulizer (Glass Expansion™) flow rate 0.4 mL/min with a standard quartz 2.5 mm torch injector. Quantification was based on a four-point calibration curve, using ^{115}In and ^{193}Ir as internal standards for Se and Hg, respectively. Quality assurance/control (QA/QC) was monitored by including one blank, DOLT-4 in triplicate, and one sample in triplicate within each batch of 36 samples. Recovery of Hg_T and Se_T were 88 ± 1.6 and 92 ± 2.6 %, respectively (*n = 18*); Hg_T and Se_T concentrations in the blanks were 0.30 ± 0.05 mg kg^{-1} dry weight (dw) and 0.21 ± 0.06 mg kg^{-1} dw for a 10 mg sample, respectively (*n = 17*). Detection limits were 0.001 and 0.01 mg kg^{-1} dw for a 10 mg sample, for Hg_T and Se_T, respectively.

Mercury speciation analysis was carried out following the method of Krey et al. (2012). In brief, Hg species were extracted in triplicate from approximately 10 mg dw sample using a solution made up of 0.1% hydrochloric acid, 0.1% 2-mercaptoethanol (Sigma), and 0.15% potassium chloride (Sigma) in ultrapure H_2O. Samples were sonicated for 30 minutes at 35 °C. Mercury species were collected from the sample by centrifugation at 3000 rpm for ten minutes at 30 °C, the supernatant was saved and the pellet was rinsed by centrifugation with extraction solution (same procedures as before). The combined supernatants were centrifuged at 4000 rpm for 10 minutes at 24 °C, filtered through a 0.45 μm filter and analyzed using HPLC (Agilent 1200 series HPLC system, Agilent Technologies Canada Inc., Mississauga, ON, Canada), equipped with

autosampler, quaternary pump, and 100 ml injection loop. A ZORBAX Eclipse XDB-C18 Column (2.1 x 50 mm, 5 μm) was connected to an ICP-MS (Agilent Technologies^{TM}, 7500 CX). Fifty μL of sample was injected into the guard column at 0.45 mL/min; the mobile phase used was an isocratic mixture of 94% mobile phase (0.1% (v/v) 2-mercaptoethanol and 0.06 mol L^{-1} ammonium acetate (Fluka) plus 6% methanol (Sigma). The instrument parameters were as follows: plasma power (1550 W), nebulizer gas flow rate (Argon, 1.05 L/min), micromist nebulizer (Glass Expansion^{TM}) and standard quartz 2.5 mm torch injector. Acquisition was carried out over 500 seconds and Retention Times (RT) were 1.78 and 2.14 for MeHg and Hg, respectively; Hg isotope *m/z* monitored was 202, with ^{193}Ir used as an internal standard. Quantification was based on five point external calibration curves for Hg and MeHg. With this method, the labile fraction of iHg (iHg_{labile}) bound weakly to proteins or thiols was extracted; however, Hg associated with strong structural proteins (Hg_{bound}) may not be extracted efficiently with this method (Wang et al., 2007). One blank and DOLT-4 in triplicate were included in each sample batch (n = 33) to ensure quality within and between batches. Recovery of total MeHg and iHg ($MeHg_T$ and iHg_T) in DOLT-4 were 130 ± 4.7% and 98.9 ± 5.8 % (n = *28*), respectively. Both $MeHg_T$ and iHg_T concentrations were below the level of detection in all blanks analyzed (n = *7*). The concentration of Hg_{bound} was estimated by subtracting the concentration of $MeHg_T$ and iHg_{labile} from the concentration of Hg_T (Equation 1).

$$[Hg_{bound}] = [Hg_T] - ([MeHg_T] + [iHg_{labile}]) \qquad \text{(Eq 1)}$$

The temporal lobe samples collected in 2008 were further analyzed for MeHg and Se speciation at the Ultra-Clean Trace Elements Laboratory at the University of Manitoba, as detailed

elsewhere (Lemes and Wang, 2009; 2011). In brief, 100 mg of freeze-dried brain tissue was extracted, in a 15-ml polypropylene centrifuge tube, with 20 mg trypsin in a 10-ml acetate buffer solution (0.05 M ammonium acetate with 0.5% sodium dodecyl sulphate; pH = 8). The extraction was carried out at 37 ^{o}C inside an Isotemp oven (Fisher Scientific) on a tube rotator with rotisserie at 20 rpm for 4 h in the dark to prevent any possible photochemical reaction during the process. The extractant was centrifuged and the supernatant was decanted and filtered through a 0.2 μm pore-size hydrophilic polypropylene membrane (Pall), and diluted 10 times with ultrapure water. The diluted extractant was then analyzed for MeHg and Se speciation using reversed phase HPLC-ICP-MS (Lemes et al., 2011).

Quality assurance and quality control of the MeHg speciation analysis was monitored by analyzing the certified reference material DOLT-3 (CRM; dogfish liver, National Research Council of Canada). Speciation analysis showed that MeHg in DOLT-3 was present exclusively as $CH_3HgSCys$ with a concentration of 1.41± 0.16 mg/g (dry wt; n=9), which agreed very well with the certified $MeHg_T$ value of 1.59 ± 0.12 mg/g (dry wt). Because no CRM was available for individual MeHg species, further quality assurance/quality control for the MeHg speciation analysis in the beluga temporal lobe tissue samples was performed by comparing the sum of all the MeHg species ($MeHg_\Sigma$) against the $MeHg_T$ concentration analyzed independently by HPLC-ICP-MS, as described above. The temporal lobe showed a good agreement for $MeHg_\Sigma$ / $MeHg_T$ = 75.2 ± 20.5 % (n=10) (Lemes et al., 2011).

The analytical method for selenium speciation monitored the following analytes: inorganic selenite Se(IV) and selenate Se(VI)), and organic selenomethionine (SeMet), methylselenocysteine (CH_3SeCys), and selenocystine (CysSeSeCys). Details can be found elsewhere (Hu et al., 2009).

As shown in Figure 3.2 below, some of the speciation chromatograms showed a slight shift in the retention time. This was because analyses were done on different days with different batches of columns and mobile phases. However, the retention times of MeHg species were corrected on a daily basis using the standards before and after the sample analysis.

For total Hg analysis of kidney, liver, muktuk, and muscle, samples were weighed to approximately 0.15 g wet weight (ww). Samples were digested with a hydrochloric/nitric acid mixture (Aqua Regia) heated to 90 °C. The digested samples were analyzed for Hg_T by Cold Vapour Atomic Absorption spectroscopy (CVAAS) (Armstrong and Uthe, 1971). The detection limit was 0.005 mg kg^{-1}.

Data analysis

The concentrations of Hg_T, Se_T, $MeHg_T$ and iHg_{labile} were recovery-corrected based on the recovery of DOLT-4, prior to data analysis. All brain values were converted from dw to ww concentrations based on the measured moisture for each sample.

Data are reported as the median, followed by range in parentheses unless otherwise stated. All

statistical analyses were conducted using STATA v12 (College Station, Texas) except for non-linear regressions for exponential relationships that were conducted using GraphPad Prism for Mac v6.0b (La Jolla, CA). A p-value ≤ 0.05 was selected to indicate a statistically significant result for all analyses, and all statistical tests were two-tailed. Scheffe *post hoc* multiple means comparison test was used to test differences in means following one-way analysis of variance. Pearson and Spearman coefficients were used to analyze parametric and non-parametric data, respectively. Linear regressions of ln-transformed Hg_T data were used to assess the relationship between cerebellar Hg_T concentrations and blood, liver, kidney, muscle and muktuk Hg_T concentrations.

Results and Discussion

Mercury concentration and distribution

Total Hg concentrations in five brain regions are provided in Table 3.1. The difference in Hg_T between brain regions was statistically significant in the 37 whales for which we had samples available for all five brain regions ($p < 0.05$). Total Hg concentrations increased in the following order: Spinal cord (SC)^{a} < Brain stem (BS)^{a,b} < Frontal lobe (FL)^{b} < Cerebellum (C)^{b} < Temporal lobe (TL)^{b} (different superscripts designate brain regions that had significantly different Hg_T concentrations from each other). Relative Hg_T concentrations in discrete brain regions normalized to Hg_T in SC were: 1 : 1.6 : 1.9 : 2.3 : 2.8, respectively.

Total Hg concentrations in brain tissue of beluga whales were similar to those reported in dolphins (*G. griseus*, *S. coeruloalba* and *T. truncates*) (Capelli et al., 2008; Meador et al., 1999), and much higher than concentrations reported for other mammals such as wild river otter (*Lutra lutra*) (Basu et al., 2005d), polar bear (*Ursus maritimus*) (Krey et al., 2012), Arctic sledgedogs (Hansen and Danscher, 1995), wild mink (*Mustela vison*) (Basu et al., 2005a) and raccoon (*Procyon lotor*) (Porcella et al., 2004) (Table 3.2). Interspecies differences in brain Hg_T concentrations cannot be explained simply by trophic position. For example, polar bears are top predators in the Arctic and are known to bioaccumulate high concentrations of Hg in their livers (Rush et al., 2008), yet the concentrations of Hg_T in cerebellar cortex samples of polar bears (*n* = *22*, age: 2 to 9 yr for 13 animals) harvested in Nunavik, northern Québec were 0.23 ± 0.07 mg kg^{-1} dw (mean ± se, approx 0.06 ± 0.02 mg kg^{-1} ww) (Krey et al., 2012). Therefore, interspecies variation in Hg_T concentration in the brain may be associated with different rates of MeHg excretion.

Due to the absence of Hg toxicity data pertaining to cetaceans, we compared Hg_T concentrations in beluga brain tissue to reported concentrations associated with neurotoxicity in humans, primates and fish-eating wildlife. The lethal concentration of Hg_T in human brain tissue was 16.8 mg kg^{-1} ww in the cerebellum of one individual (Eto et al., 1999). Dietary MeHg dosing studies on primate species indicated that Hg intoxication was associated with brain Hg_T concentrations between 6.0 and 12.0 mg kg^{-1} ww (Berlin et al., 1975a; Evans et al., 1977; Luschei et al., 1977; Stinson et al., 1989). Furthermore, concentrations below ~2 to 5 mg kg^{-1} ww were likely below thresholds of overt MeHg intoxication in mink (Suzuki, 1979; Wobeser et al., 1976). In this

study, one whale (1%) had cerebellar Hg_T above 16.8 mg kg^{-1}, four whales (5%) had cerebellar Hg_T that exceeded 6.0 mg kg^{-1} and 44 whales (53%) had cerebellar Hg_T below 2 mg kg^{-1}. In the temporal lobe, Hg_T exceeded 16.8 mg kg^{-1} in three whales (3%), 6 mg kg^{-1} ww in 11 whales (14%) and was below 2 mg kg^{-1} in 38 whales (47%). The results from this study indicate that at least 14% of the beluga whales had Hg_T concentrations in the temporal lobe exceeding concentrations associated with intoxication, but 47 to 53% had brain Hg_T concentrations below thresholds of overt intoxication, assuming that the toxic threshold concentration for beluga is similar to other mammalian species.

Hg and age

The ages of whales were the following (mean ± se): 26 ± 8 yr in 2006 (*n = 47*), 33 ± 15 yr in 2008 (*n = 19*) and 23 ± 5 yr in 2010 (*n = 15*), and ranged from 0 (full-term fetus) to 60 yr. Males were predominantly sampled in all years; only three females were sampled in 2006, three females (including one pregnant female) were sampled in 2008 and no females were sampled in 2010. The concentration of Hg_T was positively correlated to age in cerebellum ($p < 0.05$, *n = 74;* Figure 3.3). The lowest Hg_T concentration was observed in the full term fetal cerebellum (0.05 mg kg^{-1} ww). The median concentrations of cerebellar Hg_T were 0.77 and 3.37 mg kg^{-1} ww in the youngest (first quartile: 0-19 yr) and oldest (fourth quartile: 30 – 60 yr), respectively. Interestingly, the most elevated cerebellar Hg_T concentrations (15.0 mg kg^{-1} ww and 16.9 mg kg^{-1} ww) were not observed in the oldest whales, but in whales that were 47 yr and 36 yr, respectively. We are unsure about the reason for the elevated Hg in these two whales; however, these concentrations may reflect differences in feeding behaviour (Loseto et al., 2008a) and

therefore greater exposure to MeHg in these whales compared to their older counterparts. Elevated Hg_T concentrations observed in brain tissue from beluga whales could be explained in part by accumulation of Hg throughout their long lifespans.

The developing brain is extremely sensitive to Hg exposure (Clarkson and Magos, 2006). Maternal transfer of Hg to her fetus was investigated by collecting tissue samples from one female beluga (23 yr) and her full term male fetus. Total Hg concentrations in fetal tissues were (mg kg^{-1} ww, n=1): SC= 0.02, C = 0.05, FL = 0.07, TL = 0.06 and liver = 0.28. Hg_T in the fetal brain ranged from 3 to 14% of maternal brain Hg_T, with the frontal lobe bearing the most elevated Hg for the fetus compared to the temporal lobe for the mother. Previously reported Hg_T concentrations in one St. Lawrence Estuary neonate were 0.049 mg kg^{-1} ww in brain tissue and 0.145 mg kg^{-1} ww in liver (Gauthier et al., 1998). Therefore, the Hg_T concentrations observed in the full-term fetus from this study were similar to the concentrations observed from the neonate, although in general, adult belugas from this area have more elevated Hg_T concentrations than their counterparts in the Arctic (Wagemann et al., 1990). The clinical significance of *in utero* exposure on the developing beluga brain remains to be studied.

Hg speciation

Between 34 and 44% of Hg_T in the four brain regions was soluble Hg (17 to 20% $MeHg_T$ and 18 to 28% iHg_{labile}) and the remaining 56 to 66% was bound to cell membranes (Table 3.3). Of the soluble fraction, 32 to 41% was organic Hg ($MeHg_T$) and 59 to 68% was iHg_{bound}. The concentration of $MeHg_T$ was positively correlated to concentration of Hg_T in all brain regions (p

≤ 0.05): SC ($r_s = 0.80, p < 0.0001, n = 19$), FL ($r_s = 0.77, p = 0.0001, n = 19$), C ($r_s = 0.68, p = 0.0005, n = 22$) and TL ($r_s = 0.73, p = 0.0001, n = 22$). The percent $MeHg_T$ of Hg_T decreased exponentially with increasing Hg_T concentration ($p \leq 0.05$) in SC ($R^2 = 0.95$; t½ = 0.18 mg kg^{-1}), FL ($R^2 = 0.93$; t½ = 0.31 mg kg^{-1}), C ($R^2 = 0.96$; t½ = 0.37 mg kg^{-1}) and TL ($R^2 = 0.94$; t½ = 0.37 mg kg^{-1}) (Figure 3.4). The concentrations of iHg_{labile} were consistently higher than $MeHg_T$ in all brain regions, with the exception of the full-term fetus, which had 100% $MeHg_T$ and one adult whale with the lowest brain Hg_T concentration measured.

The ratio of organic mercury expressed as a percentage of total mercury in brain samples varied greatly across species and suggests interspecies differences in *in situ* demethylation of MeHg in the brain (Table 3.2). Previous studies have suggested that MeHg may be demethylated in the brains of cetaceans (Augier et al., 1993; Cardellicchio et al., 2002; Joiris et al., 2001; Meador et al., 1999). Unlike cetaceans, the majority of brain Hg_T was composed of MeHg in polar bears (Basu et al., 2009; Krey et al., 2012), raccoons (Porcella et al., 2004), and mink (Basu et al., 2005b).

Demethylation of MeHg followed by the formation of compounds similar to mercury selenide (HgSe) was linked to successful detoxification of Hg in organic Hg-exposed humans (Korbas et al., 2010). The low percentage of $MeHg_T$ in brain tissue from beluga whales could be indicative of demethylation taking place in the four brain regions analyzed. When we compared the concentration of $MeHg_T$ to threshold levels, we found that 100% of the whales had $MeHg_T$ concentrations below 2 mg kg^{-1} in the four brain regions analyzed ($n = 19$ *to* 22). Therefore, if

MeHg is indeed the neurotoxic form of Hg, biological monitoring of beluga whales and other cetaceans must focus on the concentration of MeHg in brain tissue to evaluate risk of Hg exposure.

Co-accumulation of Hg_T and Se_T

The molar concentration of Se_T was significantly predicted by molar Hg_T concentration in all brain regions ($p < 0.001$). The stoichiometric relationship of Hg and Se co-accumulation in the BS was $[Se_T] = 1.0 \times [Hg_T] + 7.1$ ($r^2 = 0.98$, $n = 39$), SC was $[Se_T] = 1.1 \times [Hg_T] + 5.5$ ($r^2 = 0.95$, $n = 77$), FL was $[Se_T] = 0.98 \times [Hg_T] + 7.1$ ($r^2 = 0.98$, $n = 77$), C was $[Se_T] = 1.1 \times [Hg_T] + 6.0$ ($r^2 = 0.97$, $n = 83$) and TL was $[Se_T] = 1.0 \times [Hg_T] + 7.0$ ($r^2 = 0.98$, $n = 81$) for whales harvested in 2006, 2008 and 2010. The median and range of molar ratios of Hg:Se in the five brain regions were: BS = 0.48 (0.08-0.88), SC = 0.50 (0.03-0.87), FL = 0.55 (0.08 – 0.96), C = 0.60 (0.06 – 0.93) and TL = 0.58 (0.08-1.01) (Table 3.1). Molar concentrations of Se_T consistently exceeded Hg_T in the five brain regions analyzed. The molar ratio of Hg:Se was less than one, in 80 of 81 whales analyzed. These consistent linear relationships between Hg and Se accumulation strongly suggest that an interaction occurs between these elements in the brains of belugas. The binding of iHg to Se in a 1:1 ratio has been observed in the liver of high trophic level mammals, and may partially protect organisms from MeHg-associated toxicity (Dietz et al., 2013). Selenium is an essential element in the nervous system, it is both a micronutrient and antioxidant, and adverse biological effects occur when bioavailable Se is below (deficiency) or above (toxicity) thresholds (Khan and Wang, 2009). It was suggested that the toxicity of MeHg could be linked to its high binding affinities with Se, limiting the bioavailability of Se for Se-dependent enzyme activity in

brain tissues (Watanabe et al., 1999). Our findings that the molar concentration of Se_T consistently exceeded Hg_T (i.e. Hg:Se < 1:1) in different beluga brain regions suggest that this hypothesis of limited availability of Se is not applicable.

Previous studies have suggested that a bio-transformation process may occur in the brain, initiated by the demethylation of MeHg followed by the formation of inert HgSe granules (Nigro and Leonzio, 1996). Selenium may also reduce the toxicity of Hg by reducing oxidative stress of Hg, forming inert compounds with Hg and forming bis(MeHg) selenide (Khan and Wang, 2010). In beluga brains, 30 to 40 % of Hg_T was $MeHg_T$ and iHg_{labile}, and the remainder was an unidentified Hg moiety. An unknown peak (U1) was observed in almost all temporal lobe samples at the same retention time as a Se peak. Direct identification of the chemical nature of U1 was not successful due to the low concentrations involved. However, based on retention times of known Hg and MeHg compounds, U1 cannot be the inert HgSe compound (Lemes et al., 2011); instead, it is most likely a mercuric cysteinate ($Hg(SCys)_2$) or its selenocysteinate analogue ($Hg(SeCys)_2$). Peaks identified as U2 are attributed to an organoseleno compound (Lemes et al., 2011). The co-accumulation of Hg and Se and the detection of an unidentified Hg moiety in the temporal lobe, suggests that Se may indeed play a role in the detoxification of Hg in the brains of beluga whales.

A previous study showed that no Hg peak was detected along the Se peaks in the Se speciation method (Lemes et al., 2011). This suggests that Hg or MeHg is not present as a complex of the identified Se species, such as SeMet, CH_3SeCys, or inorganic Se (IV or VI). However, two sets

of Hg and Se peaks overlapped in the MeHg speciation method (Figure 3.2), though the identities of the compounds remain elusive.

The presence of elevated Hg in the brain tissue of beluga whales and other cetaceans may be due to the demethylation of MeHg and co-accumulation of Hg and Se. Co-exposure to Se and Hg has been linked to increased uptake of MeHg in the brain (Chen et al., 1975); and the half-life of iHg in the brain of primates was estimated to be 230 to 540 d compared to 37 d for MeHg (Vahter et al., 1995). Therefore, although Se is likely involved in the detoxification of Hg in the brains of belugas, dietary exposure to both Hg and Se may contribute to the elevated concentrations of Hg detected in the beluga brains in this study.

Tissue distribution of Hg

The distribution of Hg in frequently sampled organs of beluga whales could provide insight into neurotoxicological risk of Hg exposure, and contribute to biomonitoring studies. Mercury concentration in the cerebellum could be predicted by liver Hg concentration ($\ln(Hg_{cerebellum}) = 0.77 \times \ln(Hg_{liver}) - 1.5$; $r^2 = 0.73$; $p < 0.05$; $n = 49$), kidney Hg concentration ($\ln(Hg_{cerebellum}) = 0.88 \times \ln(Hg_{kidney})$; $r^2 = 0.64$; $p < 0.05$; $n = 17$), muscle Hg concentration ($\ln(Hg_{cerebellum}) = 1.3 \times \ln(Hg_{muscle}) + 0.63$; $r^2 = 0.57$; $p < 0.05$; $n = 46$) and blood Hg concentration ($\ln(Hg_{cerebellum}) = 1.7 \times \ln(Hg_{blood}) - 4.7$; $r^2 = 0.32$; $p < 0.05$; $n = 17$). Therefore, Hg_T concentrations measured in frequently sampled organs could be used to predict Hg_T concentration in cerebellum and other brain regions. Total Hg concentrations measured in blood samples from beluga whales suggest

that half of the whales analyzed in 2008 had Hg_T concentrations exceeding the lowest effect level for Hg_T in blood (200 ng mg^{-1}) from the Minamata and Iraqi outbreaks (Clarkson, 1997).

4. Conclusions

A combination of bioaccumulation and biomagnification of MeHg and retention of demethylated inorganic Hg may account for elevated Hg_T concentrations observed in brain tissue of beluga whales. Although demethylation and possible detoxification appear to occur in beluga whales, the effect of Hg exposure on brain function is not known. Total Hg concentrations in some beluga whales exceeded thresholds of toxicity reported for humans and primates; therefore, it is possible that Hg exposure in beluga whales from the eastern Beaufort Sea could be associated with neurotoxicity. Future work will involve the investigation of neurochemical disruption and behavioural variation associated with Hg exposure in this whale population. Protecting beluga whales from Hg exposure and associated toxicity is essential to protecting ecosystem health, including the health of humans who rely on belugas for cultural and physical well-being (Sejersen, 2001; Tyrrell, 2007).

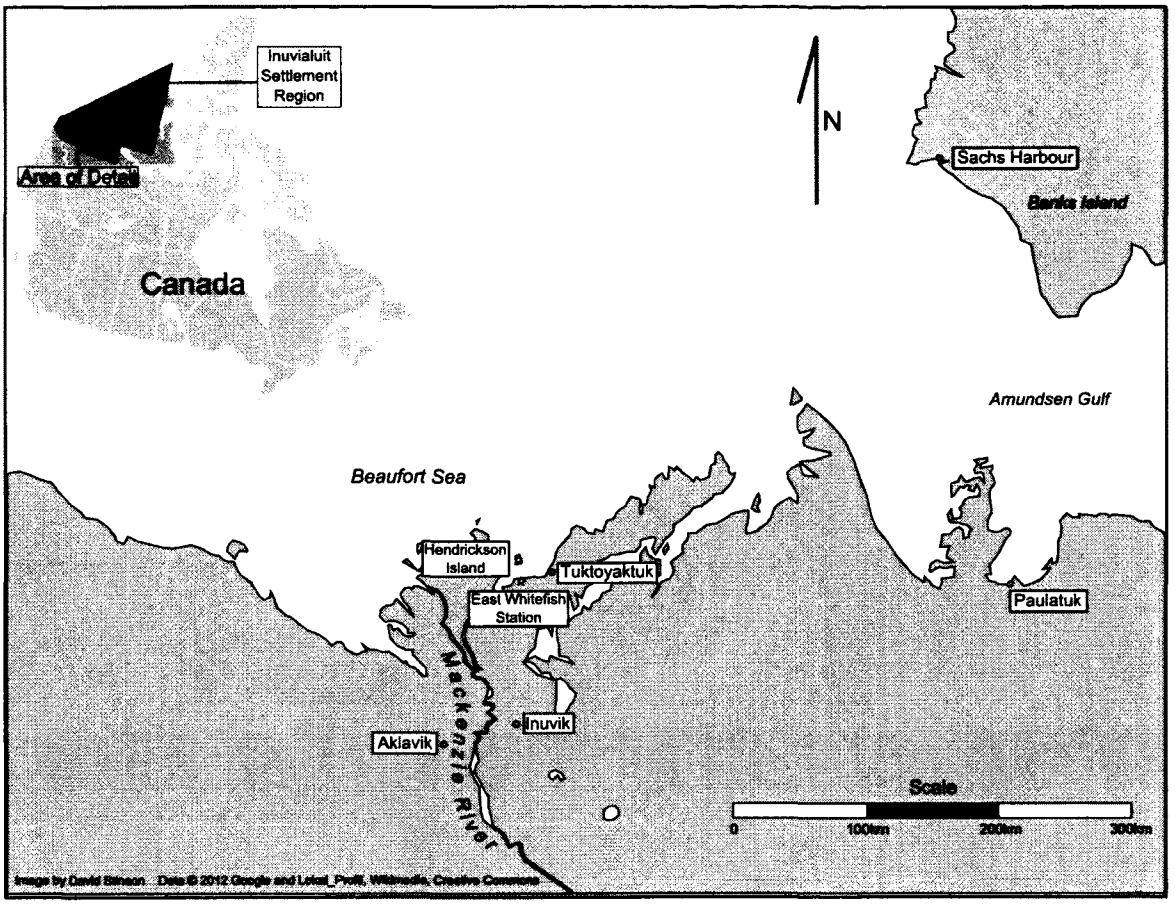


Figure 3.1 Map of the Inuvialuit Settlement Region. Sampling sites (Hendrickson Island and East Whitefish Station) and summering habitat of the Eastern Beaufort Sea beluga whale population (Amundsen Gulf and southern Beaufort Sea).

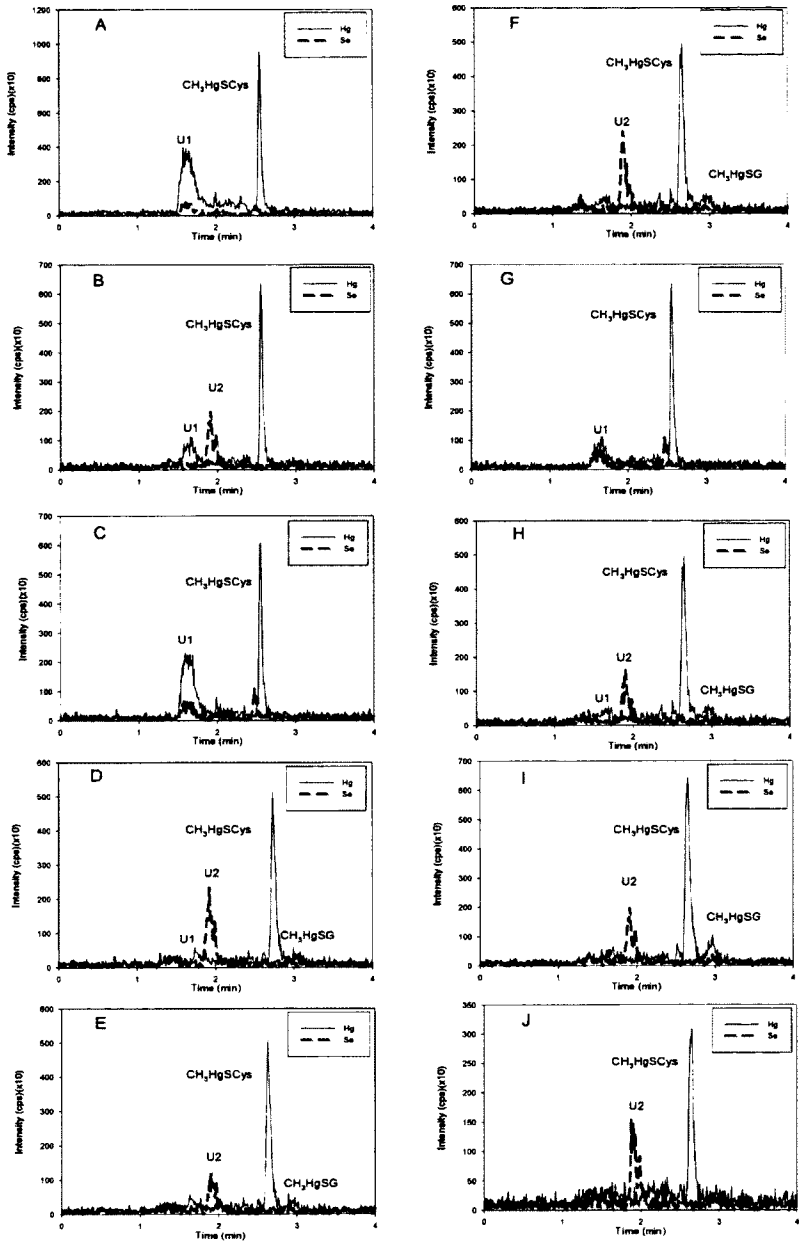


Figure 3.2 Speciation of Hg and Se. Reverse phase HPLC-ICP-MS chromatograms showing the peaks of Hg (in black) and Se (in red) species in temporal lobe samples of ten beluga whales (A-J) from the Western Canadian Arctic, 2008. U1 and U2 denote two unidentified Hg and Se peaks, respectively.

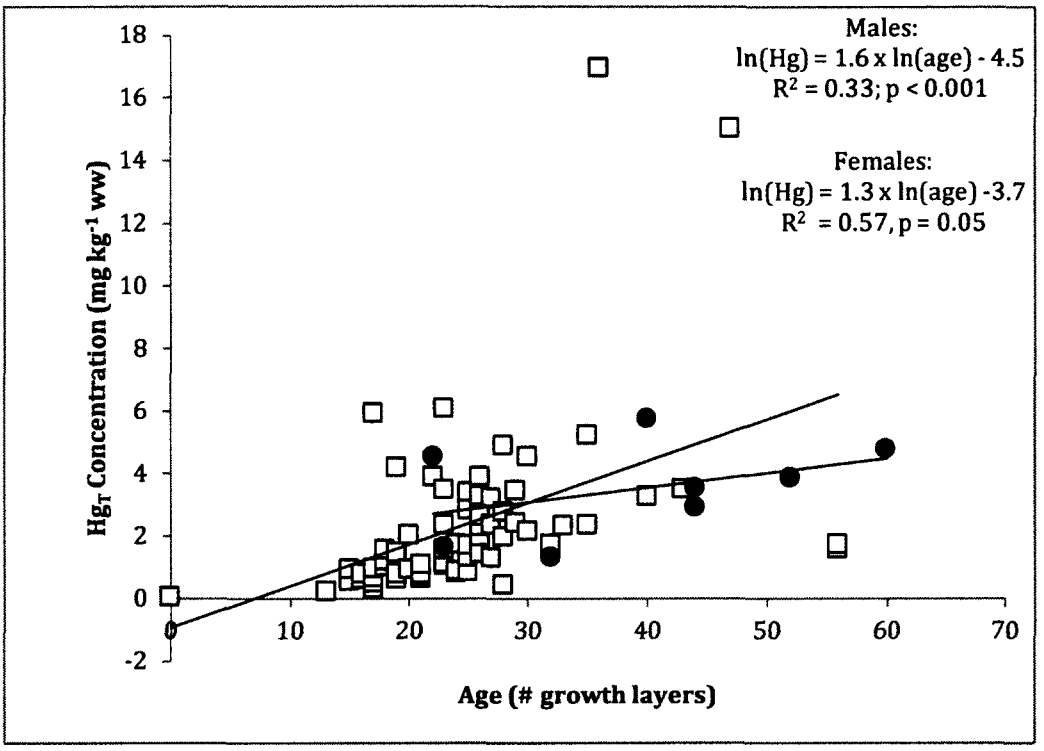


Figure 3.3 Relationship between age and cerebellar Hg_T concentration. Positive relationship between age and cerebellar Hg_T concentration in male (square) and female beluga whales (filled circle) (*n = 76)*.

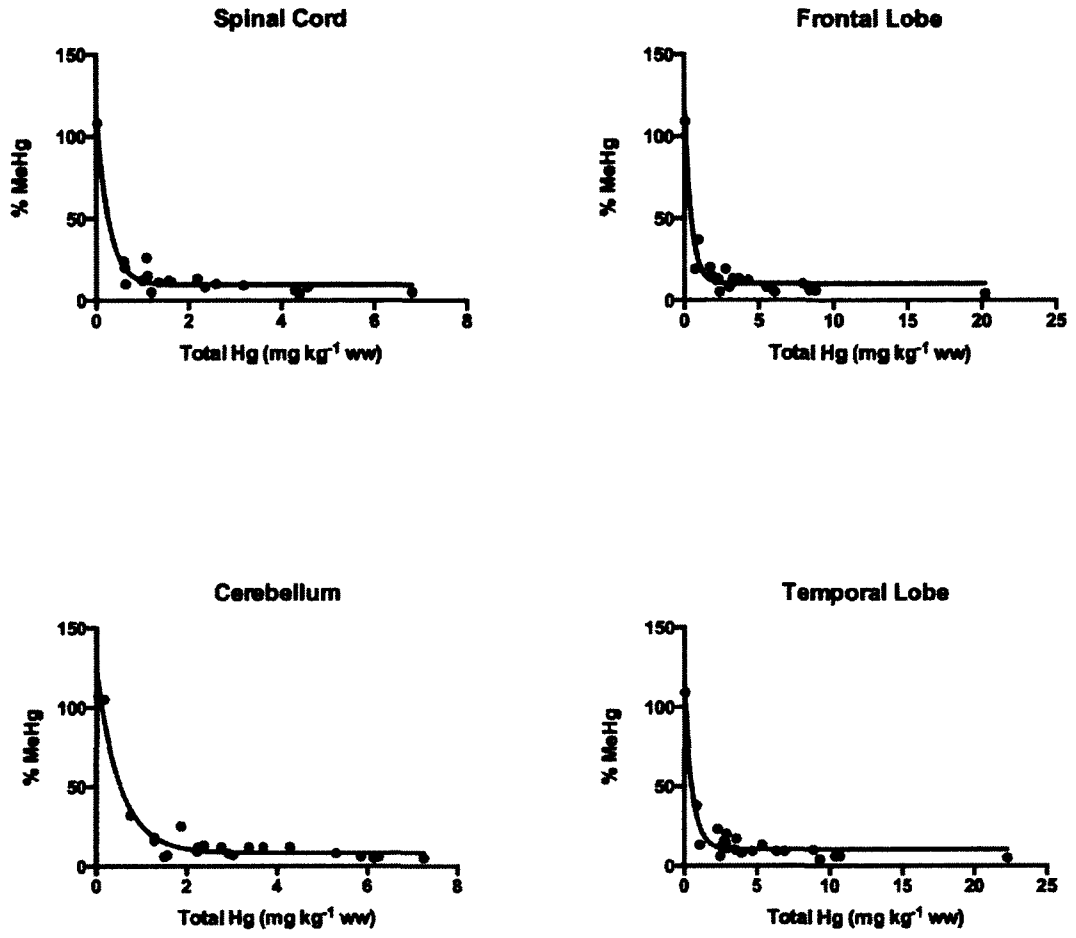


Figure 3.4 The non-linear relationship (exponential one phase decay) between percent MeHg and Hg_T concentrations. The percent MeHg decreased exponentially with increasing Hg_T in the spinal cord (*n = 19*), frontal lobe (*n = 19*), cerebellum (*n = 22*) and temporal lobe (*n = 22*) in brain samples collected from belugas (fetus, juvenile and adult whales) in 2008.

Table 3.1 Concentrations (median and range, ww) of total Hg, Se and molar ratio of Hg:Se in beluga whales sampled during the summer harvests in the western Canadian Arctic in 2006, 2008 and 2010.

Tissue	n	Hg_T (mg kg^{-1} or ng mL^{-1})		Se_T (mg kg^{-1})		Molar Ratio Hg:Se	
		Median	Range	Median	Range	Median	Range
Brain stem	39	1.32	0.13-15.2	1.22	0.56 – 6.79	0.48	0.08 – 0.88
Spinal Cord	77	1.25	0.02 – 11.1	0.98	0.32 – 5.05	0.50	0.03 – 0.87
Cerebellum	83	1.84	0.05 – 16.9	1.20	0.28 – 7.20	0.60	0.06 – 0.93
Temporal Lobe	81	2.34	0.06 – 22.6	1.50	0.30 – 10.4	0.58	0.08 – 1.01
Frontal Lobe	77	1.84	0.07 – 21.9	1.26	0.51 – 8.98	0.55	0.08 – 0.96
Liver	50	19.1	0.28 – 108	8.15	0.84 – 37.5	0.93	0.13 – 2.74
Kidney	23	4.90	0.12 – 10.9	3.48	1.13 – 6.62	0.50	0.04 – 0.95
Muktuk	24	0.35	0.07 – 0.88	3.63	0.62 – 5.97	0.04	0.01 – 0.16
Muscle	57	1.10	0.11 – 3.39	0.35	0.19 – 0.86	1.21	0.23 – 2.79
Whole Blood	17	200	77.4 – 515	N/A	N/A	N/A	N/A

Reported as mg kg^{-1} wet weight for solid samples and ng mL^{-1} for whole blood, n refers to total number of samples analyzed. Blood density is approx 1.02 mg μL^{-1}. Blood, kidney and muktuk samples were only available for 2008. Muktuk refers to blubber and outer skin layers. N/A denotes no sample available.

Table 3.2 The concentrations of Hg_T, and percent MeHg reported for brain tissue from different mammalian wildlife species. Approximate wet weight (ww) concentrations were calculated when necessary based on reported moisture content in brain tissue.

Species	Location	[Hg_T] (mg kg^{-1} ww)	MeHg (%)	Reference
Risso's dolphin (*G. griseus*)	Northwest Mediterranean	~ 0.71 – 39.1	8 – 51	(Capelli et al., 2008)
Striped dolphin (*S. coeruloalba*)		~ 0.99, 7.26	34, 90	
Bottlenose dolphin (*T. truncates*)		~ 0.21, 12.5	61, N/A	
Polar bear (*Ursus maritimus*)	Nunavik, QC, CA	~ 0.06 ± 0.02	100	(Krey et al., 2012)
Raccoon (*Procyon lotor*)	FL, USA	0.286	96	(Porcella et al., 2004)
Wild mink (*Mustela vison*)	NS, ON and YT, CA	~ 0.07 – 4.90	88.8 ± 15.4	(Basu et al., 2005a)
Wild river otter (*Lutra lutra*)	NS and ON, CA	0.02 – 3.58	68 ± 34.8 – 78 ± 30.4	(Basu et al., 2005c)

Table 3.3 Concentrations (median, range) of labile Hg species ($MeHg_T$ and iHg_{labile}) and iHg species complexed to proteins or selenium in the cerebellum, temporal lobe, frontal lobe and spinal cord of fetal, juvenile and adult beluga whales (*n = 22*) sampled on Hendrickson Island in 2008. The percentages of each Hg fraction over total Hg measured are presented as median and range.

Brain region	N	Labile Hg Species (mg kg^{-1} ww)		Bound Hg (mg kg^{-1} ww)
		MeHg	iHg	
Cerebellum	22	0.27 (0.04 – 0.53) 12% (5 – 107%)	0.56 (nd – 1.05) 20% (0 – 39%)	1.70 (nd – 6.07) 68% (0 – 84%)
Temporal lobe	22	0.44 (0.04 – 1.05) 11% (4 – 109%)	0.74 (nd – 1.59) 19% (0 – 27%)	2.50 (nd – 19.9) 71% (0 – 89%)
Frontal lobe	19	0.34 (0.08 – 0.85) 12% (4 – 109%)	0.70 (0.01 – 1.55) 20% (7 – 41%)	1.98 (nd – 17.9) 66% (0 – 88 %)
Spinal cord	19	0.18 (0.03 – 0.43) 11% (4 – 108%)	0.45 (nd – 1.05) 29% (0 – 44%)	0.88 (nd – 5.78) 56% (0 – 85%

5. Acknowledgements

This study was funded by NSERC Discovery (HMC and FW), the Fisheries Joint Management Committee (SKO), and Northern Contaminants Program (FW, GS). SKO was the recipient of a NSERC Doctoral award, Nasivvik Doctoral award, NSTP Training Fund and UNBC travel awards. We thank M. Gillingham for guidance with statistical analyses, D. Stinson for mapping, A. Essler, A. Montgomery, S. Krause, A. Krey, G. Prkachin and A. Shaw for assistance in the laboratory, and L. Loseto, M. Noel, F. Pokiak, N. Pokiak, D. Sydney, R. Felix, K. Nuyaviak, B. Voudrach, R. Walker, K. Snow, C. Pokiak, Inuvialuit hunters, Tuktoyaktuk and Inuvik HTCs, and the DFO for their support in the field.

Bridge

In the previous chapter, I identified that mercury (Hg) concentrations in brain tissue from beluga whales sampled from the Eastern Beaufort Sea population between 2006 and 2010 frequently exceeded concentrations previously associated with neurotoxicity and neurochemical disruption. Beluga whales in the Arctic may be at risk of neurochemcial disruption associated with MeHg exposure given that neurochemical variation was associated with MeHg exposure in wildlife and laboratory animals (Basu et al., 2005a; Basu et al., 2005c; Basu et al., 2009; Scheuhammer et al., 2008). Therefore, in the next two chapters I use neurochemical and molecular biomarkers to assess the potential disruption of neurological signaling pathways associated with MeHg exposure in beluga whales.

Chapter 4. Mercury and selenium exposure is associated with molecular and neurochemical biomarkers of Arctic beluga whales (*Delphinapterus leucas*).

Names and Affiliations of Authors:
1. Sonja K. Ostertag
Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, British Columbia, Canada, V2N 4Z9
ostertag@unbc.ca

2. Alyssa C. Shaw ♦
Deceased

3. Niladri Basu
Faculty of Agricultural and Environmental Sciences
Macdonald Campus, McGill University
Ste-Anne-de-Bellevue, Quebec, Canada H9X 3V9
Niladri.Basu@Mcgill.ca

4. Hing Man Chan
Center for Advanced Research in Environmental Genomics, University of Ottawa,
Ottawa, Ontario, Canada K1N 6N5
laurie.chan@uottawa.ca

♦ A. Shaw passed away on August 29, 2011

Abstract

Elevated concentrations of mercury (Hg) were found in beluga whales (*Delphinapterus leucas*) from the eastern Beaufort Sea population but effects of Hg on brain chemistry are not known. Brain tissue samples from the cerebellum and temporal cortex of 35 harvested beluga whales from Hendrickson Island, Canada were collected in 2008 and 2010. Neurochemical and molecular biomarkers were measured with radioligand binding assays and quantitative Real Time polymerase-chain-reaction, respectively. Total Hg (Hg_T) and selenium (Se_T) concentrations were analyzed using inductively coupled plasma mass spectrometry (ICP-MS); methylmercury (MeHg) and labile inorganic Hg (iHg_{labile}) were measured via high performance liquid chromatography ICP-MS. Total Hg concentration ranged from 1.7 to approx. 113 mg kg^{-1} dry weight (dw) in cerebellum and 2.6 – 113 mg kg^{-1} dw in temporal cortex. Total Hg, MeHg and Se_T were negatively associated with γ-amminobutyric acid type A receptor ($GABA_A$-R) binding in the cerebellum ($p \leq 0.05$). The expression of mRNA for $GABA_A$-R subunit α2 was negatively associated with Hg_T and iHg_{labile} concentration ($p \leq 0.05$). Furthermore, $GABA_A$-R binding was positively correlated to mRNA expression for $GABA_A$-R α2 subunit, and negatively correlated to the expression of mRNA for $GABA_A$-R α4 subunit ($p \leq 0.05$). Inorganic Hg was negatively associated with the expression of N-methyl-D-aspartate receptor (NMDA-R) subunit 2b mRNA expression in the cerebellum ($p \leq 0.05$). Based on these results, the $GABA_A$-R might be more sensitive to Hg, MeHg and iHg_{labile} exposure than the NMDA-R. These results suggest that variation of molecular and/or biochemical components of the GABAergic and glutamatergic signaling pathways were associated with MeHg exposure in beluga whales.

Introduction

Contaminants have been monitored for decades in the eastern Beaufort Sea (EBS) beluga whale (*Delphinapterus leucas*) population in the western Canadian Arctic (Fisk et al., 2005). Mercury (Hg) is of particular concern in this population of beluga whales due to elevated concentrations observed in samples collected in the 1990s (Wagemann et al., 1998). Furthermore, total Hg (Hg_T) concentrations in brain tissue of beluga whales sampled from the EBS population between 2006 and 2010 exceeded concentrations previously associated with neurotoxicity and neurochemical disruption (median Hg_T concentration was 2.34 mg kg^{-1} wet weight (range, 0.06 to 22.6 mg kg^{-1} ww) in temporal lobe (n = 81)) (Ostertag et al., 2013). For example, the concentration of Hg_T in brain tissue associated with lethal, acute onset MeHg poisoning was 16.8 mg kg^{-1} ww in the cerebellum of one adult human (Eto et al., 1999), and Hg_T concentrations between 4.1 and 15.9 mg kg^{-1} ww in brain tissue of captive mink (Wobeser et al., 1976) and between 6.6 and 18.0 mg kg^{-1} ww in primate species were associated with clinical symptoms of poisoning (e.g. visual impairment, lesions in the cerebral cortex, anorexia and incoordination) (Berlin et al., 1975a; Evans et al., 1977; Shaw et al., 1975). Mercury concentrations below ~2 to 5 mg kg^{-1} ww were likely below thresholds for overt clinical symptoms of MeHg toxicity in mink (Suzuki, 1979; Wobeser et al., 1976); however, Hg_T concentrations ranging from 1.5 ± 0.34 to 15.4 ± 3.9 mg kg^{-1} dry weight (dw) in brain tissue were associated with neurochemical variation in captive mink (Basu et al., 2010; Basu et al., 2007c; Basu et al., 2006b).

Methylmercury can pass the blood brain barrier and placenta to exert toxic effects on the central nervous system of adults and fetuses (Clarkson and Magos, 2006; Magos and Clarkson, 2006).

Overall, the toxicity of MeHg has been linked to its reactivity with sulfhydryl groups (Chakrabarti et al., 1998). Examples of how MeHg appears to exert its neurotoxicity include the disruption of intracellular calcium homeostasis (Anner et al., 1992; Sirois and Atchison, 2000; Yee and Choi, 1996), alteration of neurotransmission (Arakawa et al., 1991; Atchison and Hare, 1994; Narahashi et al., 1994; Vidal et al., 2007; Yuan et al., 2005) and causing oxidative stress (Yee and Choi, 1996; Young et al., 2002). Neurochemical changes are potential indicators of neurological harm because they may precede functional or structural damage of the nervous system (Manzo et al., 2001). Previous studies have suggested that the interaction of MeHg or inorganic Hg (iHg, Hg^{2+}) with cysteine residues may inhibit, stimulate or damage components of the dopaminergic (Gomes et al., 1976), cholinergic (Castoldi et al., 1996), GABAergic (Huang and Narahashi, 1997; Narahashi et al., 1994), and glutamatergic (Albrecht and Matyja, 1996) signaling pathways. In wildlife studies, MeHg exposure was associated with significant reductions in γ-aminobutyric acid type A receptor ($GABA_A$-R) binding in lab-exposed mink (*Mustela vison*) (Basu et al., 2010) and decreased N-methyl-D-aspartate receptor (NMDA-R) binding in several brain regions of MeHg-exposed bald eagles (*Haliaeetus leucocephalus*) (Rutkiewicz et al., 2011; Scheuhammer et al., 2008), mink (Basu et al., 2007c), and polar bears (*Ursus maritimus*) (Basu et al., 2009). It is believed that such MeHg-associated neurochemical changes may have consequences to the whole organism as these receptors are components of the main inhibitory and excitiatory pathways in the central nervous system, and they play important roles in animal behaviour, memory and motor function (Popescu, 2005; Reis et al., 2009; Vicini and Ortinski, 2004).

Arctic beluga whales may accumulate elevated concentrations of Hg_T in their brains due to their diet, long lifespans and the demethylation of MeHg (Ostertag et al., 2013). Previous studies have indicated that Hg_T concentrations in brain tissue from Arctic beluga whales may exceed mean concentrations observed in other Arctic biota (e.g. polar bears, seals and humans) (Dietz et al., 2013). Although 14 % of Arctic beluga whales had Hg concentrations that exceeded 6.0 mg kg^{-1} ww (Ostertag et al., 2013), a concentration associated with toxicity in feeding trials (Berlin et al., 1975a; Evans et al., 1977; Luschei et al., 1977), the potential neurotoxicity of MeHg exposure has not been explored to date in beluga whales. Previous research has shown that Hg concentrations were lower in the cerebellum than the temporal cortex of Arctic beluga whales (Ostertag et al., 2013); however, the cerebellum is particularly sensitive to MeHg exposure and destruction of cerebellar granule cells characterizes MeHg intoxication in humans and wildlife (Basu and Head, 2010; Clarkson and Magos, 2006). Demethylation of MeHg in Arctic beluga whales is likely, and labile iHg concentrations were consistently higher than MeHg concentrations in the temporal cortex, cerebellum, frontal cortex and spinal cord (Ostertag et al., 2013). Given that iHg cannot easily cross the blood-brain barrier, the presence of iHg in the brain is likely due to in-situ demethylation of MeHg (Clarkson and Magos, 2006). The brain is expected to be more sensitive to MeHg than iHg, based on brain pathology and symptoms associated with MeHg poisoning (Magos et al., 1985). Our previous findings of co-accumulation of Se and Hg suggested that Se may play a role in MeHg detoxification in beluga whales (Ostertag et al., 2013). Therefore, the potential neurotoxicity of elevated Hg_T requires further study in beluga whales, and should take into account Hg speciation and postential detoxification associated with Se co-accumulation.

The objective of this study was to characterize the relationship between concentrations of different chemical forms of Hg (Hg_T, MeHg and iHg), total selenium (Se_T), and the molar ratio of Hg to Se, and various neurochemical and molecular biomarkers in different brain regions of beluga whales. The overall hypothesis was that environmentally relevant Hg concentrations currently found in brain tissue of Arctic beluga whales would be associated with neurochemical and molecular variation in components of the GABAergic and glutamatergic signaling pathways.

Methods

Sample collection

Harvested beluga whales were sampled on Hendrickson Island, NT, Canada in 2008 ($n = 20$) and 2010 ($n = 15$) as described previously (Ostertag et al., 2013). Prior to each sampling season, a research permit and scientific license to fish were obtained from the Aurora Research Institute and Department of Fisheries and Oceans (DFO), respectively. The sampling program was approved and supported by the Tuktoyaktuk and Inuvik Hunters and Trappers Committees for all sampling years.

Following the beluga harvest, the animal head was removed from the body by severing the joint between the skull and atlas with a knife according to the instructions given by Noel Raymond, an Inuvialuit beluga harvester (personal communication, July 1, 2006). The brain was removed from the skull using an autopsy saw (MOPEC®, Elmira, Michigan) and subsamples were collected and frozen within 3 h of animal death. Subsamples of cerebellum and temporal cortex were collected from harvested beluga whales in 2008 and 2010 to measure metal concentrations

and speciation, neurochemical biomarkers and mRNA expression. Samples (~ 0.5 – 3 g) for metals and speciation analyses were frozen at ~ -20 °C, samples for neurochemical analyses (~ 0.5-1 g) were flash-frozen and stored in liquid nitrogen, and samples (~ 0.07 g) for mRNA expression assays were flash-frozen in 2008. In 2010, samples were placed in RNALater™ at approx 4 °C for 24 h prior to freezing at -20 °C. Sample numbers varied for the different assays due to subsample availability for the separate analyses. Age was estimated by counting individual growth layer groups in the dentine from a thin section of tooth at the Freshwater Institute, Winnipeg, MB (Stewart, 2006). The concentrations of Hg_T, iHg_{labile}, MeHg and Se_T were determined previously for the cerebellar cortex and temporal cortex (Ostertag et al., 2013). Chemical forms of Hg were analyzed following the method developed by Krey et al. (2012) as described previously (Ostertag et al., 2013).

Membrane preparation

For receptor binding assays, membrane preparations were prepared by homogenizing frozen brain tissue (~ 2 g ww) in Na/K buffer (10 mL/g tissue) with a tissue tearer for 30 s using a previously described method (Basu et al., 2005c). Endogenous ligands were removed by centrifugation three times with Na/K buffer and membranes were re-suspended in buffer, aliquoted, flash frozen in liquid nitrogen and stored at -80 °C. Protein concentration was determined with the Bradford assay and bovine serum albumin was used as the standard.

Receptor binding assays

Receptor binding assays were adapted from previous studies by Basu et al. (Basu et al., 2010; Basu et al., 2007c; Basu et al., 2009). In brief, 30 μg of membrane preparation was re-suspended in buffer and added to a microplate containing a 1.0 mM GF/B glass filter (Millipore, Boston, MA, USA). Membrane protein was suspended in 100 μL Tris buffer (pH 7.4; NMDA: buffer contained 100 μM glycine and glutamate) and incubated for 30 min on ice with [^{3}H]-flunitrazepam ([^{3}H]-FNP; 0.5 nM) or 120 min at room temp with [^{3}H]-MK-801 (16 nM), respectively for GABA and NMDA. All incubation steps were carried out with gentle shaking and binding reactions were ended by vacuum filtration. The filters were rinsed three times with buffer and were then soaked for 72 h in 25 μL OptiPhase Supermix Cocktail (PerkinElmer). Radioactivity retained by the filters was quantified in a microplate detector (Wallac Microbeta, PerkinElmer) and counting efficiency was approx 40%. Specific binding was calculated as the difference between radioligand binding in the presence and absence of inhibitor: 20 μM clonazepam for $GABA_A$-R, and 100 μM MK-801 for NMDA-R. Receptor binding is reported as fmol of radioisotope bound per mg of membrane protein (fmol mg^{-1}).

Expression of mRNA

Total RNA was extracted from approx. 80 mg samples using 1 mL Trizol using the Qiagen RNeasy^{TM} Lipid Tissue kit. Total RNA was treated with Ambion^{TM} DNase | buffer (6 μL), rDNAse | (1 μL) and DNase Inactivation reagent (6 μL), prior to quantification of RNA-40 using a spectrophotometer (Nanodrop, ND-1000). Taqman reverse transcription reagents (Applied Biosystems) were used for the generation of complementary DNA (cDNA) for all samples. A

master mix made up of 10 μL 10 X Taqman Reverse Transcriptase buffer, 22 μL $MgCl_2$, 20 μL random hexamers (50 μM), 2 μL RNase inhibitors, 2.5 μL reverse transcriptase was made that was sufficient for all samples for one brain region (each sample: 1 μg RNA, total volume of 100 μL). Concurrently, the identical reaction was performed without reverse transcriptase, to ensure the absence of genomic DNA (No Reverse Transcriptase (NRT) control). The following thermocycler parameters were used for the generation of cDNA archive: 25 °C for 10 min, 37 °C for 60 min, and 95 °C for 5 min (DYAD™, DNA Engine).

Primer pairs were designed within conserved nucleotide regions of the genes of interest based on the alignment of genes from multiple species (human, cow, goat, pig, primate (chimpanzee and macaque), dog, cat and sheep) using the National Centre for Biotechnology Information (NCBI) website. These primers were used in polymerase chain reaction (PCR) reactions on cDNA from beluga cerebellum ($n = 2$) to develop species-specific primers and probes. The PCR products were direct-sequenced at UBC Okanagan (Fragment Analysis and DNA Sequencing Services, Kelowna, CA), which were then used to design species-specific primers. Chromatograms were edited and the retrieved sequences were run through the NCBI website (Standard Nucleotide BLAST) to ensure they matched the conserved sequences of genes. The sequence obtained for NMDA-2c did not match the conserved sequence; therefore, the data for this target gene are not presented in this study. Species-specific primers and fluorogenic probes (IDT, Coralville, Iowa) were designed using Genscript RT-PCR primer design (Table 4.1).

All PCR runs were performed under identical conditions using the 7300 Real-Time PCR System (Applied Biosystems). Real time assays were performed in 96-well optical plates in duplicate. Simplex assays were run with iTaq™ Supermix with ROX kit (Biorad). PCR mastermix was prepared and each 25 μL reaction contained 12.5 μL iTaq mix, 500 nM of appropriate forward and reverse primers, 250 nM probes and 2.5 μL template for cerebellar cortex and 5.0 μL template for temporal cortex samples. The thermocycle program included an enzyme activation step at 95 °C (10 min) and 40 cycles of 95 °C (15 sec) and 60 °C (1 min).

Standard curves were generated for all genes from serial dilutions of cDNA to calculate efficiencies for target and reference genes. The mean C_T value for the three lowest Hg-exposed animals was used as the control. Pooled sample was included in triplicate for each plate to monitor inter-plate variability of s9 expression (reference gene). No-RT samples were included in each plate to ensure the absence of genomic DNA. The difference in thermal cycles for the control and sample were calculated for each target gene and reference (s9). Fold changes for each sample and target gene were calculated relative to the reference gene s9 (Pfaffl, 2001).

RNA purity and quality was evaluated using a NanoDrop (ND-1000, NanoDrop Technologies, USA). The optical density (OD) ratio of 260 nm/280 nm wavelengths was used as an indicator of RNA quality, with ratios greater than 1.8 indicating good RNA quality (Fleige and Pfaffl 2006). RNA integrity was evaluated using Experion (Bio-Rad Laboratories, USA). The ratio of 28S:18S and RNA Quality Indicator (RQI) value were used to evaluate degradation, with a 28S:18S ratio of 2.0 (Fleige and Pfaffl, 2006) or RQI of 10 indicating perfect integrity (Taylor et al., 2009).

Data Analysis

Mercury concentrations were log-transformed to meet assumptions of normality and homogeneity of variance. Fold-change ratios and receptor densities were log-transformed when necessary to meet assumptions of normal distribution and homogeneity of variance. Non-parametric tests were used when necessary if transformations were unable to improve the fit of data. The relationship between neurochemistry and the concentration of Hg_T, Hg species and Se was explored using Pearson correlation coefficients followed by multiple linear regression analysis. A backwards stepwise approach was used to evaluate the statistical significance of age, Hg (Hg_T, iHg_{labile}, MeHg, Se_T, and Hg:Se) and sampling year on neurochemical or molecular biomarker. We removed influential outliers after examination of studentized residuals, leverage and Cook's D influence. Pearson correlation coefficients were calculated for the expression of target genes and corresponding receptor binding levels. Correlations (Pearson and Spearman), F-values, Wilcoxon rank-sum test, and t-tests were considered to be statistically significant if $p \leq 0.05$. The sample size for females was <10 and could reduce our ability to detect a difference in biomarkers associated with gender. Data in tables and graphs are displayed in the original scale of measurement.

Results

The harvested whales ranged in estimated age from 16 to 60 yr, and the median age was 27 yr. Total Hg concentration ranged from 1.7 to ~ 113 mg kg^{-1} dw in cerebellum and 2.58 – 113 mg kg^{-1} dw in temporal cortex and were correlated between brain regions ($r = 0.9$, $n = 28$). Twenty whales were sampled in 2008 (16 male, 4 female) and 15 whales were sampled in 2010 (15

males, 0 females). Total Hg, MeHg and Se concentrations were higher in the temporal cortex than cerebellum, but the concentration of iHg_{labile} and molar ratio of Hg to Se did not vary between brain regions. Age was correlated to Hg_T and Se_T in the cerebellum and temporal cortex, iHg_{labile} and Hg_T to Se_T stoichiometric ratio in the temporal cortex but not cerebellum. The concentrations of iHg_{labile} and MeHg represented 38 % (11 – 89 %) and 36 % (11 - 104 %) of total Hg in cerebellum and temporal cortex, respectively. The remaining Hg was not identified or quantified in this study because it was not soluble in the extraction solution used for speciation analysis (Ostertag et al., 2013).

In the cerebellum, non-specific binding represented 35% and 45% of total binding for $GABA_A$-R and NMDA-R, respectively. In the temporal cortex, non-specific binding represented 11% and 34% of total binding for $GABA_A$-R and NMDA-R, respectively. Inter-plate variability ranged from 11 to 15% for NMDA-R and $GABA_A$-R binding assays. The optical density ratio was ≥ 1.8 for all RNA samples indicating acceptable RNA purity. The ratio of 28S:18S was < 2 and RNA quality index (RQI) was < 10 for a subset of samples analyzed via Experion (BioRad). The median and range of 28S:18S and RQI were 0.83 (0.3-1.87) and 6.9 (3.7-8.9), respectively for a subset of samples ($n = 12$), which indicated acceptable RNA quality. The Ct values for the internal control gene (s9), did not vary with age or Hg concentration in the cerebellum or temporal cortex, therefore it was considered a suitable internal control for data analysis. There was no amplification of NRT control following RT PCR.

GABAergic signaling pathway

Receptor binding was higher in the temporal cortex (median, range: 177, 137 – 291 fmol mg^{-1} protein) than cerebellum (median, range: 37, 11 - 113 fmol mg^{-1} protein for $GABA_A$-R ($z = -4.3$, $p < 0.0001$). Total Hg and iHg_{labile} concentrations were negatively correlated to $GABA_A$-R binding in the cerebellum (Figure 4.1A; $r = -0.49$, $p < 0.001$), but not temporal cortex (Figure 4.1B; $r = -0.51$, $p < 0.01$)). Gender was not associated with differences in $GABA_A$ receptor binding in either cerebellum or temporal cortex, and age was not correlated to $GABA_A$ receptor binding in either brain region (data not shown). Receptor binding levels for $GABA_A$ were significantly higher in samples collected in 2010 than 2008 ($t = -3.89$, $DF = 29$, $p < 0.001$) for cerebellum samples but not temporal cortex samples ($t = -0.7306$, $DF = 22$, $p > 0.05$). The results from three backward stepwise multiple regressions that included Hg_T, MeHg or iHg_{labile}, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed $GABA_A$ receptor binding levels in the cerebellum (Table 4.2): 1.8 + 0.17(year) -0.21(log(Hg)), 1.3 + 0.33(year) - 0.25(log(MeHg)) + 0.008(age), and 1.6 + 0.19(year)* -0.25(log(iHg)). Of the Hg species tested as predictor variables, only Hg and MeHg were found to be significant (Table 4.2). The models tested did not significantly predict $GABA_A$-R binding levels for the temporal cortex (Table 4.2).

In the cerebellum and temporal cortex, Hg_T was negatively correlated to the expression of $GABA_A$-R α2 mRNA (Figure 4.2A: cerebellum: $r = -0.48$, $p < 0.01$, temporal cortex: $r = -0.56$, $p < 0.01$). Methylmercury ($r = -0.44$, $p < 0.05$) and iHg_{labile} ($r = -0.43$, $p < 0.05$) concentrations were also correlated to $GABA_A$-R α2 mRNA expression in the temporal cortex (Figures 4.2B

and 4.2C), but not cerebellum. Gender was not associated with differences in mRNA expression for the target genes analyzed. The expression of mRNA for $GABA_A$ α4 was positively correlated to age in the cerebellum ($r = 0.45, p < 0.05$), but age was negatively associated with $GABA_A$ α2 in temporal cortex ($r = -0.38, p < 0.05$). There were differences in mRNA expression between sampling years for $GABA_A$ α2 (cerebellum: $z = -4.572$, $p < 0.0001$; and temporal cortex: $t = -3.91$, $DF = 28$, $p < 0.001$) and $GABA_A$ α4 (cerebellum: $t = 5.14$, $DF = 26$, $p < 0.0001$; temporal cortex: $z = -2.75$, $p < 0.01$). The results from three backward stepwise multiple regressions that included Hg_T, MeHg or iHg_{labile}, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed $GABA_A$ α2 mRNA expression (Table 4.2) in the cerebellum: -300 + 0.15(year) – 0.15(log(Hg)), -326 + 0.16(year) – 0.13(log(MeHg)) and -340 + 0.17(year) – 0.16(log(iHg)), and in the temporal cortex: -283 + 0.14(year) – 0.10(log(Hg)), -315 + 0.16(year) - 0.26(log(MeHg)), and -304 + 0.15(year) – 0.09(log(iHg)). Of the Hg species tested as predictor variables, only Hg_T was a significant predictor for $GABA_A$ α2 mRNA expression in the cerebellum, and MeHg was a significant predictor of $GABA_A$ α2 mRNA expression in the temporal cortex (Table 4.2). However, iHg_{labile} was not a significant predictor of $GABA_A$ α2 mRNA expression ($p < 0.1$) (Table 4.2). Only sampling year was a significant predictor of $GABA_A$ α4 mRNA expression in both brain regions (data not shown).

Selenium ($r = -0.49, p < 0.01$) and the stoichiometric ratio of Hg to Se ($r = -0.40, p < 0.05$) were correlated to $GABA_A$-R binding in the cerebellum (Figures 4.1C and 4.1D), but not temporal cortex. The results from two backward stepwise multiple regressions that included Se_T or the

stoichiometric ratio of Hg to Se, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted $GABA_A$-R binding levels in the cerebellum (Table 4.2): 2.0 + 0.20(year) – 0.49(log(Se)) + 0.0067(age), and 1.4 + 0.23(year) – 0.39(log(HgSe)). The stoichiometric ratio of Hg to Se was correlated to $GABA_A$-R α2 mRNA expression in the temporal cortex but not cerebellum (Figure 4.2D; r = -0.43, $p < 0.05$). Total Se concentration was also correlated to mRNA expression for $GABA_A$-R α2 in both brain regions (Figure 4.2E; cerebellum: r = -0.57, $p < 0.01$; temporal cortex: r = -0.59, $p < 0.001$), and $GABA_A$-R α4 mRNA expression (Figure 4.2F; r = -0.49, $p < 0.01$) in the temporal cortex. The results from two backward stepwise multiple regressions that included Se_T or the stoichiometric ratio of Hg to Se, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed $GABA_A$-R α2 mRNA expression (Table 4.2) in the cerebellum: -334 + 0.17(year) – 0.11(log(HgSe)) and -290 + 0.14(year) – 0.23(log(Se)); and, in the temporal cortex: -319 + .16(year) – 0.16(log(HgSe)) and -264 + 0.13(year) - 0.18(log(Se)). However, of the selenium-related predictor variables tested, only the concentration of Se in the cerebellum was a significant predictor of log-transformed $GABA_A$-R α4 mRNA expression.

Inconsistent relationships between mRNA expression for target genes and receptor binding or enzyme activity were observed. A significant positive correlation between $GABA_A$-R α2 mRNA expression and $GABA_A$ receptor density was observed in the cerebellum (3A; r = 0.39, $p < 0.05$). A negative correlation was found between $GABA_A$-R α4 mRNA expression and $GABA_A$-R density in the cerebellum (Figure 4.3B; r = -0.38, $p < 0.1$). Receptor binding and mRNA

expression were not correlated in the temporal cortex for either the target gene, nor the receptor analyzed (data not shown).

Glutamatergic Signaling Pathway

Binding levels were higher in the temporal cortex than cerebellum for NMDA-R ($z = -3.5$, $p < 0.001$). Total Hg was negatively correlated to NMDA-R binding in the temporal cortex ($r = -0.42$, $p < 0.05$) but not cerebellum (Figure 4.4A); however, MeHg and iHg were not correlated to NMDA-R binding. Gender was not associated with differences in NMDA-R binding in either cerebellum or temporal cortex. Age was not significantly correlated to NMDA-R binding in either brain region. Sampling year was associated with statistically significant differences in NMDA-R binding levels in the temporal cortex ($t = -2.36$, $p = 0.03$), but not cerebellum ($t = -0.69$, $DF = 29$, $p = 0.50$). The results from three backward stepwise multiple regressions that included Hg_T, MeHg or iHg_{labile}, in combination with animal age and sampling year, for both brain regions, indicated that only the following models tested for the temporal cortex (Table 4.3) were significant predictors of NMDA-R binding levels: -55 + 0.029(year) – 0.11(log(Hg)), -110 + 0.055(year) -0.12(log(MeHg)) and -63 + 0.033(year) – 0.15(log(iHg)). However, the Hg_T, MeHg and iHg_{labile} were not significant predictors of log-transformed NMDA-R binding levels in the temporal cortex.

The expression of NMDA-R 2b was not correlated to Hg, MeHg or iHg_{labile} in either brain region (data not shown). Gender was not associated with differences in mRNA expression for NMDA-R 2b. The expression of mRNA for NMDA-R 2b was positively correlated to age in the cerebellum

($r = 0.37, p = 0.053$) but not temporal cortex. There were statistically significant differences in mRNA expression and sampling year for NMDA-R 2b for the cerebellum ($t = 5.99$, $DF = 28$, $p < 0.0001$) but not temporal cortex. The results from three backward stepwise multiple regressions that included Hg_T, MeHg or iHg_{labile}, in combination with animal age and sampling year, for both brain regions, indicated that only the following models tested for the cerebellum (Table 4.3) were significant predictors of mRNA expression for NMDA-R subunit 2b: 352 - 0.17(year) - 0.16(log(Hg)), 319 - 0.16(year) – 0.13(log(MeHg)) and 347 -0.17(year) – 0.27(log(iHg)). Of the Hg species tested as predictor variables, only iHg_{labile} was a significant predictor of NDMA-R 2b mRNA expression in the cerebellum (Table 4.3). Furthermore, the expression of mRNA for NMDA-R 2b and NMDA-R binding levels were not correlated in the temporal cortex or cerebellum (data not shown).

Selenium concentration was correlated to NMDA-R binding in the temporal cortex but not cerebellum (Figure 4.4B, $r = -0.43$, $p < 0.05$). The molar ratio of Hg to Se was not correlated to NMDA-R binding in either brain region (data not shown). The results from two backward stepwise multiple regressions that included Se_T or the stoichiometric ratio of Hg to Se, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed NMDA-R binding levels in the temporal cortex (Table 4.3): -63 + 0.039(year) – 0.28(log(HgSe)) and -56 + 0.029(year) – 0.15(log(Se)). However, the stoichiometric ratios of Hg to Se, and the concentrations of Se_T were not significant predictors of NMDA-R binding levels in either brain region. Selenium concentration and the molar ratio of Hg to Se were not correlated to NMDA-R subunit 2b mRNA

expression either brain region (data not shown). The results from two backward stepwise multiple regressions that included Se_T or the stoichiometric ratio of Hg to Se, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed NMDA-R 2b mRNA expression in the cerebellum (Table 4.3): 325 -0.16(year) - 0.35(log(HgSe)) and 360 - 0.16(year) - 0.30(log(Se)) + 0.003(age). However, of the selenium-related predictor variables tested, only Se_T was a significant predictor of NMDA-R 2b mRNA expression in the cerebellum.

Table 4.1. Sequences of primers and probes used for real time PCR.

Target gene	Primer sequence		Probe
	Fw	Rvs	
NMDA-R subunit 2b	ACAAGCGCTACTTCAGGGAC AA	TGCGGCGAGGTCTCCTT	TGCGGGACTTCTACCTGGACCAGTTC
NMDA-2c	CCTTCTACAGGCACCTACTGA ATG	GGTACCCACCAGGGCTGAA	CCTGGGAGGGCCGGGACTTCTC
$GABA_A$-R subunit α2	TGTCCAATGCACTTGGAGGAT	CGCATAGCTGCCAAATTTC A	CGAATCCAGGATGATGGGACTCTGCT
$GABA_A$-R subunit α4	TGTCCCATGAGATTGGTGGAT	CATCTCACTCTTCGGATAG GCATA	CATGCATGCCCTTTGAAATTTGGGAG
S9	GCTGCTGACGCTGGATGAG	CGCAGCAGGGCATTGC	AAGACCCGCGGCGTCTGTTTGAA

Table 4.2. Summary of backwards stepwise linear multiple regression analysis for the cerebellum and temporal cortex with $GABA_A$ receptor binding to [3H]-FNP, and mRNA expression (fold change) for $GABA_A$ α2 and α4 as the three outcome variables tested. The predictor variables tested included total mercury (Hg_T), methylmercury (MeHg), labile inorganic mercury (iHg_{labile}), total selenium (Se_T), or the molar ratio of Hg_T to Se_T, and sampling year, and animal age. Adjusted R squared values and F-values are provided for the models as indicated.

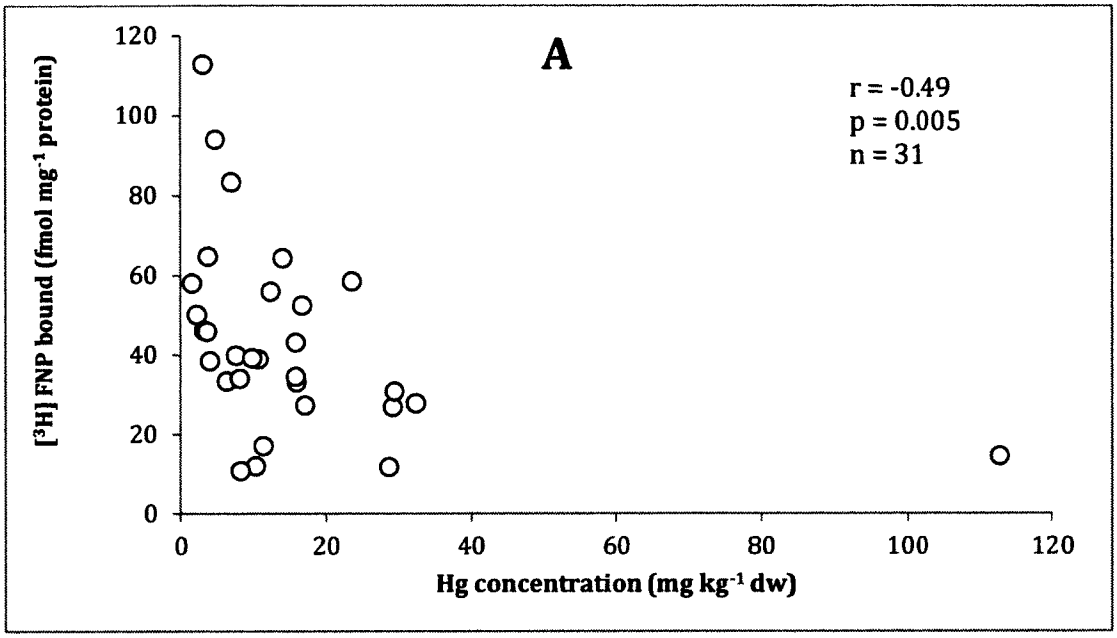
Biomarker	Cerebellum				Temporal Cortex			
	Adjusted R^2	Intercept	Slope	F-value	Adjusted R^2	Intercept	Slope	F-value
$GABA_A$ Receptor	0.4	1.8	0.17(year)^t -0.21(log(Hg))*	$F_{2,24}$ = 8.9*	0.3	3.3	-0.001(year) - 0.0.5(log_Hg)	$F_{2,21}$ = 0.62
	0.4	1.3	0.33(year)* - 0.25(log(MeHg))* + 0.008(age)	$F_{3,18}$ = 6.6*	0.2	-21	0.01(year) – 0.04(log_MeHg)	$F_{2,21}$ = 0.34
	0.3	1.6	0.19(year)* -0.25(log(iHg))	$F_{2,24}$ = 7.6*	0.2	-6.9	0.004(year) – (0.05(log_iHg))	$F_{2,21}$ = 0.45
	0.3	1.4	0.23(year)* – 0.39(log(HgSe))	$F_{2,24}$ = 7.2*	0.08	7.2	-0.002(year) – 0.05(log_HgSe)	$F_{2,20}$ = 0.07
	0.5	2.0	0.20(year)* – 0.49(log(Se))* + 0.0067(age)	$F_{3,19}$ = 7.3*	0.07	21	-0.009(year) – 0.11(log(Se))	$F_{2,21}$ = 1.05
$GABA_A$-R α2 mRNA	0.8	-300	0.15(year)* – 0.15(log(Hg))*	$F_{2,24}$ = 50*	0.8	-283	0.14(year)* – 0.10(log(Hg))	$F_{2,24}$ = 19.8*
	0.8	-326	0.16(year)* – 0.13(log(MeHg))	$F_{2,25}$ = 37.7*	0.8	-315	0.16(year)* - 0.26(log(MeHg))*	$F_{2,25}$ = 24.2*
	0.8	-340	0.17(year)* – 0.16(log(iHg))^t	$F_{2,25}$ = 44*	0.8	-304	0.15(year)* – 0.09(log(iHg))	$F_{2,25}$ = 19.8*
	0.8	-334	0.17(year)* – 0.11(log(HgSe))	$F_{2,26}$ = 37.1*	0.7	-319	0.16(year)* – 0.16(log(HgSe))	$F_{2,25}$ = 19.6*
	0.8	-290	0.14(year)* – 0.23(log(Se))*	$F_{2,24}$ = 54*	0.8	-264	0.13(year)* - 0.18(log(Se))	$F_{2,25}$ = 21.8*

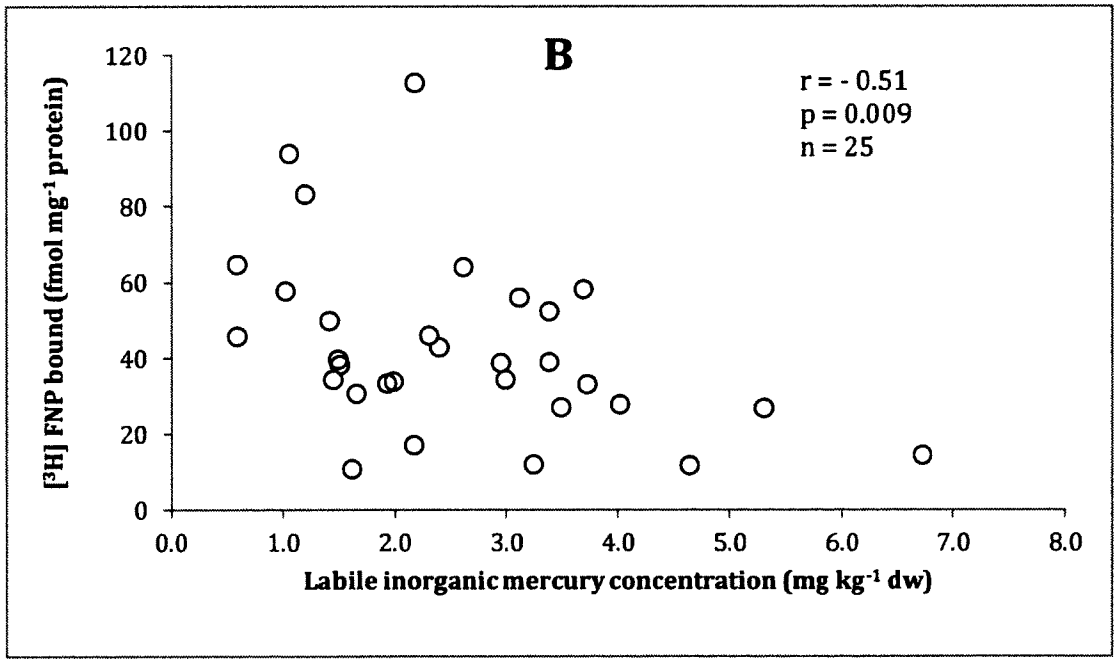
*$p \leq 0.05$
$^t \leq 0.10$

Table 4.3. Summary of backwards stepwise linear multiple regression analysis for the cerebellum and temporal cortex with NMDA receptor binding to [3H]-801, and mRNA expression (fold change) for NMDA subunit 2b as the three outcome variables tested. The predictor variables tested included total mercury (Hg_T), methylmercury (MeHg), labile inorganic mercury (iHg_{labile}), total selenium (Se_T), or the molar ratio of Hg_T to Se_T, and sampling year, and animal age. Adjusted R squared values and F-values are provided for the models indicated.

Biomarker	Cerebellum				Temporal Cortex			
	Adjusted R^2	Intercept	Slope	F-value	Adjusted R^2	Intercept	Slope	F-value
NMDA Receptor	0.2	2.9	0.07(year) + 0.03(log(Hg)) – 0.002(age)	$F_{3,19}$ = 0.71	0.6	-55	0.029(year) – 0.11(log(Hg))^t	$F_{2,19}$ = 5.7*
	0.1	2.9	0.06(year) + 0.09(log(MeHg)) – 0.002(age)	$F_{3,19}$ = 0.83	0.5	-110	0.055(year)* - 0.12(log(MeHg))	$F_{2,19}$ = 4.1*
	0.2	2.9	0.08(year) + 0.07(log(iHg)) – 0.002(age)	$F_{3,19}$ = 0.74	0.5	-63	0.033(year) – 0.15(log(iHg))^t	$F_{2,19}$ = 5.6*
	0.2	2.9	0.06(year) – 0.003(log(HgSe)) – 0.002(age)	$F_{3,19}$ = 0.66	0.5	-76	0.039(year) – 0.28(log(HgSe))^t	$F_{2,19}$ = 5.5*
	0.2	2.8	0.08(year) + 0.07(log(Se)) - 0.002(age)	$F_{3,19}$ = 0.77	0.5	-56	0.029(year) – 0.15(log(Se))^t	$F_{2,19}$ = 5.3*
NMDA 2b mRNA	0.8	352	-0.17(year)* -0.16(log(Hg))	$F_{2,24}$ = 26.1*	0.1	-28	0.01(year) – 0.02(log(Hg))	$F_{2,26}$ = 0.54
	0.8	319	-0.16(year)* – 0.13(log(MeHg))	$F_{2,24}$ = 19.5*	0.1	-48	0.02(year) + 0.2(log(MeHg))	$F_{2,27}$ = 0.85
	0.8	347	-0.17(year)* – 0.27(log(iHg))*	$F_{2,24}$ = 28.1*	0.1	-29	0.01(year) – 0.05(log(iHg))	$F_{2,27}$ = 1.1
	0.8	325	-0.16(year)* - 0.35(log(HgSe))	$F_{2,24}$ = 24.8*	0.05	-39	0.02(year) – 0.05(log(HgSe))	$F_{2,27}$ = 0.95
	0.8	360	-0.16(year) - 0.30(log(Se))* + 0.003(age)	$F_{3,20}$ = 15.8*	0.1	-44	0.02(year) – 0.008(log(Se))	$F_{2,27}$ = 0.84

*$p \leq 0.05$
$^t \leq 0.10$


A
r = -0.49
p = 0.005
n = 31
[3H] FNP bound (fmol mg-1 protein)
Hg concentration (mg kg-1 dw)
0
20
40
60
80
100
120


B
r = - 0.51
p = 0.009
n = 25
[3H] FNP bound (fmol mg-1 protein)
Labile inorganic mercury concentration (mg kg-1 dw)
0
20
40
60
80
100
120
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0

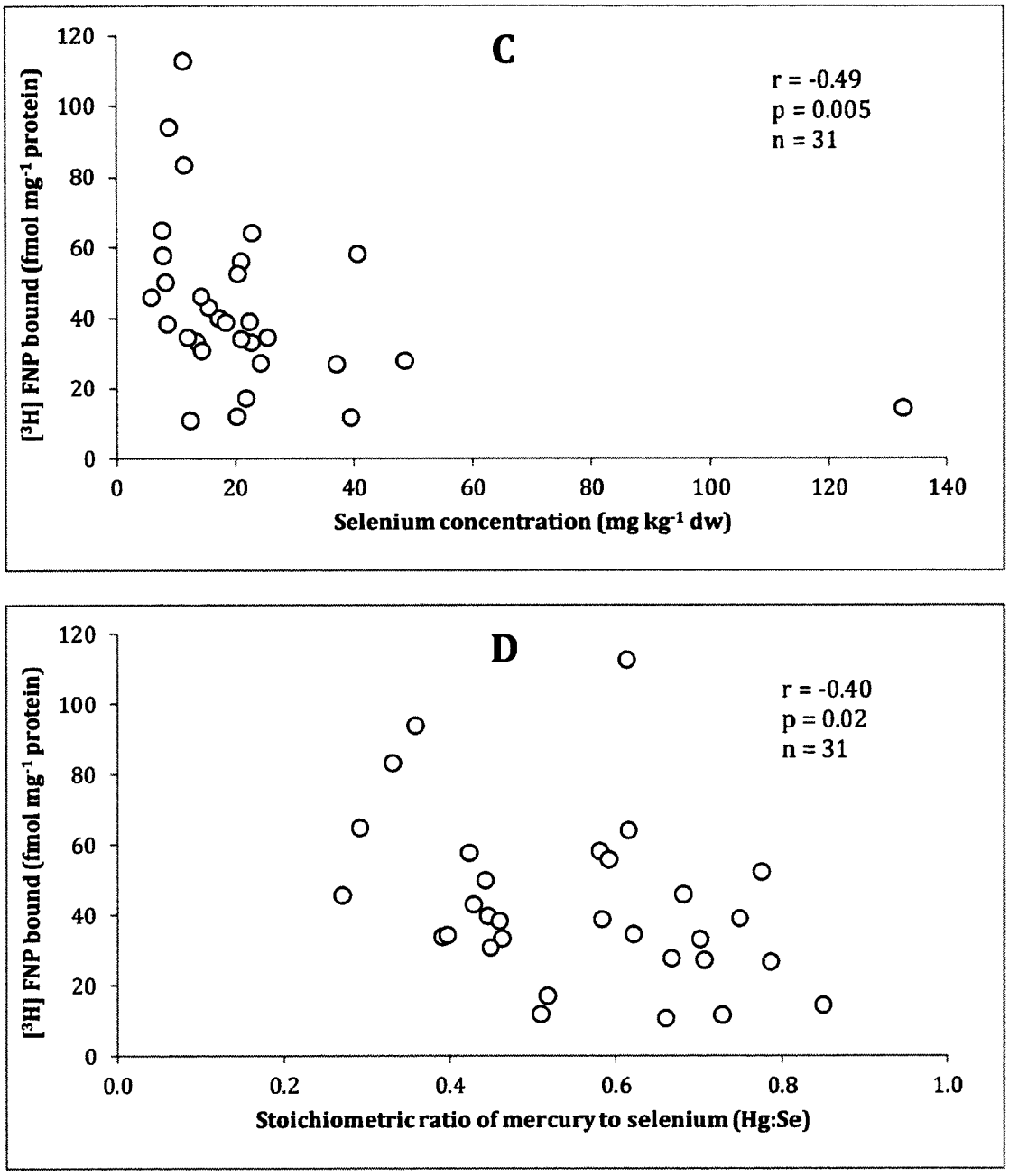
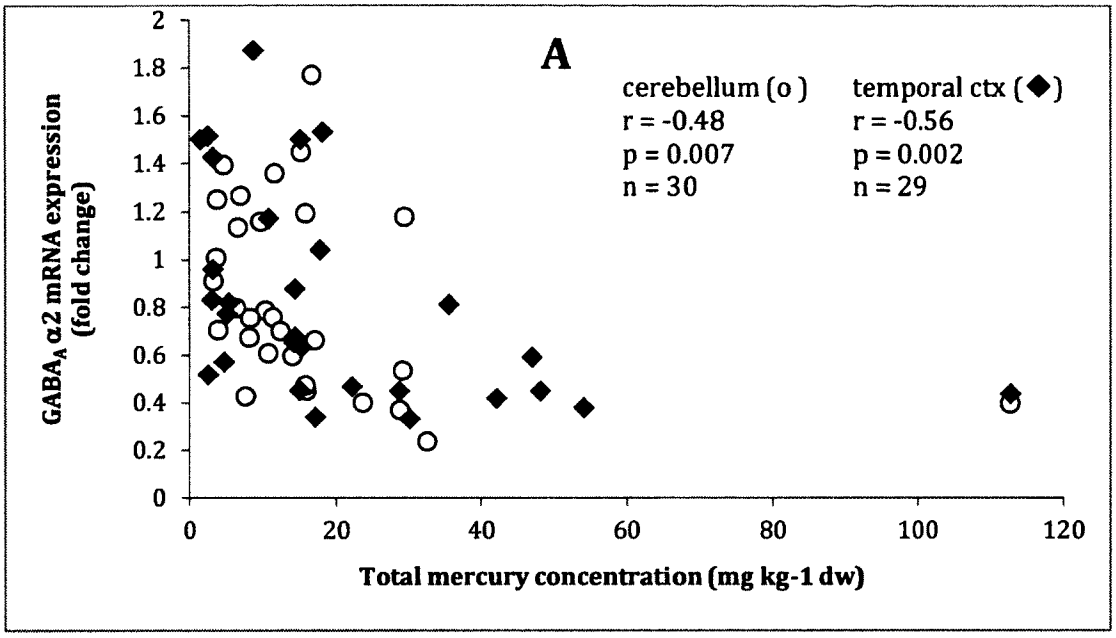
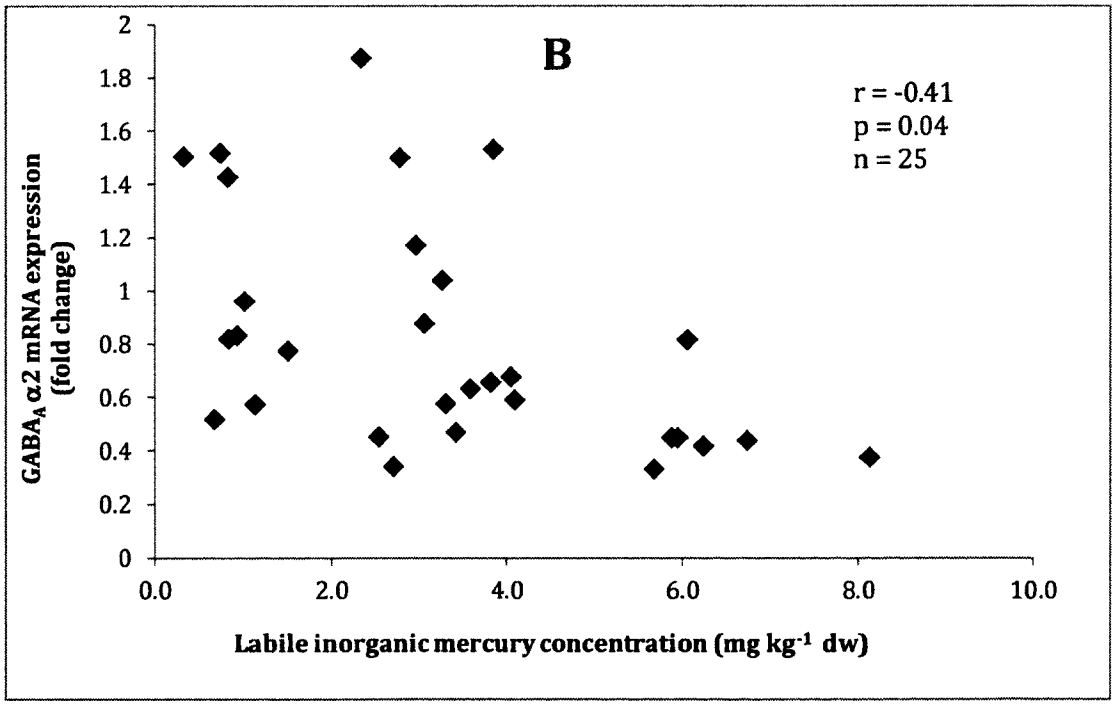
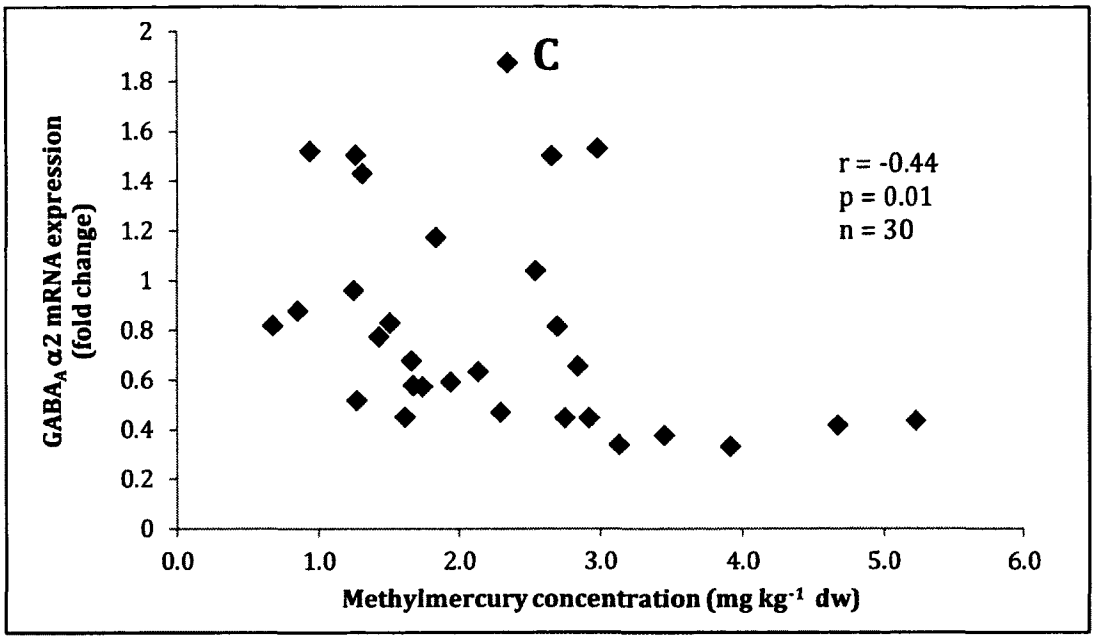
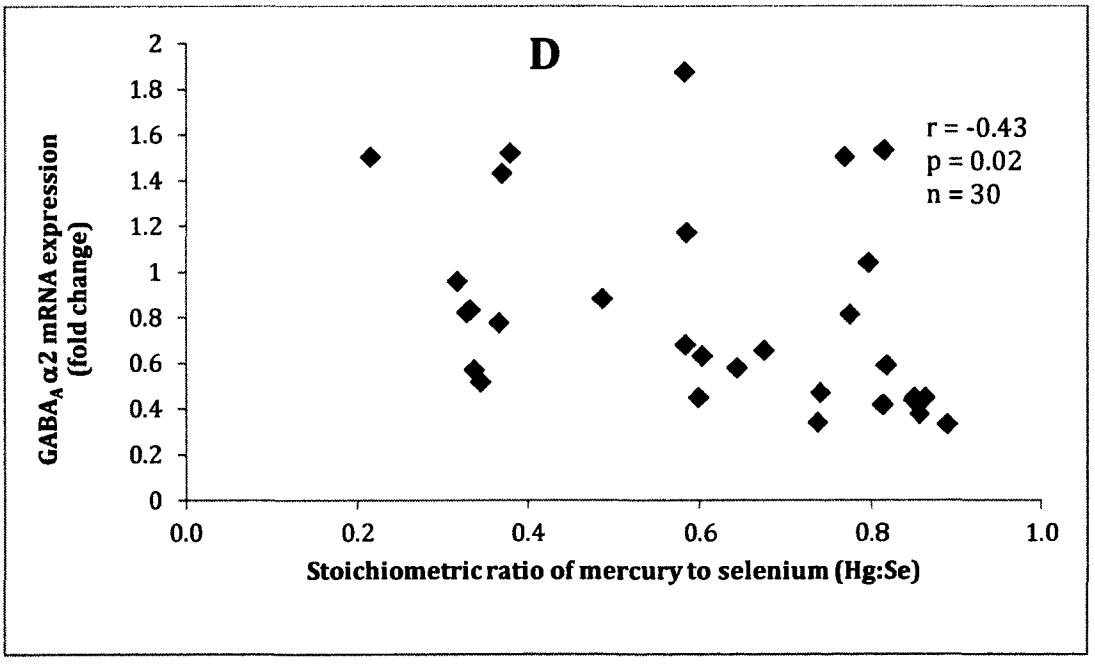


Figure 4.1. Correlations between $GABA_A$ receptor binding and mercury concentration (A), labile inorganic mercury concentration (B), selenium concentration (C), and stoichiometric ratio of mercury to selenium (D) in the cerebellar cortex of beluga whales (*Delphinapterus leucas*).


A
cerebellum (o)
r = -0.48
p = 0.007
n = 30
temporal ctx (◆)
r = -0.56
p = 0.002
n = 29
GABA$_A$ α2 mRNA expression (fold change)
Total mercury concentration (mg kg-1 dw)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
20
40
60
80
100
120


B
r = -0.41
p = 0.04
n = 25
GABA$_A$ α2 mRNA expression (fold change)
Labile inorganic mercury concentration (mg kg^{-1} dw)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0.0
2.0
4.0
6.0
8.0
10.0


C
r = -0.44
p = 0.01
n = 30
GABA$_A$ α2 mRNA expression (fold change)
Methylmercury concentration (mg kg^{-1} dw)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0.0
1.0
2.0
3.0
4.0
5.0
6.0


D
r = -0.43
p = 0.02
n = 30
GABA$_A$ α2 mRNA expression (fold change)
Stoichiometric ratio of mercury to selenium (Hg:Se)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0.0
0.2
0.4
0.6
0.8
1.0

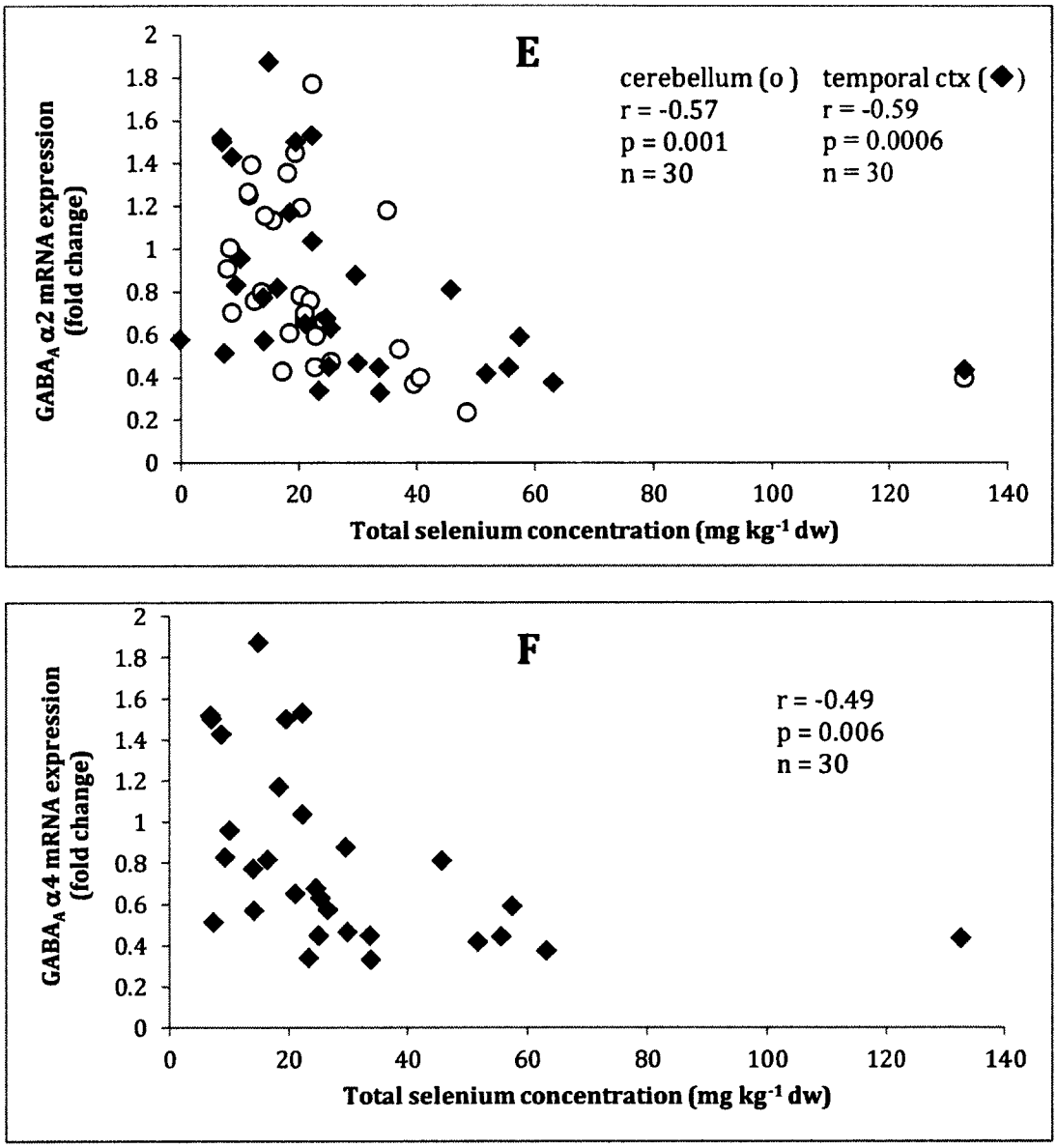


Figure 4.2. Correlations between mRNA expression of $GABA_A$ subunit α2 (fold change) and total mercury concentration (A, mg kg^{-1} dw), labile inorganic mercury concentration (B), methylmercury concentration (C), stoichiometric ratio of mercury to selenium (D) and selenium concentration (E, mg kg^{-1} dw) in the cerebellum (circle, o) and temporal cortex (diamond, ◆). Correlations between mRNA expression of $GABA_A$ subunit α4 (F) and selenium concentration (mg kg^{-1} dw) in the temporal cortex (diamond, ◆). The expression of mRNA was normalized to the internal control gene S9 and fold changes were calculated based on the lowest-exposed whales (*n = 3*).

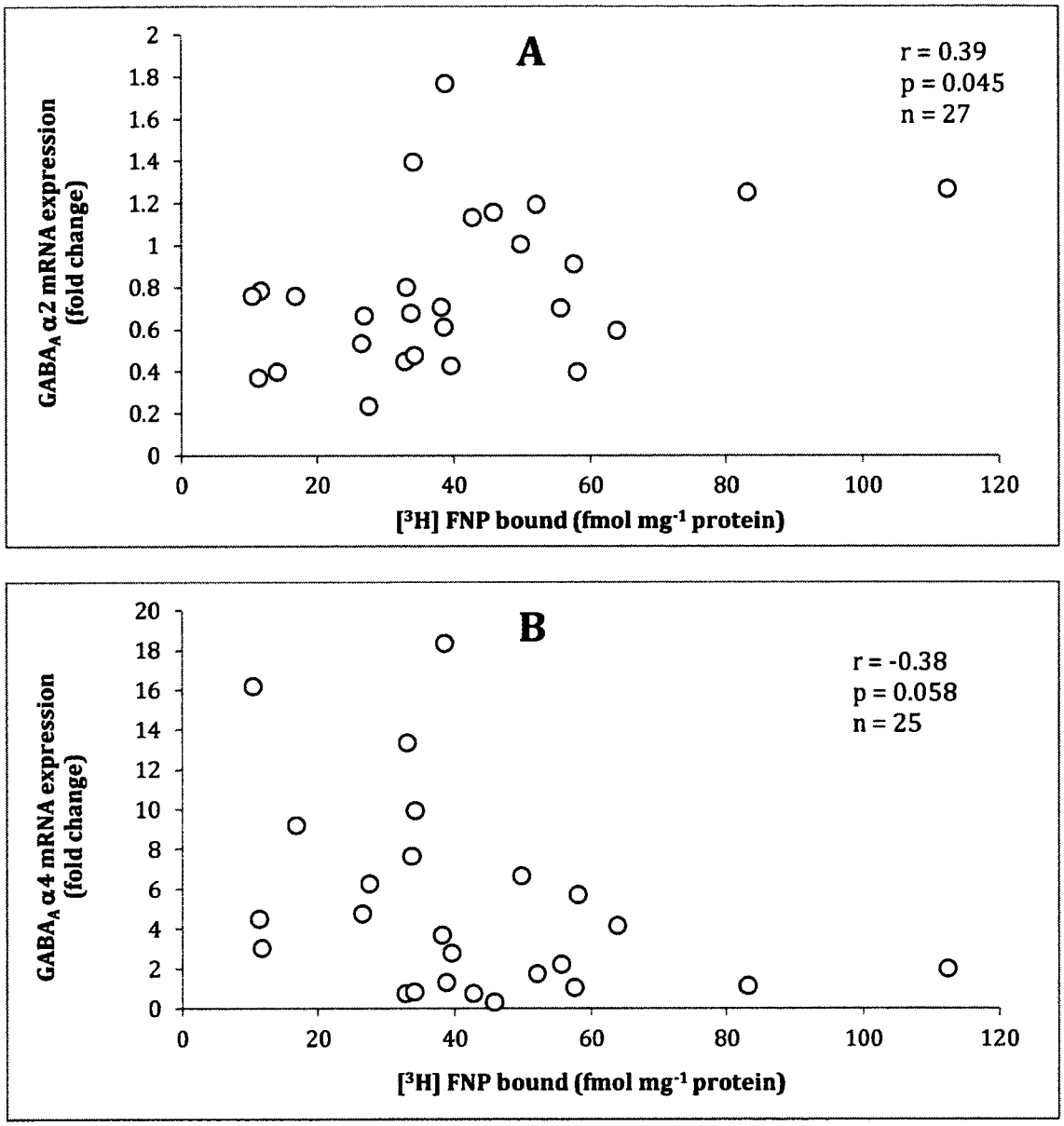


Figure 4.3. Correlations between mRNA expression for target genes and corresponding neurochemical expression for GABAergic signaling pathway (A: $GABA_A$-R α2 and GABA-R; B: $GABA_A$-R α4 and GABA-R) in the cerebellar cortex (unfilled circle, o) of beluga whales. Messenger RNA expression was normalized to the internal control gene S9 and fold changes were calculated based on the lowest-exposed whales (n = 3).

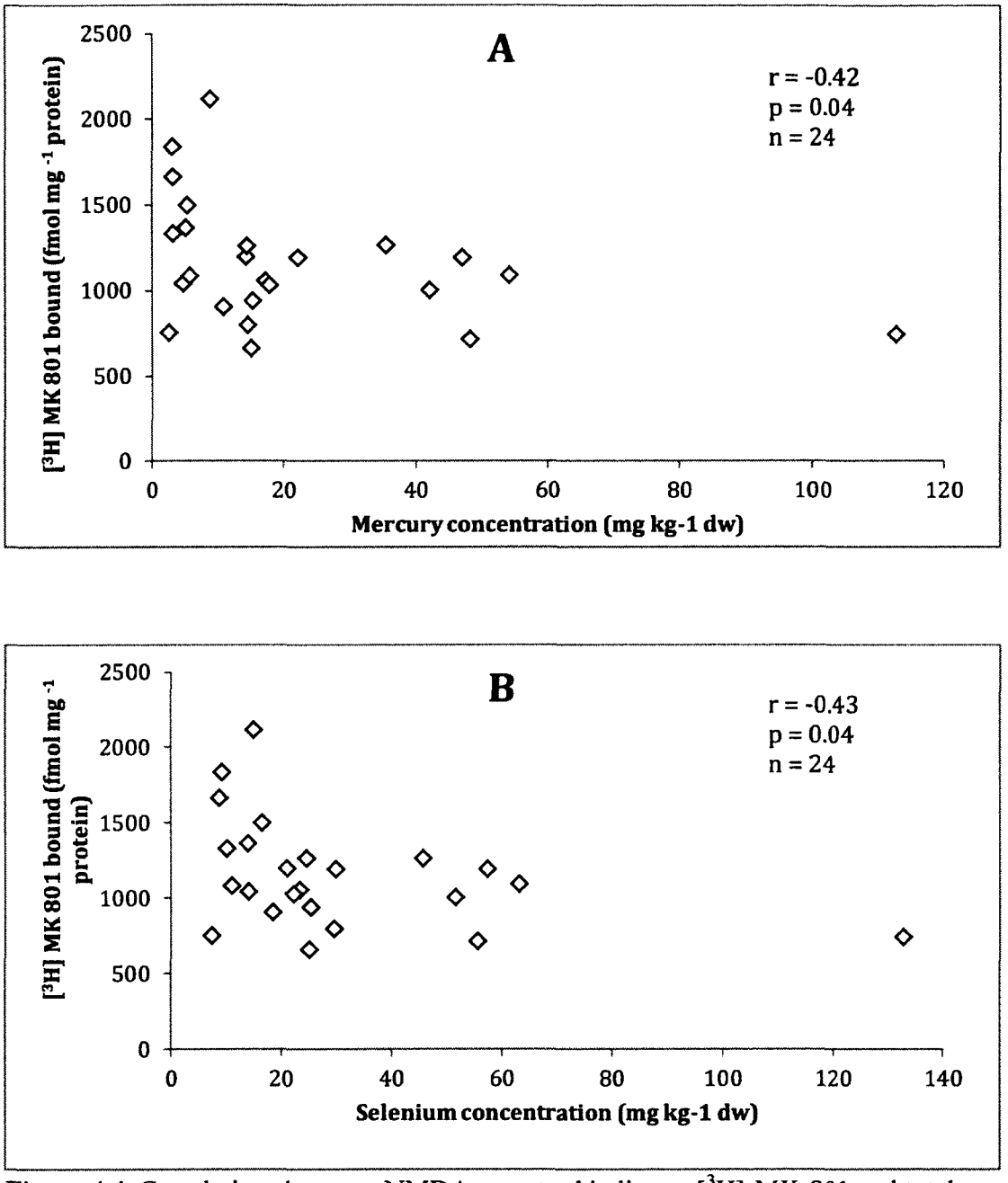


Figure 4.4. Correlations between NMDA receptor binding to [^{3}H]-MK-801 and total mercury (A) and selenium (B) concentrations (mg kg^{-1} dw) in the temporal cortex of beluga whales (*Delphinapterus leucas*).

Discussion

This is the first study to investigate the potential effects of Hg exposure in beluga whale brains at both molecular and biochemical levels. The major findings from this study were that Hg_T and Se_T concentrations, and different chemical forms of Hg, were associated with variation of both neurochemical biomarkers and mRNA expression in the cerebellar and temporal cortex of beluga whales. Total Hg, MeHg, iHg_{labile} and Se were found to be significant predictors of receptor binding and/or mRNA expression of various components of the GABAergic and glutamatergic signaling pathways. Overall, our results suggested that the $GABA_A$-R might be more sensitive to Hg, MeHg and iHg_{labile} exposure than the NMDA-R. Although Se may detoxify MeHg in the brain, we did not observe a relationship between Se_T co-accumulation with Hg that would suggest a protective effect of Se for either signaling pathway, at a neurochemical or molecular level. Furthermore, the cerebellum may be more sensitive than the temporal cortex to perturbation of molecular and neurochemical components from the GABAergic signaling pathway. Regional differences were less apparent for molecular and neurochemical components of the glutamatergic signaling pathway analyzed. The potential mechanisms of action and physiological outcomes of these findings are discussed in relation to previous captive and wildlife animal studies, and *in vitro* studies.

GABAergic signaling pathway

Disruption of GABAergic signaling has been proposed as a basis for many neuropsychiatric disorders and pathologies (Reis et al., 2009). Gamma-butyric acid-A receptors have been linked to sedation, sleep, anxiety, seizures and amnesia (Vicini and Ortinski, 2004). The activity of the

$GABA_A$-R is modulated by benzodiazepines (Chebib and Johnston, 1999), and drugs that enhance the function of the benzodiazepine binding site result in symptoms very similar to symptoms of MeHg toxicity, including ataxia, lack of muscular incoordination, motor disturbance and decreased Purkinje cell activity (Concas et al., 1983). The negative relationship between $GABA_A$ receptor levels and Hg_T and MeHg in the cerebellar cortex but not temporal cortex was consistent with findings from captive mink in which GABA-R binding to [^{3}H]-Muscimol in cerebellum but not occipital cortex were negatively associated with dietary exposure to MeHg (Basu et al., 2010). To our knowledge, the negative relationships between mRNA expression for $GABA_A$ $\alpha 2$, and Hg concentration (Hg_T and MeHg) and Se_T in the cerebellar and temporal cortex, are the first reported observations of Hg-associated variation in mRNA expression for subunits of the $GABA_A$-R.

Disruption of the GABAergic signaling pathway has been associated with dietary exposure to MeHg and *in vitro* exposure to MeHg and iHg. In acute MeHg feeding trials, MeHg-exposed rats had increased benzodiazepine binding sites, but not GABA binding sites in various brain regions (Concas et al., 1983; Corda et al., 1981). Methylmercury and iHg may disrupt GABAergic signaling through the inhibition of GABA uptake by astrocytes, and the modulation of GABA-induced inward currents (Arakawa et al., 1991; Narahashi et al., 1994). Inhibition of the $GABA_A$ receptor could potentially lead to an excitatory effect (Sunol et al., 2008); therefore, receptors and enzymes that mediate GABAergic signaling are tightly regulated to protect neurons from excitotoxicity (Reis et al., 2009). Decreasing $GABA_A$ receptor levels could occur to maintain homeostasis of GABAergic signaling following Hg exposure (Basu et al., 2010). However,

changes in the function of the $GABA_A$ system may adversely affect synaptic transmission if the balance between excitatory and inhibitory synapses is not maintained (Narahashi et al., 1994). Furthermore, balancing the excitatory and inhibitory signaling pathways may have an energetic cost to the whole organism (Basu et al., 2010).

Glutamatergic signaling pathway

NMDA receptors are involved in the formation and maintenance of synapses and are also required for learning and memory processes (Popescu, 2005). Glutamate binds to the NMDA-R and is the principle excitatory neurotransmitter in the brain; therefore, excessive amounts of glutamate in the synaptic space can cause overstimulation of neurons (Fitsanakis and Aschner, 2005). Previous studies have found significant negative relationships between Hg_T concentration and NMDA-R in polar bears (Basu et al., 2009), wild mink (Basu et al., 2007c), loons and eagles (Scheuhammer et al., 2008). The negative relationship observed for mRNA expression of NMDA-R 2b and iHg_{labile} concentration are consistent with findings that MeHg exposure in rats was associated with a decrease in mRNA expression and protein densities of NMDA subunits 1, 2A and 2B (Xu et al., 2013).

The activation of NMDA receptors was found to play a key role in glutamate-associated excitotoxicity of astrocyte cultures (Albrecht and Matyja, 1996). Methylmercury and/or Hg^{2+} interacted with the glutamatergic signaling pathway by increasing extracellular glutamate (Juarez et al., 2002), increasing the release of dopamine (Faro et al., 2002; Vidal et al., 2007), and increasing Ca^{2+} influx and apoptosis of cerebellar granule cells (Rossi et al., 1997). Furthermore,

NMDA-R has been associated with MeHg-associated apoptosis in the cerebral cortex of rats; pre-treatment of rats with the NMDA-R antagonist MK-801 reduced apoptotic cells compared to the MeHg-treated group (Xu et al., 2013). Therefore, downregulation of NMDA-R subunits may protect glutamatergic neurons from MeHg-associate excitotoxicity. Excessive NMDA-R activity promotes seizures and contributes to neuronal loss, which in turn is associated with hypoxia, brain injury and neurodegenerative diseases (Popescu, 2005).

Messenger RNA expression and neurochemistry

We observed inconsistent correlations between neurochemistry and the expression of mRNA for associated target genes. Given the lack of data specific to beluga whales, we provide possible explanations for the correlations observed based on the available literature. The positive correlation observed between mRNA expression and receptor binding suggests that downregulation of mRNA transcription for $GABA_A$-R α2 (cerebellum) and NMDA-R 2b (temporal cortex) was associated with a decrease in $GABA_A$-R and NMDA-R binding, respectively. However, increased mRNA transcription for $GABA_A$ α4 was negatively correlated to $GABA_A$-R binding to [^{3}H]-FNP. The decrease in $GABA_A$-R binding associated with increased $GABA_A$-R α4 mRNA expression may not actually be due to a decoupling of mRNA transcription and translation, but instead the insensitivity of receptors composed of the α4 subunit to FNP (Whittemore et al., 1996). An inverse relationship between the expression of mRNA and density of polypeptides for $GABA_A$–R α2 and α4 was observed in cortical cells isolated from mouse fetuses and exposed to ethanol (chronic or chronic intermittent); however, mRNA expression was positively associated with polypeptide density for subunit α2 and α4

(Sheela Rani and Ticku, 2006). Our findings suggest that $GABA_A$-R binding and/or composition may be modulated by Hg exposure.

Potential detoxification of mercury by selenium

Previous research has linked Se accumulation in dolphins to potential detoxification of Hg (Cardellicchio et al., 2002). However, in this study, the molar ratio of Hg to Se did not predict receptor binding levels or mRNA expression levels for either the GABAergic or glutamatergic signaling pathways. We would have expected the molar ratio of Hg to Se to be associated with the biomarkers explored in this study, if Se was indeed detoxifying MeHg. Therefore, the potential protective effects of Se must be further explored in future studies.

Acknowledgements

This study was funded by NSERC Discovery (HMC) and the Fisheries Joint Management Committee (SKO). SKO was the recipient of a NSERC Doctoral award, Nasivvik Doctoral award, NSTP Training Fund and UNBC travel awards. We thank A. Philibert and M. Gillingham for guidance with statistical analyses, E. Montie for sharing dissection methods, A. Essler, A. Montgomery, S. Krause, L. Rose, A. Krey, G. Prkachin, J. Rutkiewicz, K. Mittal, and P. Dornbos for assistance in the laboratory, and L. Loseto, M. Noel, F. Pokiak, N. Pokiak, D. Sydney, R. Felix, K. Nuyaviak, B. Voudrach, R. Walker, K. Snow, C. Pokiak, Inuvialuit hunters, Tuktoyaktuk and Inuvik HTCs, and the DFO for their support in the field.

Chapter 5. Methylmercury and selenium exposure were associated with biomarkers of the cholinergic and dopaminergic signaling pathways in Arctic beluga whales (*Delphinapterus leucas*).

Names and Affiliations of Authors:
1. Sonja K. Ostertag
Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, British Columbia, Canada, V2N 4Z9
ostertag@unbc.ca

2. Hing Man Chan
Center for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5
laurie.chan@uottawa.ca

Abstract

There are increasing concerns about potential subclinical impacts of chronic methylmercury (MeHg) exposure in Arctic wildlife and human populations. The objective of this study was to characterize the relationship between MeHg and selenium (Se) exposure, and various neurochemical and molecular biomarkers in different brain regions of beluga whales. Samples were collected in 2008 (n = 20) and 2010 (n = 15) on Hendrickson Island, NT, Canada, from hunter-harvested beluga whales. The concentrations of total mercury (Hg_T) and Se, and labile inorganic Hg (iHg_{labile}) and MeHg, were determined by inductively coupled plasma Mass Spectrometry and high performance liquid chromatography, respectively. Receptor binding levels for the muscarinic acetylcholine receptor (mAChR) were quantified using radioligand-binding assays with a Chameleon^{TM} liquid scintillation counter. Total monoamine oxidase activity was quantified using spectrofluorometery (Chameleon^{TM}). Quantitative Real Time Polymerase Chain Reaction was used to quantify the relative expression of mRNA for mAChR subtype m1 and MAO-A. Total Hg concentrations ranged from 1.7 to ~ 113 mg kg^{-1} dw in cerebellar cortex and 2.58 – 113 m g kg^{-1} dw in temporal cortex. In the temporal cortex, mAChR binding was negatively associated with the ratio of Hg to Se; MAO activity was negatively associated with the concentrations of Hg, MeHg, iHg and molar ratio of Hg_T to Se_T. The relative expression of mRNA for mAChR subtype m1 was statistically significantly associated with the concentrations of Hg, MeHg, iHg and the ratio of Hg to Se in the cerebellum (temporal cortex samples unavailable). These results suggest that MeHg exposure was associated with neurochemical variation of key components of the cholinergic and dopaminergic signaling pathways in the temporal cortex, and molecular variation of mAChR subtype m1 in the

cerebellum. Furthermore, these results suggest that accumulation of Se_T may provide protective effects on mAChR and MAO activity. The response of beluga whales to MeHg exposure at a physiological and population level remains to be elucidated.

Introduction

Organisms that are high in the Arctic marine food web have elevated mercury (Hg) levels due to the length and complexity of Arctic food webs (Fisk et al., 2005). In mammals, methylmercury (MeHg) is transported across the intestinal mucosa and is able to cross the blood brain barrier (Aschner and Aschner, 1990). The brain is highly sensitive to MeHg, and elevated exposure has been associated with adverse neurological outcomes including ataxia, constriction of the visual field and damage to the auditory region of the temporal lobe (Clarkson, 1997; Ekino et al., 2007). Concerns have been raised about the subclinical impacts of chronic MeHg exposure in Arctic wildlife and human populations (Dietz et al., 2013). Total Hg (Hg_T) concentrations in brain tissue from Arctic beluga whales ranged from 1.7 to ~ 113 mg kg^{-1} dw in cerebellum and 2.6 – 113 mg kg^{-1} dw in temporal cortex (Ostertag et al., 2013). In beluga whales harvested in 2008 and 2010, variation of molecular and/or biochemical components of the GABAergic and glutamatergic signaling pathways was associated with MeHg exposure in different brain regions (Ostertag et al., in review). Components of the cholinergic and dopaminergic signaling pathways are known to be sensitive to MeHg exposure (Basu et al., 2005a; Basu et al., 2006a; Basu et al., 2007b; Basu et al., 2006b; Beyrouty et al., 2006; Chakrabarti et al., 1998; Coccini et al., 2000; Coccini et al., 2007; Stamler et al., 2006); therefore, the effect of the Hg and Hg species on these signaling pathways needs further study in beluga whales.

Beluga whales from the eastern Beaufort Sea beluga whale population migrate to the southern Beaufort Sea during the summer and are harvested by Inuvialuit for food (Harwood and Smith, 2002). Contaminant levels have been monitored in this population in collaboration with the

Fisheries Joint Management Board, local Hunters and Trappers Committees and harvesters periodically since 1984 and yearly since 2001 (Lockhart et al., 2005). Results from this sampling program indicate that age-adjusted Hg concentration estimates (13.1 yr) in beluga whale liver from the Mackenzie Delta peaked in 1996 (29.04 mg kg^{-1} wet weight (ww)) and have since decreased. However, recent Hg concentrations (2002: 13.45 mg kg^{-1} ww) are higher than they were in the 1980s (7.76 and 5.56 mg kg^{-1} ww in 1981 and 1984, respectively) (Lockhart et al., 2005). Furthermore, contemporary Hg concentrations in beluga whale tissue have increased by an order of magnitude in the eastern Beaufort Sea population since preindustrial times (Outridge et al., 2002). Mercury concentrations ranged from 0.02 to 22.6 mg kg^{-1} ww in brain tissue collected from beluga whales harvested in 2006, 2008 and 2010 in the Mackenzie Delta Estuary, and the percentage of MeHg and labile iHg (iHg_{labile}) ranged from 4 to 109% and 0 to 89% of total Hg concentration, respectively (Ostertag et al., 2013). The significant relationship between Hg_T and total selenium (Se_T) in these beluga brain samples suggested potential detoxification of MeHg via demethylation followed by binding to Se (Ostertag et al., 2013). However, the relationships observed between co-accumulation of Se_T and Hg_T, and neurochemical or molecular biomarkers in beluga whales did not suggest a protective effect of Se for either GABAergic or glutamatergic signaling pathways (Ostertag et al., in review).

Although an adult human suffered Hg poisoning with a Hg_T concentration of 16.8 mg kg^{-1} in the cerebellum (Eto et al., 1999), dietary MeHg dosing studies on primate species indicated that intoxication was associated with Hg_T concentrations in brain tissue ranging from 6.0 to 12.0 mg kg^{-1} ww (Berlin et al., 1975b; Evans et al., 1977; Luschei et al., 1977). Concentrations of MeHg

below ~ 2–5 mg kg^{-1} ww were likely below thresholds of overt MeHg intoxication in mink (Suzuki, 1979; Wobeser et al., 1976). However, Hg_T concentrations measured in the central nervous system (CNS) of Arctic beluga whales exceeded concentrations associated with neurochemical variation in wildlife and avian studies (Basu et al., 2005c; Basu et al., 2007b; Basu et al., 2007c; Basu et al., 2009; Basu et al., 2005e; Rutkiewicz et al., 2011; Scheuhammer et al., 2008).

Chronic exposure to MeHg has been associated with significant increases in muscarinic acetylcholine receptor (mAChR) binding in wild mink (*Mustela vison*) (Basu et al., 2008; Basu et al., 2006b), loons (*Gavia immer*) and eagles (*Haliaeetus leucocephalus*) (Scheuhammer et al., 2008), and decreased mAChR levels in wild river otters (*Lontra canadensis*) (Basu et al., 2005c). Furthermore, decreased monoamine oxidase (MAO) activity was associated with Hg exposure in experimental studies (Beyrouty et al., 2006) and human populations (Stamler et al., 2006). Monoamine oxidase plays an important role in the metabolism of neurotransmitters; therefore, exposure to MeHg could affect the normal turnover rates of neurotransmitter amines in exposed animals (Chakrabarti et al., 1998). Given that biochemical changes may be an indicator of early-stage effects before the manifestation of disease (Manzo et al., 1996), the objective of this study was to characterize the relationship between different chemical forms of Hg, Se_T and Hg to Se molar ratio, and various neurochemical and molecular biomarkers in different brain regions of beluga whales.

Methods

Sample collection

Samples were collected in 2008 (n = 20) and 2010 (n = 15) on Hendrickson Island, NT, Canada, from hunter-harvested beluga whales, as described elsewhere (Ostertag et al., 2013). Appropriate research permits and licenses were obtained from the Aurora Research Institute and Department of Fisheries and Oceans (DFO) and the sampling program was approved and supported by the Tuktoyaktuk and Inuvik Hunters and Trappers Committees. Subsamples of cerebellum and temporal cortex were collected, and samples (~ 0.5 – 3 g) for metals and speciation analyses were frozen at ~ -20 °C, and samples for neurochemical analyses (~ 0.5-1 g) were flash-frozen and stored in liquid nitrogen. Samples (~ 0.07 g) for mRNA expression assays were flash-frozen in 2008 and were placed in RNALater™ at approx 4 °C for 24 h prior to freezing at -20 °C in 2010. Sample numbers varied for the different assays due to subsample availability for the separate analyses.

Mercury and Selenium analyses

The concentrations of Hg_T, iHg_{labile}, MeHg and Se_T were determined previously for the brain regions analyzed (Ostertag et al., 2013). Briefly, following a modified acid digestion (Armstrong and Uthe, 1971) total metals were analyzed by inductively coupled plasma Mass Spectrometry (Agilent Technologies, 7500 CX). The chemical forms of Hg were analyzed via high performance liquid chromatography (HPLC; Agilent 1200 series HPLC system, Agilent Technologies Canada Inc., Mississauga, ON, Canada), equipped with autosampler, quaternary pump, and 100 ml injection loop. A ZORBAX Eclipse XDB-C18 Column (2.1 x 50 mm, 5 μm)

was connected to an inductively coupled mass spectrometer (ICP-MS; Agilent Technologies™, 7500 CX), following extraction using a method developed by Krey et al. (2012).

Receptor binding assays

Receptor binding assays were adapted from previous studies by Basu et al. (2010; 2007c; 2006b), following the preparation of membrane homogenate as previously described (Basu et al., 2005c). Protein concentration was determined with the Bradford assay and bovine serum albumin was used as the standard. In brief, thirty μg of membrane preparation was re-suspended in buffer and added to a 96 well plate (Costar™). Membrane protein was suspended in 100 μL of 50 mM sodium phosphate buffer (pH 7.4) and incubated with [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB; 1.6 nM for cerebellum, 3.2 nM temporal cortex) for 60 min. Incubation was carried out with gentle shaking and binding reactions were ended by vacuum filtration. Sample was filtered through glass fiber (Inotech) using a cell harvester (Inotech), the filter was washed 4 times with ice-cold NaK buffer. The filter was set in an Omnifilter microplate (PerkinElmer), dried for 60 min under a heat lamp before 25 μL scintillation cocktail (Universol™, MP Biomedicals) was added. Radioactivity retained by the filters was quantified immediately by a Chameleon™ liquid scintillation counter (Hidex, Turku, Finland) with approx 19% counting efficiency. Specific binding was calculated as the difference between radioligand binding in the presence and absence of 100 μM atropine, a mAChR inhibitor. Receptor binding is reported as fmol of radioisotope bound per mg of membrane protein (fmol mg^{-1}).

Enzyme activity

Total monoamine oxidase activity was analyzed according to a previously described protocol (Nam et al., 2010; Zhou et al., 1997). In brief, samples were homogenized in Na/K buffer (10 mL g^{-1} tissue), 0.05% Triton-X-100 was added to the homogenate and the sample was sonicated for 30 s at setting 10 (Sonic dismembrator: Model 100, Fisher Scientific), prior to centrifugation (12 000 x g, 8 °C for 10 minutes). Homogenate (25 μg protein) was incubated with buffer for 30 min and enzyme activity was quantified following 15 min incubation with 10 mM 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red reagent), 1000 mM Tyramine, 200 mU horseradish peroxidase. Fluorescence (540 nm excitation and 590 nm emission) of resorufin (end product) was read every five min for 80 min with a Chameleon™ spectrofluorometer (Hidex). Enzyme activities were expressed as nmol resorufin formed per minute per microgram protein (ng μg^{-1} min^{-1}) based on the standard curve of resorufin product. Each sample was assayed in triplicate.

Expression of mRNA

Real time Polymerase Chain Reaction (RT-PCR) expression of mRNA for the mAChR subtype m1 (mAChR m1) and MAO-A target genes followed the RNA extraction and cDNA archive procedures outlined previously (Ostertag et al., in review). Species-specific primers and fluorogenic probes (IDT, Coralville, Iowa) were designed using Genscript RT-PCR primer design (Table 5.1). In brief, total RNA was extracted using Trizol with the Qiagen RNeasy™ Lipid Tissue kit. Total RNA was treated with Ambion™ DNase | buffer (6 μL), rDNAse | (1 μL) and DNase Inactivation reagent (6 μL), prior to quantification of RNA-40 using a spectrophotometer (Nanodrop, ND-1000). Taqman reverse transcription reagents (Applied

Biosystems) were used for the generation of complementary DNA (cDNA) for all samples. The identical reaction was performed concurrently without reverse transcriptase, to ensure the absence of genomic DNA. The thermocycler parameters for the generation of cDNA archive were as follows: 25 °C for 10 min, 37 °C for 60 min, and 95 °C for 5 min (DYAD™, DNA Engine).

All PCR runs were performed under identical conditions using the 7300 Real-Time PCR System (Applied Biosystems) as described previously (Ostertag et al., in review). Simplex assays were run with iTaq™ Supermix with ROX kit (Biorad). The thermocycle program included an enzyme activation step at 95 °C (10 min) and 40 cycles of 95 °C (15 sec) and 60 °C (1 min). The efficiencies for target genes and reference genes were calculated using standard curves generated for all genes from serial dilutions of cDNA. Fold changes for each target and sample gene were calculated relative to the reference gene s9 (Pfaffl, 2001) and the mean C_T value for the three lowest Hg-exposed animals was used as the control. Inter-plate variability of s9 expression (reference gene) was monitored through the inclusion of pooled sample in triplicate for each plate. The absence of genomic DNA was ensured through the inclusion of No-RT samples in each plate.

We used a NanoDrop (ND-1000, NanoDrop Technologies, USA) to evaluate RNA purity and quality. Optical density (OD) ratios (260 nm/280 nm wavelengths) greater than 1.8 indicated good RNA quality (Fleige and Pfaffl, 2006). The ratio of 28S:18S and RNA Quality Indicator (RQI) value were used to evaluate degradation via Experion (Bio-Rad Laboratories, USA); a

28S:18S ratio of 2.0 (Fleige and Pfaffl, 2006) or RQI of 10 indicated perfect integrity (Taylor et al., 2009).

Data Analysis

Mercury concentrations, fold-change ratios, receptor density and enzyme activity were log-transformed when necessary to meet assumptions of normal distribution and homogeneity of variance. The relationship between neurochemical and molecular biomarker, and the concentration of Hg, Hg species and Se was explored through multiple regression analysis. A backwards-stepwise approach was used to evaluate the following predictor variables: age, sampling year, and Hg_T, iHg_{labile}, MeHg, Se_T, or molar ratio of Hg to Se. The outcome variables used were mAChR binding, MAO activity and MAO-A mRNA expression in both brain regions, and mAChR m1 mRNA expression in the cerebellum. We removed influential outliers after examination of studentized residuals, leverage and Cook's D influence. Pearson correlation coefficients were calculated for the expression of target genes and corresponding receptor or enzyme. Correlations (Pearson and Spearman), F-values and t-tests were considered to be statistically significant if $p \leq 0.05$. Data in tables and graphs are displayed in the original scale of measurement.

Results

Twenty whales were sampled in 2008 (16 male, 4 female) and 15 beluga whales were sampled in 2010 (15 males, 0 females) and ranged from an estimated age of 16 to 60 yr (median age = 27 yr). The small sample size for females limited our ability to assess differences in Hg concentration/speciation, neurochemistry and mRNA expression based on gender. In the

cerebellum, MeHg concentrations ranged from 0.5 to 5.2 mg kg^{-1} dry weight (dw, median: 2.6 mg kg^{-1}), labile inorganic Hg (iHg_{labile}) concentrations ranged from 0.6 to 6.7 mg kg^{-1} dw (median: 2.6 mg kg^{-1} dw), selenium (Se_T) concentrations were 6 to 133 mg kg^{-1} dw (median: 19 mg kg^{-1} dw) and the ratios of Hg_T to Se_T were 0.2 to 0.9 (median: 0.6) (Table 5.2). The molar ratio of Hg to Se was correlated to Hg_T concentration in both the cerebellum ($r = 0.87$, $p < 0.0001$) and temporal cortex ($r = 0.92$, $p < 0.0001$).

The OD ratio was ≥ 1.8 for all RNA samples; therefore, RNA purity was considered acceptable. The ratio of 28S:18S was < 2 and the RQI was < 10 for a subset of samples analyzed via Experion (BioRad). The median and range of 28S:18S and RQI were 0.83 (0.3-1.87) and 6.9 (3.7-8.9), respectively for a subset of samples (*n = 12*), which indicated acceptable RNA quality. The Ct values for the internal control gene (s9) did not vary with age or Hg concentration in the cerebellum or temporal cortex. There was no amplification of NRT control following RT PCR.

Cholinergic signaling pathway

Receptor binding levels were greater in the temporal cortex than cerebellum for mAChR ($z = -4.1, p < 0.0001$). Non-specific binding represented 60% and 15% of total binding in the cerebellar and temporal cortex, respectively. Inter-plate variability ranged from 21-23% for mAChR assays. Muscarinic AChR binding to [^{3}H]-QNB was not correlated to Hg_T, MeHg or iHg concentration in either brain region (data not shown). Furthermore, gender was not associated with differences in mAChR binding in either cerebellum or temporal cortex (data not shown). However, age was negatively correlated with mAChR binding to [^{3}H]-QNB in temporal cortex (Figure 5.1, $r = -0.58, p < 0.05$), but not cerebellum. The results from three backward

stepwise multiple regressions that included Hg_T, MeHg or iHg_{labile}, in combination with animal age and sampling year, for both brain regions, indicated that mAChR binding was not significantly predicted by the models tested (Table 5.3).

The expression of mRNA for mAChR m1 was not correlated to concentrations of Hg_T, MeHg, or iHg_{labile}. Furthermore, age was not correlated to mRNA expression for mAChR m1, and there were no significant differences in mRNA expression for mAChR m1 with sampling year or animal gender. The results from three backward stepwise multiple regressions that included Hg_T, MeHg or iHg_{labile}, in combination with animal age and sampling year, for both brain regions, indicated that the following models were significant ($p < 0.05$) predictors of log-transformed mRNA expression in the cerebellum (Table 5.3; data not available for temporal cortex): 230 - 0.1(year) -0.7(log(Hg)) + 0.01(age), 120 - 0.06(year) – 0.6(log(MeHg)) + 0.01(age), and 204 - 0.2(year) -0.8(log(iHg)) + 0.04(age). However, the expression of mRNA for mAChR m1 was not correlated to mAChR binding to [^{3}H]-QNB in the cerebellum (data for temporal cortex unavailable).

Selenium concentration and the molar ratio of Hg to Se were not correlated to mAChR binding (data not shown). However, the results from two backward stepwise multiple regressions that included Se_T or the molar ratio of Hg to Se, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed mAChR binding in the temporal cortex (Table 5.3): -140 + 0.07(year) + 0.3(log(HgSe)) - 0.004(age), and -110 + 0.06(year) - 0.06(log(Se)) – 0.003(age). However, only

the molar ratio of Hg to Se, animal age and year were significant predictors of mAChR binding in the temporal cortex. At a molecular level, the expression of mRNA for mAChR m1 was not correlated to the molar ratio of Hg to Se or to Se_T concentration (data not shown). Yet, the results from two backward stepwise multiple regressions that included Se_T or the molar ratio of Hg to Se, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed mRNA expression mAChR m1 in the cerebellum (Table 5.3): 207 - 0.1(year) – 1.2(log(HgSe)) + 0.01(age). The molar ratio of Hg to Se was the only significant predictor ($p < 0.05$) of log-transformed mAChR mRNA expression in the cerebellum.

Dopaminergic signaling pathway

Total MAO ($z = -2.5$, $p = 0.01$) activity was greater in temporal cortex (median, range; 352 nM μg^{-1} min^{-1}, 227 - 592 nM μg^{-1} min^{-1}) than cerebellum (median, range; 161 nM μg^{-1} min^{-1}, 67 - 854 nM μg^{-1} min^{-1}). Total MAO activity was not correlated to Hg_T, iHg_{labile} or MeHg in either brain region. Gender was not associated with differences in total MAO activity in either cerebellum or temporal cortex. However, age was positively correlated to total MAO activity in the temporal cortex but not cerebellum (Figure 5.2; $r = 0.50$, $p < 0.05$). Sampling year was associated with statistically significant differences in total MAO activities in the cerebellum ($t = -3.91$, $DF = 28$, $p < 0.001$), but not temporal cortex ($t = 0.99$, $DF = 23$, $p < 0.35$).

The results from three backward stepwise multiple regressions that included Hg_T, MeHg or iHg_{labile}, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed total MAO activity in

the cerebellum: -460 + 0.2(year) + 0.3(log(Hg)) + 0.01(age), -434 + 0.2(year) + 0.1(log(MeHg)) + 0.01(age), and -395 + 0.2(year) + 0.4(log(iHg)) + 0.01(age); and, in the temporal cortex: 2.5 + 0.007(age) - 0.1 (log(Hg)), 2.5 + 0.005(age) -0.3(log(MeHg)), and 2.4 + 0.007(age) - 0.2(log(iHg)). In the temporal cortex, only animal age, Hg_T, MeHg and iHg_{labile} in the temporal cortex were significant predictors of log transformed MAO activity (Table 5.4). In the cerebellum, only sampling year was a significant predictor of MAO activity (Table 5.4).

Total Hg, MeHg and iHg were not correlated to the expression of mRNA for MAO-A in either brain region analyzed (data not shown). Gender was not associated with differences in mRNA expression for the target genes analyzed (data not shown). The results from three backward stepwise multiple regressions that included Hg_T, MeHg or iHg_{labile}, in combination with animal age and sampling year, for both brain regions, indicated that none of the models that we tested predicted log-transformed mRNA expression for MAO-A (Table 5.4). Furthermore, the expression of MAO-A mRNA was not correlated to MAO-A activity in the cerebellum or temporal cortex.

Monoamine oxidase activity was not correlated to molar Hg to Se ratio or Se concentration in either brain region (data not shown). In contrast, Se_T concentration was negatively correlated to mRNA expression for MAO-A in the cerebellum, but not temporal cortex (Figure 5.3; r = -0.36, $p \leq 0.05$). The results from two backward stepwise multiple regressions that included Se_T or the molar ratio of Hg to Se, in combination with animal age and sampling year, for both brain regions, indicated that the following models significantly ($p < 0.05$) predicted log-transformed

MAO-T activity in the cerebellum (Table 5.4): -452 + 0.2(year) + 0.2(log(HgSe)) + 0.01(age), -491 + 0.3(year) + 0.3(log(Se)) + 0.01(log(Se)); and, in the temporal cortex (Table 5.4): 2.2 + 0.007(age) -0.4(log(HgSe)) and 2.5 + 0.006(age) -0.1(log(Se)). However, of the selenium-related predictor variables, only the Hg to Se molar ratio was a significant predictor of MAO-T activity in the temporal cortex. Furthermore, multiple linear regression models that we tested did not significantly predict mRNA expression MAO-A (Table 5.4).

Table 5.1. Sequences of primers and probes used for real time PCR.

Target gene	Primer sequence		Probe
	Fw	Rvs	
MAO-A	GGCCAGGAACGGAAGTTTGT	CCCCGAGGAGGTGCATTA	TGGATCTGGTCAAGTAAGCG AGCGG
mAChR subtype m1	GCAACGCCTCGGTCATG	GCCGGGTCACGGAGAAGTA	CGGTCAACAACTACTTCCTG CTGAGCCTG
S9	GCTGCTGACGCTGGATGAG	CGCAGCAGGGCATTGC	AAGACCCGCGGCGTCTGTTT GAA

Table 5.2. Descriptive statistics for total mercury (Hg), methylmercury (MeHg), labile inorganic mercury (iHg_{labile}) and selenium (Se_T) concentrations and stoichiometric ratio of Hg_T to Se_T in the cerebellum and temporal cortex from beluga whales sampled at Hendrickson Island, NT, Canada in 2008 and 2010.

Variable	**Descriptive Statistics**	
	Temporal Cortex	Cerebellum
Sample number (*n*)	31	35
Total Hg concentration ($mg\ kg^{-1}$ dw)	15.0 (1.5 – 113)	10.6 (1.7 – ~ 113)
MeHg concentration ($mg\ kg^{-1}$ dw)	1.9 (0.68 – 5.2)	1.4 (0.45 – 5.2)
iHg_{labile} concentration ($mg\ kg^{-1}$ dw)	3.1 (0.33 – 8.1)	2.6 (0.60 – 6.7)
Se_T concentration ($mg\ kg^{-1}$ dw)	22.4 (7.0 – 133)	19.1 (6.1 – 133)
Hg:Se	0.6 (0.2 – 0.9)	0.6 (0.2 – 0.9)

Table 5.3. Results from backward stepwise multiple regressions conducted for both brain regions, with binding of [^{3}H]-QNB to the muscarinic acetylcholine receptor (mAChR) and mRNA expression of mAChR subtype m1 as the outcome variables. Concentrations of mercury (total mercury (Hg_T)), methylmercury (MeHg), labile inorganic mercury (iHg)), molar ratio of Hg to Se (HgSe), or selenium (Se) concentration, and sampling year and animal age were tested as predictor variables.

	Cerebellum				Temporal cortex			
Dependent variable	Adjusted R^2	Intercept	Slope	F-value	Adjusted R^2	Intercept	Slope	F-value
mAChR	-0.05	-44	0.02(year) + 0.01(log(Hg)) + 0.001 (age)	$F_{3,20} = 0.6$	0.45	-110	0.06(year)* + 0.05 (log(Hg)) – 0.003 (age)*	$F_{3,18} = 6.8$*
	-0.03	-38	0.02(year) + 0.06(log(MeHg)) + 0.001(age)	$F_{3,20} = 0.8$	0.33	-95	0.05 (year)* – 0.08(log(MeHg)) – 0.003 (age)	$F_{3,13} = 3.6$*
	-0.04	-33	0.02(year) – 0.03(log(iHg)) + 0.001(age)	$F_{3,20} = 0.7$	0.43	-200	0.1 (year)* + 0.2(log(iHg)) – 0.01(age)^t	$F_{3,18} = 6.8$*
	-0.03	-47	0.02(year) + 0.07(log(HgSe)) + 0.001(age)	$F_{3,20} = 0.7$	0.59	-140	0.07(year)* + 0.3(log(HgSe))* - 0.004(age)*	$F_{3,17} = 10.6$*
	-0.05	-37	0.02(year) -0.01(log(Se)) + 0.001(age)	$F_{3,20} = 0.6$	0.46	-110	0.06(year)* - 0.06(log(Se)) – 0.003(age)	$F_{3,18} = 6.6$*
mAChR m1 mRNA	0.38	230	-0.1(year)^t -0.6 (log(Hg))* + 0.01(age)	$F_{3,19} = 5.4$*	NA			
	0.26	120	-0.06(year) – 0.6(log(MeHg))* + 0.01(age)	$F_{3,20} = 3.7$*				
	0.25	204	-0.1(year) -0.7(log(iHg))* + 0.01(age)	$F_{3,17} = 3.3$*				
	0.42	207	-0.1(year) – 1.2(log(HgSe))* + 0.01(age)	$F_{3,21} = 6.8$*				
	0.16	230	-0.1(year) -0.6(log(Se))^t + 0.007(age)	$F_{3,22} = 2.6$				

* $p \leq 0.05$
$^t p \leq 0.10$

Table 5.4. Results from backward stepwise multiple regressions conducted for both brain regions, with total monoamine oxidase (MAO) activity and mRNA expression of MAO A as the outcome variables. Concentrations of mercury (total mercury (Hg_T)), methylmercury (MeHg), labile inorganic mercury (iHg)), molar ratio of Hg to Se (HgSe), or selenium (Se) concentration, and sampling year and animal age were tested as predictor variables.

	Cerebellum				Temporal cortex			
Dependent variable	Adjusted R^2	Intercept	Slope	F-value	Adjusted R^2	Intercept	Slope	F-value
Total MAO	0.3	-460	0.2(year)* + 0.3(log(Hg)) + 0.01(age)	$F_{3,20} = 3.6$*	0.35	2.5	0.007(age)* - 0.1 (log(Hg))*	$F_{2\,20} = 7.02$*
	0.25	-434	0.2(year)* + 0.1(log(MeHg)) + 0.01(age)	$F_{3,21} = 3.7$*	0.47	2.5	0.005(age)* -0.3(log(MeHg))*	$F_{2,20} = 10.7$*
	0.26	-395	0.2(year) + 0.4(log(iHg)) + 0.01(age)	$F_{3,15} = 3.2$*	0.41	2.4	0.007(age)* - 0.2(log(iHg))*	$F_{2,20} = 8.81$*
	0.25	-452	0.2(year)* + 0.2(log(HgSe)) + 0.01(age)	$F_{3,21} = 3.7$*	0.48	2.2	0.007(age)* -0.4(log(HgSe))*	$F_{2,20} = 11.2$*
	0.27	-491	0.3(year)* + 0.3(log(Se)) + 0.01(log(Se))	$F_{3,21} = 3.9$*	0.27	2.5	0.006(age)* -0.1(log(Se))	$F_{2,20} = 5.1$*
MAO-A mRNA	0.01	-2.3	0.001(year) – 0.05(log(Hg)) – 0.002(age)	$F_{3,19} = 1.1$	-0.09	-33	0.02(year) + 0.04(log(Hg)) – 0.001(age)	$F_{3,22} = 0.28$
	-0.11	-6.8	0.003(year) + 0.06(log(MeHg)) – 0.001(age)	$F_{3,23} = 0.2$	-0.11	-19	0.001(year) + 0.002(MeHg) – 0.0003(age)	$F_{3,22} = 0.11$
	0.01	28	-0.01(year) – 0.07(log(iHg)) – 0.004(age)	$F_{3,18} = 1.1$	-0.07	-31	0.02(year) + 0.05(log(iHg)) – 0.001(age)	$F_{3,22} = 0.3$
	-0.11	-10	0.01(year) – 0.1(log(HgSe)) – 0.0003(age)	$F_{3,23} = 0.14$	-0.07	-27	0.01(year) + 0.1(log(HgSe)) – 0.001(age)	$F_{3,22} = 0.5$
	-0.02	11	-0.005(year) -0.14(log(Se)) + 0.0002(age)	$F_{3,23} = 0.9$	-0.01	-27	0.01(year) + 0.04(log(Se)) – 0.001(age)	$F_{3,22} = 0.2$

*$p \leq 0.05$
$^t p \leq 0.10$

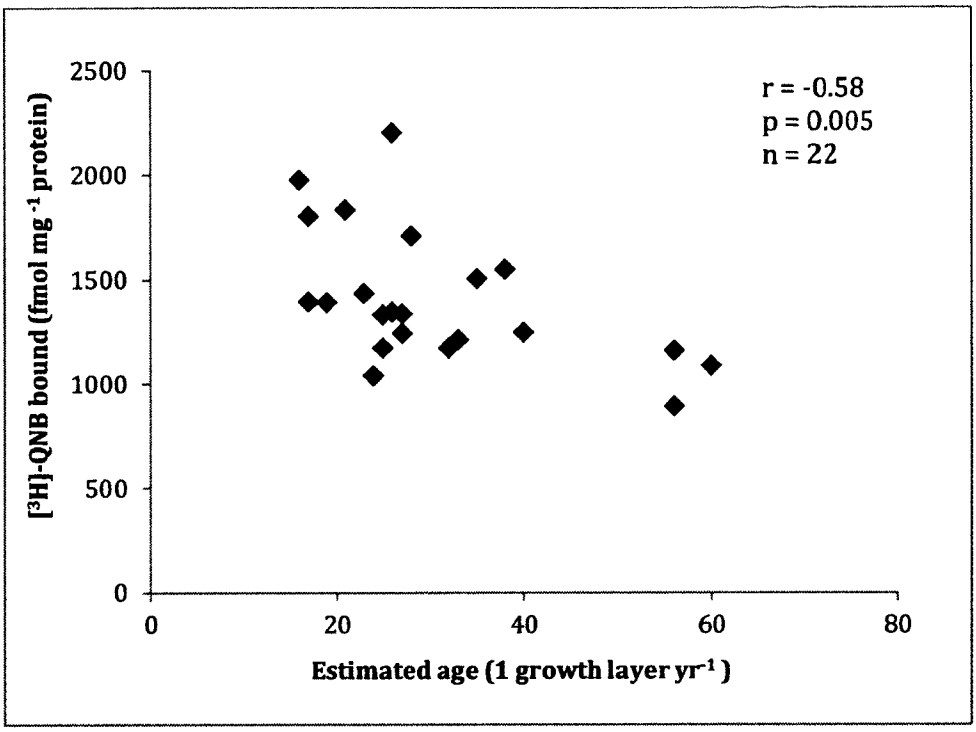


Figure 5.1. The correlation between muscarinic acetylcholine receptor binding to [^{3}H]-QNB and estimated age, based on tooth analysis (one growth layer per year), in the temporal cortex of beluga whales (*Delphinapterus leucas*).

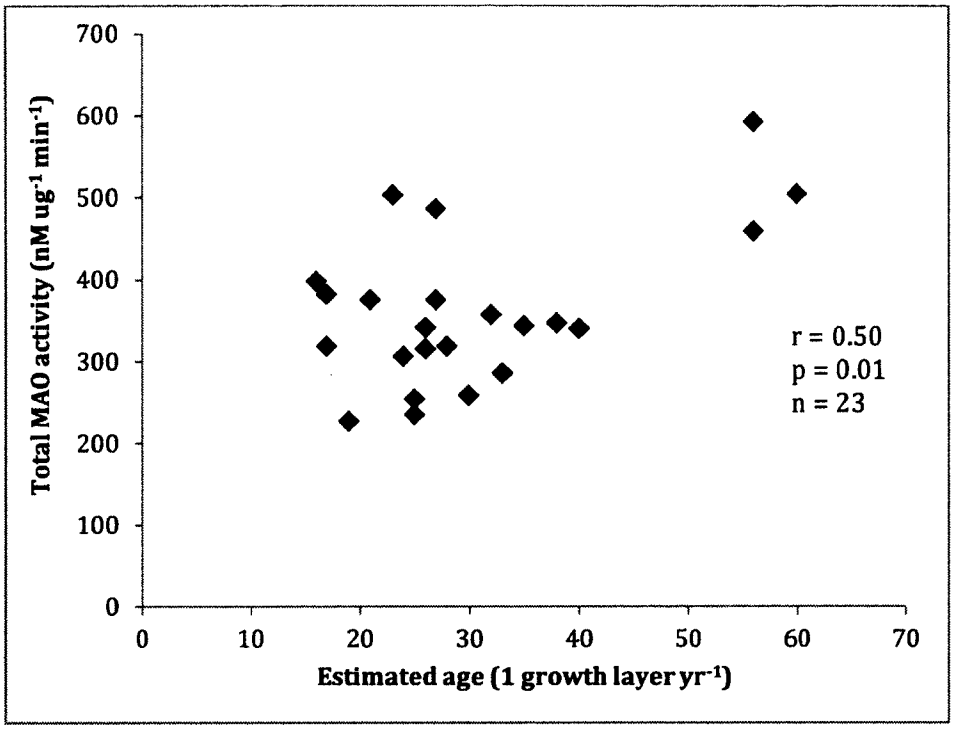


Figure 5.2. The correlation between total monoamine oxidase activity and estimated age, based on tooth analysis (one growth layer per year), in the temporal cortex of beluga whales (*Delphinapterus leucas*).

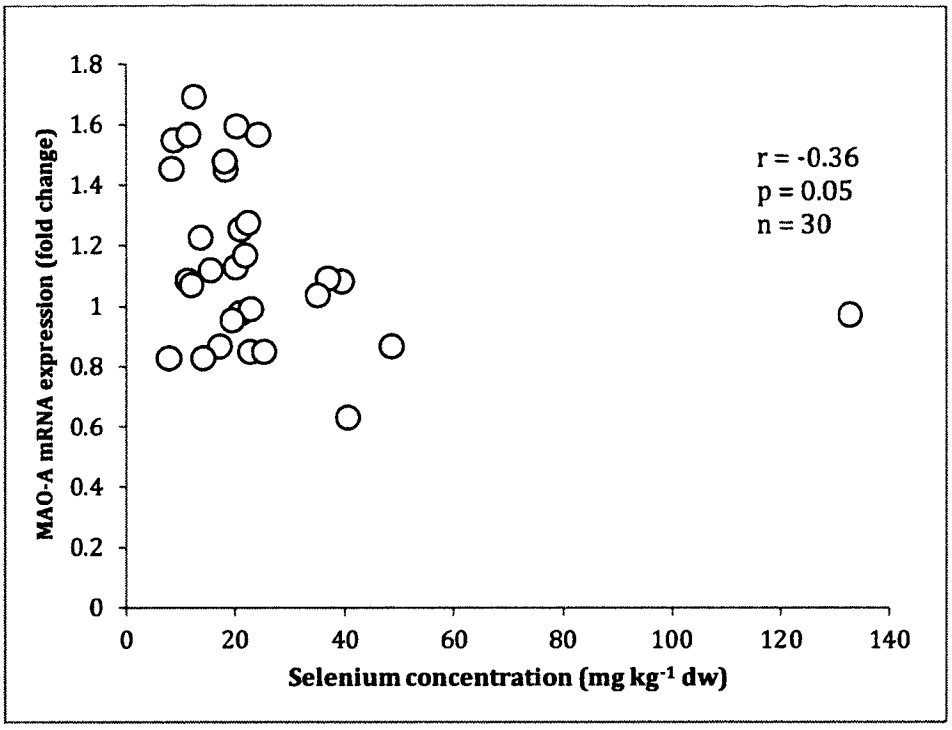


Figure 5.3. The correlation between mRNA expression (fold change) and selenium concentration in the cerebellum of beluga whales (*Delphinapterus leucas*). The expression of mRNA was normalized to the internal control gene S9 and fold changes were calculated based on the lowest-exposed whales ($n = 3$).

Discussion

The major findings from this study were that MeHg exposure was negatively associated with total MAO activity in the temporal cortex, and mRNA expression of the mAChR subtype M1 in the cerebellum, of beluga whales. Furthermore, our results suggest that co-accumulation of Se and Hg was associated with variation in MAO activity, mAChR binding and mRNA expression for mAChR m1. Variation observed in components of both the cholinergic and dopaminergic signaling pathways in association with an increase in the molar ratio of Hg to Se, suggests that Se may provide a protective effect from Hg exposure for these two signaling pathways. We did not observe a relationship between mRNA expression and receptor binding or enzyme activity, which may have been due to varied post-transcriptional mechanisms that convert mRNA to protein, variation in *in vivo* half- lives of proteins, and the error and noise associated with protein and mRNA experiments (Greenbaum et al., 2003). Furthermore, the prolonged time to collect wildlife samples may have resulted in the degradation of RNA. Between 33 and 48 percent of mRNA transcripts may have decayed during sample collection and preservation (approx. 3 hrs) based on reported hourly decay rates that ranged from 0.085 to 0.221 for mRNA transcripts from human cells (Yang et al., 2003). The post-mortem interval in our study may have had limited impact on overall mRNA integrity, given that a decrease in the 28S/18S ribosomal RNA ratio was only observed in mouse brain samples kept at ambient temperatures for 36 hrs after death (Catts et al., 2005).

Overall, the results from this study complement previous findings (Ostertag et al., in review), and taken together, suggest that MeHg exposure may indeed be of toxicological concern for beluga whales from the eastern Beaufort Sea population. We explore the

potential mechanisms of action and physiological outcomes of these findings in relation to previous captive and wildlife animal studies, and *in vitro* studies.

Cholinergic signaling pathway

Cholinergic signaling pathways have been linked to essential physiological processes including learning, memory, stress response and modulation of sensory information (Reis et al., 2009). The mAChR may play a critical role in physiological processes including thermoregulation, motor function and feeding (Bymaster et al., 2003; Wess, 2004). Our findings indicated a statistically significant positive relationship between mAChR density and the molar ratio of Hg to Se in the temporal cortex, with a slight negative relationship between age and mAChR density. The relationships between mAChR binding and Hg (+), MeHg (-), iHg (+) and Se (-) concentrations were not statistically significant. Therefore, these results suggest that Se may play a role in modulating the interaction between Hg and the mAChR. It is possible that as the ratio of Hg to Se increased, the concentration of unbound Hg increased, allowing Hg to interact more effectively with sulfhydryl groups and cause a homeostatic response at a neurochemical or molecular level. In the cerebellum there was not a significant relationship between Hg concentration, Hg to Se ratio, and mAChR binding; however, at a molecular level, the expression of mRNA for mAChR m1 was negatively associated with Hg species (Hg_T, MeHg, iHg) and the ratio of Hg to Se. The lack of relationship between mAChR binding and expression of mRNA for mAChR m1 may be due to differences in agonist-induced receptor internalization and downregulation (Thangaraju and Sawyer, 2011).

In previous studies, an increase in the density of mAChR was observed following MeHg dosing of rats *in vivo* (Coccini et al., 2000; Coccini et al., 2007; Costa, 1988; Rajanna et al., 1997) and a positive relationship between Hg and mAChR levels was reported for wild mink (Basu et al., 2005a), loons and eagles (Scheuhammer et al., 2008). A negative relationship between MeHg and mAChR density was found in the cerebral cortex of river otters (Basu et al., 2005c) and in rats exposed prenatally to MeHg (Zanoli et al., 1994). A previous avian study found that Hg exposure was not correlated to relative mRNA expression of nicotinic receptor α-7 in herring gulls with low brain Hg levels ranging from 0.14 – 2.0 μg g^{-1} dw (Rutkiewicz et al., 2010). Interestingly, we observed a negative relationship between mRNA expression for mAChR m1 and Hg concentration and speciation (Hg_T, MeHg, iHg and Hg to Se ratio) in the cerebellum. The concentration of Hg and Hg species in beluga cerebellum (median, range; Hg_T: 10.6 mg kg^{-1} dw, 1.7 – 113 mg kg^{-1} dw) were much greater than those observed in herring gulls. Therefore, the different relationships between MeHg exposure and mRNA expression observed in beluga and herring gulls may be due to differences in Hg exposure, differences in sensitivity of the nicotinic receptor α-7 and mAChR m1 to Hg exposure, or other undetermined differences.

Methylmercury was found to inhibit agonist binding to m1 and m2 muscarinic receptors in rat brain cortical membranes (Castoldi et al., 1996). The binding of agonists and antagonists to extracellular cysteine residues modulates muscarinic receptor activity; therefore, MeHg may modify mAChR activity by binding to this critical region of the receptor and competitively inhibiting mAChR binding (Limke et al., 2004). The binding of MeHg to mAChR has been linked to disruption of Ca^{2+} in cerebellar granule cells, and

has been suggested as a cause of cell-regulated death (apoptosis) or the downregulation of mAChR (Limke et al., 2004). Three subtypes of the mAChR (m1, m3 and m5) are coupled to G proteins of the $G_{q/11}$ family, which mediate the activation of phospholipase C and subsequent release of Ca^{2+} (Ehlert and Thomas, 1995). Therefore, the decrease in mRNA expression for mAChR m1 associated with MeHg exposure could be explained by downregulation of the mAChR, to protect the cerebellum from a disruption in Ca^{2+} homeostasis.

Dopaminergic signaling pathway

Monoamine oxidase plays an integral role in maintaining the homeostasis of key neurotransmitters in the CNS and other organs (Bortolato and Shih, 2011). Our findings suggest that total MAO activity may be negatively associated with Hg_T, MeHg, iHg concentrations, and ratio of Hg to Se in the temporal cortex. This is consistent with findings of decreased MAO activity with Hg_T and MeHg in the cerebral cortex but not cerebellum of wild river otters (Basu et al., 2007b). The statistically significant effect of sampling year on MAO activity in the cerebellum may have obscured a relationship between enzyme activity and Hg concentration or speciation in this brain region. Another explanation for the regional difference in MAO activity and Hg exposure may be that the temporal cortex had more elevated concentrations of Hg, iHg_{labile} and MeHg compared to the cerebellum. The results from this study suggest that the ratio of Hg to Se is a significant predictor of MAO activity in the temporal cortex; therefore, Se may play a role in reducing Hg availability and toxicity in beluga whales.

The lack of relationship between mRNA expression for MAO-A and total MAO activity, and mRNA expression for MAO-A and Hg concentration or speciation, suggests that MeHg exposure may result in disruption of enzyme function but not mRNA transcription. For example, the decrease in total MAO activity in the temporal cortex associated with Hg concentration may have occurred due to alteration of the mitochondrial structure (Franco et al., 2007; Shenker et al., 1999), leading to decreased MAO activity without related changes in mRNA transcription for the MAO-A target gene. The slight positive relationship between age and MAO-T activity was consistent with findings that in general, MAO-B activity increases with age in humans, although MAO-A activity remains stable with age (Nicotra et al., 2004).

Exposure to MeHg has been associated with alterations of neurotransmitter amine metabolism in the central nervous system (Chakrabarti et al., 1998). Monoamine oxidase is located on the outer mitochondrial membrane and catalyzes the oxidative deamination of monoamine neurotransmitters (e.g. dopamine), neuro-modulators and hormones (Bortolato and Shih, 2011). Methylmercury may exert an effect on MAO either by directly binding to thiol groups on the enzyme or by altering mitochondrial function (Chakrabarti et al., 1998). Previous studies have found decreased mitochondrial activity associated with MeHg exposure in mitochondrial-enriched fractions of mouse cerebrum (Franco et al., 2007; Meinerz et al., 2011), and striatal synaptosomes from rats (Dreiem and Seegal, 2007). A decrease in MAO activity was observed in the cerebellum and cortex of rats exposed to MeHg (Chakrabarti et al., 1998), and the intrastriatal administration of MeHg in rats was associated with a concentration-related increase in striatal output of dopamine (Faro et al., 2003). Disruption of MAO activity could lead to

downstream impacts on neuronal signaling pathways that involve monoamines that are associated with fight-or-flight response, emotion, motor activity and cognition (Beyrouty et al., 2006). Clinical observations indicate that disturbance of dopaminergic neurotransmission is related to psychiatric symptoms (Reis et al., 2009).

Variation of mRNA expression and neurochemical biomarkers associated with Hg exposure in beluga whales may provide complementary evidence of potential disruption of neurosignaling pathways. Our results suggest that MAO and mAChR may be more impacted by MeHg exposure in the temporal cortex than the cerebellum, which may be due to more elevated concentrations of Hg, MeHg and iHg_{labile} present in the temporal cortex. Taken together with our previous findings (Ostertag et al., in review), the neurochemical components of the cholinergic, dopaminergic and GABAergic signaling pathways may be good candidates as biomarkers of neurochemical disruption in cetaceans. The relationships observed in this study between the molar ratio of Hg to Se, and components of the cholinergic and dopaminergic signaling pathways confirms that including this ratio or the Se to Hg ratio in wildlife studies is important for evaluating the biological effects of MeHg exposure (Burger et al., 2013). Furthermore, the results from our study support the use of both molecular and neurochemical biomarkers to improve our understanding of potential effects of MeHg exposure on wildlife. However, to fully understand the relationship between mRNA and protein expression, requires a better understanding of the dynamics of protein synthesis and degradation (Greenbaum et al., 2003). There is mounting evidence that chronic MeHg exposure in beluga whales is associated with variation in components of diverse neurosignaling pathways (Ostertag et al., in review). These results suggest that MeHg exposure may lead to neurochemical

changes associated with maintaining homeostasis in the CNS. The impact of chronic MeHg exposure for beluga health requires further study.

Acknowledgements

This study was funded by NSERC Discovery (HMC) and the Fisheries Joint Management Committee (SKO). SKO was the recipient of a NSERC Doctoral award, Nasivvik Doctoral award, NSTP Training Fund and UNBC travel awards. We thank A. Philibert and M. Gillingham for guidance with statistical analyses, E. Montie for sharing dissection methods, A. Essler, A. Montgomery, S. Krause, L. Rose, A. Krey, and G. Prkachin for assistance in the laboratory, and L. Loseto, M. Noel, F. Pokiak, N. Pokiak, R. Felix, K. Nuyaviak, B. Voudrach, R. Walker, K. Snow, C. Pokiak, Inuvialuit hunters, Tuktoyaktuk and Inuvik HTC, and the DFO for their support in the field.

Bridge

In the previous three chapters we assessed the neurotoxicological risk of methylmercury exposure through the comparison of brain mercury concentrations with threshold levels and the use of molecular and neurochemical biomarkers. We found that concentrations of Hg in brain tissue exceeded thresholds of adverse effect, and were also associated with neurochemical and molecular variation. A particular challenge in risk assessments is determining causal linkages between biological responses to toxicant exposure in wildlife studies. Harvesters' observations of beluga whales may provide a unique and valuable line of evidence to determine linkages between toxicant exposure and biological response. Therefore, for the next study, we worked with Inuvialuit beluga harvesters to document their observations of the behaviour of beluga whales during harvesting activities. Our primary objectives were to develop a questionnaire to document local observations of beluga whale behaviour during harvesting activities and to assess whether Hg levels were associated with differences in beluga whale behaviour.

Chapter 6. Inuvialuit observations of beluga whale (*Delphinapterus leucas*) link mercury exposure and behaviour during harvesting activities.

Names and Affiliations of Authors:
1. Sonja K. Ostertag
Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, British Columbia, Canada, V2N 4Z9
ostertag@unbc.ca

3. Hing Man Chan *
Center for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5
laurie.chan@uottawa.ca

Abstract

Beluga whales in the Beaufort Sea are facing many environmental changes associated with global climate change and pollution. Harvesters' observations of beluga whales' behaviour may provide key insights into linkages between toxicant exposure and biological response. The objective of this study was to determine if there were differences in the behaviour of beluga whales associated with mercury (Hg) exposure. We developed and administered a questionnaire ($n = 11$) to document hunters' observations of beluga whale behaviour during harvesting activities. The participation rate was 73 % and all respondents had ten or more years of beluga hunting experience. Mercury concentrations were measured in cerebellar cortex samples using inductively-coupled plasma mass spectrometry, and ranged from 0.32 to 3.27 mg kg^{-1} wet weight (ww; median, 0.77 mg kg^{-1} ww). There was no evidence that the amount of time required to harpoon the whales was associated with Hg concentrations. However, we detected slight differences in beluga whales' use of evasive strategies associated with Hg exposure. Fewer whales exhibited evasive behaviour when they had higher than median Hg than those with lower than median Hg (evasive behaviour and > median Hg: $n = 1$; no evasive behaviour and > median Hg: $n = 3$). The small sample size precluded the use of statistical tests, but suggests that behaviour could vary with Hg exposure in this population of beluga whales. We recommend that Inuvialuit harvesters' observations of beluga whale behaviour are documented in future community-based monitoring studies. In the long term, combining traditional ecological knowledge and traditional scientific knowledge may provide greater insight into how environmental change could impact this beluga population.

Introduction

Anthropogenic activities including climate change (ACIA, 2005), industrial development (Prowse et al., 2009), shipping (AMSA, 2009) and long range transport of pollutants (AMAP, 2011; Dietz et al., 2013; Muir and de Wit, 2010) are causing rapid changes to the Arctic. These changes pose a serious threat to the conservation of Arctic marine mammals (as reviewed by Huntington (2009)). Recent studies on beluga whales (*Delphinapterus leucas*) from the Eastern Beaufort Sea beluga population in the western Canadian Arctic, found concentrations of mercury (Hg) in brain tissue exceeded thresholds of adverse effect (Ostertag et al., 2013) and were also associated with neurochemical variation (Ostertag et al., *Submitted;* Ostertag et al., in review). A particular challenge in risk assessments is determining causal linkages between biological responses to toxicant exposure in wildlife studies. The use of multiple lines of evidence, including field observational studies may increase confidence in the conclusions of risk assessments (EPA, 1998). Inuvialuit travel every summer to traditional whaling camps along the Beaufort Sea coast to harvest beluga whales (Harwood et al., 2002). Harvesters' observations of beluga whales may provide a unique and valuable line of evidence to determine linkages between toxicant exposure and biological response. In the long term, combining traditional ecological knowledge (TEK) and traditional scientific knowledge (TSK) may provide useful insight into how environmental change is impacting Arctic ecosystems.

As a result of global pollution, Hg concentrations in Arctic marine mammals have increased by an order of magnitude since the preindustrial period (Dietz et al., 2009). The toxic effects of methylmercury (MeHg) are primarily due to its ability to cross the blood-

brain barrier (Aschner and Aschner, 1990) and damage the central nervous system (Clarkson and Magos, 2006). Methylmercury is the principle form of Hg consumed by Arctic beluga whales (Loseto et al., 2008a); Arctic beluga whales have also been identified as being particularly vulnerable compared to other Arctic marine mammals to MeHg exposure, due to elevated concentrations of total Hg measured in brain tissue compared to other species (Dietz et al., 2013). Mercury exposure in beluga whales from the western Canadian Arctic exceeded levels associated with neurotoxicity (Ostertag et al., 2013) and showed variation in components of neurochemical signaling pathways (Ostertag et al., *Submitted;* Ostertag et al., in review). Biochemical changes may be an indicator of early-stage effects before the manifestation of disease (Manzo et al., 1996). If beluga whales are equally sensitive to Hg toxicity as primates and mink, we would expect clinical symptoms to arise when total Hg concentrations in brain tissue exceeded 6.0 to 12.0 mg kg^{-1} wet weight (ww; Berlin et al., 1975b; Evans et al., 1977; Luschei et al., 1977; Stinson et al., 1989).

Beluga whales inhabit the northern coasts of Alaska, Canada, Greenland and Norway (Jefferson, 2008). Summering populations are concentrated in western Hudson Bay (WHB) and eastern Beaufort Sea (EBS) (Jefferson, 2008) and Inuit continue to highly value beluga whales as a source of food (Harwood and Smith, 2002). Beluga whale monitoring has been taking place in the Mackenzie Delta since the 1970s (Harwood et al., 2002) and TEK has been fundamental to the success of this program through the provision of high quality samples from harvesters' catch. However, harvesters' observations have been minimally included in this program. Scientific and local observations of environmental change can be brought together to identify new avenues

for further exploration, compare observations from different scales and discuss potential mechanisms that explain both sets of observations (Huntington et al., 2004). Previous studies have documented Inuit observations of beluga whale migration, feeding behaviour, calving, response to disturbance, changes in prey quantity and quality, and health (Carter and Nielsen, 2011; Fernandez-Gimenez et al., 2006; Huntington et al., 1999; Kilabuk, 1998; Mymrin et al., 1999). Elders and hunters have also provided possible explanations for changes in blubber thickness, prey availability, migration patterns and feeding behaviour based on their observations (e.g. Carter and Nielsen, 2011; Huntington et al., 1999; Kilabuk, 1998). Although TEK about beluga whales has been documented in the Arctic, to date, linking observed behaviour to body burden of contaminants or physiological parameters has not been attempted. Such integration may provide insight into the potential effects of neurotoxicants (e.g. MeHg) on beluga whale behaviour.

There have been increasing calls to include or consider TEK in decision-making and environmental assessment in the north (Bennett, 2012). We chose to use a broad definition of TEK as "knowledge gathered and maintained by groups of people, based on intimate experience with their environment" (Huntington et al., 2004). One approach for including TEK in assessing ecosystem changes includes observations of animal behaviour to assess animal health (Huntington et al., 2004). Traditional ecological knowledge has provided valuable information about marine mammals in the Arctic (Carter and Nielsen, 2011; Ferguson et al., 2012) and co-management (Dowsley, 2009). Inuvialuit knowledge and wisdom about beluga whales has been documented and confirms that hunters' and elders' knowledge of beluga whale behaviour and predation is

associated with decades of observations (Byers and Roberts, 1995). Indigenous hunters and elders of Chukotka, Russia, shared their observations made while hunting beluga whales or pursuing walrus (*Odobenus rosmarus divergens*) and seals (*Phoca* spp. and *Erignathus barbatus)* of beluga whale diving behaviour, feeding, migration, communication and response to disturbance (Mymrin et al., 1999).

In this study, we worked with Inuvialuit harvesters to document their observations of beluga whale behaviour made during harvesting activities. Our primary objectives were to develop a questionnaire to document local observations of beluga whale and to assess whether differences in Hg concentrations were associated with beluga whale behaviour. We hypothesized that if whales were experiencing Hg-associated neurotoxicity, whales with higher concentrations of brain Hg would behave abnormally, be harpooned more quickly and/or exhibit different evasive strategies during the hunt.

Methods

Study area, people and context

This study was a component of the Hendrickson Island Beluga Study, which was a collaborative study of beluga whales from the Eastern Beaufort Sea population. We worked closely with Inuvialuit harvesters from Tuktoyaktuk (69.44° N, 133.03° W), a coastal community of 930 people, located on the Beaufort Sea in the Inuvialuit Settlement Region (ISR), western Canada. In 2010, beluga whales were sampled and harvesters' observations were collected on Hendrickson Island (69.50° N, 133.59° W), a

small island located in the southern Beaufort Sea, approximately 20 km west of Tuktoyaktuk (Figure 6.1).

Inuvialuit have a long history of hunting beluga whales during their summer migration through the Mackenzie River Estuary. The beluga whale is known as qilalugaq in Inuvialuktun, the Indigenous language of the Inuvialuit. Based on the archeological record, beluga whales made up approximately half of the diet of pre-contact Mackenzie Inuit (Friesen and Arnold, 1995). Beluga hunting typically occurs in the month of July, when beluga whales migrate through the warm waters of the Mackenzie Delta Estuary (Harwood et al., 2002). Beluga hunting in the ISR typically occurs from 4.6 m long aluminum boats and hunters harpoon the whale before killing it, to make retrieval easier (Harwood et al., 2002). The total annual number of landed beluga whales on the shores of the Beaufort Sea and Amundsen Coast was 111 between 1990 and 1999 (Harwood et al., 2002). Beluga whales from this population are also harvested by residents of some coastal villages in Alaska (average 64 per year between 1995 and 2000) and possibly by residents of Chukotka (Harwood et al., 2002). Beluga whales travel through Kugmallit Bay in the Mackenzie River Estuary during their summer migrations. The shallow waters in this bay make it an ideal location for harvesters to track and hunt beluga whales. Hunters from Tuktoyaktuk butcher beluga whales on Hendrickson Island following the hunt and generally return to Tuktoyaktuk immediately after butchering the whale to process the muktuk (skin and blubber) and to prepare mipku (dry meat).

Sampling

Brain sampling occurred immediately following the harvest on the shores of Hendrickson Island, after receiving informed consent from the harvesters. Details about sample collection and analysis are provided elsewhere (Ostertag et al., in review; Ostertag et al., 2013). Briefly, brain samples were collected on Hendrickson Island and the concentration of Hg was analyzed by inductively-coupled plasma mass spectrometry (Agilent Technologies, 7500 CX) following a modified acid digestion (Armstrong and Uthe, 1971). Permission to collect samples was obtained from the Tuktoyaktuk Hunters' and Trappers' Committee and the Aurora Research Institute prior to sampling. Ethics approval to carry out this research was received from the Research Ethics Board at University of Northern British Columbia.

Questionnaire

A questionnaire was developed to document harvesters' observations of beluga whale behaviour during harvesting activities. We chose to use a questionnaire to document harvesters' observations about the behaviour of beluga whales to enable comparison between whales, for efficient documentation in a field environment and to increase participation (Huntington, 2000). Nine questions were asked; three questions were multiple choice, two questions required one-word answers and four questions were open-ended questions. Topics covered were the beluga hunting experience of the harvesters, the weather and seasonal conditions for hunting and the behaviour of the beluga during the hunt. Questions about beluga whale behaviour ranged from general questions such as "was there anything unusual about this whale's behaviour", to more specific questions

about the time it took harpoon the whale. Questions about behaviour were both multiple choice ("was there anything unusual about this whale's behaviour") and open-ended such as "how would you describe this whale's behaviour" and "how did this beluga act when you were hunting it" to ensure that more detailed information could be recorded. Questions were included about harvesters' beluga-hunting experience, perceived weather conditions for harvesting, and seasonal changes in beluga harvesting. The Tuktoyaktuk Hunters and Trappers Committee gave permission for S. Ostertag to administer this questionnaire on Hendrickson Island, with consenting harvesters.

Respondents

Generally, two hunters, a harpooner and a boat driver worked together to hunt one beluga whale. Occasionally, Elders accompany the harpooner and boat driver during the hunt. The questionnaire was administered to the boat drivers ($n = 10$) or in one case, an Elder ($n = 1$), after the whale was brought to shore and the butchering was complete. The participants provided informed consent prior to completing the questionnaire. The questions were read aloud and S. Ostertag documented the answers. The drivers were all fluent in English. Of the 15 whales harvested during the 2010 field season, observations were recorded for 11 whales for the purpose of this study. All participants provided informed consent and completed the questionnaire within 10 to 20 minutes.

Data analysis

The results from the questionnaire were entered into Microsoft® Excel (v 12.3.5). For each whale, the observations were cross-referenced with Hg concentration data for brain

tissue. Beluga whales were categorized as having brain Hg concentrations greater or lesser than the median concentration measured. The responses were categorized as follows: weather conditions (good or excellent, fair or poor), time to harpoon (more or the same time to harpoon, less time to harpoon), general behaviour (normal, unusual), swimming speed (fast, medium speed) and observed evasive strategies (yes: turned or charged; no: did not turn or charge, straightforward to harvest) (Table 6.1).

The small sample size made statistical analysis unfeasible. Therefore, we grouped whales based on behaviour, 'time to harpoon', and evasive strategies for whales with relative Hg concentration measured in brain tissue, to have a visual representation of the potential relationship between behaviour and Hg exposure. To assess variables that were associated with the 'time to harpoon' the whale, we investigated the potential relationships between weather conditions, water level and Hg exposure, with the relative time to harpoon the whale.

Results

Mercury concentrations

The median Hg concentration in the cerebellar cortex was 0.77 mg kg^{-1} ww, and ranged from 0.32 to 3.27 mg kg^{-1} ww; therefore, none exceeded threshold levels for clinical symptoms from primate studies, which are between 6.0 and 12.0 mg kg^{-1} ww (Berlin et al., 1975b; Evans et al., 1977; Luschei et al., 1977; Stinson et al., 1989). Five beluga whales had Hg concentrations between 1.0 and 4.0 mg kg^{-1} ww, which is the range in which we would expect to observe biochemical changes but not clinical symptoms (Basu

et al., 2007a; Basu et al., 2008; Suzuki, 1979; Wobeser et al., 1976). Overall, the concentrations of Hg in brain tissue were below levels of clinical symptoms.

Response rate

The participation rate was 73 %. All respondents had ten or more years of beluga hunting experience (Figure 6.2). In general, the driver had more hunting experience than the harpooner, which may be associated with the skill and knowledge required to successfully track beluga whales in the murky waters of the Mackenzie Delta Estuary. The respondents compared the behaviour of the harvested whale to the behaviour of previously observed whales. The questionnaire was an efficient and effective mechanism for experienced hunters to share their observations about the whale's behaviour with the beluga researchers at Hendrickson Island in 2010. Respondents generally provided detailed responses to the questions that were asked.

General observations

All of the whales behaved normally when they were being hunted. For ten of the eleven whales, no unusual observations were noted about their behaviour (Table 6.1). For example, one whale that was described to behave normally "just tried to get away from the hunters" and another whale that did not exhibit unusual behaviour "must be a healthy whale". However, one whale that behaved normally prior to harpooning, and took approximately the same amount of time to harpoon compared to other whales, began to act differently after it was harpooned. For example, it "wouldn't leave the side of the boat after harpooning" and it was "swimming fast, in circles around the boat" (Elder,

Tuktoyaktuk). This was the first time that this behaviour was observed in this Elders's experience. This whale did not have an elevated concentration of brain Hg (0.8 mg kg^{-1} ww) and was median-aged (approx 16 yo) (Figure 6.3). The cause of this abnormal behaviour was not determined; however this whale also had a large number of nematodes in the auditory canal (personal obs).

Time to harpoon

Harvesters responded that 6 whales took less time to harpoon compared to other whales, and five whales took more or the same amount of time to harpoon (Table 6.1). Of the five whales with 'higher than median' Hg, three took less time to harpoon, and two took more or the same time to harpoon (Figure 6.4). Three whales with 'lower than median' Hg took less time to harpoon and the remaining three whales in this category took more or the same time to harpoon. In this study, therefore, the data indicates that whales with higher than median Hg were not harpooned in less time than whales with lower than median Hg concentration.

Evasive strategies

Based on the recorded observations about whale behaviour, we compared Hg concentrations in the whales that were "easy or straight forward to hunt" and those that used evasive strategies such as turning and charging. Four respondents described the whales as turning, charging or hiding during the hunt (Table 6.1). Three whales did not exhibit evasive behaviour and were straightforward to harpoon. Two whales "didn't charge... didn't give any trouble" or "never turned on us". Four whales were

straightforward to harpoon, but of these four, two also turned or charged; therefore, we grouped them with whales that exhibited evasive behaviour. The likely reason that these two whales were straightforward to harvest was that they were harvested during good weather and low tide and one was "impossible to lose" and the other whale was "easy to follow, after finding out what it would do". Although the sample size was very small, fewer whales exhibited evasive strategies with higher than median Hg ($n = 1$) than with lower than median Hg ($n = 3$; Figure 6.5). Furthermore, whales with lower than median Hg exhibited evasive strategies more frequently ($n = 3$) than not ($n = 1$).

Weather

The weather was predominantly good or excellent for hunting during harvesting activities (n = 7) compared to poor or fair (n = 3). Good weather was described as being calm or a little choppy with low water. Poor or fair weather was described as being windy with high water, "a bit choppy" or "water a bit high". Harvesters provided explanations for differences in the time to harpoon, which included weather conditions or water level. For example, the low tide made it "impossible to lose sight of the whale because it had a good wake on it", shallow water made it easier to see the whale, and "[the] weather was good for tracking". Whales that took more time to harpoon were in deeper water "so harder" to harpoon, "harder to track than other whales in its group... hiding lots.... waited for a long time before coming up for air", "easy to follow after finding out what it would do". Overall, whales took less time to harpoon when the weather was good ($n = 5$) and the water was low ($n = 4$), and took more or the same time to harpoon when the weather was poor or fair ($n = 2$) and the water was high ($n = 2$). There were also cases where whales

took less time to harpoon in poor weather ($n = 1$) and more time to harpoon in good weather ($n = 2$). One harvester stated that it was a 'little harder' to harvest the whale on July 18 than earlier in the season. Whales that were harvested later in the season may have been faster than whales harvested earlier; respondents observed whales to be 'fast' on July 13, 18 and 22.

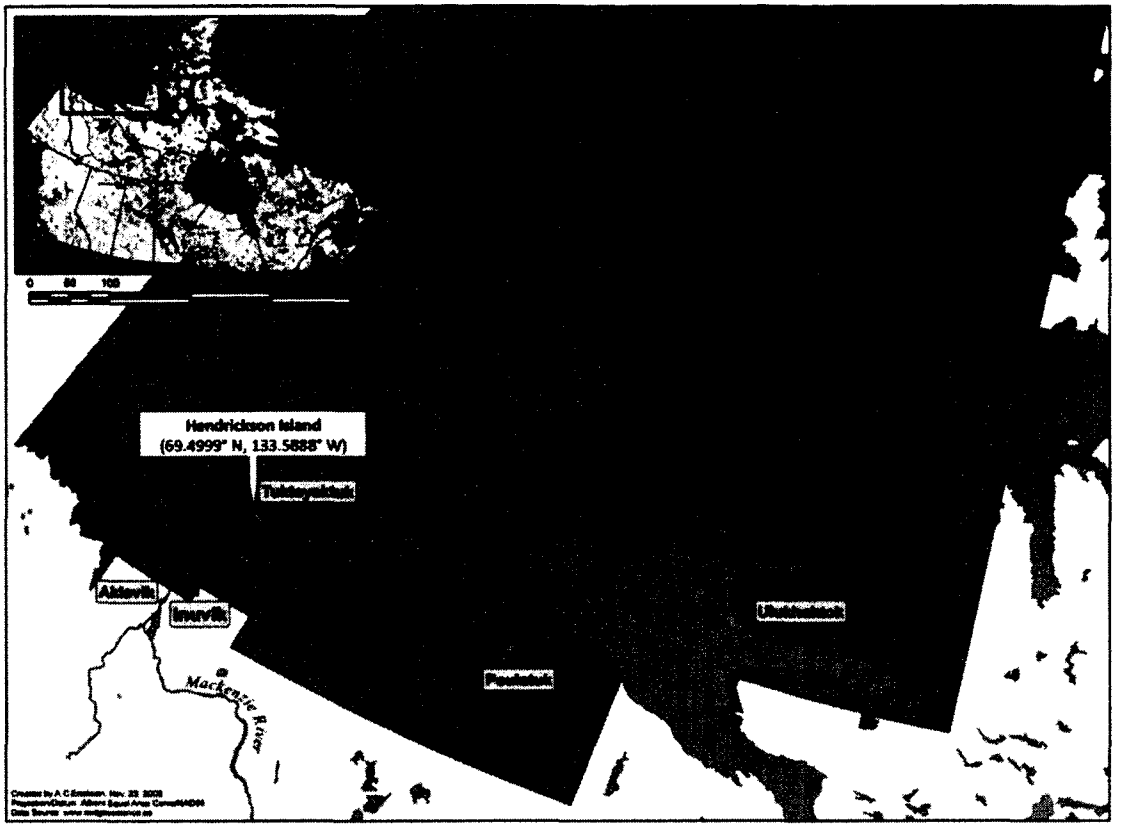


Figure 6.1. This map depicts the location of Hendrickson Island, a traditional beluga-harvesting site in the Inuvialuit Settlement Region, NT (adapted from Wesche et al., 2011).

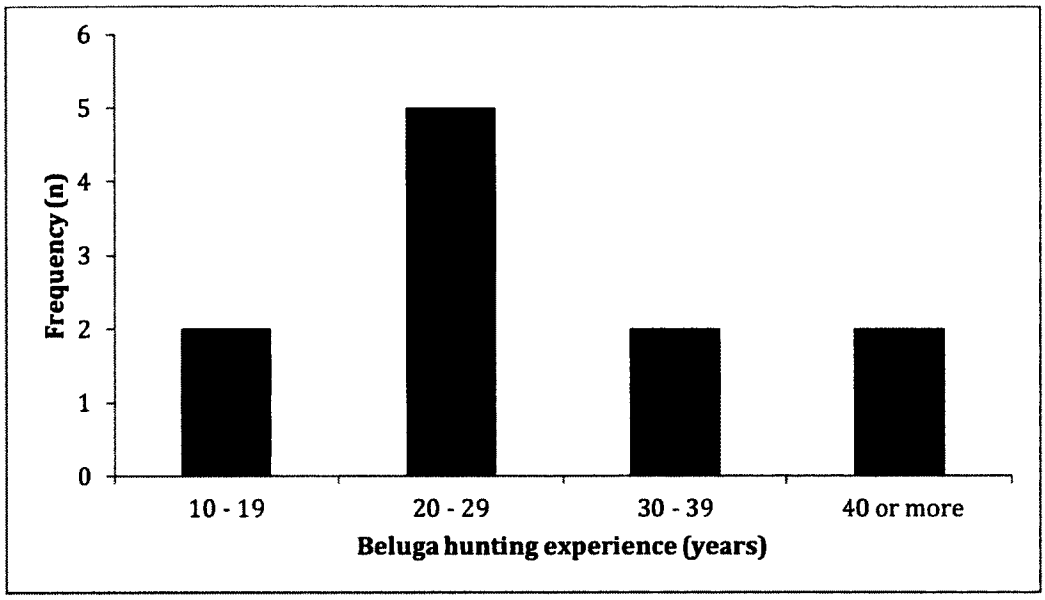


Figure 6.2. Beluga hunting experience (years) of the 11 participants of this study. Harvesters were counted each time they hunted a beluga and responded to the questionnaire. Therefore, some harvesters are counted more than once.

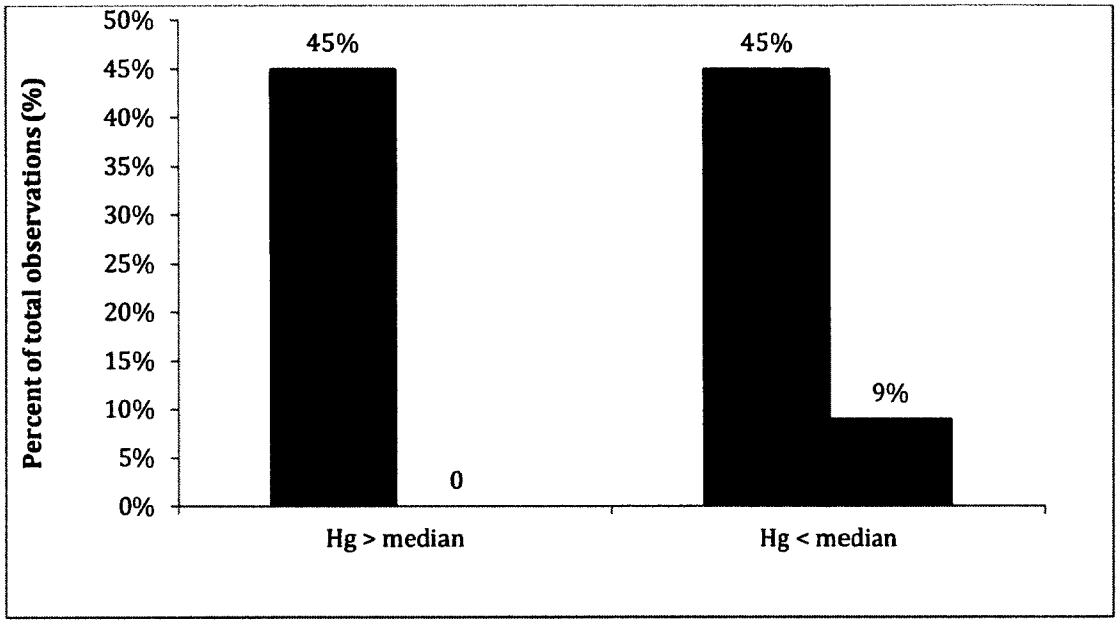


Figure 6.3. Harvesters' observation of normal (black column) and unusual behaviour (gray column) in whales during the harvest (*n = 11*), based on mercury (Hg) exposure (above or below the median Hg concentration measured). Median Hg concentration was 0.77 mg kg^{-1} wet weight.

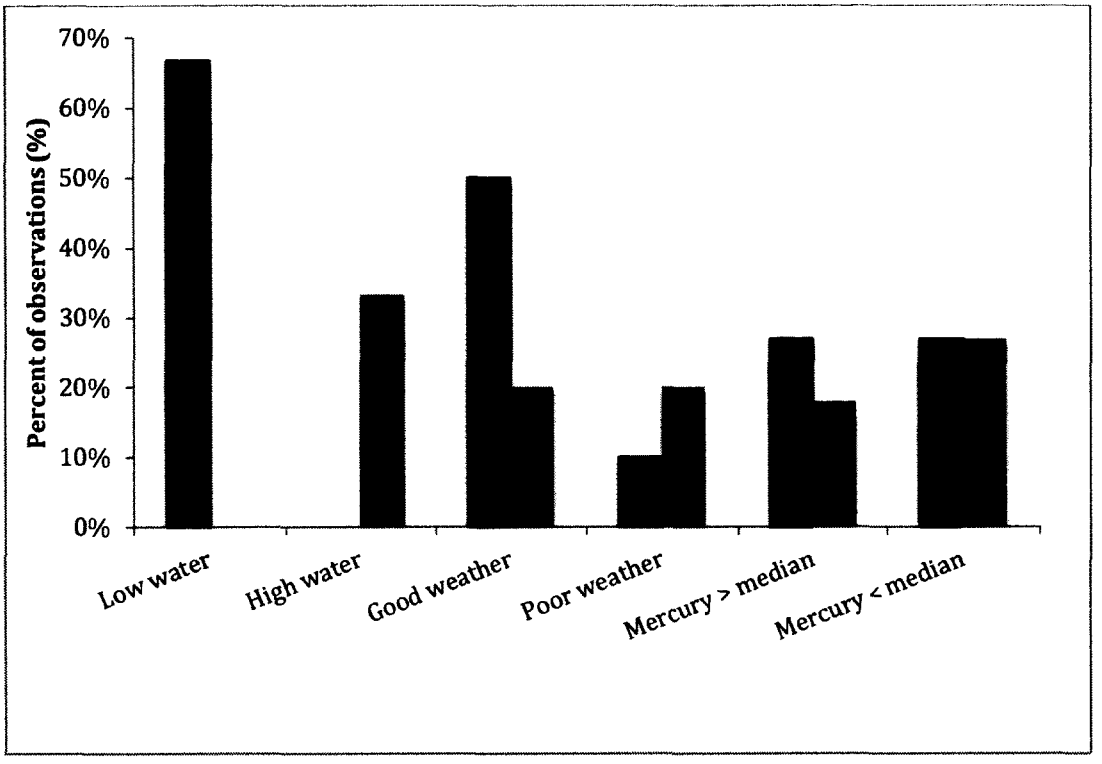


Figure 6.4. Variables that may have affected time to harpoon and harvesters observations (black column = less time to harpoon; gray column = more or the same time to harpoon). Median mercury concentration in cerebellar cortex was 0.77 mg kg^{-1} ww.

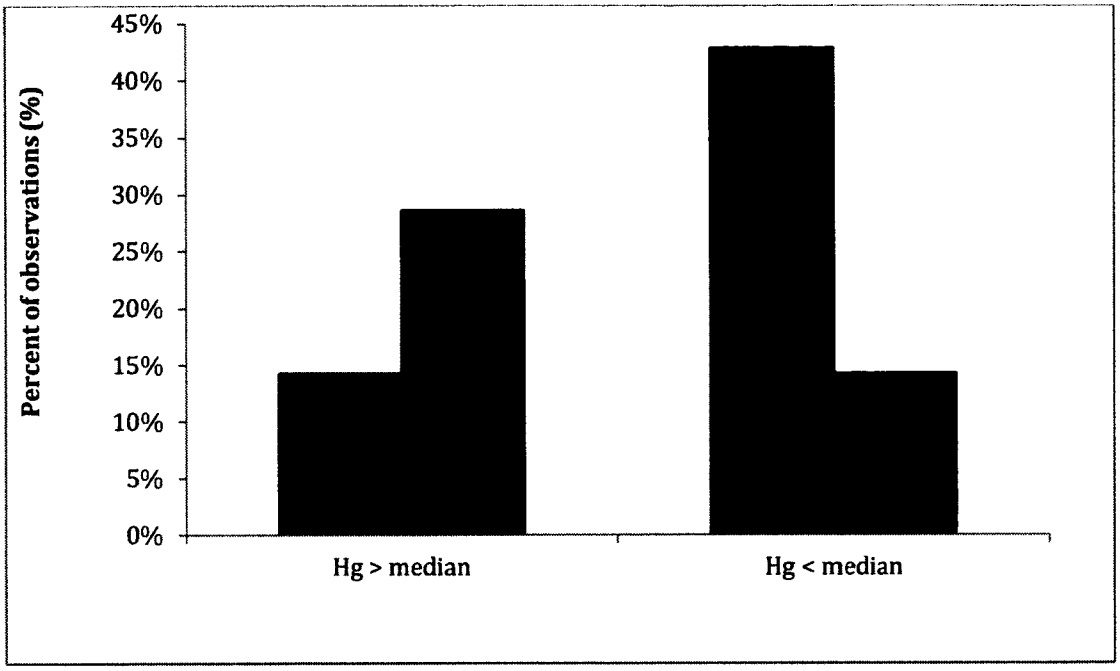


Figure 6.5. Observations of evasive strategies (n = 7) demonstrated during beluga harvest and related mercury (Hg) exposure (more or less than median Hg). Whales that used evasive strategies (turning or diving; black column) or did not use evasive strategies (straightforward, did not turn; gray column) are presented according to Hg exposure. Median Hg concentration in cerebellar cortex was 0.77 mg kg^{-1} ww.

Table 6.1. Observations of beluga whale behaviour during harvesting activities (*n = 11*).

Behaviour	Observation		
	Yes	No	Blank
"Less time to harpoon"	6	5	0
Normal	10	1	0
Swam fast	4	1	6
Straight forward	4	2	5
Turned or charged	4	2	5

Discussion

To our knowledge, this is the first time that behavioural observations and contaminant data were linked directly for individual beluga whales. The use of a questionnaire provided the opportunity for harvesters to have their observations of beluga whales documented and cross-referenced with Hg-exposure data. Although conclusions about potential neurotoxicity associated with Hg could not be established, the key finding was that beluga whales with higher Hg were observed to use evasive strategies less frequently during the hunt than whales with lower Hg.

Integration of multiple lines of evidence

Mercury concentrations in brain tissue from the harvested whales were below the lowest observable adverse effects levels documented for primates (Berlin et al., 1975b; Evans et al., 1977; Luschei et al., 1977; Stinson et al., 1989). We would expect clinical symptoms of Hg intoxication to include the loss of motor coordination (Bellum et al., 2012), abnormal movements and convulsions (Takeuchi et al., 1977), loss of balance (Farina et al., 2005), and reduced passive avoidance. The harvesters did not observe any abnormal movements or behaviour in the beluga whales prior to harpooning, which suggests that Hg exposure in the eleven whales analyzed in this study were not exhibiting clinical symptoms of Hg intoxication. The difference in evasive strategies observed in whales with more elevated Hg concentrations was consistent with behavioural symptoms of Hg toxicity including the loss of motor coordination. This study suggests that documenting observations of evasive strategies may provide a sensitive measure of changes in beluga behaviour that could be associated with Hg exposure. However, conclusions based on these results are limited by the small sample size. Further study into the potential effects

of Hg on beluga whale behaviour are merited given that the animal behaviour represents the integration of sensory, motor and associative functions of the nervous system (Tilson and Cabe, 1978).

In general, the results from this study suggest that Hg concentrations in brain tissue ranging from 0.32 to 3.27 mg kg^{-1} ww were not associated with unusual behaviour and 'time to harpoon'. Many variables could likely affect how quickly a beluga is harpooned including weather and timing. To harpoon a beluga, the boat driver has to track the beluga by following the wake that it creates in the water. Therefore, the association between weather and 'time to harpoon' reflects the importance of good weather for hunting success and limits the usefulness of 'time to harpoon' as an indicator of the motor and cognitive function of belugas.

Behavioural observations as complementary line of evidence

Behaviour has been monitored as an indicator of stress in dogs (Bergeron et al., 2002) and macaques (Bercovitch and Clarke, 1995), as a response to physiological differences in rhesus monkeys (Laudenslager et al., 1999) or as a modulator of the neuroendocrine and reproductive effects of dominance interactions in baboons (Sapolsky, 1993; Sapolsky and Mott, 1987). Behavioural studies have also been used to complement physical examinations and blood analyses to assess the fitness of stranded dolphins (Sampson et al., 2012). Beluga whale behaviour is particularly difficult to document in the wild because beluga whales spend approximately 85 % of their time below water (Kingsley et al., 2001). Furthermore, beluga whales are a protected species in Canadian waters under

the Fisheries Act and may not be disturbed except during fishing activities (Regulation 7, Marine mammal regulations, Fisheries Act (Canada, 1993)). Therefore, documenting the behaviour of beluga whales during hunting activities provides a unique opportunity to capture detailed information without disturbing the animals unnecessarily. Combining behavioural observations with physiological data would be very difficult or impossible to achieve without the collaboration of Inuvialuit harvesters. Given that the behavioural observations provided a complementary line of evidence regarding potential effects of Hg on beluga whales, we suggest that harvesters' observations of beluga whale behaviour be used to complement, where possible, the physiological and toxicological parameters measured.

This study documented one case in which a beluga whale was observed to behave very unusually after it was harpooned. To our knowledge, this was the first incidence in which abnormal behaviour in beluga was documented during harvesting activities. The use of the questionnaire provided the only opportunity for the observations of abnormal beluga behaviour to be documented in the Hendrickson Island beluga-monitoring program. Observations about abnormal behaviour are valuable to document in beluga monitoring because they could signal a potential risk to individual and population health, if increased parasite infestation occurred due to environmental change. Nematodes and flukes may be located within the external auditory system of beluga whales and narwhal, and there is speculation that nematodes and flukes could affect echolocation and cause mass strandings (Vlasman and Campbell, 2004). During brain sampling, parasites were commonly observed in the auditory canals and brain tissue (pers. obs). Therefore, future

studies should systematically document the presence of this parasite to monitor its presence, intensity of infestation and animal behaviour.

Bridging TEK, local observations and science

We recommend that future monitoring be expanded to include harvesters' observations in the ISR. Beluga whales have been harvested for centuries in the Mackenzie Delta Estuary and harvesters' observations may provide key information about changes occurring in the marine ecosystem. In this study, harvesters were willing to respond to a brief questionnaire at the harvest camp on Hendrickson Island. Questionnaires have been identified as one of many methods for documenting TEK (Huntington, 2000). The importance of recording harvesters' observations at the harvest site is that it allows these observations to be cross-referenced with health indicators and contaminant data for the same individual animal. The strength of questionnaires is that they provide consistency and allow comparisons to be made between respondents and over time; however, semi-directed interviews provide a greater depth and breadth of knowledge and may reveal unanticipated information (Huntington, 2000). Conducting semi-directed interviews in this particular field camp would be challenging based on the short time that harvesters spend at Hendrickson Island following the hunt. Given that ethnographic, participatory and iterative methods may be more respectful and constructive approaches to engaging with Indigenous communities and local knowledge-holders (Thornton and Scheer, 2012), researchers may benefit from carrying out interviews in a location where harvesters are spending more time (i.e. in a permanent settlement or at a harvest camp), provided that these observations could be cross-referenced with other data that is collected.

Bringing together TEK and TSK in this study fit into developments at regional, national and international levels to include TEK in resource management and decision-making. In recent decades, there has been increasing recognition that Aboriginal knowledge could contribute to co-management and environmental impact assessments (Usher, 2000). This was the outcome of advocacy, negotiation of comprehensive land claims across the north, and the development of formal Environmental Impact Assessments and review processes, in addition to legal developments within the Supreme Court of Canada and lower court rulings (Usher, 2000). In northern Canada, Indigenous knowledge is recognized in the Northwest Territories as "a valid and essential source of information about the natural environment and its resources" (Territories, 2005). Internationally, specific recommendations to establish marine and Arctic programmes that include the use of TEK for the conservation of biodiversity were presented during the workshop on traditional knowledge and biological diversity (Programme, 1997). More recently, efforts have been made to integrate or bridge TEK with TSK in Arctic ecological research (Gagnon and Berteaux, 2009; Gilchrist et al., 2005; Huntington et al., 2004).

Bridging or linking western science with TEK has been recognized as particularly important when "identifying problems related to hazardous wastes and industrial pollution"(Wavey, 1993). To effectively bridge these ways of knowing, southern scientists may not simply impose their views (Stevenson, 1996), but must support "the development of permanent technical, scientific and support capacity under the control and direction of Indigenous peoples" (Wavey, 1993). Challenges that have been identified in bridging TEK and TSK include the sharing of power (Berkes, 1993) and ownership of data (Wavey, 1993). Benefits that arise from bridging TSK and TEK

include the fact that people who spend long periods of time on the land will see things "more often, for longer, and at more different times and places than is normally the case for scientists" (Usher, 2000).

The observations that Inuvialuit beluga harvesters shared reflected knowledge gained from decades of observing beluga during harvesting activities and travel in the Mackenzie Delta Estuary. Traditional Ecological Knowledge is comprised not only of factual knowledge about the environment and use of the environment (past and present), but also values about the environment and a culturally-based cosmology (Usher, 2000). This study represents the documentation of factual information gathered from experience beluga harvesters; however, this will hopefully be the starting point for greater inclusion of TEK and local observations in beluga monitoring.

Limitations

Although the results from this study do not suggest that the whales sampled for this study were at risk of Hg-associated toxicity, these conclusions should not be extrapolated to the eastern Beaufort Sea beluga population. Limitations in this study include the small sample size, low Hg concentrations measured in these whales compared to previously sampled beluga from the eastern Beaufort Sea population and lack of specificity in the questions included in the questionnaire. Furthermore, relying solely on a questionnaire for documenting TEK of beluga whales limited the type and quantity of data that was gathered.

The small sample size was due in part to the challenges associated with combining biological sampling of belugas with the harvester-questionnaires. One of us (SO) was not able to administer the questionnaire to three harvesters ($n = 3$) due to challenges associated with collecting samples for laboratory analyses and administering the questionnaire before the hunters departed HI. The weather in the Mackenzie Delta changes rapidly; there are stronger and more frequent winds experienced by Inuvialuit in the summer, which reduces travel safety and shortens hunting trips in the summer (Wesche and Chan, 2010). Therefore, there was very little time to administer the questionnaire following the harvest. Furthermore, very few beluga whales were harvested during the 2010 sampling season compared to the 2006 and 2008 sampling season ($n = 24 - 30$ *whales*).

Beluga whales harvested in 2008 had more elevated brain Hg concentrations than beluga whales harvested in 2010 (Ostertag et al., *Submitted*). The difference in Hg concentrations observed in 2008 and 2010 could be due to the younger age of beluga whales harvested in 2010, and the small sample size ($n = 15$) that reduced the sampling of whales at the extremes of Hg exposure. Increasing the number of field seasons and sampling sites, and training whale monitors to administer questionnaires would increase sample size and provide greater understanding of changes occurring in the eastern Beaufort Sea beluga population.

The use of a questionnaire provided a rapid method for documenting harvesters' observations following the hunt. Overall, the questionnaire was effective for documenting general observations such as 'time to harpoon the whale' and whether the whale behaved

normally. The use of open-ended questions provided valuable additional information that was more specific about the whale's behaviour. However, the use of open-ended questions made it challenging to compare the specific observations for different whales, due to variability in the types of observations that harvesters shared. This further reduced the sample size for the analysis of specific behavioural differences in whales related to Hg exposure. Including input from harvesters and community members on the content of the questionnaire may have increased the specificity of questions regarding behaviour. This could have resulted in more comparable responses regarding the specific behaviour of the harvested whales.

Results were presented to the harvesters in 2012; however, harvesters were not involved in the interpretation of results. The observations documented in this study were analyzed semi-qualitatively, based on the dominant scientific paradigm, which reflects an imbalance of power between the researchers and knowledge-holders (Stevenson 1996). We acknowledge that this study is merely a starting point towards a greater inclusion of TEK in beluga monitoring research in the ISR.

Conclusions

We recommend that scientists and Inuvialuit knowledge-holders continue to work together to include TEK in beluga monitoring programs in the ISR. We suggest that semi-directed interviews and focus groups be conducted with harvesters, elders, whale monitors and youth prior to the field season to identify observations that should be documented. Future studies that aim to bridge TEK and TSK are encouraged to use less

extractive methods of documenting TEK and local observations through the use of participatory research methods. Furthermore, additional methods for documenting and sharing TEK and TSK need to be identified through partnerships between researchers, harvesters and co-management boards. A participatory approach to community-based monitoring is recommended, in which whale monitors, Elders, harvesters and youth are equal participants in the documentation and sharing of beluga TEK. Issues of data ownership, interpretation of results and research control must be negotiated in future ecological monitoring in the ISR to ensure the equal distribution of power in decision-making.

This study clearly shows that harvesters' observations of beluga behaviour can quickly be documented following the harvest. The observations made by harvesters offered valuable information about beluga behaviour. This study suggested a possible relationship between observed evasive strategies and Hg exposure; therefore, the link between specific behaviour during harvesting and Hg exposure should be studied further. Beluga harvesters have a unique ability to observe whale behaviour during the hunt, and these observations could be documented and compared to contaminant exposure and other parameters in future studies. Further inclusion of TEK and local observations of beluga whales in monitoring programs will improve our understanding of how environmental change may affect beluga whales from the eastern Beaufort Sea beluga population.

Beluga whales in the Beaufort Sea face many challenges associated with increased shipping, offshore oil and gas exploration, climate change and contaminants exposure. A community based participatory research approach would facilitate co-learning and would

bring together different knowledge-holders to monitor changes in the health of beluga whales and the ecosystem to which they belong. A collaborative and community-based approach can guide the ethical and respectful inclusion of TEK in monitoring programs. Research partnerships should continue to be developed to bridge TSK and TEK to monitor the effects of environmental change on beluga health at individual and population levels.

Acknowledgements

This research presents the observations made by Inuvialuit beluga whale harvesters from Tuktoyaktuk in July 2010. We thank the hunters for sharing their knowledge for this research. We thank Frank and Nellie Pokiak for making this research possible by providing continuity in the beluga-sampling program at Hendrickson Island from 2001 to 2013. This study was possible due to funding provided by the Fisheries Joint Management Committee (L. Loseto, S. Ostertag, P. Ross) and NSERC Discovery (HMC). SKO was the recipient of a NSERC Doctoral award, Nasivvik Doctoral award, NSTP Training Fund and UNBC travel awards. We thank A. Essler, A. Montgomery, S. Krause, A. Krey, and G. Prkachin for assistance in the laboratory, and L. Loseto, M. Noel, D. Sydney, R. Felix, K. Nuyaviak, B. Voudrach, R. Walker, K. Snow, C. Pokiak, J. Pokiak, Inuvialuit hunters, Tuktoyaktuk and Inuvik HTCs, and the DFO for their support in the field.

Chapter 7. Conclusions and Recommendations

Objectives and Significance

The goal of this dissertation was to further our understanding of the toxicological risk posed by methylmercury (MeHg) exposure for beluga whales in the western Canadian Arctic, through community-based research methods. I worked closely with community research assistants, harvesters and youth to collect high quality samples, while also providing mentoring and training opportunities to northerners. To assess the toxicological risk of MeHg exposure in harvested beluga whales, I measured total mercury (Hg_T), MeHg and labile inorganic Hg (iHg_{labile}) in various brain regions of beluga whales and compared these concentrations to threshold levels. I used a biomarker approach to evaluate if Hg exposure was associated with neurochemical and molecular variation of components from the dopaminergic, γ-aminobutyric acid (GABA), glutamatergic and cholinergic signaling pathways. Finally, I documented harvesters' observations of beluga whale behaviour and compared the behaviour of whales with higher than median and lower than median Hg concentrations. The overall hypothesis was that Hg_T, iHg_{labile} and MeHg concentrations would exceed threshold levels, and would be associated with behavioural, neurochemical and/or molecular variation, if MeHg exposure was of toxicological concern for this population of beluga whales.

The overarching goals of this research and the Hendrickson Island Beluga Study were to carry out respectful and inclusive research in the Inuvialuit Settlement Region that would increase research capacity, respond to community concerns and questions, and develop research partnerships for long-term beluga monitoring in the region. Efforts were made

throughout the study period to engage youth, communicate effectively and provide training opportunities for northerners. The efforts made to involve community members and especially youth in the research process fostered good working relationships and strengthened the beluga-monitoring program.

This dissertation has direct implications for policies at a regional and international level. In the Inuvialuit Settlement Region, the Fisheries Joint Management Committee is particularly concerned with ensuring that the beluga population is managed to "provide for a harvest that generates the greatest net benefit to the Inuvialuit while ensuring the long-term sustainability of beluga in the Canadian Beaufort Sea" (FJMC, 2001). Therefore, research that furthers our understanding of the potential negative impacts of MeHg exposure in beluga may have management implications. Internationally, there have been increasing efforts to develop a legally-binding treaty on Hg emissions, and governments recently agreed to the text of a global, legally binding document to reduce mercury emissions. High quality contaminants research carried out in the Arctic and the advocacy of Arctic peoples contributed to the success of the Stockholm Convention, which regulates the production and use of 21 organic chemical substances (Watt-Cloutier, 2003). Therefore, expanding our understanding of the potential neurotoxicity of MeHg exposure in beluga whales will feed into policies to reduce Hg emissions and use.

This dissertation represents an extension of previous studies focused on Hg accumulation and distribution in beluga whales from the western Canadian Arctic (Lockhart et al., 2005; Outridge et al., 2002; Outridge et al., 2009; Wagemann et al., 1990; Wagemann et al., 1998). We have expanded on the state of knowledge of Hg accumulation in the

central nervous system (CNS) of beluga whales, and we have provided additional information about the forms of Hg present in the CNS and the relationship between Hg and selenium accumulation in five brain regions. This dissertation increases our knowledge about how Hg accumulation in beluga whale CNS compares to other taxa, and provides possible explanations for elevated Hg accumulation in the CNS of beluga whales.

To our knowledge, this work represents the first use of neurochemical and molecular biomarkers to assess potential neurotoxicity associated with MeHg exposure in cetaceans. Furthermore, contaminant exposure assessments are rarely, if ever, combined with behavioural studies of wildlife; therefore, this dissertation provides a unique example of how behavioural observations can be linked to MeHg exposure studies in beluga whales. This method may be adapted and replicated in situations where hunter-harvested animals are sampled for contaminants analysis. The overall significance of this work is that it provides the first species-specific analysis of the potential risk of MeHg exposure for beluga whales.

The toxicological risk of MeHg exposure

Mercury exposure thresholds

Total Hg concentrations in some beluga whales exceeded thresholds of toxicity reported for humans and primates; therefore, it is possible that Hg exposure in beluga whales from the eastern Beaufort Sea could be associated with neurotoxicity. At least 14% of the beluga whales had Hg_T concentrations higher than levels of observable adverse effect (6.0 mg kg^{-1} wet weight (ww)) in primates. The concentration of MeHg (range: 0.03 to

1.05 mg kg^{-1} ww) was positively associated with Hg_T concentration, and was below levels of observable effect in all animals sampled. The positive association between selenium (Se) and Hg_T in all brain regions suggests that Se could play a role in the detoxification of MeHg in the brain.

Neurochemical and molecular variation associated with mercury exposure

Total Hg concentrations (1.7 to 113 mg kg^{-1} dw), MeHg (0.5 – 5.2 mg kg^{-1} dw) and iHg_{labile} (0.6 to 6.7 mg kg^{-1} dw) in the samples analyzed exceeded the concentrations of Hg associated with neurochemical variation in wild mink (Hg_T = 0.27 to 18.84 mg kg^{-1} dw; MeHg = 0.26 to 13.52 mg kg^{-1} dw) (Basu et al., 2005a), river otter (Hg_T = 0.09 to 14.31 mg kg^{-1} dw; iHg = 0.00 to 10.65 mg kg^{-1} dw; organic Hg = 0.08 – 8.54 mg kg^{-1} dw) (Basu et al., 2005c), polar bear (Hg_T = 0.11 to 0.87 mg kg^{-1} dw) (Basu et al., 2009), common loons (Hg_T = 0.2 to 68 mg kg^{-1} dw) and bald eagles (Hg_T = 0.3 to 23 mg kg^{-1} dw) (Scheuhammer et al., 2008). My findings suggested that $GABA_A$-R binding was negatively associated with Hg and MeHg concentrations, NMDA-R binding was negatively associated with Hg_T and and iHg_{labile} concentrations (nss), and MAO activity was negatively associated with Hg_T, MeHg and iHg_{labile} concentrations (Table 7.1). Overall, these findings were consistent with results from previous avian and wildlife studies (Basu et al., 2005a; Basu et al., 2007b; Basu et al., 2008; Basu et al., 2007c; Basu et al., 2009; Rutkiewicz et al., 2011; Scheuhammer et al., 2008).

The results from our analysis of relative mRNA transcription levels for target genes for mAChR subtype m1, $GABA_A$-R α2 and NMDA-R 2b were negatively associated with

Hg_T, MeHg and/or iHg_{labile} concenterations (Table 7.1). The expression of mRNA for NMDA-2b and $GABA_A$ subunit α2 target genes were positively correlated to receptor binding levels of the NMDA-R and $GABA_A$-R, respectively. Furthermore, mRNA expression for $GABA_A$ subunit α4 was negatively correlated to $GABA_A$ receptor binding levels, which could be explained by the lack of binding affinity of the radioligand used ($[^3H]$-FNP) and the $GABA_A$ receptor if it contains the α4 subunit.

Selenium co-accumulation with Hg_T may provide protective effects for MAO activity and mAChR binding, based on the relationship between Hg_T and Se_T molar ratio and receptor binding. Futhermore, mRNA expression for mAChR m1 was also significantly associated with the molar ratio of Hg_T to Se_T. In contrast, the results from the GABA-R or NMDA-R binding assays did not indicate a significant relationship between receptor binding and the molar ratio of Hg_T to Se_T. Therefore, potential protective effects of Hg and Se co-accumulation for the glutamatergic and GABAergic signaling pathways were less evident in these studies.

To our knowledge, these were the first reported data on neurochemical or molecular variation associated with MeHg exposure in beluga whales, or cetaceans in general. In general, the decrease in receptor binding and mRNA expression for target genes from neurosignaling pathways associated with Hg_T, MeHg and/or iHg_{labile} concentration may be explained by signaling pathways being downregulated, in response to increased stimulation (Duman et al., 1994). These results suggest that although MeHg is demethylated and possibly detoxified through an interaction with Se, current MeHg

exposure may nonetheless be of toxicological concern for this population of beluga whales.

Table 7.1. Significant predictors (total mercury, Hg; methylmercury, MeHg; labile inorganic Hg, iHg_{labile}) of neurochemical and molecular variation in brain tissue from harvested beluga whales.

Biomarker		Total Hg	MeHg	iHg_{labile}
Neurochemical biomarker	$GABA_A$-R	↘ *	↘ *	-
	NMDA-R	↘ t	-	↘ t
	Muscarinic ACh-R	-	-	-
	MAO activity	↘ *	↘ *	↘ *
Molecular biomarker	$GABA_A$-R α2	↘ *	↘ *	↘ t
	$GABA_A$-R α4	-	-	-
	NMDA-R 2b	-	-	↘ *
	Muscarinic AChR m1	↘ *	↘ *	↘ *
	MAO activity	-	-	-

* $p \leq 0.05$
t $p \leq 0.1$
- $p > 0.1$

Evasive behaviour during hunt and mercury exposure

Behavioural changes associated with Hg toxicosis in one wild river otter were ataxia, scleral injection (red eyes) and lack of fleeing response (Sleeman et al., 2010). One cat with 16.4 mg kg^{-1} total Hg from White Dog, ON developed convulsions, ataxia, jumping

and circling around ('dancing') after it was fed fish entrails from the English River (Takeuchi et al., 1977). Therefore, we would expect a lack of motor coordination (ataxia), convulsions and abnormal behaviour from whales suffering mercury toxicosis. Our findings suggested a possible relationship between beluga whales' use of evasive strategies and Hg exposure. Given that the harvesters observed the whales behaving normally during the hunt, it is unlikely that the Hg exposure in the eleven whales analyzed was associated with overt toxicity like that seen in the intoxicated wild river otter and cat studies. However, a larger study would allow further exploration of the potential effect of Hg accumulation on beluga behaviour. Beluga harvesters have a unique ability to observe whale behaviour during the hunt, and these observations could be documented and compared to contaminant exposure and other parameters in future studies. Overall, the inclusion of traditional ecological knowledge and local observations of beluga whales in monitoring programs would improve our understanding of how environmental change may affect beluga whales from the eastern Beaufort Sea population.

Limitations

Assessing the significance of the relationships between Hg exposure and neurochemical and molecular biomarkers is limited by a number of factors. Specifically, this study lacked negative controls and had small sample sizes in each sampling year because samples were collected opportunistically from harvested beluga. Furthermore, sampling brain tissue from hunter-harvested whales affected the quality of samples due to the time between whale death and sampling (~ 20 min to 1 h), the time required to remove the

brain from the harvested beluga (~ 30 min), and the additional time to sub-sample the brain tissue (30-60 min). Differences in neurochemistry and mRNA expression between sampling years may have been due to sample instability over time, differences in Hg exposure between years, differences in age between years, or other differences in whale physiology between years. Other physiological differences in whales that could also impact neurochemistry and mRNA expression were not explored in this study due to limitations in sample size, and the lack of baseline information about beluga whale physiology and neurological signaling pathways.

Community-based research approach

The research process relied on collaboration within the research team and with northern partners to sample beluga whales during the field season on Hendrickson Island. Having a multi-disciplinary and multi-institutional team made it possible to have more frequent and extended contact between the team and our northern partners. Fieldwork on Hendrickson Island provided an important interface for the research team and community members from Tuktoyaktuk. During fieldwork, the researchers had the unique opportunity to live at a traditional whaling site and to learn about Inuvialuit culture, traditions, and way of life. Equally importantly, the researchers could learn first-hand from the local sampling team, youth, whale monitors and harvesters about the value of beluga and other country foods for Inuvialuit health and well-being. Finally, throughout the field season, the researchers were reminded of the responsibilities and privilege associated sampling and studying beluga whales in the ISR.

The research team relied on a number of strategies to increase connectivity across the worlds of academia and the Arctic. For example, the team connected to their northern

research partners through a diversity of communication strategies ranging from teleconference and phone calls, emails, research reports and pamphlets. The best way to connect remotely was through emails and phone calls directly to the THTC. The research team also supported the participation of mentoring students and northern research partners at scientific conferences and workshops in Ottawa, ON, Victoria, BC, and Montreal, QC. The best way for the research team to maintain communication and dialogue with Arctic partners and organizations was through community visits that included meetings, classroom presentations and family visits.

Communication

During fieldwork, the research team communicated with harvesters about the research that was taking place on Hendrickson Island. Unfortunately due to time restraints, dialogue was generally brief between researchers and harvesters. The intention for organizing a 'Sharing Knowledge' results workshop was for the Science Team to present their research findings to community members, provide a place to address and discuss questions from community members about our research, engage youth in learning about belugas and beluga research, and to discuss the future direction of beluga research/monitoring in the Inuvialuit Settlement Region. Organizing this workshop required an extensive time commitment for the research team and Tuktoyaktuk HTC resource person. This investment by the team and HTC were made due to the sense of responsibility to adequately respond to the community's questions following a three-year study period and ten-year beluga-monitoring program. The communication event in Tuktoyaktuk succeeded in bringing together diverse knowledge-holders and stakeholders

from the Hendrickson Island Beluga Study. The workshop and gatherings provided the opportunities for the research team to answer many of questions about belugas and beluga health that were raised by harvesters and their families.

Following the 'Sharing Knowledge about Belugas' workshop, the questions that arose from community members were summarized and the research team prepared answers to these questions. The final report from the Hendrickson Island Beluga Study aimed to present information about beluga whales based on the scientific studies that took place at Hendrickson Island, knowledge gained from the scientific literature and the knowledge held by Inuvialuit in Tuktoyaktuk, NT. The report was then transformed into a photo book using photos from fieldwork, conferences and community meetings, using iPhoto (Apple®). The draft book was sent to northern research partners and the Tuktoyaktuk HTC to receive additional feedback prior to publication. Community input was incorporated into the book and the final book was printed (250 copies) and distributed to harvesters, youth and community organizations in 2013.

Conclusions and future research

The weight-of-evidence from these studies suggests that Hg concentrations are indeed reaching levels associated with sub-clinical changes in neurochemistry in beluga whales from the eastern Beaufort Sea beluga population. Therefore, current MeHg exposure is of toxicological concern for beluga whales from this population. Although the response of beluga whales to MeHg exposure at a physiological and population level remains to be elucidated, further study is warranted to address potential adverse outcomes (e.g., tissue

pathologies, behavioural changes, motor impairment) associated with Hg toxicity in this population of beluga whales. Another stressor that needs to be considered for this population a warming Arctic, which may have detrimental consequences for beluga whales due to increased Hg loading of the Beaufort Sea from the Mackenzie River (Leitch et al., 2007), changes in ice regimes, decreased availability of food (e.g. Arctic cod), and increased risk of predation (IPCC, 2007). Climate change may also affect beluga whales and other Arctic marine mammals indirectly, by increasing exposure to ships strikes and noise associated with human activities such as shipping, fishing and industry in the Arctic (Huntington, 2009). To date, harvest levels by subsistence harvesters is sustainable and represents a removal of less than 0.6% of the estimated population (Harwood and Smith, 2002).

Given that beluga whales are unable to change their diet to reduce exposure to MeHg, global efforts are required to reduce emissions of Hg to protect Arctic beluga whales from MeHg exposure and potential intoxication. Furthermore, continued monitoring of beluga populations is required to ensure that changes in population size or health are documented early and mitigation efforts can be effective if possible.

References

Abd-Elfattah AS, Shamoo AE. Regeneration of a functionally active rat brain muscarinic receptor by D-penicillamine after inhibition with methylmercury and mercuric chloride. Mol Pharmacol 1981; 20: 492-7.

Aberg B, Ekman L, Falk R, Greitz U, Persson G, Snihs JO. Metabolism of methyl mercury (203Hg) compounds in man. Arch Environ Health 1969; 19: 478-84.

ACIA. Arctic Climate Impact Assessment: Cambridge University Press, 2005.

ACUNS. Ethical principles for the conduct of research in the North. Association of Canadian Universities for Northern Studies, 2003.

Adams DH, Sonne C, Basu N, Dietz R, Nam DH, Leifsson PS, et al. Mercury contamination in spotted seatrout, Cynoscion nebulosus: an assessment of liver, kidney, blood, and nervous system health. Sci Total Environ 2010; 408: 5808-16.

Albrecht J, Matyja E. Glutamate: a potential mediator of inorganic mercury neurotoxicity. Metab Brain Dis 1996; 11: 175-84.

Alvarez-Flores CM, Heide-Jorgensen MP. A risk assessment of the sustainability of the harvest of beluga (Delphinapterus leucas (Pallas 1776)) in West Greenland. Ices J Mar Sci 2004; 61: 274-286.

AMAP. AMAP Assessment 2002: human health in the Arctic Monitoring and Assessment Programme (AMAP). Oslo, Norway, 2003, pp. 137.

AMAP. AMAP Assessment 2011: Mercury in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, 2011, pp. xiv+193.

AMSA. Arctic Marine Shipping Assessment 2009 Report. Arctic Council, 2009, pp. 188.

Anner BM, Moosmayer M, Imesch E. Mercury blocks Na-K-ATPase by a ligand-dependent and reversible mechanism. Am J Physiol 1992; 262: F830-6.

Arakawa O, Nakahiro M, Narahashi T. Mercury modulation of GABA-activated chloride channels and non-specific cation channels in rat dorsal root ganglion neurons. Brain Res 1991; 551: 58-63.

Armstrong JAJ, Uthe JF. Semi-automated determination of mercury in animal tissue. At Absorpt Newsl 1971; 10: 101-103.

Arya M, Shergill IS, Williamson M, Gommersall L, Arya N, Patel HR. Basic principles of real-time quantitative PCR. Expert Rev Mol Diagn 2005; 5: 209-19.

Aschner M, Aschner JL. Mercury neurotoxicity: mechanisms of blood-brain barrier transport. Neurosci Biobehav Rev 1990; 14: 169-76.

Atchison WD, Hare MF. Mechanisms of methylmercury-induced neurotoxicity. Faseb J 1994; 8: 622-9.

Atwell L, Hobson KA, Welch HE. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Can J Fish Aquat Sci. 1998; 55: 1114-1121.

Augier H, Benkoel L, Chamlian A, Park WK, Ronneau C. Mercury, zinc and selenium bioaccumulation in tissues and organs of Mediterranean striped dolphins Stenella coeruleoalba meyen. Toxicological result of their interaction. Cell Mol Biol (Noisy-le-grand) 1993; 39: 621-34.

Bach AW, Lan NC, Johnson DL, Abell CW, Bembenek ME, Kwan SW, et al. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. Proc Natl Acad Sci U S A 1988; 85: 4934-8.

Barnes EM, Jr. Use-dependent regulation of GABAA receptors. Int Rev Neurobiol 1996; 39: 53-76.
Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, et al. Arctic contaminants: sources, occurrence and pathways. Sci Total Environ 1992; 122: 1-74.
Basu N, Head J. Mammalian wildlife as complementary models in environmental neurotoxicology. Neurotoxicol Teratol 2010; 32: 114-9.
Basu N, Klenavic K, Gamberg M, O'Brien M, Evans D, Scheuhammer AM, et al. Effects of mercury on neurochemical receptor-binding characteristics in wild mink. Environ Toxicol Chem 2005a; 24: 1444-50.
Basu N, Klenavic K, Gamberg M, O'Brien M, Evans D, Scheuhammer AM, et al. Effects of mercury on neurochemical receptor-binding characteristics in wild mink. Environ Toxicol Chem 2005b; 24: 1444-50.
Basu N, Kwan M, Chan HM. Mercury but not organochlorines inhibits muscarinic cholinergic receptor binding in the cerebrum of ringed seals (Phoca hispida). J Toxicol Environ Health A 2006a; 69: 1133-43.
Basu N, Scheuhammer A, Grochowina N, Klenavic K, Evans D, O'Brien M, et al. Effects of mercury on neurochemical receptors in wild river otters (Lontra canadensis). Environ Sci Technol 2005c; 39: 3585-91.
Basu N, Scheuhammer A, Grochowina N, Klenavic K, Evans D, O'Brien M, et al. Effects of mercury on neurochemical receptors in wild river otters (Lontra canadensis). Environ Sci Technol 2005d; 39: 3585-91.
Basu N, Scheuhammer AM, Bursian SJ, Elliott J, Rouvinen-Watt K, Chan HM. Mink as a sentinel species in environmental health. Environ Res 2007a; 103: 130-44.
Basu N, Scheuhammer AM, Evans RD, O'Brien M, Chan HM. Cholinesterase and monoamine oxidase activity in relation to mercury levels in the cerebral cortex of wild river otters. Hum Exp Toxicol 2007b; 26: 213-20.
Basu N, Scheuhammer AM, Rouvinen-Watt K, Evans RD, Grochowina N, Chan LH. The effects of mercury on muscarinic cholinergic receptor subtypes (M1 and M2) in captive mink. Neurotoxicology 2008; 29: 328-34.
Basu N, Scheuhammer AM, Rouvinen-Watt K, Evans RD, Trudeau VL, Chan LH. In vitro and whole animal evidence that methylmercury disrupts GABAergic systems in discrete brain regions in captive mink. Comp Biochem Physiol C Toxicol Pharmacol 2010; 151: 379-85.
Basu N, Scheuhammer AM, Rouvinen-Watt K, Grochowina N, Evans RD, O'Brien M, et al. Decreased N-methyl-D-aspartic acid (NMDA) receptor levels are associated with mercury exposure in wild and captive mink. Neurotoxicology 2007c; 28: 587-93.
Basu N, Scheuhammer AM, Rouvinen-Watt K, Grochowina N, Klenavic K, Evans RD, et al. Methylmercury impairs components of the cholinergic system in captive mink (Mustela vison). Toxicol Sci 2006b; 91: 202-9.
Basu N, Scheuhammer AM, Sonne C, Letcher RJ, Born EW, Dietz R. Is Dietary Mercury of Neurotoxicological Concern to Wild Polar Bears (Ursus Maritimus)? Environmental Toxicology and Chemistry 2009; 28: 133-140.
Basu N, Stamler CJ, Loua KM, Chan HM. An interspecies comparison of mercury inhibition on muscarinic acetylcholine receptor binding in the cerebral cortex and cerebellum. Toxicol Appl Pharmacol 2005e; 205: 71-6.
Bellum S, Thuett KA, Bawa B, Abbott LC. The effect of methylmercury exposure on behavior and cerebellar granule cell physiology in aged mice. J Appl Toxicol 2012.

Bennett T. Monitoring environmental conditions using participatory photo-mapping with Inuvialuit knowledge holders in the Mackenzie Delta Region, Northwest Territories. School of Environmental Studies. M.A. University of Victoria, 2012, pp. x+174.
Bercovitch FB, Clarke AS. Dominance rank, cortisol concentrations, and reproductive maturation in male rhesus macaques. Physiol Behav 1995; 58: 215-21.
Bergeron R, Scott SL, Emond JP, Mercier F, Cook NJ, Schaefer AL. Physiology and behavior of dogs during air transport. Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire 2002; 66: 211-216.
Berkes F. Traditional ecological knowledge in perspective. Ottawa: IDRC, 1993.
Berlin M, Carlson J, Norseth T. Dose-dependence of methylmercury metabolism. A study of distribution, biotransformation and excretion in the squirrel monkey. Arch Environ Health 1975a; 30: 307-313.
Berlin M, Grant CA, Hellberg J, Hellstrom J, Schultz A. Neurotoxicity of methylmercury in squirrel monkeys. Cerebral cortical pathology, interference with scotopic vision, and changes in operant behavior. Arch Environ Health 1975b; 30: 340-8.
Bernhoft A, Skaare JU. Levels of selected individual polychlorinated-biphenyls in different tissues of harbor seals (Phoca vitulina) from the southern coast of Norway. Environmental Pollution 1994; 86: 99-107.
Berntssen MH, Aatland A, Handy RD. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (Salmo salar) parr. Aquat Toxicol 2003; 65: 55-72.
Berry MJ, Ralston NV. Mercury toxicity and the mitigating role of selenium. Ecohealth 2008; 5: 456-9.
Beyrouty P, Stamler CJ, Liu JN, Loua KM, Kubow S, Chan HM. Effects of prenatal methylmercury exposure on brain monoamine oxidase activity and neurobehaviour of rats. Neurotoxicol Teratol 2006; 28: 251-9.
Birnbaum LS, Tuomisto J. Non-carcinogenic effects of TCDD in animals. Food Addit Contam 2000; 17: 275-88.
Bjorkman L, Mottet K, Nylander M, Vahter M, Lind B, Friberg L. Selenium concentrations in brain after exposure to methylmercury: relations between the inorganic mercury fraction and selenium. Arch Toxicol 1995; 69: 228-34.
Bortolato M, Shih JC. Behavioral outcomes of monoamine oxidase deficiency: preclinical and clinical evidence. Int Rev Neurobiol 2011; 100: 13-42.
Braune BM, Outridge PM, Fisk AT, Muir DC, Helm PA, Hobbs K, et al. Persistent organic pollutants and mercury in marine biota of the Canadian Arctic: an overview of spatial and temporal trends. Sci Total Environ 2005; 351-352: 4-56.
Brookes N, Kristt DA. Inhibition of amino acid transport and protein synthesis by HgCl2 and methylmercury in astrocytes: selectivity and reversibility. J Neurochem 1989; 53: 1228-37.
Burger J, Jehl JRJ, Gochfeld M. Selenium:mercury molar ratio in eared grebes (Podiceps nigricollis) as a possible biomarker of exposure Ecological Indicators 2013; 34: 60-68.
Byers T, Roberts LM. Harpoons and Ulus: collective wisdon and traditions of Inuvialuit regarding the beluga ("qilalugaq") in the Mackenzie River estuary. Byers Environmental Studies, Winnipeg, MB, 1995.

Bymaster FP, McKinzie DL, Felder CC, Wess J. Use of M1-M5 muscarinic receptor knockout mice as novel tools to delineate the physiological roles of the muscarinic cholinergic system. Neurochem Res 2003; 28: 437-42.
Canada Go. Marine Mammal Regulations. In: Justice Do, editor. SOR/93-56, 1993.
Capelli R, Das K, De Pellegrini R, Drava G, Lepoint G, Miglio C, et al. Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios Sci Total Environ 2008; 390: 569-78.
Cardellicchio N, Decataldo A, Di LA, Misino A. Accumulation and tissue distribution of mercury and selenium in striped dolphins (Stenella coeruleoalba) from the Mediterranean Sea (southern Italy). Environ Pollut 2002; 116: 265-71.
Carter BTG, Nielsen EA. Exploring ecological changes in Cook Inlet beluga whale habitat though traditional and local ecological knowledge of contributing factors for population decline. Marine Policy 2011; 35: 299-308.
Castoldi AF, Candura SM, Costa P, Manzo L, Costa LG. Interaction of mercury compounds with muscarinic receptor subtypes in the rat brain. Neurotoxicology 1996; 17: 735-41.
Castoldi AF, Coccini T, Manzo L. Neurotoxic and molecular effects of methylmercury in humans. Rev Environ Health 2003; 18: 19-31.
Catts VS, Catts SV, Fernandez HR, Taylor JM, Coulson EJ, Lutze-Mann LH. A microarray study of post-mortem mRNA degradation in mouse brain tissue. Brain Res Mol Brain Res 2005; 138: 164-77.
Chakrabarti SK, Loua KM, Bai C, Durham H, Panisset JC. Modulation of monoamine oxidase activity in different brain regions and platelets following exposure of rats to methylmercury. Neurotoxicol Teratol 1998; 20: 161-8.
Chan HM, Kim C, Khoday K, Receveur O, Kuhnlein HV. Assessment of dietary exposure to trace metals in Baffin Inuit food. Environ Health Perspect 1995; 103: 740-6.
Chebib M, Johnston GA. The 'ABC' of GABA receptors: a brief review. Clin Exp Pharmacol Physiol 1999; 26: 937-40.
Chen RW, Lacy VL, Whanger PD. Effect of selenium on methylmercury binding to subcellular and soluble proteins in rat tissues. Res Commun Chem Pathol Pharmacol 1975; 12: 297-308.
Clarkson TW. The toxicology of mercury. Crit Rev Clin Lab Sci 1997; 34: 369-403.
Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 2006; 36: 609-62.
Coccini T, Randine G, Candura SM, Nappi RE, Prockop LD, Manzo L. Low-level exposure to methylmercury modifies muscarinic cholinergic receptor binding characteristics in rat brain and lymphocytes: physiologic implications and new opportunities in biologic monitoring. Environ Health Perspect 2000; 108: 29-33.
Coccini T, Randine G, Castoldi AF, Acerbi D, Manzo L. Methylmercury interaction with lymphocyte cholinergic muscarinic receptors in developing rats. Environ Res 2007; 103: 229-37.
Concas A, Corda MG, Salis M, Mulas ML, Milia A, Corongiu FP, et al. Biochemical changes in the rat cerebellar cortex elicited by chronic treatment with methyl mercury. Toxicol Lett 1983; 18: 27-33.

Condon RG, Collings P, Wenzel G. The best part of life: subsistence hunting, ethnicity and economic adaptation among young adult Inuit males. Arctic 1995; 48: 31-46.
Corda MG, Concas A, Rossetti Z, Guarneri P, Corongiu FP, Biggio G. Methyl mercury enhances [3H]diazepam binding in different areas of the rat brain. Brain Res 1981; 229: 264-9.
COSEWIC. COSEWIC assessment and update status report on the beluga whale *Delphinapterus leucas* in Canada. In: Canada CotSoEWi, editor, Ottawa, 2004, pp. ix + 70 pp.
Costa LG. Interactions of neurotoxicants with neurotransmitter systems. Toxicology 1988; 49: 359-66.
Costa LG, Fattori V, Giordano G, Vitalone A. An in vitro approach to assess the toxicity of certain food contaminants: methylmercury and polychlorinated biphenyls. Toxicology 2007; 237: 65-76.
Council NR. Toxicological effects of methylmercury. Washington, DC: National Academy Press, 2000.
Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science 1993; 262: 689-95.
Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: diversity, development and disease. Curr Opin Neurobiol 2001; 11: 327-35.
Dalley JW, Everitt BJ. Dopamine receptors in the learning, memory and drug reward circuitry. Semin Cell Dev Biol 2009; 20: 403-10.
Day B. Renewable resources of the Beaufort Sea for our children: perspectives from an Inuvialuit Elder. Arctic 2002; 55: 1-3.
Dewailly E, Ayotte P, Bruneau S, Laliberte C, Muir DC, Norstrom RJ. Inuit exposure to organochlorines through the aquatic food chain in arctic quebec. Environ Health Perspect 1993; 101: 618-20.
DFO. Northern Quebec (Nunavik) Beluga (*Delphinapterus leucas*). DFO Sciences Stock Report E4-01 2001.
DFO. Proceedings of the workshop on the St. Lawrence beluga - review of carcass program. DFO Can. Sci. Advis. Sec. Proceed. Ser. 2007; 2007/005.
DFO. Recovery strategy for the beluga whale (*Delphinapterus leucas*) St. Lawrence Estuary population in Canada. *Species at Risk Act* Recovery Strategy Series. In: Canada FaO, editor, Ottawa, 2012, pp. 88 p + X pp.
Dietz R, Outridge PM, Hobson KA. Anthropogenic contributions to mercury levels in present-day Arctic animals--a review. Sci Total Environ 2009; 407: 6120-31.
Dietz R, Riget F, Cleemann M, Aarkrog A, Johansen P, Hansen JC. Comparison of contaminants from different trophic levels and ecosystems. Science of the Total Environment 2000; 245: 221-231.
Dietz R, Sonne C, Basu N, Braune B, O'Hara T, Letcher RJ, et al. What are the toxicological effects of mercury in Arctic biota? Sci Total Environ 2013; 443: 775-90.
Dowsley M. Community clusters in wildlife and environmental management: using TEK and community involvement to improve co-management in an era of rapid environmental change. Polar Research 2009; 28: 43-59.
Dreiem A, Seegal RF. Methylmercury-induced changes in mitochondrial function in striatal synaptosomes are calcium-dependent and ROS-independent. Neurotoxicology 2007; 28: 720-6.

Duman RS, Heninger GR, Nestler EJ. Molecular psychiatry. Adaptations of receptor-coupled signal transduction pathways underlying stress- and drug-induced neural plasticity. J Nerv Ment Dis 1994; 182: 692-700.
Ehlert FJ, Thomas EA. Functional role of M2 muscarinic receptors in the guinea pig ileum. Life Sci 1995; 56: 965-71.
Ekino S, Susa M, Ninomiya T, Imamura K, Kitamura T. Minamata disease revisited: an update on the acute and chronic manifestations of methyl mercury poisoning. J Neurol Sci 2007; 262: 131-44.
EPA U. Guidelines for ecological risk assessment, 1998, pp. 188.
Eto K, Takizawa Y, Akagi H, Haraguchi K, Asano S, Takahata N, et al. Differential diagnosis between organic and inorganic mercury poisoning in human cases--the pathologic point of view. Toxicol Pathol 1999; 27: 664-71.
Evans JL, Garman RH, Weiss B. Methylmercury: exposure duration and regional distribution as determinants of neurotoxicity in non-human primates. Toxicol Appl Pharmacol 1977; 41: 15-33.
Farina M, Franco JL, Ribas CM, Meotti FC, Missau FC, Pizzolatti MG, et al. Protective effects of Polygala paniculata extract against methylmercury-induced neurotoxicity in mice. J Pharm Pharmacol 2005; 57: 1503-8.
Faro LR, do Nascimento JL, Alfonso M, Duran R. Protection of methylmercury effects on the in vivo dopamine release by NMDA receptor antagonists and nitric oxide synthase inhibitors. Neuropharmacology 2002; 42: 612-8.
Faro LR, Duran R, Do Nascimento JL, Perez-Vences D, Alfonso M. Effects of successive intrastriatal methylmercury administrations on dopaminergic system. Ecotoxicol Environ Saf 2003; 55: 173-7.
Ferguson SH, Higdon JW, Westdal KH. Prey items and predation behavior of killer whales (Orcinus orca) in Nunavut, Canada based on Inuit hunter interviews. Aquat Biosyst 2012; 8: 3.
Fernandez-Gimenez ME, Huntington HP, Frost KJ. Integration or co-optation? Traditional knowledge and science in the Alaska Beluga Whale Committee. Environmental Conservation 2006; 33: 306-315.
Fisk AT, de Wit CA, Wayland M, Kuzyk ZZ, Burgess N, Letcher R, et al. An assessment of the toxicological significance of anthropogenic contaminants in Canadian arctic wildlife. Sci Total Environ 2005; 351-352: 57-93.
Fitsanakis VA, Aschner M. The importance of glutamate, glycine, and gamma-aminobutyric acid transport and regulation in manganese, mercury and lead neurotoxicity. Toxicol Appl Pharmacol 2005; 204: 343-54.
FJMC. Beaufort Sea Beluga Management Plan, 2001.
Fleige S, Pfaffl MW. RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med 2006; 27: 126-39.
Fournier M, Degas V, Colborn T, Omara FO, Denizeau F, Potworowski EF, et al. Immunosuppression in mice fed on diets containing beluga whale blubber from the St Lawrence estuary and the Arctic populations. Toxicol Lett 2000; 112-113: 311-7.
Franco JL, Braga HC, Stringari J, Missau FC, Posser T, Mendes BG, et al. Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. Chem Res Toxicol 2007; 20: 1919-26.
Freeman MM, Bogoslovskaya L, Caulfield RA, Egede I, Krupnik II, Stevenson MG. Inuit, whaling, and sustainability Walnut Creek: Altamira Press, 1998.

Friesen TM, Arnold CD. Zooarchaeology of a focal resource - dietary importance of beluga whales to the precontact Mackenzie Inuit. Arctic 1995; 48: 22-30.
Furgal CM, Powell S, Myers H. Digesting the message about contaminants and country foods in the Canadian North: A review and recommendations for future research and action. Arctic 2005; 58: 103-114.
Gagnon CA, Berteaux D. Integrating Traditional Ecological Knowledge and Ecological Science: a Question of Scale. Ecology and Society 2009; 14.
Gauthier JM, Pelletier E, Brochu C, Moore S, Metcalfe CD, Béland P. Environmental contaminants in tissues of a neonate St Lawrence Beluga Whale (Delphinapterus leucas). Mar Pollut Bull 1998; 36: 102-108.
Gearheard A, Shirley J. Challenges in community-research relationships: learning from natural science in Nunavut. Arctic 2007; 60: 62-74.
Gilchrist G, Mallory M, Merkel F. Can local ecological knowledge contribute to wildlife management? Case studies of migratory birds. Ecology and Society 2005; 10.
Gladden JG, Ferguson MM, Friesen MK, Clayton JW. Population structure of North American beluga whales (Delphinapterus leucas) based on nuclear DNA microsatellite variation and contrasted with the population structure revealed by mitochondrial DNA variation. Mol Ecol 1999; 8: 347-63.
Gomes B, Kloepfer HG, Oi S, Yasunobu KT. The reaction of sulfhydryl reagents with bovine hepatic monoamine oxidase. Evidence for the presence of two cysteine residues essential for activity. Biochim Biophys Acta 1976; 438: 347-57.
Green AR, Youdim MB. Effects of monoamine oxidase inhibition by clorgyline, deprenil or tranylcypromine on 5-hydroxytryptamine concentrations in rat brain and hyperactivity following subsequent tryptophan administration. Br J Pharmacol 1975; 55: 415-22.
Greenbaum D, Colangelo C, Williams K, Gerstein M. Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol 2003; 4: 117.
Grimsby J, Chen K, Wang LJ, Lan NC, Shih JC. Human monoamine oxidase A and B genes exhibit identical exon-intron organization. Proc Natl Acad Sci U S A 1991; 88: 3637-41.
Hamilton M, Scheuhammer A, Basu N. Mercury, selenium and neurochemical biomarkers in different brain regions of migrating common loons from Lake Erie, Canada. Ecotoxicology 2011; 20: 1677-83.
Hansen JC, Danscher G. Quantitative and qualitative distribution of mercury in organs from arctic sledgedogs: an atomic absorption spectrophotometric and histochemical study of tissue samples from natural long-termed high dietary organic mercury-exposed dogs from Thule, Greenland. Pharmacol Toxicol 1995; 77: 189-95.
Harris EW, Ganong AH, Cotman CW. Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. Brain Res 1984; 323: 132-7.
Harwood LA, Norton P, Day B, Hall PA. The harvest of beluga whales in Canada's western Arctic: Hunter-based monitoring of the size and composition of the catch. Arctic 2002; 55: 10-20.
Harwood LA, Smith TG. Whales of the Inuvialuit settlement region in Canada's Western Arctic: An overview and outlook. Arctic 2002; 55: 77-93.
Held P. Horseradish Peroxidase (HRP) Determination Using Amplex Red and Synergy HT Multi-Mode Microplate Reader. In: Instruments B, editor. Tech Resources - Application Notes, 2003.

Hoekstra PF, O'Hara TM, Fisk AT, Borga K, Solomon KR, Muir DC. Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort-Chukchi Seas. Environ Pollut 2003; 124: 509-22.

Hovelsrud GK, McKenna M, Huntington HP. Marine mammal harvests and other interactions with humans. Ecol Appl 2008; 18: S135-47.

Huang CS, Narahashi T. Mercury chloride modulation of the GABAA receptor-channel complex in rat dorsal root ganglion neurons. Toxicol Appl Pharmacol 1996; 140: 508-20.

Huang CS, Narahashi T. The role of phosphorylation in the activity and mercury modulation of GABA-induced currents in rat neurons. Neuropharmacology 1997; 36: 1631-40.

Hunter D, Russell DS. Focal cerebellar and cerebellar atrophy in a human subject due to organic mercury compounds. J Neurol Neurosurg Psychiatry 1954; 17: 235-41.

Huntington H, Callaghan T, Fox S, Krupnik I. Matching traditional and scientific observations to detect environmental change: a discussion on Arctic terrestrial ecosystems. Ambio 2004; Spec No 13: 18-23.

Huntington HP. Using Traditional Ecological Knowledge in science: Methods and applications. Ecological Applications 2000; 10: 1270-1274.

Huntington HP. A preliminary assessment of threats to arctic marine mammals and their conservation in the coming decades. Marine Policy 2009; 33: 77-82.

Huntington HP, Buckland C, Elim C, Koyuk C, Lay CP, Shaktoolik C. Traditional knowledge of the ecology of beluga whales (Delphinapterus leucas) in the eastern Chukchi and northern Bering Seas, Alaska. Arctic 1999; 52: 49-61.

IPCC. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007. Cambridge, United Kingdom and New York, NY, USA.: Cambridge University Press, , 2007.

ITK, NRI. Negotiating research relationships with inuit communities: a guide for researchers. Ottawa and Iqaluit: Inuit Tapiriit Kanatami and Nunavut Resarch Institute, 2007.

Jefferson TA, Karczmarski, L., Laidre, K., O'Corry-Crowe, G., Reeves, R.R., Rojas-Bracho, L., Secchi, E.R., Slooten, E., Smith, B.D., Wang, J.Y. & Zhou, K. Delphinapterus leucas. IUCN 2012. IUCN Red List of Threatened Species., 2008.

Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 1987; 325: 529-31.

Joiris CR, Holsbeek L, Bolbat D, Gascard C, Stanev T, Komakhidze A, et al. Total and organic mercury in the Black Sea harbour porpoise Phocoena phocoena relicta. Mar Pollut Bull 2001; 42: 905-11.

Juarez BI, Martinez ML, Montante M, Dufour L, Garcia E, Jimenez-Capdeville ME. Methylmercury increases glutamate extracellular levels in frontal cortex of awake rats. Neurotoxicol Teratol 2002; 24: 767-71.

Juarez BI, Portillo-Salazar H, Gonzalez-Amaro R, Mandeville P, Aguirre JR, Jimenez ME. Participation of N-methyl-D-aspartate receptors on methylmercury-induced DNA damage in rat frontal cortex. Toxicology 2005; 207: 223-9.

Kelly BC, Ikonomou MG, Blair JD, Gobas FA. Hydroxylated and methoxylated polybrominated diphenyl ethers in a Canadian Arctic marine food web. Environ Sci Technol 2008; 42: 7069-77.

Khan MA, Wang F. Mercury-selenium compounds and their toxicological significance: toward a molecular understanding of the mercury-selenium antagonism. Environ Toxicol Chem 2009; 28: 1567-77.
Khan MAK, Wang F. Chemical demethylation of methylmercury by selenoamino acids. Chem Res Toxicol 2010; 23: 1202-1206.
Kilabuk P. A study of Inuit knowledge of the southeast Baffin beluga. In: Board NWM, editor, Iqaluit, NT, 1998.
Kingsley MCS, Gosselin S, Sleno GA. Movements and dive behaviour of belugas in northern Quebec. Arctic 2001; 54: 262-275.
Kirk JL, St Louis VL, Hintelmann H, Lehnherr I, Else B, Poissant L. Methylated mercury species in marine waters of the Canadian high and sub Arctic. Environ Sci Technol 2008; 42: 8367-73.
Kodavanti PR. Neurotoxicity of persistent organic pollutants: possible mode(s) of action and further considerations. Dose-Response 2005; 3.
Komulainen H, Keranen A, Saano V. Methylmercury modulates GABAA receptor complex differentially in rat cortical and cerebellar membranes in vitro. Neurochemical Research 1995; 20: 659-662.
Korbas M, O'Donoghue JL, Watson GE, Pickering IJ, Singh SP, Myers GJ, et al. The chemical nature of mercury in human brain following poisoning or environmental exposure. ACS Chem Neurosci 2010; 1: 810-8.
Krahn MM, Ylitalo GM, Burrows DG, Calambokidis J, Moore SE, Gosho M, et al. Organochlorine contaminant concentrations and lipid profiles in eastern North Pacific gray whales (Eschrichtius robustus). Journal of Cetacean Research and Management 2001; 3: 19-29.
Krey A, Kwan M, Chan HM. Mercury speciation in brain tissue of polar bears (Ursus maritimus) from the Canadian Arctic. Environ Res 2012; 114: 24-30.
Lakowicz JR. Principles of Fluorescence Spectroscopy. New York, NY: Springer, 2006.
Laudenslager ML, Rasmussen KL, Berman CM, Lilly AA, Shelton SE, Kalin NH, et al. A preliminary description of responses of free-ranging rhesus monkeys to brief capture experiences: Behavior, endocrine, immune, and health relationships. Brain Behavior and Immunity 1999; 13: 124-137.
Lehmler HJ, Robertson LW, Garrison AW, Kodavanti PR. Effects of PCB 84 enantiomers on [3H]-phorbol ester binding in rat cerebellar granule cells and 45Ca2+-uptake in rat cerebellum. Toxicol Lett 2005; 156: 391-400.
Leitch DR, Carrie J, Lean D, Macdonald RW, Stern GA, Wang F. The delivery of mercury to the Beaufort Sea of the Arctic Ocean by the Mackenzie River. Science of the Total Environment 2007; 373: 178-195.
Lemes M, Wang F. Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. J Anal Atom Spectrom 2009; 24: 663-668.
Lemes M, Wang F, Stern GA, Ostertag SK, Chan HM. Methylmercury and selenium speciation in different tissues of beluga whales (Delphinapterus leucas) from the western Canadian Arctic. Environ Toxicol Chem 2011; 30: 2732-8.
Letcher RJ, Bustnes JO, Dietz R, Jenssen BM, Jorgensen EH, Sonne C, et al. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci Total Environ 2010; 408: 2995-3043.

Leysen J, Langlois X, Heylen L, Lammertsma A. Receptors: Binding Assays. In: Stolerman I, Price L, editors. Encyclopedia of Psychopharmacology. Springer-Verlag, Heidelberg, 2010.
Limke TL, Bearss JJ, Atchison WD. Acute exposure to methylmercury causes Ca2+ dysregulation and neuronal death in rat cerebellar granule cells through an M3 muscarinic receptor-linked pathway. Toxicol Sci 2004; 80: 60-8.
Lindberg S, Bullock R, Ebinghaus R, Engstrom D, Feng X, Fitzgerald W, et al. A synthesis of progress and uncertainties in attributing the sources of mercury in deposition. Ambio 2007; 36: 19-32.
Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-8.
Lockhart WL, Stern GA, Wagemann R, Hunt RV, Metner DA, DeLaronde J, et al. Concentrations of mercury in tissues of beluga whales (Delphinapterus leucas) from several communities in the Canadian Arctic from 1981 to 2002. Sci Total Environ 2005; 351-352: 391-412.
Loseto LL, Stern GA, Connelly TL, Deibel D, Gemmill B, Prokopowicz A, et al. Summer diet of beluga whales inferred by fatty acid analysis of the eastern Beaufort Sea food web. J Exp Mar Biol Ecol 2009; 374: 12-18.
Loseto LL, Stern GA, Deibel D, Connelly TL, Prokopowicz A, Lean DRS, et al. Linking mercury exposure to habitat and feeding behaviour in Beaufort Sea beluga whales. J Marine Syst 2008a; 74: 1012-1024.
Loseto LL, Stern GA, Ferguson SH. Size and biomagnification: How Habitat selection explains beluga mercury levels. Environ Sci Technol 2008b; 42: 3982-8.
Luque SP, Ferguson SH. Age structure, growth, mortality, and density of belugas (Delphinapterus leucas) in the Canadian Arctic: responses to environment? . Polar Biol 2010; 33: 163-178.
Luschei R, Mottet NK, Shaw CM. Chronic methylmercury exposure in the monkey (*Macaca mulatta*). Arch Environ Health 1977; 32: 126-131.
Mackey EA, Becker PR, Demiralp R, Greenberg RR, Koster BJ, Wise SA. Bioaccumulation of vanadium and other trace metals in livers of Alaskan cetaceans and pinnipeds. Arch Environ Contam Toxicol 1996; 30: 503-12.
Magos L, Brown AW, Sparrow S, Bailey E, Snowden RT, Skipp WR. The comparative toxicology of ethyl- and methylmercury. Arch Toxicol 1985; 57: 260-7.
Magos L, Clarkson TW. Overview of the clinical toxicity of mercury. Ann Clin Biochem 2006; 43: 257-68.
Magos L, Webb M. The interactions of selenium with cadmium and mercury. Crit Rev Toxicol 1980; 8: 1-42.
Manzo L, Artigas F, Martinez E, Mutti A, Bergamaschi E, Nicotera P, et al. Biochemical markers of neurotoxicity. A review of mechanistic studies and applications. Hum Exp Toxicol 1996; 15 Suppl 1: S20-35.
Manzo L, Castoldi AF, Coccini T, Prockop LD. Assessing effects of neurotoxic pollutants by biochemical markers. Environ Res 2001; 85: 31-6.
Martin RS, Witt MLI, Pyle DM, Mather TA, Watt SFL, Bagnato E, et al. Rapid oxidation of mercury (Hg) at volcanic vents: Insights from high temperature thermodynamic models of Mt Etna's emissions. Chemical Geology 2011; 283: 279-286.

Mathieu A, Payne JF, Fancey LL, Santella RM, Young TL. Polycyclic aromatic hydrocarbon-DNA adducts in beluga whales from the Arctic. J Toxicol Environ Health 1997; 51: 1-4.

Meador JP, Ernest D, Hohn AA, Tilbury K, Gorzelany J, Worthy G, et al. Comparison of elements in bottlenose dolphins stranded on the beaches of Texas and Florida in the Gulf of Mexico over a one-year period. Arch Environ Contam Toxicol 1999; 36: 87-98.

Meinerz DF, de Paula MT, Comparsi B, Silva MU, Schmitz AE, Braga HC, et al. Protective effects of organoselenium compounds against methylmercury-induced oxidative stress in mouse brain mitochondrial-enriched fractions. Braz J Med Biol Res 2011; 44: 1156-63.

Mergler D, Anderson HA, Chan LH, Mahaffey KR, Murray M, Sakamoto M, et al. Methylmercury exposure and health effects in humans: a worldwide concern. Ambio 2007; 36: 3-11.

Michalke B, Halbach S, Nischwitz V. JEM spotlight: metal speciation related to neurotoxicity in humans. J Environ Monit 2009; 11: 939-54.

Muir DC, de Wit CA. Trends of legacy and new persistent organic pollutants in the circumpolar arctic: overview, conclusions, and recommendations. Sci Total Environ 2010; 408: 3044-51.

Mymrin NI, Huntington HP, Community Novoe C, Community S, Community U, Community Y. Traditional knowledge of the ecology of beluga whales (Delphinapterus leucas) in the northern Bering Sea, Chukotka, Russia. Arctic 1999; 52: 62-70.

Nam DH, Adams DH, Flewelling LJ, Basu N. Neurochemical alterations in lemon shark (Negaprion brevirostris) brains in association with brevetoxin exposure. Aquat Toxicol 2010; 99: 351-9.

Narahashi T, Ma JY, Arakawa O, Reuveny E, Nakahiro M. GABA receptor-channel complex as a target site of mercury, copper, zinc, and lanthanides. Cell Mol Neurobiol 1994; 14: 599-621.

Newland MC, Paletz EM. Animal studies of methylmercury and PCBs: what do they tell us about expected effects in humans? Neurotoxicology 2000; 21: 1003-27.

Newland MC, Reed MN, LeBlanc A, Donlin WD. Brain and blood mercury and selenium after chronic and developmental exposure to methylmercury. Neurotoxicology 2006; 27: 710-20.

Nicotra A, Pierucci F, Parvez H, Senatori O. Monoamine oxidase expression during development and aging. Neurotoxicology 2004; 25: 155-65.

Nigro M, Leonzio C. Intracellular storage of mercury and selenium in different marine vertebrates. Mar Ecol Prog Ser 1996; 135: 137-43.

Nriagu JO, Pacyna JM. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 1988; 333: 134-9.

O'Hammill MO, Lesage V, Gosselin F-F, Bourdages H, de March BGE, Kingsley MCS. Evidence for a decline in Northern Quebec (Nunavik) belugas. Arctic 2004; 57: 183-195.

Ostertag S, Shaw AC, Chan HM. Methylmercury and selenium exposure were associated with biomarkers of the cholinergic and dopaminergic signaling pathways in arctic beluga whales (Delphinapterus leucas).. Environmental Research *Submitted.*

Ostertag SK, Shaw AC, Basu N, Chan HM. Mercury and selenium exposure is associated with molecular and neurochemical biomarkers in two brains regions of arctic beluga whales (Delphinapterus leucas). Environ. Sci. Technol. in review.
Ostertag SK, Stern GA, Wang F, Lemes M, Chan HM. Mercury distribution and speciation in different brain regions of beluga whales (Delphinapterus leucas). Sci Total Environ 2013; 456-457: 278-86.
Outridge PM, Hobson KA, McNeely R, Dyke A. A comparison of modern and preindustrial levels of mercury in the teeth of beluga in the Mackenzie Delta, Northwest Territories, and walrus at Igloolik, Nunavut, Canada. Arctic 2002; 55: 123-132.
Outridge PM, Hobson KA, Savelle J. Long-term changes of mercury levels in ringed seal (Phoca hispida) from Amundsen Gulf, and beluga (Delphinapterus leucas) from the Beaufort Sea, western Canadian Arctic. Science of the Total Environment 2009; 407: 6044-6051.
Outridge PM, Macdonald RW, Wang F, Stern GA, Dastoor AP. A mass balance inventory of mercury in the Arctic Ocean. Environmental Chemistry 2008; 5: 89-111.
Penschuck S, Paysan J, Giorgetta O, Fritschy JM. Activity-dependent regulation of GABAA receptors. Ann N Y Acad Sci 1999; 868: 654-66.
Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001; 29: e45.
Popescu G. Mechanism-based targeting of NMDA receptor functions. Cell Mol Life Sci 2005; 62: 2100-11.
Porcella DB, Zillioux EJ, Grieb TM, Newman JR, West GB. Retrospective study of mercury in raccoons (Procyon lotor) in south Florida. Ecotoxicology 2004; 13: 207-21.
Programme UE. Report of the workshop on traditional knowledge and biological diversity, 1997.
Prowse TD, Furgal C, Chouinard R, Melling H, Milburn D, Smith SL. Implications of climate change for economic development in northern Canada: energy, resource, and transportation sectors. Ambio 2009; 38: 272-81.
Rajanna B, Chetty CS, Rajanna S, Hall E, Fail S, Yallapragada PR. Interaction of metals with muscarinic cholinoceptor and adrenoceptor binding, and agonist-stimulated inositol phospholipid hydrolysis in rat brain. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1997; 116: 111-6.
Rapley R. Recombinant DNA and genetic analysis. In: Wilson K, Walker J, editors. Principles and techniques of biochemistry and molecular biology. Cambridge University Press, Cambridge, UK, 2010, pp. 744.
Reis HJ, Guatimosim C, Paquet M, Santos M, Ribeiro FM, Kummer A, et al. Neuro-transmitters in the central nervous system & their implication in learning and memory processes. Curr Med Chem 2009; 16: 796-840.
Rhind SM. Anthropogenic pollutants: a threat to ecosystem sustainability? Philos Trans R Soc Lond B Biol Sci 2009; 364: 3391-401.
Richards J. Closing the Aboriginal/non-Aboriginal education gaps. In: Institute CDH, editor. C.D. Howe Institute Backgrounder (social policy). 116. C.D. Howe Institute, 2008, pp. 13.

Riget F, Moller P, Dietz R, Nielsen TG, Asmund G, Strand J, et al. Transfer of mercury in the marine food web of West Greenland. Journal of Environmental Monitoring 2007; 9: 877-883.
Riget F, Muir D, Kwan M, Savinova T, Nyman M, Woshner V, et al. Circumpolar pattern of mercury and cadmium in ringed seals. Sci Total Environ 2005; 351-352: 312-22.
Rossi AD, Viviani B, Zhivotovsky B, Manzo L, Orrenius S, Vahter M, et al. Inorganic mercury modifies Ca2+ signals, triggers apoptosis and potentiates NMDA toxicity in cerebellar granule neurons. Cell Death Differ 1997; 4: 317-24.
Rush S, Borga K, Dietz R, Born E, Sonne C, Evans T, et al. Geographic distribution of selected elements in the livers of polar bears from Greenland, Canada and the United States. Environ Pollut 2008; 153: 616-618.
Rutkiewicz J, Nam DH, Cooley T, Neumann K, Padilla IB, Route W, et al. Mercury exposure and neurochemical impacts in bald eagles across several Great Lakes states. Ecotoxicology 2011; 20: 1669-76.
Rutkiewicz J, Scheuhammer A, Crump D, Jagla M, Basu N. Investigation of spatial trends and neurochemical impacts of mercury in herring gulls across the Laurentian Great Lakes. Environ Pollut 2010; 158: 2733-7.
Sampson K, Merigo C, Lagueux K, Rice J, Cooper R, Weber ES, III, et al. Clinical assessment and postrelease monitoring of 11 mass stranded dolphins on Cape Cod, Massachusetts. Marine Mammal Science 2012; 28: E404-E425.
Sapolsky RM. Stres, social status, ad reproductive physiology in free-living baboons. In: Crews D, editor. Psychobiology of reproductive behaviour. Prentice Hall, Inc, Englewood Cliffs, New Jer, 1993.
Sapolsky RM, Mott GE. Social subordinance in wild baboons is associated with suppressed high density lipoprotein-cholesterol concentrations: the possible role of chronic social stress. Endocrinology 1987; 121: 1605-10.
Scheuhammer AM, Basu N, Burgess NM, Elliott JE, Campbell GD, Wayland M, et al. Relationships among mercury, selenium, and neurochemical parameters in common loons (Gavia immer) and bald eagles (Haliaeetus leucocephalus). Ecotoxicology 2008; 17: 93-101.
Sejersen F. Hunting and management of beluga whales (*Delphinapterus leucas*) in Greenland: changing strategies to cope wtih new national and local interests. Arctic 2001; 54: 431-443.
Shapiro AM, Chan HM. Characterization of demethylation of methylmercury in cultured astrocytes. Chemosphere 2008; 74: 112-8.
Shaw CM, Mottet NK, Body RL, Luschei ES. Variability of neuropathologic lesions in experimental methylmercurial encephalopathy in primates. Am J Pathol 1975; 80: 451-70.
Sheela Rani CS, Ticku MK. Comparison of chronic ethanol and chronic intermittent ethanol treatments on the expression of GABA(A) and NMDA receptor subunits. Alcohol 2006; 38: 89-97.
Shenker BJ, Guo TL, O I, Shapiro IM. Induction of apoptosis in human T-cells by methyl mercury: temporal relationship between mitochondrial dysfunction and loss of reductive reserve. Toxicol Appl Pharmacol 1999; 157: 23-35.
Shih JC, Chen K, Ridd MJ. Role of MAO A and B in neurotransmitter metabolism and behavior. Pol J Pharmacol 1999; 51: 25-9.

Sirois JE, Atchison WD. Effects of mercurials on ligand- and voltage-gated ion channels: a review. Neurotoxicology 1996; 17: 63-84.
Sirois JE, Atchison WD. Methylmercury affects multiple subtypes of calcium channels in rat cerebellar granule cells. Toxicol Appl Pharmacol 2000; 167: 1-11.
Skaare JU, Larsen HJ, Lie E, Bernhoft A, Derocher AE, Norstrom R, et al. Ecological risk assessment of persistent organic pollutants in the arctic. Toxicology 2002; 181-182: 193-7.
Skov H, Christensen JH, Goodsite ME, Heidam NZ, Jensen B, Wahlin P, et al. Fate of elemental mercury in the arctic during atmospheric mercury depletion episodes and the load of atmospheric mercury to the arctic. Environ Sci Technol 2004; 38: 2373-2382.
Sleeman JM, Cristol DA, White AE, Evers DC, Gerhold RW, Keel MK. Mercury poisoning in a free-living northern river otter (Lontra canadensis). J Wildl Dis 2010; 46: 1035-9.
Smith JC, Allen PV, Turner MD, Most B, Fisher HL, Hall LL. The kinetics of intravenously administered methyl mercury in man. Toxicol Appl Pharmacol 1994; 128: 251-6.
Sonne C, Aspholm O, Dietz R, Andersen S, Berntssen MH, Hylland K. A study of metal concentrations and metallothionein binding capacity in liver, kidney and brain tissues of three Arctic seal species. Sci Total Environ 2009; 407: 6166-72.
Staff NDL. Principles and Applications of Liquid Scintillation Counting. National diagnostics, 2004.
Stamler CJ, Abdelouahab N, Vanier C, Mergler D, Chan HM. Relationship between platelet monoamine oxidase-B (MAO-B) activity and mercury exposure in fish consumers from the Lake St. Pierre region of Que., Canada. Neurotoxicology 2006; 27: 429-36.
Stern GA, Macdonald CR, Armstrong D, Dunn B, Fuchs C, Harwood L, et al. Spatial trends and factors affecting variation of organochlorine contaminants levels in Canadian Arctic beluga (Delphinapterus leucas). Sci Total Environ 2005; 351-352: 344-68.
Stevenson MG. Indigenous knowledge in environmental assessment. Arctic 1996; 49: 278-291.
Stewart REA. Bomb radiocarbon dating calibrates beluga (Delphinapterus leucas) age estimates. Can J Zool 2006; 84: 1840-1852.
Stinson CH, Shen DM, Burbacher TM, Mohamed MK, Mottet NK. Kinetics of methyl mercury in blood and brain during chronic exposure in the monkey Macaca fascicularis. Pharmacol Toxicol 1989; 65: 223-30.
Sunol C, Babot Z, Fonfria E, Galofre M, Garcia D, Herrera N, et al. Studies with neuronal cells: From basic studies of mechanisms of neurotoxicity to the prediction of chemical toxicity. Toxicol In Vitro 2008; 22: 1350-5.
Suzuki T. Dose-effect and dose-response relationships of mercury and its derivatives. In: Nriagu JO, editor. The biogeochemistry of Mercury in the Environment. Elsevier/North-Holland Press, New York, 1979, pp. 399-431.
Takeuchi T, D'Itri FM, Fischer PV, Annett CS, Okabe M. The outbreak of Minamata disease (methyl mercury poisoning) in cats on Northwestern Ontario reserves. Environ Res 1977; 13: 215-28.

Taylor S, Wakem M, Dijkman G, Alsarraj M, Nguyen M. A practical approach to RT-qPCR - publishing data that conform to the MIQE guidelines. Amplification. Bio-Rad Laboratories, Inc, Hercules, CA, 2009.
Territories GoN. Traditional Knowledge. 53, 2005, pp. 6.
Thangaraju A, Sawyer GW. Comparison of the kinetics and extent of muscarinic M1-M5 receptor internalization, recycling and downregulation in Chinese hamster ovary cells. Eur J Pharmacol 2011; 650: 534-43.
Thornton TF, Scheer AM. Collaborative Engagement of Local and Traditional Knowledge and Science in Marine Environments: A Review. Ecology and Society 2012; 17.
Tilson HA, Cabe PA. Strategy for the assessment of neurobehavioral consequences of environmental factors. Environ Health Perspect 1978; 26: 287-99.
Tomy GT, Budakowski W, Halldorson T, Helm PA, Stern GA, Friesen K, et al. Fluorinated organic compounds in an eastern Arctic marine food web. Environ Sci Technol 2004; 38: 6475-81.
Tyrrell M. Sentient beings and wildlife resources: Inuit, beluga whales and wildlife management regimes in the Canadian Arctic. Human Ecology 2007; 35: 575-586.
Usher PJ. Traditional ecological knowledge in environmental assessment and management. Arctic 2000; 53: 183-193.
Vahter ME, Mottet NK, Friberg LT, Lind SB, Charleston JS, Burbacher TM. Demethylation of methyl mercury in different brain sites of Macaca fascicularis monkeys during long-term subclinical methyl mercury exposure. Toxicol Appl Pharmacol 1995; 134: 273-84.
Van Oostdam J, Donaldson SG, Feeley M, Arnold D, Ayotte P, Bondy G, et al. Human health implications of environmental contaminants in Arctic Canada: A review. Sci Total Environ 2005; 351-352: 165-246.
Vicini S, Ortinski P. Genetic manipulations of GABAA receptor in mice make inhibition exciting. Pharmacol Ther 2004; 103: 109-20.
Vidal L, Duran R, Faro LE, Campos F, Cervantes RC, Alfonso M. Protection from inorganic mercury effects on the in vivo dopamine release by ionotropic glutamate receptor antagonists and nitric oxide synthase inhibitors. Toxicology 2007; 238: 140-146.
Vlasman KL, Campbell GD. Field guide: diseases and parasites of marine mammals of the Eastern Arctic. Vol 22. Guelph, ON: Canadian Cooperative Wildlife Health Centre: Newsletters and Publications, 2004.
Wagemann R, Stewart PW, Beland P, Desjardins C. Heavy metals and selenium in tissues of beluga whales, *Delphinapterus leucas*, from the Canadian Arctic and the St. Lawrence estuary. Can Bull Fish Aquat Sci 1990; 224: 191-206.
Wagemann R, Trebacz E, Boila G, Lockhart WL. Methylmercury and total mercury in tissues of arctic marine mammals. Sci Total Environ 1998; 218: 19-31.
Wang M, Feng W, Shi J, Zhang F, Wang B, Zhu M, et al. Development of a mild mercaptoethanol extraction method for determination of mercury species in biological samples by HPLC-ICP-MS. Talanta 2007; 71: 2034-9.
Watanabe C, Yoshida K, Kasanuma Y, Kun Y, Satoh H. In utero methylmercury exposure differentially affects the activities of selenoenzymes in the fetal mouse brain. Environ Res 1999; 80: 208-14.
Watt-Cloutier S. The Inuit journey to a POPs-free world. Montreal: McGill, 2003.

Wavey R. International workshop on indigenous knowledge and community-based resource management: keynote address. Ottawa: IDRC, 1993.
Weijs L, Das K, Neels H, Blust R, Covaci A. Occurrence of anthropogenic and naturally-produced organohalogenated compounds in tissues of Black Sea harbour porpoises. Marine Pollution Bulletin 2010; 60: 725-731.
Wesche SD, Chan HM. Adapting to the impacts of climate change on food security among Inuit in the Western Canadian Arctic. Ecohealth 2010; 7: 361-73.
Wess J. Molecular biology of muscarinic acetylcholine receptors. Crit Rev Neurobiol 1996; 10: 69-99.
Wess J. Muscarinic acetylcholine receptor knockout mice: novel phenotypes and clinical implications. Annu Rev Pharmacol Toxicol 2004; 44: 423-50.
Whittemore ER, Yang W, Drewe JA, Woodward RM. Pharmacology of the human gamma-aminobutyric acidA receptor alpha 4 subunit expressed in Xenopus laevis oocytes. Mol Pharmacol 1996; 50: 1364-75.
Wobeser G, Nielsen N, Schiefer B. Mercury and mink II: experimental methylmercury intoxication. Can J Comp Med 1976; 40: 34-45.
Xu B, Xu Z, Deng Y, Liu W, Yang H, Wei YG. MK-801 protects against intracellular Ca(2+) overloading and improves N-methyl-D-aspartate receptor expression in cerebral cortex of methylmercury-poisoned rats. J Mol Neurosci 2013; 49: 162-71.
Yee S, Choi BH. Oxidative stress in neurotoxic effects of methylmercury poisoning. Neurotoxicology 1996; 17: 17-26.
Yang E, van Nimwegen E, Zavolan M, Rajewsky N, Schroeder M, Magnasco M, et al. Decay rates of human mRNAs: correlation with functional characteristics and sequence attributes. Genome Res 2003; 13: 1863-72.
Yokel RA, Lasley SM, Dorman DC. The speciation of metals in mammals influences their toxicokinetics and toxicodynamics and therefore human health risk assessment. J Toxicol Environ Health B Crit Rev 2006; 9: 63-85.
Young YH, Chuu JJ, Liu SH, Lin-Shiau SY. Neurotoxic mechanism of cinnabar and mercuric sulfide on the vestibulo-ocular reflex system of guinea pigs. Toxicol Sci 2002; 67: 256-63.
Yuan Y, Otero-Montanez JK, Yao A, Herden CJ, Sirois JE, Atchison WD. Inwardly rectifying and voltage-gated outward potassium channels exhibit low sensitivity to methylmercury. Neurotoxicology 2005; 26: 439-54.
Zanoli P, Truzzi C, Veneri C, Braghiroli D, Baraldi M. Methyl mercury during late gestation affects temporarily the development of cortical muscarinic receptors in rat offspring. Pharmacol Toxicol 1994; 75: 261-4.
Zhou M, Diwu Z, Panchuk-Voloshina N, Haugland RP. A stable nonfluorescent derivative of resorufin for the fluorometric determination of trace hydrogen peroxide: applications in detecting the activity of phagocyte NADPH oxidase and other oxidases. Anal Biochem 1997; 253: 162-8.

Licence No. 14717
File No. 12 402 846
May 11, 2010

2010
Northwest Territories Scientific Research Licence

Issued by:	**Aurora Research Institute – Aurora College** Inuvik, Northwest Territories
Issued to:	Ms. Sonja K Ostertag University of Northern British Columbia 3333 University Way Prince George, BC V2N 4Z9 Canada Phone: (250) 960-5676 Fax: (250) 960-5418 Email: ostertag@unbc.ca
Affiliation:	University of Northern British Columbia
Funding:	Fisheries Joint Management Committee
Team Members:	Laurie Chan; Gary Stern; Peter Ross; Marie Noel; Stephen Raverty; Lisa Loseto
Title:	**Linking Neurochemistry to Contaminant Exposure in Belugas of the Mackenzie Delta**
Objectives:	To collect brain samples from beluga whales harvested in the ISR for contaminant and brain analyses to establish whether a link exists between contaminant exposure and brain chemistry.
Dates of data collection:	June 15, 2010 to August 15, 2010
Location:	Hendrickson Island (69 degrees N, 134 degrees W) 30 km from Tuktoyaktuk

Licence No.14717 expires on December 31, 2010
Issued in the Town of Inuvik on May 11, 2010

*** original signed ***

Pippa Seccombe-Hett,
Director, Aurora Research Institute

Appendix 3. Harvester questionnaire/informed consent form

Information Letter and Consent Form

Neurochemical changes and behavioral effects associated with mercury exposure in beluga whales (*Delphinapterus leucas*) in the Mackenzie Delta

You are invited to participate in a study entitled *Neurochemical and behavioral changes associated with mercury exposure in beluga whales (Delphinapterus leucas) in the Mackenzie Delta* that is being conducted by Dr. Laurie Chan and his PhD student Sonja Ostertag from the University of Northern British Columbia (UNBC). This project is part of Sonja Ostertag's PhD research in the Natural Resources and Environmental Studies Program at UNBC. The project is supported by the Tuktoyaktuk Hunters and Trappers Committee and is funded by the Natural Sciences and Engineering Research Council of Canada and the Fisheries Joint Management Committee.

Purpose and Objectives

Mercury comes to the Canadian Arctic from air pollution. Mercury is toxic to the brain and can affect the health of wildlife and humans. We want to study the effects of mercury on beluga whales. The objective is to see if a higher level of mercury in the brain is related to changes in brain chemistry and behavioral changes observed by Inuit hunters.

Importance of this Research

Results of this study will help us understand whether pollution is affecting the health of beluga whales.

Participants Selection

You are being asked to participate in this study because you have experience in hunting beluga whales and your knowledge of beluga behavior.

What is involved

If you agree to voluntarily participate in this research, your participation will include filling out the questionnaire regarding the behavior of the whale that you harvested today.

Inconvenience and risks

It will take about 10 minutes to do the questionnaire. There is no perceived risk associated with completing this questionnaire.

Benefits

The community as a whole may benefit from knowing more about effects of pollution on an important food source. Your observations provide important information about whether any observed changes in brain chemistry are also linked to unusual or abnormal animal behavior. This study will also help to champion for the control of pollution.

Compensation

You will not receive compensation for participating in this study.

Voluntary Participation
Your participation in this research is voluntary. If you do decide to participate, you may withdraw at any time without any consequences or any explanation. If you do withdraw from the study your responses to the questionnaire will not be used.

Anonymity
We will record your name in case of follow up questions but your name will not be linked with any of the data or presented in any way. Your responses will be linked to the ID of the beluga you harvested only for the analysis of the data. The whale IDs will be changed when we present/report the results of this study to make sure that your responses cannot be linked to you.

Confidentiality
We will destroy the record of your participation within one year. During this year, your name record will be kept in a locked file cabinet in Dr. Chan's office.

Dissemination of results
Results of this study will be shared with the community and regional contaminants committee before being published in scientific reports and conference presentations.

Disposal of Data
In 2015, written interviews will be shredded and in 2020, the electronic version will be erased.

Contacts
If you have any questions, you can contact Sonja Ostertag at 250 960 5676 or ostertag@unbc.ca, or her supervisor Dr. Laurie Chan at 250 960 5237 or lchan@unbc.ca at any time.

If you have any complaints, you may contact the Human Research Ethics Office at the University of Northern British Columbia (Office of research, Ethics Coordinator Debbie Krebs, phone 250 960 5650, email krebsd@unbc.ca) any time.

Consent
I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____
Printed Name of Subject

_____ _____
Signature of Subject Date

_____ _____
Signature of Witness Date

Please retain a copy of this letter for your reference.

Questions for Beluga Hunters

Beluga ID _____ Date _____
When did you first start hunting whales?
Hunter: _____ Driver: _____

Weather Conditions:

_____
_____

Hunting Conditions
1. How were the weather conditions for hunting today? excellent good fair poor
2. How does harvesting whales now compare with earlier this season?

_____

Harvesting this whale
3. How did this beluga act when you were hunting it?

_____
_____

4. How does that compare with other belugas you have hunted?

_____
_____

5. Did you find that it took more less the same amount of time to harpoon this whale compared to other whales? Why do you think it took _____ time to harvest this whale compared to others?

_____
_____

Behaviour
6. How would you describe this whale's behaviour?

_____
_____
_____
_____

7. Was there anything unusual about this whale's behaviour? Yes No Unsure

Appendix 5. Job application and contract for mentoring students

Application: Field Assistant on Hendrickson Island

Job Description

- ☐ Participate in the beluga health research program taking place in the ISR
- ☐ Assist with beluga sampling on Hendrickson Island from July 1 st to July 20th
- ☐ Learn about human activities that could affect beluga health
- ☐ Initiate your own project of interest
- ☐ Participate in camp maintenance and logistics on Hendrickson Island
- ☐ Engage youth back in Tuktoyaktuk and the ISR in beluga research
- ☐ Earn $100/day if this is your first time working on the bel uga research team, $150/day if you have previous research experience.
- ☐ Travel to university/government labs to learn about sample preparation and analysis (optional)
- ☐ Attend a conferences in the fall/winter 2010 (optional)

Name: _____ Phone Number: _____

Why do you want this job? _____

_____

_____

_____

_____

How often do you go camping? Frequently S ometimes Rarely Never

Are you currently in: High School College University Other: _____

Are you interested in research? YES NO Have you helped researchers before? YES NO

Have you worked with children/youth? YES NO

Do you want to attend college or university? YES NO

What are your future goals? _____

_____

_____

_____

Please contact Marie Noel (250-363-6414) or Rebecca Pokiak if you have any questions

Project Description

The Beluga Health Research Program aims to bring together scientists and local knowledge-holders to study the health of the Eastern Beaufort beluga population. The research team is composed of scientists, graduate students and local researchers from Tuktoyaktuk who will share their knowledge of beluga health.

As in the past several years, a group of researchers will be on Hendrickson Island this summer to collect beluga samples. We are interested in studying the possible effects of contaminant exposure on beluga health. We will collect samples of blubber, blood, liver, brains, reproductive units and diseased tissues from harvested belugas. Samples will be analyzed in university and government labs in BC and Manitoba.

A research team led by Myrna and Rebecca Pokiak will be collecting information from hunters and their families in Tuktoyaktuk to learn about belugas and their health.

We would like to hire one or two students, between the ages of 16 and 29 to work with us this summer. In addition to field experience, there may also be opportunities to travel to our labs and to conferences in the fall/winter 2010/2011.

Research Team

Dr. Stephen Raverty is a veterinarian from the animal health care center in BC. He will collect samples for the assessment of illness and disease in the harvested whales.

Sonja Ostertag is a PhD candidate at the University of Northern British Columbia, Prince George. She will collect brains to evaluate the effects of contaminants on brain chemistry.

Marie Noel is a PhD candidate at the University of Victoria and will collect the blood of belugas to examine their health.

Frank and Nellie Pokiak are participants of a monitoring program for Fisheries and Oceans Canada. They sample tissue for contaminants and record their observations.

Myrna and Rebecca Pokiak are leading a research program in Tuktoyaktuk to gather knowledge from residents about beluga whales and their health.

Beluga Health Research Team
Student Employment Agreement

This Agreement made between the following two parties as of _____ (Date)

BETWEEN:

_____

(Student funded by Sharing Knowledge on Belugas and Beluga Health Team)

And

_____

(Representative [Sonja Ostertag] for Sharing Knowledge on Belugas and Beluga Health Team)

The two parties agree to the following arrangement from June 30, 2010 to July 20, 2010:

The student will:

1. Assist and participate in all sampling activities taking place on Hendrickson Island between June 30 and July 20 (weather dependent)
2. Assist with camp duties including cooking, washing dishes and camp clean up
3. Carry out an independent project with guidance from the research team
4. Be available to work at any time of the day or night to sample whales
5. Remain on Hendrickson Island between June 30 and July 20 (weather dependent) unless there is a family emergency or the agreement has been terminated

The Beluga Health Research Team (Marie Noel, Sonja Ostertag, Stephen Raverty, Lisa Loseto) will:

1. Mentor and teach the student the sampling procedures
2. Provide guidance on designing and carrying out the independent project with the student
3. Provide a safe and supportive learning environment to the student
4. Provide transportation to and from Hendrickson Island

If the student at any time does not agree to the terms of this agreement, the contract can be terminated.
By signing below, both individuals agree to the arrangements of this agreement.

_____ _____
(Student) (Date)

_____ _____
(Representative [Sonja Ostertag] (Date)

_____ _____
(Witness) (Date)