Mercury and Selenium in Beluga Teeth: Tools for Biomonitoring and Dietary Exposure Assessment

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Abstract

Beluga teeth are evaluated as biomonitors of heavy metal accumulation in beluga soft tissues and contaminant exposure in people who consume beluga as part of a traditional diet. Selenium, which protects marine mammals from the toxic effects of mercury, was measured in beluga teeth for the first time using hydride-generation atomic fluorescence spectrometry. Tooth selenium concentrations are shown to be moderately strong predictors of liver and muscle selenium, validating the use of teeth as a selenium biomonitor. Dietary exposure to mercury from the consumption of beluga was compared between historic and modern Mackenzie Delta Inuit populations, based on measured mercury concentrations in archeological beluga teeth and modern beluga tissues. Despite higher mercury levels in modern beluga, estimated average mercury exposure from the consumption of beluga is higher for pre-industrial Inuit populations than for modern Inuit populations, due to the significantly decreased average consumption of beluga among the modern population.

Résumé

Des dents de beluga sont évaluées pour prévoir l'accumulation des métaux lourds dans les tissus mous, et l'évaluation d'exposition diététique pour les personnes qui consomment le beluga en tant qu'élément d'un régime traditionnel. Le sélénium, qui protège les mammifères marins contre les effets toxiques du mercure, a été mesuré dans des dents de beluga pour la première fois. C'était trouvé que le sélénium dans les dents est un facteur prédictif modérément efficace de sélénium dans le foie et les muscles, validant l'utilisation des dents comme biomoniteurs de sélénium. L'exposition diététique au mercure venant de la consommation du beluga a été comparée entre les populations d'Inuit historiques et modernes, à l'aide des dents de beluga archéologiquement préservées et des tissus de beluga modernes. Malgré le plus haut nivelle de mercure dans les baleines modernes, l'évaluation de l'ingestion diététique du mercure venant de la consommation de baleine de beluga ont été plus hauts pour les populations historiques que pour les populations modernes, à cause d'une diminution de la consommation moyenne de beluga parmi la population moderne.

Contribution of Authors

This thesis is comprised of two manuscripts. These manuscripts describe the research that was undertaken during two parallel studies. In these studies, the candidate developed the research methods, measured Se in all tooth samples, and managed, analyzed and interpreted the data. The construction of both manuscripts was the responsibility of the candidate.

The supervisors, Laurie H.M. Chan and Murray Humphries, and committee member Peter Outridge, co-authored both manuscripts. The supervisors guided the candidate in the development of the research questions and methods. They also provided direction in the interpretation of results to realize greater depth and clarity. Both manuscripts were critiqued by all supervisors and committee members. The manuscript "Reconstructing historical mercury exposure from beluga whale consumption among Inuit in the Mackenzie delta" has been conditionally accepted for publication in the Journal of Ethnobiology, for the autumn 2006 volume. The manuscript "Teeth as biomonitors of selenium concentrations in the tissues of beluga whales" has not yet been submitted for publication.

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List of Abbreviations

dw	dry weight
GLG	growth layer group
Hg	mercury
HG-AFS	Hydrogen Generation Atomic Fluorescence Spectrometry
MeHg	methylmercury
MNI	Minimum Number of Individuals
NIST	National Institutes of Standards and Technology
PTDI	Provisional Tolerable Daily Intake
Se	selenium
SRM	Standard Reference Material
TF	traditional foods
WHO	World Health Organization
ww	wet weight

Chapter 1: Introduction

1.1 Rationale and overall objectives:

Mercury (Hg) is a naturally occurring element that is released into the environment via natural and anthropogenic sources. Natural sources of atmospheric elemental Hg include degassing of the earth's surface, volcanic eruptions, mineral deposits, forest fires, and emissions from water surfaces. Major anthropogenic sources are metal production, chlor-alkali and pulp industries, waste treatment, and wood burning. Hg has no function in the mammalian body, therefore all forms of Hg are toxic to some degree. The organic form of Hg (methylmercury, MeHg) is absorbed into the body at a greater rate than inorganic Hg, and is thus of greater concern when considering Hg contamination.

Mercury contamination in the Canadian Arctic has, over the past several decades, become a problem of significant concern. Soft tissue analysis of marine mammals, such as beluga (*Delphinapterus leucas*), indicate an increasing trend of Hg contamination (Wagemann et al. 1996). Beluga, like many marine mammals, often exhibit extremely high levels of Hg (Muir et al. 1999). This occurs due to the combined factors of bioaccumulation (the transfer of a chemical from water or dietary sources into an organism) and biomagnifications (the increase in concentration of a chemical with increasing trophic levels). Beluga are slow-growing, long-lived organisms, and occupy a high trophic level.

Despite these elevated Hg concentrations, often particularly high in the liver, there have been very few cases of observed metal intoxication in high-trophic marine mammals (Cuvin-Aralar and Furness 1991a). This suggests an effective metal detoxification mechanism at work. There is substantial evidence that selenium (Se) plays an important role in protecting marine mammals against the toxic effects of Hg and other heavy metals. Hg has been measured in the teeth of beluga, and found to be a good biomonitor of soft tissue Hg levels in liver, kidney, muscle and muktuk (the skin and blubber eaten by Inuit) (Outridge et al. 2000a). Teeth are useful for measuring the accumulation of trace elements because they exhibit slow, incremental growth, and are not remodeled during life. This preserves the trace elements that have been deposited within each growth layer throughout the life of the animal. In addition, teeth tend to retain their chemical composition post-mortem, so archaeological tooth samples can be used to give information about metal contamination in the past (Grandjean and Jorgensen 1990). Although Se has been measured in the teeth of humans and rats (Hadjimarkoes and Bonhorst 1959; Shearer 1975), it has never been measured in a marine mammal species. Considering the purported important detoxification role of Se in marine mammals, there is interest in characterizing the relationship between tooth Se and soft tissue Se in a marine mammal species.

Because beluga accumulate Hg to such a significant degree, they can act as indicators of Hg contamination in the Arctic marine ecosystem. Beluga can therefore be used to study changes in Hg contamination over time. The construction of retrospective temporal trends of Hg in arctic biota can indicate the extent to which the degree of contamination in traditional food systems has changed over time.

Changes in Hg levels in beluga will have potentially significant effects on the people who consume these animals. While there have been several studies describing Hg exposure in modern Arctic populations, up to now there have been no investigations into how exposure may have changed in the last several centuries. It is important to know how changing inputs of Hg into the marine ecosystem affect the Inuit who harvest beluga and other marine animals as part of their traditional subsistence lifestyle. The Inuit living in the Mackenzie Delta have consumed large amounts of beluga for many centuries (Stefansson 1914; Friesen and Arnold 1995a). If the current Hg levels are more heavily influenced by anthropogenic sources of pollution, modern Inuit are potentially being exposed to higher rates of Hg from traditional foods than their ancestors were. Conversely, if the current rate of Hg loading in the arctic environment is a natural phenomenon, the Inuit may have been exposed to similar levels of Hg in their traditional diet for thousands of years (Outridge et al. 2002). Establishing estimates of dietary exposure to Hg from beluga whale for both the modern and historic (pre-Euroamerican contact) Mackenzie Inuit allows for classification of risk for these potentially vulnerable populations.

This thesis reports the findings of two related projects examining, respectively, the use of Se in beluga teeth as indicators for soft tissue Se accumulation, and the use of archaeologically preserved beluga teeth as a tool for analysing dietary exposure to Hg in historic Inuit populations. The objectives of this thesis are: 1) to determine a method for measuring Se in beluga whale teeth, and to characterize the relationship between selenium in teeth and soft tissue selenium concentrations; and 2) to calculate the importance of beluga whales in the pre-contact diet of the Inuit population in the Mackenzie Delta, to estimate the corresponding dietary exposure to Hg from this important food source, and to compare the consumption and exposure of the historic Inuit population with modern Inuit beluga consumption and Hg exposure.

1.2 Literature Review

1.2.1 Beluga whales:

The beluga whale belongs to the Monodontidae, a family of ancient whales whose members primarily inhabit Arctic waters, though a small population is also found in the St. Lawrence river (Stewart and Stewart 1989). Beluga are distinguished by white adult colouration and the absence of a dorsal fin. Calves are born dark grey, but are completely white by the time they reach adulthood. Sexual maturity is obtained, among females, between four and seven years, and among males after eight or nine years (Gurevich 1980). Adult beluga whales range in length from 3.0-4.5 m, although there are observed regional differences in size (Sergeant 1973; DFO 2000). The whales can weigh between 1000 and 2000 kg; males are significantly larger than females.

Sergeant (1973) estimated a natural lifespan of the beluga, based on counting dentinal layers in the teeth, to be 20-25 years; more recent studies, however, have proposed a lifespan of 35-40 years (DFO 2000). Beluga teeth are formed by alternating layers of opaque and translucent dentin; these alternating bands are called growth layer groups (GLGs). Two growth layer groups are deposited per year (Brodie 1969; Goren et al. 1987; Brodie et al. 1990). Brodie et al (1990) hypothesized that growth layer groups are deposited on a biannual basis in beluga as a result of seasonal cycles in hormonal secretion. This regular deposition rate allows for age determination of beluga teeth.

Beluga are migratory, and organize into herds, which range in size from several dozen to several thousand individuals. They feed on small fish, crustaceans and benthic animals such as molluscs and worms (Seaman et al. 1982). This broad feeding spectrum allows for adaptation to a variety of marine ecologic regimes.

The distribution of the beluga is circumpolar. Population studies have concluded that beluga form regional groupings (Sergeant 1973; Fraker 1980; Gurevich 1980; Smith and Martin 1994). Four main populations of beluga are found in the North American Arctic; these populations are found off the Alaskan coast and the Beaufort Sea, in the Central Arctic and Hudson Bay, off the coast of West Greenland, and in the St. Lawrence Estuary. The largest of these groups is the Beaufort stock. A recent survey of Alaskan marine mammal stock estimated a large Beaufort beluga population of approximately 39 000 (Harwood and Smith 2002). This group of whales over-winters in the Bering Sea, and congregates in the summer months in the Beaufort Sea (Fraker 1980). From late June to early August, many whales gather in the Mackenzie River estuary, while others remain offshore (Harwood et al. 1996). Beluga tend to feed mainly in offshore areas where prey are more abundant, and use estuarine conditions for moulting and for nurturing of young calves (Caron and Smith 1990; Frost and Lowry 1990; Harwood et al. 1996).

As high-end trophic consumers, beluga may accumulate relatively high levels of contaminants, such as organic pollutants and Hg. Mercury contamination, in particular, has become a concern in this species. Within the arctic food web, Hg levels have biomagnified through several trophic levels before ingestion by beluga whales. Beluga are slow-growing and long-living organisms, causing contaminants to bioaccumulate over a long period of time (Muir et al. 1999). The potential for high levels of Hg contamination within beluga populations is therefore considerable.

1.2.2 Mercury in the environment:

Mercury (Hg) is a naturally occurring element that is released into the environment via natural and anthropogenic sources. The most common forms of Hg are elemental (Hg°), ionic (Hg^{2+}, bound to chloride, sulfide, or organic acids) and methylmercury (MeHg). Mercury is transformed from one form to another by processes such as oxidation, reduction and methylation. Elemental Hg cycles through the environment by fluxing through reserves in the atmosphere, oceans, and land and lakes. Natural sources of atmospheric elemental Hg include degassing of the earth's surface, volcanic eruptions, mineral deposits, forest fires, and emissions from water surfaces. Major anthropogenic sources include metal production, chlor-alkali and pulp industries, waste treatment, and wood burning. The residence time of Hg° in the atmosphere is around one year; during this time, elemental Hg is redistributed through long-range atmospheric transport and via ocean currents, and is often deposited (in the ionic form, Hg^{2+}) far from its source of origin (Bard 1999; Muir et al. 1999). As a result, Hg contamination can affect even the most remote locations. Lake sediment records indicate that atmospheric inputs of Hg have tripled in the past 150 years, indicating that two thirds of the Hg now in the atmosphere is of anthropogenic origin (Morel et al. 1998). Transported Hg is removed from the atmosphere by the processes of precipitation, gas absorption, and dry deposition.

All forms of Hg are toxic to some degree in the mammalian body. Methylmercury is almost completely absorbed from the diet, whereas the dietary absorption of inorganic Hg is typically less than 10%. The higher absorption rate of MeHg results in an increased collection of the contaminant in the target organs. Methylmercury is therefore of greater concern when considering Hg contamination. Sulphate-reducing bacteria methylate most Hg in natural waters, a process which occurs mainly in anoxic waters and sediment (Morel et al. 1998). Methylmercury is a particularly dangerous toxin that bioaccumulates in organisms and biomagnifies along the food chain. Bioaccumulation is the transfer of a chemical from water or dietary sources into an organism. Bioaccumulation results in animals retaining the contaminants ingested throughout their lifetime. The toxin level is therefore often strongly correlated with age. Biomagnification is the increase in concentration of a chemical with increasing trophic levels. Accumulation of MeHg in higher trophic organisms results mostly from the ingestion of contaminated foods, rather than direct uptake from water (Muir et al. 1999).

1.2.3 Mercury in humans:

Much of what is known about Hg poisoning comes from studies of large-scale outbreaks of MeHg poisoning from contaminated food. The two largest outbreaks occurred in Minamata, Japan (1953-1965) and Iraq (1971-1972). In fact, MeHg poisoning is often referred to as ‘Minamata disease’, named after the location of the first large outbreak.

Studies following the contamination events in Japan and Iraq have reported on the health effects of MeHg poisoning. MeHg crosses the blood-brain barrier and destroys cells in the cerebellum and the cortex, resulting in multiple neurological abnormalities. Abnormal gait, ataxia, constriction of the visual field, emotional instability and dysarthria are the primary symptoms reported (Watanabe and Satoh 1996). Serious cases are likely to exhibit mental confusion, drowsiness and stupor. Coma and death may also occur. The severity of symptoms is dose-dependent (Bakir et al. 1973; Lebel et al. 1998). Due to its affinity for protein sulfhydryl groups, and its tendency to accumulate in fatty tissues, MeHg has a long half-life in the human body, generally from 50 to 70 days (Mahaffey 1999). As a result, the body burden of MeHg can potentially reach high levels.

Human fetuses are particularly sensitive to MeHg because it crosses the placental barrier during pregnancy. Infants may also be exposed through consumption of breast milk, which often contains elevated levels of MeHg. In the North American population, Hg measured in women's breast milk under normal conditions contains 1.4 - 1.7 μg/g total Hg, of which 50 to 55% is MeHg (Oskarsson et al. 1996; Abadin et al. 1997). Fetuses appear to be five to 10 times more sensitive to Hg than adults (Bakir et al. 1973; Mahaffey 1999). Women of childbearing age are therefore considered to be an 'at risk' population, due to the potential of damage to their children.

Because 95% of humans' MeHg exposure is through fish and marine mammal consumption, populations that eat large amounts of these animals are at elevated risk for adverse effects (Mahaffey 1999). Such populations include coastal populations and indigenous peoples worldwide. Fish and marine mammals are a large part of the traditional diet of many of Canada's Indigenous populations. The consumption of traditional foods varies within populations depending on age, income and occupation. Exposure to contaminants through the consumption of traditional fish and marine mammals is dependent on the frequency of consumption, as well as the contamination level of the resource (Gerstenberger and Dellinger 2002). In areas where contamination is elevated, many Indigenous community members have dietary intake levels that exceed Health Canada recommendations, even when traditional foods do not provide the majority of total energy intake (Chan and Receveur 2000).

1.2.4 Selenium in the mammalian body:

Selenium is a natural element that is essential in human and animal diets and is, in low doses, non-toxic in most forms. Selenium is efficiently transferred from soil to plants to animals and humans, so geographical variations in the amount of Se in the soil available for uptake by plants accounts for much of the variation in human Se intake (Levander 1987). Before the middle of the 20^{th} century, Se was known only as a poison; however, it is now known that Se is required for normal enzyme function and contributes to animal growth and metabolism (Schwarz and Foltz 1957). A deficiency in Se intake can have adverse effects on health, such as impaired immune and thyroid function, reduced reproductive capacity, and negative mood states (Raymon 2000). Two of the 22 primary amino acids, selenomethionine and selenocysteine, contain Se as part of their basic structure. Selenomethionine functions mainly as an unregulated storage compartment for Se, and replaces methionine in the formation of some proteins (Raymond and Ralston 2004). Selenomethionine is the primary form of dietary Se (Schrauzer 2000). Selenocysteine is incorporated into many proteins, known collectively as selenoproteins, which perform important biological functions. More than twenty different selenoproteins have been isolated in animal cells, contributing to fertility, immune function, and thyroid function. Several selenoproteins, such as glutathione-peroxidases and selenoprotein P, act as antioxidants and anti-cancer agents (Holben and Smith 1999). Se also plays an important role in the detoxification of heavy metals, including Hg, in animal tissues.

In high doses, Se acts as a toxin in all animal species. The symptoms of Se toxicity vary between different species and different chemical forms, but generally excess Se affects the nervous system and may be carcinogenic in animals. In most animal species, the levels corresponding to 5-10 mg Se/kg food are toxic (Skerfving 1978). In humans, symptoms of Se toxicity include skin lesions, and digestive system and nervous system disorders (Whitney and Rolfes 1999).

1.2.5 Interactions of selenium and mercury:

Parizek and Ostadalova (1967) conducted one of the first studies examining the interaction between Hg and Se, and found that the presence of selenite significantly reduced the toxic effects of mercuric chloride ($HgCl_2$) in the kidneys of rats. This protective effect occurred only if the selenite was administered after the Hg compound, rather than before. Selenium also reduced the toxicity of chronic exposure to $HgCl_2$ (Groth et al. 1973) and MeHg in rats (Ganther et al. 1972). The interaction between Se and Hg is complex, with chemical form, order of administration, and dose affecting the outcome of feeding trials in rats and other animals (Shamberger 1983). Other studies have noted interactions between Hg and Se in terrestrial animals (Gailer et al. 2000) and aquatic species (Pelletier 1986), though the strength of the relationship differs widely among species. In field conditions, the strongest relationship between Hg and Se is found in marine mammals, which often accumulate levels of Hg and Se that would normally be considered toxic to terrestrial species, yet seem to suffer no toxicological effects.

Koemen et al. (1973; 1975) were the first to hypothesize a potential protective role played by Se within marine mammals. They found an Hg to Se molar ratio of 1:1 within the livers of seals, porpoises and dolphins. The measured concentrations of these two elements were strongly correlated, indicating a near-perfect linear relationship between Hg and Se in the liver. Because these marine mammals show no signs of Hg toxicity, and since Se had been found to mitigate the effects of Hg in rats in laboratory conditions, Koemen et al. (1973; 1975) suggested that Se might mitigate the toxicity of the Hg in marine mammals. They proposed that the 1:1 molar ratio of these two elements in the liver indicated that both elements were structurally connected through a mutual receptor. This 1:1 molar ratio of Hg and Se has subsequently been found in the livers and kidneys (but not in other tissues) of many high-trophic species of marine mammals, including beluga whales (Wagemann et al. 1990; Wagemann et al. 1998; Endo et al. 2002; Bustamante et al. 2003; Bustamante et al. 2004; Lockhart et al. 2005). However, some studies find tissue Hg:Se ratios that differ significantly from 1.0 (Hansen et al. 1990; Woshner et al. 2001). In some cases, a 1:1 molar ratio is only found in marine mammal tissues with high concentrations of Hg (above 10 ppm) (Skaare et al. 1994; Palmisano et al. 1995; Dietz et al. 2000), which suggests that the detoxification mechanism may only mobilize at higher concentrations of Hg.

A literature review by Cuvin-Araler and Furness (1991b) suggested five possible mechanisms of interaction between the two elements: 1) redistribution of Hg in the presence of Se, 2) competition for binding sites between the two elements, 3) formation of an Hg-Se complex, 4) conversion of toxic forms of Hg to less toxic forms, and 5) prevention of oxidative damage through increased activity of glutathione peroxidases. While mechanisms may be operating concurrently to some degree, the formation of an inert inorganic Hg-Se complex is the mechanism which has been investigated most thoroughly in marine mammals. Non-toxic HgSe granules (tiemannite) were first identified in the livers of dolphins by Martoja and Berry (1980). These deposits have subsequently been found in the livers of other marine mammals (Pelletier 1986; Nigro and Leonzio 1996; Lockhart et al. 2005). These studies have suggested that HgSe granules are the end product of a process of demethylation (and therefore detoxification) of MeHg occurring continuously in the liver. Evidence for a continuous demethylation process is provided by the fact that, in general, the majority (95%) of Hg in the muscle and epidermis is reported to be MeHg while Hg in the liver and kidney is mainly (90%) inorganic (Wagemann et al. 1998; Woshner et al. 2001). The formation of the inert and insoluble HgSe reduces acute Hg toxicity by preventing the bound Hg from reaching target tissue. The accumulation of HgSe in marine mammal livers explains the 1:1 molar ratio of Hg and Se detected in so many studies.

1.2.6 Spatial trends of mercury and selenium in beluga:

Several studies conducted over the past two decades have commented on spatial trends in Hg and Se accumulation across the North American Arctic. Hansen et al. (1990) collected data in 1980 in West Greenland. Measured levels of total Hg and Se were relatively low, but since the mean age of the sampled beluga is not calculated, comparison with results from other studies is difficult.

Woshner et al. (2001) provided a comprehensive analysis of metals in Alaskan beluga. Mean measured Hg and Se levels in the liver and kidney were high enough to cause toxicosis in domestic species (such as cattle and dogs), but were within ranges commonly reported for marine mammals. No differences in Hg levels were found between the sexes. Concentrations of both Hg and Se were significantly correlated with age, confirming prior research on the bioaccumulation of heavy metals in beluga. This study did not find the 1:1 molar ratio of Hg to Se considered to be characteristic in marine mammals. The authors suggest that it may not be necessary to achieve this 1:1 ratio for the animal to be protected from Hg toxicity.

A compilation of trace metal data in Alaskan marine mammals was presented by Ponce et al. (1996). Mercury and Se data were reported only in the liver of beluga. The reported hepatic total Hg and Se concentrations in their study are much lower than those reported by Woshner et al. (2001). However, because Ponce et al. (1996) did not report the ages of the sampled beluga, it is impossible to accurately compare the two studies. The data reported by Woshner et al. (2001) are therefore considered to be a more accurate portrayal of trace element levels in Alaska.

Wagemann et al. (1990; 1995; 1996) measured Hg and Se in the liver of beluga in six locations across Canada, including the Mackenzie Delta, the Eastern Arctic archipelago, and the St. Lawrence estuary. After accounting for the effects of age on metal accumulation, this series of studies indicates that the highest levels of total Hg are found in St. Lawrence beluga, likely because of the proximity of this habitat to the major industries of Southern Ontario, Quebec, and the NorthEastern United States. Within the Arctic locations, higher concentrations of total Hg were found in beluga from the Western Arctic (Mackenzie Delta) than from the Eastern Arctic locations. This difference may be due to differences in the geology of the regional oceanic drainage basins. The sedimentary rock in the Western Arctic contributes a much higher background rate of Hg deposition into the environment than the older igneous and metamorphic rock of the Eastern Canadian Shield system (Wagemann et al. 1995). In addition, mean measured hepatic Hg and Se concentrations are significantly higher in Western Arctic beluga than in Eastern Arctic animals (though concentrations measured in the St. Lawrence beluga were the highest among all the groups). In muscle and muktuk, the concentrations of MeHg were significantly higher in the Western Arctic than in the Eastern Arctic (Wagemann et al. 1998), but Se in these tissues was similar between the two regions (Wagemann et al. 1996). Hg and Se are significantly positively correlated in liver tissues, with a regression slope near 1.0 (Wagemann et al. 1998).

Lockhart et al. (2005) measured Hg and Se in liver, kidney, muscle and muktuk in beluga sampled from 13 locations across the Canadian Arctic. The liver measurements were age-adjusted to allow for comparison between locations. Although some geographical differences were found, this study did not find a consistent difference between samples from the Western and Eastern Arctic that had been reported by Wagemann et al. (1990; 1995; 1996). The authors suggested that regional differences have diminished and are no longer statistically significant. This study found a significant 1:1 molar ratio of Hg and Se in liver and kidney, but no significant relationship in muscle and muktuk.

1.2.7 Temporal trends of mercury and selenium in beluga:

Studies of temporal variation of metal contaminants in marine mammals are still sparse, mainly because metals have been examined in Arctic ecosystems for only the last several decades. Wagemann et al. (1996) found that in both the Western and Eastern Arctic, total Hg in beluga liver was significantly higher in 1993-1994 than levels obtained in 1981-1984. In addition, the slopes of the regression of liver Hg concentration and animal age showed that the rate of accumulation of Hg in the 1993-1994 liver samples was higher than the rate of accumulation from the samples harvested a decade earlier. Lockhart et al (2005) found a significant increasing temporal trend in age-adjusted beluga liver Hg harvested from the Mackenzie Delta between 1981 and 2002, but did not find consistent trends in other Arctic locations. Both of these studies found a significant 1:1 ratio of Hg and Se in the liver, indicating that Se concentrations are increasing over time concurrent with potential increases in Hg in this tissue.

Outridge et al. (2002) compared modern and pre-industrial levels of Hg in beluga teeth. The beluga teeth were collected from a Thule site in the Mackenzie Delta. This site, known as “Gupuk”, is located on the east side of Richards Island, facing into Kugmallit Bay (see Map, Appendix I). These pre-industrial teeth, dated at 1450-1650 A.D., were compared with teeth from beluga harvested in 1993 by Inuit hunters, near Richards Island. These modern beluga teeth contained Hg concentrations up to twenty times higher than the concentrations measured in the pre-industrial teeth. After regression adjustment for age, modern teeth showed an increased rate of Hg accumulation with age compared with the pre-historic teeth. These results indicate that beluga whales in the Mackenzie Delta experienced a significant increase in Hg exposure between 1450-1650 A.D. and modern times.

The authors suggested that the increase is largely due to anthropogenic contamination, since no evidence exists which would indicate such large fluctuations are attributable to naturally occurring phenomenon. It is therefore likely that the elevated Hg concentrations in modern beluga teeth reflect the increased input of industrial sources of Hg (from Eurasia or lower North America) into the Arctic in the past fifty years.

Several studies have established trace metal levels within historic human arctic inhabitants by examining mummified remains of hair (Hansen 1981; Wheatley and Wheatley 1988; Egeland et al. 1999). These studies all found that ancient mercury concentrations within the hair of people from Alaska, Greenland, and the central Canadian Arctic, respectively, are lower than the modern mercury loads in comparable regions. These studies imply that the baseline (or background) level of mercury within the Arctic is lower than the levels currently found.

1.2.8 Using teeth as a bioassay for trace minerals:

Teeth are useful for measuring the accumulation of trace elements because they exhibit slow, incremental growth, and are not remodeled during life. This preserves the trace elements that have been deposited within each growth layer throughout the life of the animal, until the tooth is shed (Ando et al. 2005). In addition, teeth tend to retain their chemical composition post-mortem, so archaeological tooth samples can be used to give information about metal contamination in the past (Grandjean and Jorgensen 1990). Measuring trace element concentrations in pre-industrial teeth allows researchers to determine how increasing environmental levels of metals affects their accumulation in human and animal bodies. Grandjean and Jorgensen (1990) note that, as with all archaeological samples, the best tooth samples are those that have been preserved in dry environments (such as deserts) and in cold or frozen conditions (such as polar regions or at high altitude).

Many trace elements have been measured in modern and archaeological human tooth samples, including lead, cadmium, zinc, and copper (Fosse and Justesen 1978; Fosse and Wesenberg 1981; Grandjean and Jorgensen 1990). Mercury has been measured in human teeth (Eide et al. 1993) and, as mentioned above, in the teeth of beluga (Outridge et al. 2000b). Although Se has been measured in the teeth of humans and rats (Hadjimarkoes and Bonhorst 1959; Shearer 1975), it has never been measured in a marine mammal species.

1.2.9 Inuit inhabitants of the Mackenzie delta:

Changes in mercury levels in beluga could have significant effects on the people who consume these animals. It is important to know how increasing inputs of mercury into the marine ecosystem affect the Inuit who harvest beluga and other marine animals as part of their traditional subsistence lifestyle. Examining the dietary habits of modern and pre-contact Inuit allows for study of the potential changes in dietary exposure to mercury this population may have experienced in the past century.

The prehistoric Inuit of the Mackenzie Delta were one of the largest Indigenous groups in the Canadian Arctic, with an estimated pre-contact population of 2,500-4,000 individuals covering a territory of several thousand square kilometres (Usher 1971; McGhee 1974). The Mackenzie Delta is a highly productive area, which is likely why it was able to sustain such a large population. Subsistence activities were based on a broad resource base, which included seals, fish, caribou, small game, and beluga. The population groups occupying territory along the East channel of the Delta in particular specialized in hunting beluga whales. Large numbers of beluga enter the shallow waters of Kugmallit Bay every summer, near the Siglit settlements of Gupuk and Kittigazuit, which were occupied in both summer and winter. During this season, Inuit congregated at summer hunting camps situated to take advantage of the beluga in the Bay.

Preliminary contact with European explorers occurred in 1799, but the Siglit resisted further associations with the foreigners until the second half of the 19th century (McGhee 1974). Trade upstream of the Delta became increasingly common after 1850, and by 1890 American whaling ships frequented the coast of the Mackenzie Delta. These whaling ships frequently brought Alaskan Inupiat along, who served aboard as whalers or servants. Concurrently, many Inupiat moved into the Mackenzie Delta over land, following the caribou herds, as the Alaskan herds were over-hunted to supply the demands of the whaling ships (Usher 1971). Although initially hostile to one another, the Siglit and the Inupiat eventually lived as neighbours in the Delta, and significant cultural exchange occurred.

The next several decades were devastating for the Siglit population; two major measles epidemics in 1900 and 1902 killed a large number of people (Usher 1971). The few Siglit who remained were integrated into the new Alaskan Inupiat communities in the Delta. The current Inuit people in the Mackenzie Delta are known as the Inuvialuit, and are considered to be mainly descended from the Inupiat that replaced the Siglit at the turn of the century (Morrison 1997).

Ethnographies compiled by European explorers, missionaries, and traders, as well as archaeological interpretation of abandoned sites, describe the nature of the prehistoric beluga hunt in the East Channel of the Mackenzie Delta. Ethnographic accounts indicate that large numbers of people congregated in temporary camps along the shore during the months of July and August to participate in large, organized beluga hunts (Stefansson 1914; Whittaker 1937; Nuligak 1966; Krech III 1989; Friesen 2004). Upon completion of the beluga hunt, the occupants would disperse to hunt caribou and seal during the short fall season. Many families would return to the large villages during the winter, occupying large, multi-family winter dwellings. Stored beluga meat and blubber harvested during the summer provided a major food source during the winter months.

All ethnographic data provide a similar account of the beluga hunt in the East Channel (Stefansson 1914; Whittaker 1937; Nuligak 1966; Krech III 1989). A look-out on high ground was continuously manned throughout the whaling season to watch for large pods of beluga venturing into the channel. Once the look-out signalled the approach of a group of whales, all the men set out in their kayaks. A temporary hunt-leader was chosen, and all other hunters followed his kayak. The boats set out in a straight line, usually spaced about 40 m apart. The line of kayaks encircled the pod of whales. When the whales were sufficiently surrounded, the leader made a signal, and the hunters would shout and slap their paddles on the surface of the water. The panicking beluga whales were easily driven towards shore, then harpooned and lanced. Dead whales were towed towards the summer camp, where they were skinned and butchered by the waiting women.

Archaeological work conducted by Friesen and Arnold (1995a) provide faunal evidence in support of the method of beluga hunting described by the ethnographic reports. The mortality profile of the beluga bones excavated from Gupuk indicates a "catastrophic" type kill, typical of drive hunting methods. In this profile, age sets appear in the same frequency as they do in the living population (usually a decrease in numbers with each increasing age set). Friesen and Arnold (1995b) also offer important evidence for the ubiquity of beluga in the Siglit diet. The authors recovered over 2000 beluga bones from a winter house at Gupuk, which were dated at 650 ± 40 years before present. To estimate the importance of beluga meat in the pre-contact diet, Friesen and Arnold (1995b) employed a technique developed by White (1953) to estimate the relative importance of each potential food source at an archaeological site. Using this technique, beluga meat is calculated as 66% of the total available meat at the site. The next most available species are ringed seals (*Phoca hispida*) and harbour seals (*Phoca vitulina*), which together represent only 7.1% of the total available meat, indicating that beluga provided a great proportion of the available meat at this site to the near exclusion of other resource types. Friesen and Arnold (1995b) suggested that beluga meat and blubber may have provided the occupants of Gupuk with at least half of their caloric intake during the half of the year the residents occupied the winter dwellings. Although summer occupancy is archaeologically invisible (with people living in tents on the beach), the authors predicted that consumption of beluga during the hunting season may have been even higher.

Ethnographic data also support Friesen and Arnold's (1995a) calculations of the relative importance of beluga meat in the winter diet of the Siglit. During the winter, Steffanson (1914) wrote that Kittigazuit families generally ate two meals per day, both of which consisted of frozen beluga, frozen fish and beluga oil. The diet appears to have been strongly dominated by beluga and fish, since no other food types were commonly consumed during the winter months. Steffanson's (1914) description of subsistence activities throughout the year provide strong support for Friesen and Arnold's (1995a) prediction that beluga meat was consumed heavily during the summer months as well as during the winter. Friesen and Arnold's (1995a) conservative estimate that beluga meat provided half the available meat for half of the year (or 25% of food intake per year), is therefore a conservative approximation of the importance of beluga to the pre-contact Siglit of the East Channel of the Mackenzie Delta. Because beluga provided such a large part of the pre-contact diet, mercury intake from this important food source may have been significant, even with low pre-industrial levels of Hg in the environment. The meat intake estimate calculated by Friesen and Arnold (Friesen and Arnold 1995a) provides a base from which dietary exposure to mercury can be calculated.

The modern Inuvialuit population is composed of around 5,000 people, most of whom live in towns such as Aklavik, Holman, Inuvik, Paulatuk, Sachs Harbour and Tuktoyaktuk. Although most people work in the communities, many still go out onto the land to hunt and fish, and the consumption of traditional foods is strongly associated with the preservation of Inuit culture (Usher 2002). Like many Canadian Indigenous communities, economic and social upheaval are common problems in the modern Mackenzie Delta towns, and the harvesting of traditional foods helps to alleviate these issues by promoting self-sufficiency, the transmission of knowledge, and spiritual satisfaction (Wheatley and Wheatley 2000). Unfortunately, in the past twenty years, the consumption of traditional foods has become associated with health risks, since elevated levels of organic contaminants and heavy metals, such as Hg, have been measured in many traditional food species (Wagemann et al. 1996; Muir et al. 1999; Braune et al. 2005).

1.3 Direction and specific objectives

The overall aim of this thesis is to assess the feasibility of using Hg and Se measurements in beluga teeth to assess contaminant levels in soft tissues and dietary exposure to at-risk populations. Appropriate methods were developed to address the following research questions: For the first study, 1) Is it possible to measure Se in beluga teeth? 2) Can Se in beluga teeth be used as a biomonitor of Se levels in relevant soft tissues? For the second study, 3) Was beluga a significant source of dietary Hg for historic Inuit populations in the Mackenzie Delta? 4) Can Hg in ancient beluga teeth be used to calculate dietary Hg exposure in historic Inuit populations? 5) Does the modern population experience a higher dietary intake of Hg from beluga sources than the historic population did, or vice-versa? 6) Which age and gender sub-populations are most at-risk for Hg exposure in both the historic and modern context?

Thus, this thesis evaluates the following core hypotheses:

1) Selenium in beluga teeth can be used as a biomonitor for soft tissue Se, especially in the storage organs, liver and kidney.

2) The historic population experienced a higher intake of dietary Hg from beluga sources than the modern population, due to much greater consumption of this food source.

The remainder of this thesis is presented in three additional chapters. Chapter 2 is a manuscript prepared for submission to the journal *Environmental Toxicology and Chemistry*. This chapter is entitled "Teeth as biomonitors of selenium concentrations in tissues of beluga whales (*Delphinapterus leucas*)." The chapter describes a method to measure Se in the teeth of beluga whales, and compares tooth Se concentrations to Se levels in the liver, kidney, muscle and muktuk, in relation to animal age. Chapter 3 is a manuscript conditionally accepted by the *Journal of Ethnobiology*, entitled "Reconstructing historical mercury exposure from beluga whale consumption among Inuit in the Mackenzie delta." This chapter presents the dietary exposure to Hg from beluga whale food sources for both the modern and historic populations in the Mackenzie Delta. Population exposure levels are compared, and discussed in the context of risk-assessment. Chapter 4 presents the overall conclusions that result from this thesis.

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Chapter 2

Teeth as biomonitors of selenium concentrations in tissues of beluga whales (*Delphinapterus leucas*)

Manuscript in preparation, for submission to the journal *Environmental Toxicology and Chemistry*

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2.1 Abstract:

There is substantial evidence that selenium (Se) plays an important role in protecting marine mammals against the toxic effects of mercury (Hg) and other heavy metals. In this study, we develop a method for measuring Se concentrations in mammal teeth, and test whether tooth Se concentrations are related to soft tissue Se in beluga (*Delphinapterus leucas*). Se in the teeth of beluga was measured using hydride generation atomic fluorescence spectrometry (HG-AFS) and compared with Se levels in the soft tissues (liver, kidney, muscle and muktuk). Tooth Se concentrations ranged from 108 ng/g to 245 ng/g dry weight. In the soft tissues, Se concentrations were highest in the liver, followed by kidney, muktuk, and muscle. There were significant linear correlations between tooth Se concentrations and animal age, tooth Se and liver and muscle Se, and hepatic Se and animal age. These results indicate that tooth Se is a moderately effective predictor of liver and muscle Se.

Keywords: selenium, mercury, beluga, teeth

2.2 Introduction:

Predatory marine mammals often exhibit higher levels of heavy metal contaminants, such as mercury (Hg), than their terrestrial counterparts (Wagemann et al. 1996; Woshner et al. 2001; Lockhart et al. 2005). This occurs because Hg is methylated much more effectively in aquatic environments than in terrestrial. In addition, marine mammals generally occupy a high trophic position in the marine ecosystem (marine trophic chains are generally longer than terrestrial chains) and they have long life spans, resulting high Hg exposure to top predators over long periods of time (Ikemoto et al. 2004). Despite the elevated Hg concentrations observed, which are particularly high in the liver, there have been very few cases of observed metal intoxication in high-trophic level marine mammals (Cuvin-Aralar and Furness 1991). This suggests the existence of an effective metal detoxification mechanism in these marine mammals.

There is substantial evidence that selenium (Se) plays an important role in protecting marine mammals against the toxic effects of Hg and other heavy metals. This detoxification mechanism may involve metals such as silver, copper, cadmium and lead (Becker et al. 1995; Ikemoto et al. 2004); however, the relationship has been best characterized between Se and Hg. Although the exact mechanisms of this interaction are not known, it has been observed that the end product of the detoxification of organic Hg is the formation of a stable and inert complex with selenium, called tiemannite (mercuric selenide, HgSe) (Martoja and Berry 1980; Cuvin-Aralar and Furness 1991). This inert complex binds the two elements in an equimolar ratio when high levels of Hg are present, and a significant correlation of Hg and Se in the liver of marine mammals has been reported in many studies (Koeman et al. 1973; Hansen et al. 1990; Wagemann et al. 1990; Dietz et al. 2000; Woshner et al. 2001).

Trace metals, such as Hg and Se, are incorporated either into the crystalline apatite structure or the protein fraction of teeth (Ando et al. 2005). Hg has been measured in the teeth of beluga whales (*Delphinapterus leucas*), and found to be a good biomonitor of soft tissue Hg levels (Outridge et al. 2000). Because teeth are not modified or reabsorbed, long-term changes in trace element body burden can be monitored, and preserved tooth samples can be used to give information about metal contamination in the past. Although Se has been measured in the teeth of humans and rats (Hadjimarkoes and Bonhorst 1959; Shearer 1975), it has never been measured in a marine mammal species. Considering the important detoxification role of Se in marine mammals, there is interest in characterizing the relationship between tooth Se and soft tissue Se in a marine mammal species. In this study, we develop a method for analysing Se concentrations in teeth and other calcified sample types, and examine the relationships between Se in beluga teeth and soft tissues, and between Se and Hg in teeth.

2.3 Methods:

Sample collection and preparation.— Beluga were harvested from the Mackenzie Delta, Northwest Territories, in 1996 and 2002, as part of an annual traditional Inuit hunt. The soft tissues (liver, muscle, muktuk and kidney) were sampled and analyzed for Hg and Se as part of a long-term assessment program into contaminants in the Canadian Arctic (Lockhart et al. 2005; AMAP , 2005). Soft tissue data for some of the harvested animals were made available for this study by Fisheries and Oceans Canada (G. Stern, pers. comm.). The second and fifth right mandibular teeth of each animal were removed for aging purposes. Beluga teeth are composed of an inner core of dentine, surrounded by thick cementum (enamel is absent). Teeth grow in incremental layers, which are deposited in both cementum and dentine on a biannual basis; these layers are used to age individuals (Brodie et al. 1990).

For this study, teeth were split vertically, from root to crown, using a Dremel high speed rotary saw with interchangeable bits. Care was taken to ensure that each half preserved all growth layers in cementum and dentine. This is critical for both aging and trace element analysis, since it ensures that all growth layers are included in age determination and that the lifetime accumulation of trace elements is accounted for. One half-tooth was sent to a Fisheries and Oceans Canada laboratory for aging, which was conducted by mounting each half-tooth in epoxy resin blocks, thin-sectioning, and counting growth layers (Outridge et al. 2000). The other half-tooth was used for Se measurement.

Using a dremel tool, the inner dentine layer was removed and discarded from each half-tooth, leaving only the cementum material. Because we ultimately wished to compare Hg and Se concentrations in these teeth, and Hg values are only available for cementum, Se was likewise only determined on cementum. Cementum sections were cleaned with a rotary grinder to remove organic tissue or other contaminating material on the outer surface of the tooth. Cross sections were taken from the thickest part of the cementum sections (ensuring equal representation of all growth layers). Samples were dipped in 10% HNO_3 for 20 seconds, then rinsed several times in distilled deonized water, and left to air-dry in a fumehood. Dried samples were weighed and stored in sterile sampling bags in preparation for digestion and analysis. Sample weights were between 0.2 and 0.4 g. In this study, tooth metal concentrations are expressed in a dry weight basis (µg/g dw). Samples were not ground to powder form to reduce the risk of contamination.

A microwave digestion was performed using a mixture of HNO_3 and H_2O_2. Each sample was placed in a microwave digestion vial with 8.0 mL concentrated HNO_3 (ACS grade) and 2.0 mL H_2O_2 (30%, ACS grade). Samples were heated in a microwave (Milestone Microwave Laboratory System model Ethos SEL with HPR-1000/10S rotor) using the following digestion program: 1) 0-85° C, 4 min; 2) 85-145°C, 10 min; 3) 145-210°C, 6 min; 4) maintain 210°C, 10 min; 5) vent, 20 min. No pre-reduction step was conducted, since previous tests had shown that this step was unnecessary, most likely due to the largely inorganic nature of the matrix. The digests were diluted to a final volume of 25 mL, using distilled deionized water.

Analysis.— Total Se was determined by hydride generation atomic fluorescence spectrometry (HG-AFS; model PSA 10.055 Millennium Excalibur). Analytical quality was controlled using the standard reference material (SRM) bonemeal (National Institutes of Standards and Technology [NIST], USA, no. 1486), which was the available SRM with a matrix most similar to that of teeth. Although this SRM is not certified for Se, it contains a suggested Se concentration of 0.13 μg/g. In addition, analytical quality was controlled by analyzing all samples in duplicate, and measuring a digest blank, a SRM and a standard spike recovery for every eight digested tooth samples. The potential for a difference in matrix effect between the bonemeal SRM and the tooth samples was tested by spiking four replicates of a bonemeal digest, a tooth sample digest, and a blank acid digest with 0.0, 0.1, 0.2, and 0.4 ppb Se.

Total Hg in the teeth was determined by cold-vapour atomic absorption (CVAA; model CETAC QuickTrace M-6000 Mercury Analyzer), using the digests prepared for Se analysis. Analytical quality was controlled by measuring random samples in duplicate, measuring several digest blanks, and measuring an oyster tissue SRM (NIST, no. 1566b) every five samples. The oyster tissue SRM contains a certified concentration of 37.1 ± 1.3 ng/g Hg. Bonemeal SRM digests were analysed along with tooth samples, but since this SRM contains no information value for Hg, it was not used for external quality control.

Statistical analyses.— Concentration data satisfied statistical assumptions of normality and heteroscedasticity, and was therefore left untransformed. Linear regression and correlation analysis was conducted between tooth Se concentrations and soft tissue Se concentrations, between tooth and soft tissue Se concentrations and tooth age estimates, and between tooth Se and tooth Hg concentrations. Subsequently, age was included as an additional predictor of tooth

Se concentration using multiple regression analysis. Sex was not considered as a factor, since almost all (90%) of the harvested animals were male. A p value of <0.05 was considered significant. All statistical analyses were performed using the program SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

2.4 Results:

Analytical quality.— The measured standard reference material Se values were consistently above the information value, with an average accuracy of 115%, and a precision of 7.0% relative standard deviation (Table 2.1). Repeatability of duplicate samples was 5.3%.

No significant matrix effect was detected using HG-AFS. The slopes of the graphs representing the value of spiked tooth sample replicates and corresponding peak height measurements (slope = 0.199) was within the standard deviation of the mean slope of four separate measurement runs of the spiked blank replicates (with corresponding peak height measurement) (mean slope = 0.207, standard deviation = 0.008) (Figure 2.1). The slope of the spiked bonemeal replicates was slightly above this standard deviation (slope = 0.223). In these graphs, since the y value represents the peak height measurement of each sample, the slopes are not expected to be 1.

The measured Hg values in the oyster tissue SRM were based on a series of four replicate samples. The average accuracy was 97%, and the precision was 4.9% relative standard deviation (Table 2.1).

Tooth selenium.— Tooth concentrations ranged from 108 ng/g to 245 ng/g Se. There is a linear correlation of tooth Se with animal age (Figure 2.2a; $r^2 = 0.50$, $p < 0.05$) and hepatic Se with animal age (Figure 2.2b; $r^2 = 0.52$, $p < 0.001$). Age was not a significant factor in the Se concentrations of the other soft tissues.

Comparison of selenium in teeth and soft tissues.— In the soft tissues, Se concentrations were highest in the liver, followed by kidney, muktuk, and muscle (Table 2.2). Simple linear regression showed that tooth Se is significantly correlated with Se concentrations in liver and muscle, but not in muktuk or kidney (Figure 2.3a, b, c, d; Table 2.3). The linear regression equations predicting tooth Se (in ng/g dry weight), from soft tissue Se (in µg/g wet weight or dry weight depending on tissue; see Table 2.2) are:

Tooth Se = 1.45 * liver Se + 126.6

Tooth Se = 352 * muscle Se + 21.3

When age is included as a co-variate in the analysis of the relationship between tooth Se and soft tissue Se, tooth Se becomes significantly correlated with muktuk Se and age, but the model remains non-significant for kidney. It appears that age accounts for most of the co-accumulation of Se in teeth and the soft tissues (liver, muscle and muktuk) (Table 2.4). The multiple regression equations predicting tooth Se (in ng/g dry weight), from soft tissue Se (in µg/g wet weight or dry weight depending on tissue; see Table 2.2), and age in years are:

Tooth Se = -0.50 * liver Se + 4.03 * age + 94.4

Tooth Se = 186.2 * muscle Se + 3.11 * age + 35.7

Tooth Se = -10.30 * muktuk + 4.32 * age + 125.7

Tooth mercury.— Concentrations of Hg in teeth ranged from 10.0 to 188.6 ng/g, with a mean measurement of 70.9 ng/g Hg (Table 2.5). There is no significant linear correlation between tooth Hg concentrations and tooth Se concentrations ($r^2 = 0.11$; $p = 0.16$). Linear regression also showed that animal age is not a significant factor in the accumulation of Hg in beluga teeth in this study (r^2 = 0.04; p = 0.45). However, when age is included as a co-variate in a multiple regression analysis of tooth Se and tooth Hg, the overall regression model becomes significant ($r^2 = 0.507$, p = 0.01). In this model, age accounts for most of the accumulation of tooth Se (p = 0.005), and the correlation with tooth Hg remains non-significant (p = 0.466).

Mercury in soft tissues.— In the soft tissues, measured Hg concentrations were highest in the liver, followed by kidney, muscle and muktuk (Table 2.5). The concentrations of total Hg measured in the liver were all above 10 nmol/g (2 µg/g), considered the point at which most Hg in the liver is present as inorganic Hg in association with Se (Dietz et al. 1990; Dietz et al. 2000). The mean liver MeHg concentration was 2.56 µg/g, with an average of 10.6% of total Hg in the liver present as MeHg. The mean molar Hg:Se ratio in the liver is 0.70 ($r^2 = 0.83$; $p < 0.001$). Linear regression shows that the mean molar liver Hg:Se ratio is significantly correlated with tooth Hg concentrations ($r^2 = 0.33$; p = 0.007), but not with tooth Se concentrations ($r^2 = 0.04$; p = 0.21). Age was a significant factor in hepatic total Hg concentrations ($r^2 = 0.48$; p = 0.002), but not in hepatic Hg:Se molar ratio ($r^2 = 0.00$; p = 0.33).

2.5 Discussion:

This study showed that hydride-generation atomic fluorescence spectrometry is an effective technique for measuring Se in beluga teeth. Fluorimetric methods have been used in the past to measure Se in hard tissues in rats and humans (e.g., Johnson and Shearer 1979), but radiative techniques such as neutron activation analysis and gamma spectrometry have been used more frequently (Nixon and Myers 1970; Retief et al. 1974; Retief et al. 1976). However, radiative techniques are not readily available to many researchers. Atomic fluorescence spectrometry represents a relatively inexpensive and precise technique for measuring Se in mammal teeth.

Selenium concentrations in teeth were correlated significantly to Se in liver and muscle, using simple linear regression analysis, with 29% and 36%, respectively, of variation in tooth Se accounted for by soft tissue Se. These results indicate that tooth Se is a moderately effective predictor of liver and muscle Se. Simple linear regression analysis showed that tooth Se concentrations were not significantly correlated with kidney and muktuk Se, indicating that tooth Se alone is a poor predictor of Se in kidney and muktuk. The linear regression relationships between tooth Se and Se in these four soft tissues are not as strong as those found by Outridge et al (2000) for tooth Hg and Hg in the same soft tissues.

Outridge et al (2000) found that Hg levels in all four soft tissues are significantly correlated with tooth Hg, with soft tissue Hg explaining 54% to 66% of variance in tooth Hg. The difference between tooth and soft tissue relationships for Se and Hg may be explained by the different roles that these two elements play in the body. Selenium is an essential element for all animals, and is found throughout the body as selenoprotein compounds. Approximately 35 different selenoproteins have been isolated from animal cells, and are particularly important as antioxidants (Raymond and Ralston 2004). Selenium is incorporated into two of the 22 primary amino acids: selenomethionine and selenocysteine, the latter of which is integrated into proteins contributing to necessary biological functions. Selenium accumulation in storage organs, such as the liver or kidneys, occurs when Se is present at concentrations which exceed metabolic requirements (Mackey et al. 1996; Holben and Smith 1999). Conversely, Hg serves no functional purpose in the animal body. It is present solely as a contaminant, and may therefore be more likely to be concentrated in storage organs. This may explain the stronger correlations of Hg levels between storage organs (teeth and soft tissues), relative to Se.

The significant correlation between tooth Se and liver Se concentrations probably occurs because Se accumulates with age in both liver and teeth, and because liver is the main storage organ for Se and for Hg. Multiple regression analysis using liver Se and age as independent variables to predict tooth Se concentrations showed that age accounted for most of the accumulation of Se in teeth (see Table 2.4). Thus, although tooth Se may be used as a biomonitor of liver Se concentrations in a sample of multi-aged individuals, it does not indicate whether individuals of a given age class have high or low levels of liver Se. Metabolic studies in humans, mammals, and fish indicate that the liver acts in the storage, metabolism, and elimination of Se (Thomson and Stewart 1974; Hilton et al. 1982; Behne and Wolters 1983). In addition, in marine mammals the deposition of tiemannite (mercuric selenide) crystals in the liver, as an end product of methylmercury detoxification, contributes to the accumulation of Se in this organ, when Hg levels are sufficiently elevated. The presence of Se in the liver of marine mammals may therefore be more of a reflection of the accumulation of Hg in this organ than an indication of Se intake in excess of physiological need.

In this study, liver Se concentrations were found to range from 6.2 to 45.2 μg/g Se (Table 2.2). This is within the ranges of values previously reported for beluga by other studies (Hansen et al. 1990; Mackey et al. 1996; Woshner et al. 2001); it is likely that the liver Se concentrations reported in these studies all occur in the context of high Hg load, since, like all predatory marine mammals, beluga often demonstrate high hepatic Hg levels, even in uncontaminated environments (Outridge et al. 2002). It is therefore likely that these levels are representative of "normal" hepatic Se levels. Similarily, it is probable that the concentrations measured in this study reflect an association of Se with inorganic Hg in the liver, since the measured total Hg concentrations in this organ are high enough (above 2 μg/g; see Table 2.5) that the majority of the Hg is present in the inorganic form as part of mercuric selenide crystals (Koeman et al. 1975; Dietz et al. 1990; Hansen et al. 1990; Dietz et al. 2000). In this study, the mean molar Hg:Se ratio in the liver was 0.70, which is below the 1:1 equimolar ratio observed by Koemen et al

(1973; 1975). However, many subsequent studies have found molar Hg:Se ratios below 1 in cetacean livers (Hansen et al. 1990; Krone et al. 1999; Outridge et al. 2000; Woshner et al. 2001). Since most marine mammals with extremely high concentrations of Hg in their tissues do not exhibit symptoms of toxicity, it may not be necessary to demonstrate a 1:1 molar ratio of Hg:Se (even in tissues with elevated levels of Hg, such as beluga liver) for the animal to be protected (Woshner et al. 2001).

Unlike in the liver, where Se acts as part of a detoxifying mechanism when Hg concentrations are elevated, the Se present in muscle plays an important metabolic role independent of Hg levels in this tissue. The association between tooth Se and muscle Se may therefore reflect the metabolic function of this element. From a physiological perspective, Se is largely associated with proteins in animal tissues, and muscle tissue is an important organ for selenoprotein activity and metabolism. Selenoprotein W, for example, plays a role in muscle metabolism (Holben and Smith 1999; Gu et al. 2000; Whanger 2000), and glutathione peroxidases and other selenoproteins act as anti-oxidants in muscle cells. In addition, Se is stored in muscle tissues as part of the amino acid selenomethionine (Butler et al. 1990; Raymond and Ralston 2004). The association between muscle Se levels and tooth Se may reflect accumulation of Se in teeth and in muscle as selenomethionine when the availability of Se in the body exceeds physiological requirements. However, multiple regression analysis using muscle Se and age as covariates to predict tooth Se concentrations showed that age was a better predictor of tooth Se accumulation than muscle Se concentrations, even though simple linear regression did not find muscle Se concentrations to be significantly correlated with age. In this case, co-accumulation of Se in beluga teeth and muscle may occur because Se is deposited into the both the teeth and the muscle tissues at a rate proportional to blood concentrations.

The situation may also be similar for selenium in muktuk. Although simple linear regression did not find significant accumulation of selenium in teeth related to either muktuk Se or age, multiple regression analysis using muktuk Se and age as covariates found that these variables together did have significant a significant explanatory effect on the accumulation of tooth Se. It is possible that the epidermis portion of muktuk (composed of skin and fat) contains metabolically active selenoproteins, and that Se is deposited into this tissue, similarly to muscle, at a rate proportional to the concentration of Se in blood.

In marine mammals, including beluga, the highest concentrations of Se are generally found in the liver, followed by the kidneys and muscle (Dietz et al. 1996; Bustamante et al. 2003; Bustamante et al. 2004). The soft tissue Se concentrations discussed in this study are in agreement with these previous findings, with the range of muktuk Se concentrations between kidney and muscle Se ranges. However, extremely high Se levels have been found in the skin (muktuk) of harbour porpoises (*Phocoena phocoena*) (Paludan-Muller et al. 1993; Dietz et al. 2000). Paludan-Muller et al (1993) suggest that these elevated levels indicate that the skin may function as part of an excretion mechanism of excess Se. Similarly elevated muktuk Se levels have not been found in other species, but the moulting of the epidermis has been suggested to contribute to the excretion of Hg and Se in beluga (Wagemann et al. 1995; Woshner et al. 2001). Wagemann et al (1996) found that Se was 10 times higher in beluga muktuk than in muscle, and the results from this study give a similar ratio (Table 2.2). Hansen et al (1990) found no significant correlations in Se concentrations between liver, kidney and muscle in beluga, and Paludan-Muller et al (1993) found none between liver, kidney, muscle and muktuk in harbour porpoises. This study, however, found significant correlations between muscle Se and kidney Se and between muscle Se and liver Se.

Selenium accumulates relatively strongly with age in beluga teeth. This accumulation occurs because teeth are stable hard tissues, and Se that is deposited over time remains in the teeth as an animal gets older. Age is also a significant factor in liver Se, with half of the variance in liver Se explained by animal age.

This age-dependent accumulation is likely due to the association of Se with Hg in the liver, since the hepatic Hg concentrations reported in this study are sufficiently high to prompt the formation of inert tiemannite. Liver Hg levels are strongly associated with age in marine mammals (Hansen et al. 1990; Becker et al. 1995; Mackey et al. 1996; Wagemann et al. 1996; Lockhart et al. 2005), and the 1:1 ratio of Hg and Se frequently found in the liver of marine mammals when hepatic Hg levels are sufficiently high, suggests that most of the Se in the liver is in association with inorganic Hg. Unlike Hg, which accumulates with age in most marine mammal soft tissues (Wagemann et al. 1998), the age accumulation of Se is tissue specific. Age accumulation of Se in tissues other than liver is inconsistent, and Se is generally not found to increase with age to the same degree in kidney, muscle and other soft tissues as in liver (Hansen et al. 1990; Wagemann et al. 1990; Paludan-Muller et al. 1993; Dietz et al. 1996). In this study, simple linear regression found that Se did not accumulate significantly with age in kidney, muscle and muktuk.

This study did not find a significant linear correlation between tooth Se and tooth Hg concentrations. Age alone was also not found to be a significant factor in the accumulation of Hg in teeth, although previous research had found tooth Hg to be significantly linearly correlated with age (Outridge et al. 2000). Multiple regression, however, shows that tooth Hg and age together do contribute to significant variation in tooth Se concentrations, though most of that variation is explained by the significant correlation between age and tooth Se. Considering the strong interaction between Hg and Se in the liver, the accumulation with age of both Hg and Se, the significant correlation between hepatic Se and tooth Se found in this study, and the significant correlation between hepatic Hg and tooth Hg found in previous research (Outridge et al. 2000), the interaction between tooth Se, tooth Hg and animal age may be a reflection of the age-dependent accumulation of inert tiemannite in the liver of beluga.

It is unclear what form of Se is stored in beluga teeth. Shearer (1975) found that Se incorporates into the protein fraction of rat teeth, generally in association with cysteine and methionine. That study found that the majority of Se in enamel and dentine was associated with protein fractions, not with the inorganic hydroxyapatite fraction of these tissues. Ando et al (2005) suggested, however, that in marine mammals most trace elements are incorporated into the inorganic hydroxyapatite structure of the teeth. Since cementum is mostly composed of inorganic material, Se in beluga teeth may be found mainly in the inorganic apatite matrix. However, cementum does contain an organic component, composed mainly of collagen (Hillson 2005), so incorporation of Se into the protein fraction of beluga teeth remains a possibility. It is also unclear how high levels of Hg in the bodies of marine mammals might affect the incorporation of Se in hard tissues. First, it is not known whether Se and Hg are stored in association with one another in marine mammal teeth. If they are, it is possible that Se is entering teeth already in association with Hg, or that the two elements form an association after both have already been incorporated into the tooth structure. If Se and Hg are stored in the teeth in the form of inorganic tiemannite, this compound is more likely to be found in the inorganic matrix, rather than the protein fractions of teeth.

There are several factors that contribute to potential variance in the determination of tooth Se. First, there are likely to be variations in Se concentrations between teeth in the same jaw, as was found Outridge et al. (2000) for Hg in beluga teeth. It is felt, however, that these variations do not distort the results in any particular direction, since the differences in measured Hg in teeth in the same jaw were not consistent across multiple animals. Second, the deposition of Se may not be homogenous within each tooth. This uncertainty was addressed by consistently removing samples from the same position between teeth.

2.6 Conclusions:

This study is the first to measure Se in the teeth of a marine mammal species, and HG-AFS is found to be an effective technique for determining Se in beluga teeth. Tooth Se is moderately effective as a predictor of liver and muscle Se, though these relationships are not as strong as those of Hg in teeth and soft tissues. Considering the important role that Se plays in the detoxification of Hg in marine mammals, the distribution of Se in the bodies of marine mammals can provide information about this protective mechanism. This study contributes to an increased understanding of the storage and metabolism of Se in marine mammals.

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2.8 Tables and Figures:

Table 2.1 Analytical quality data (accuracy and precision) for selenium in bonemeal standard reference material and for mercury in oyster tissue standard reference material (ng/g).

SRM	N	measured mean ± SD	% relative SD	information value	accuracy (% of info value)
bonemeal (NIST 1486)	8	148 ± 10.4	7.0	130	115
oyster tissue (NIST 1566b)	4	38.2 ± 1.9	4.9	37.1	103

Table 2.2 Mean, standard deviation and range of selenium concentrations in beluga teeth and soft tissue, harvested from the Mackenzie Delta (µg/g dry weight[a] or µg/g wet weight[b], N = 20*).

tissue	tooth[a]	liver[a]	muscle[b]	muktuk[b]	kidney[a]
mean	0.16	20.65	0.38	3.82	4.86
st. deviation	0.04	13.50	0.06	0.97	1.75
range	0.11-0.25	6.17-45.52	0.30-0.50	2.33-5.96	2.06-8.99

*except for kidney, where N = 12

Table 2.3 Coefficients of determination (r^2) for linear regression between selenium in beluga teeth, liver, muscle, muktuk, and kidney, harvested from the Mackenzie Delta.

tissue type	kidney	muktuk	liver	muscle	tooth
kidney	—	0.28	0.09	0.77*	0.06
muktuk	—	—	0.01	0.10	0.03
liver	—	—	—	0.21*	0.29*
muscle	—	—	—	—	0.36*
tooth	—	—	—	—	—

*significant at $p < 0.05$

Table 2.4 Coefficients of determination (r^2) and p values for the relationships between selenium in teeth, soft tissue (liver and muscle) Se concentrations, and animal age, from beluga harvested in the Mackenzie Delta.

model summary	soft tissue		
	liver	muscle	muktuk
r^2 of model	0.496	0.574	0.546
significance of model (p =)	0.008*	0.003*	0.004*
significance of age variable (p=)	0.020*	0.014*	0.001*
significance of soft tissue variable (p=)	0.945	0.132	0.232

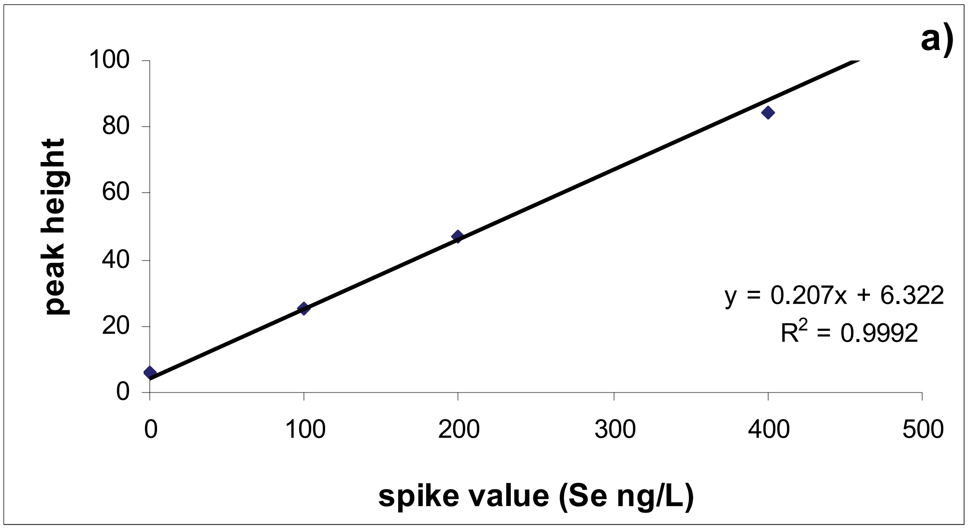
*significant at $p < 0.05$

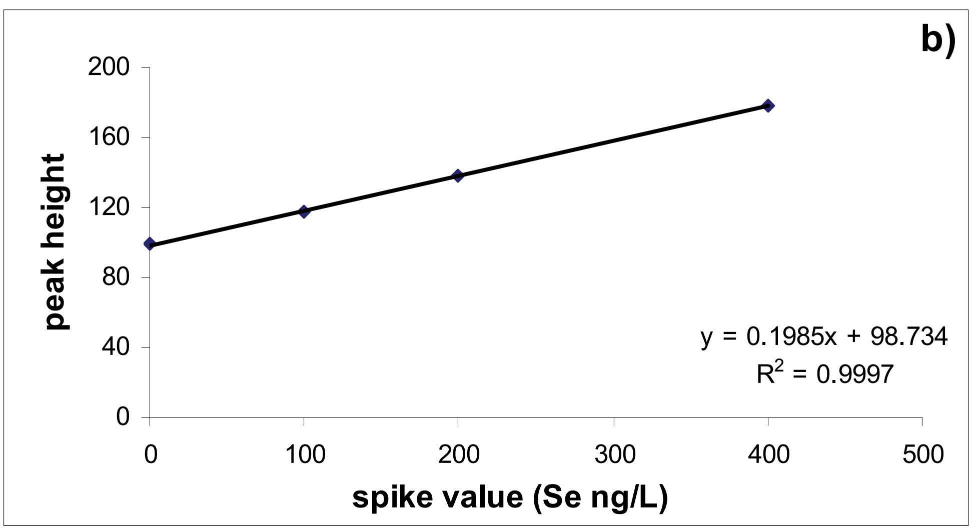
Table 2.5 Mean, standard deviation and range of total mercury concentrations in beluga teeth and soft tissue, harvested from the Mackenzie Delta (ng/g dry weight[a], μg/g dry weight[b] or μg/g wet weight[c], N = 20*).

tissue	tooth[a]	liver[b]	muscle[c]	muktuk[c]	kidney[b]
mean	70.92	40.17	1.66	0.93	8.14
st. deviation	45.35	32.78	0.61	0.35	3.47
range	10.01-188.61	3.36-96.9	0.79-3.36	0.30-1.73	2.20-13.0

*except for kidney, where N = 14

Figure 2.1 Peak height measurements of replicates spiked with 0.0, 0.1, 0.2, and 0.4 ppb Se for (a) sample blanks (mean of 4 separate runs); (b) tooth sample digest; (c) bonemeal digest. Solid line represents trendline.





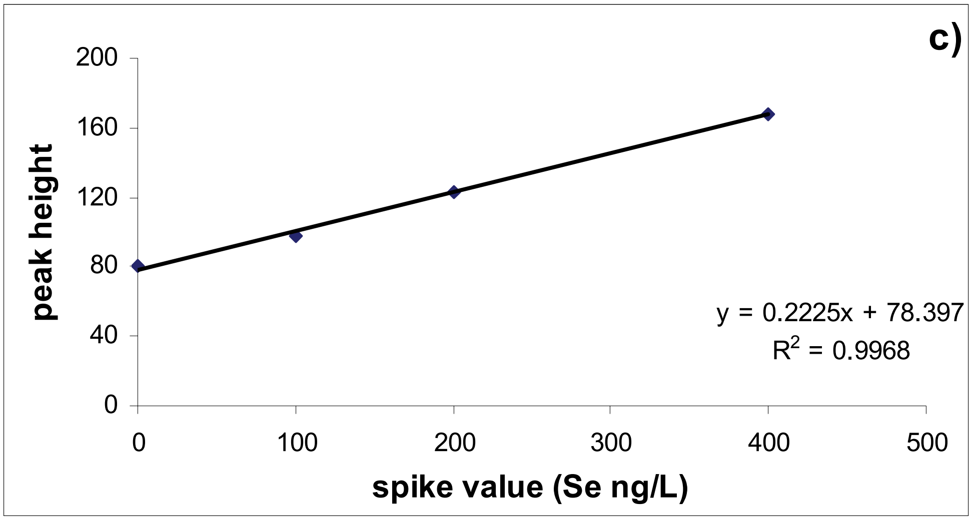
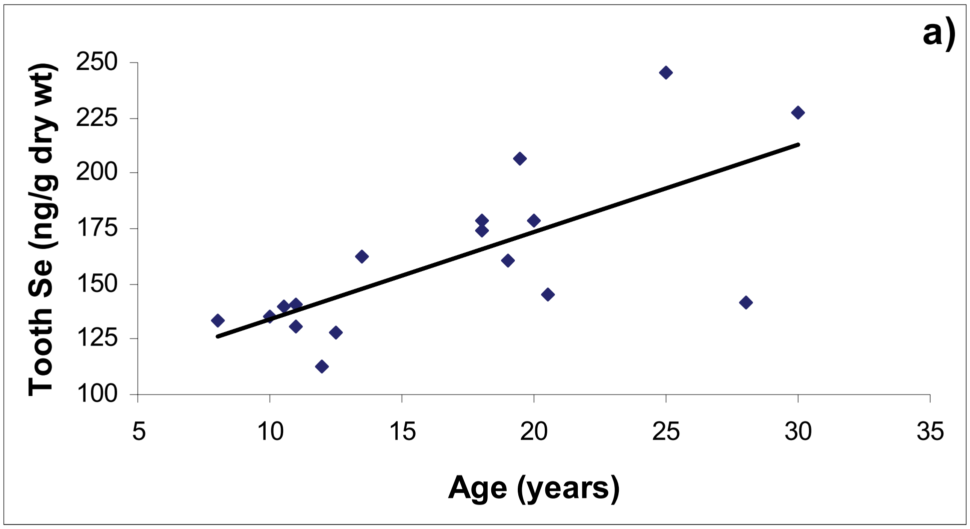

c)
200
160
120
80
40
0
peak height
y = 0.2225x + 78.397
R2 = 0.9968
0
100
200
300
400
500
spike value (Se ng/L)

Figure 2.2 Age-dependent concentrations of selenium (a) teeth and (b) liver, of beluga harvested from the Mackenzie Delta. Solid lines represent linear regressions.



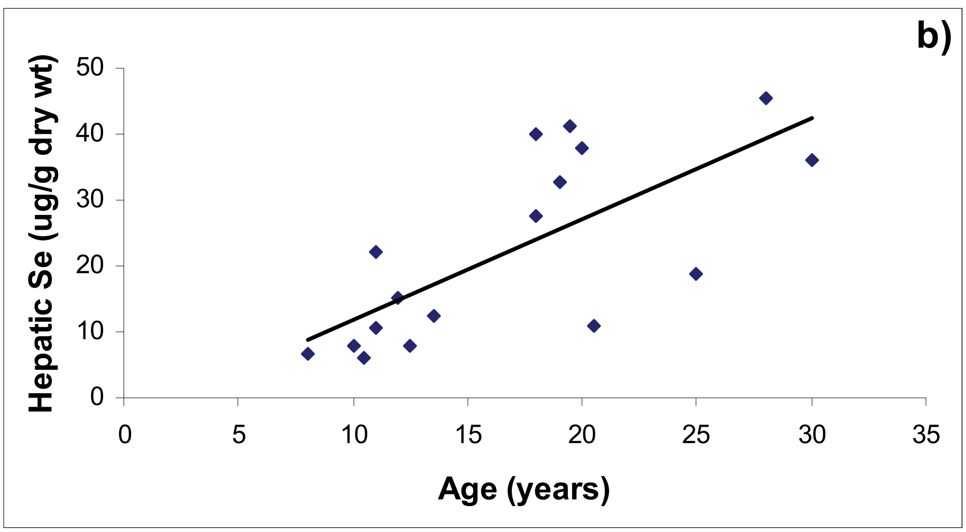
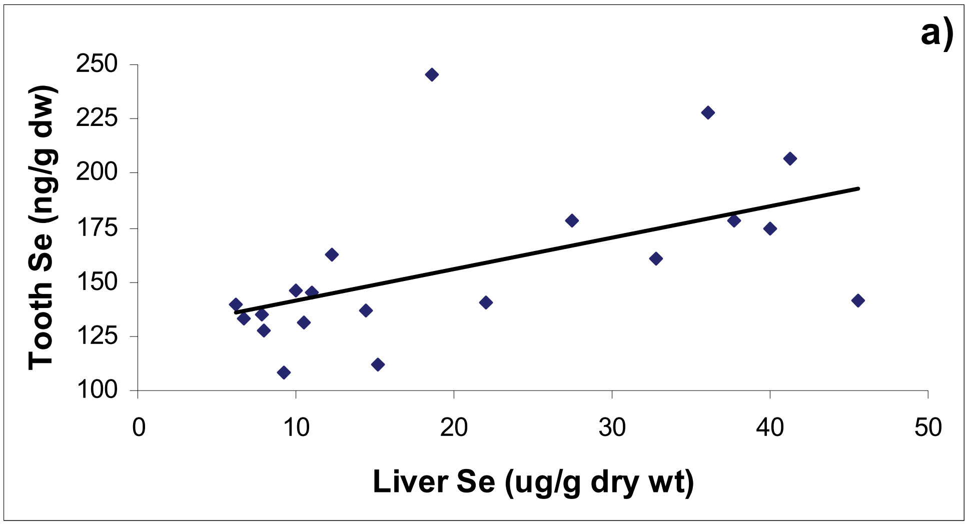
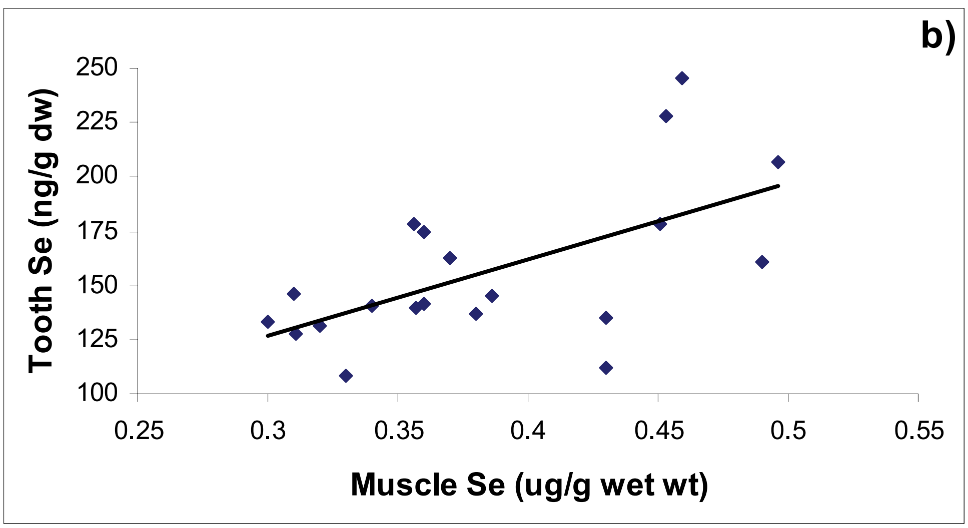
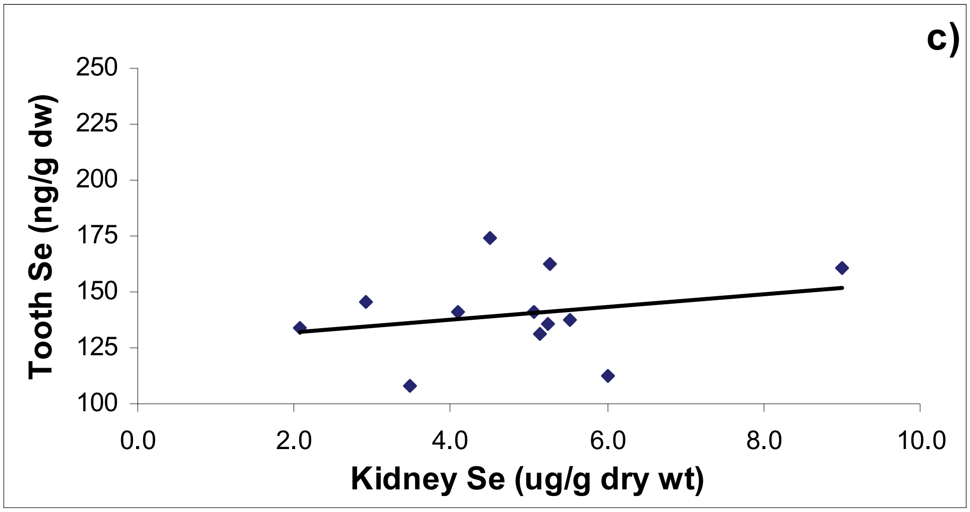
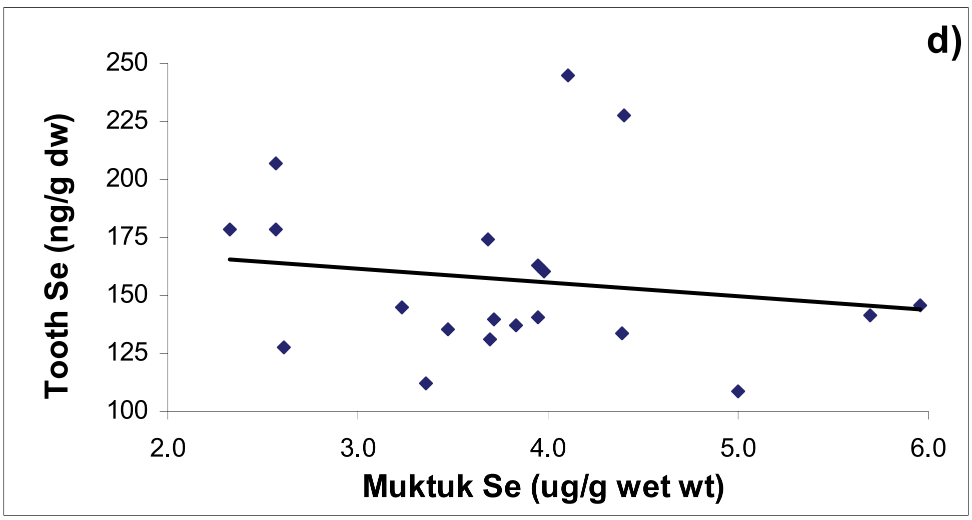


Figure 2.3 Relationship between concentrations of selenium in beluga tooth and (a) liver, (b) muscle, (c) kidney, and (d) muktuk. Solid lines represent linear regressions (see equations in text for liver and muscle).






c)
Tooth Se (ng/g dw)
250
225
200
175
150
125
100
0.0
2.0
4.0
6.0
8.0
10.0
Kidney Se (ug/g dry wt)


d)
Tooth Se (ng/g dw)
250
225
200
175
150
125
100
2.0
3.0
4.0
5.0
6.0
Muktuk Se (ug/g wet wt)

Link statement:

The results of the last chapter show that tooth Se levels are moderately effective as predictors of Se in the liver and muscle tissues of beluga whales (r^2 = 0.29 for liver; r^2 = 0.36 for muscle). Tooth Se, however, is not significantly correlated with Se in muktuk and kidney. The relationships between tooth Se and Se in the four soft tissues are not as strong as those found for tooth Hg and Hg in the same soft tissues. These results are a reflection of the different roles that Se and Hg play in the mammalian body. This study contributes to an increased understanding of the storage and metabolism of Se in marine mammals, which is important considering the critical role that Se plays in the detoxification of Hg in marine mammals.

The results of chapter 2 indicate that beluga tooth Se concentrations can be used as biomonitors of Se concentrations in liver and muscle, just as tooth Hg is effective as a biomonitor of Hg in beluga liver, muscle, muktuk and kidney. This information is useful because beluga whales are an important food source for Inuit peoples, past and present. Beluga teeth found at archaeological sites can be used to comment on the contamination levels of these animals, thereby building a temporal examination of heavy metal exposure in these animals (for example Outridge et al. 2002; Outridge et al. 2005). Assessing the heavy metal concentrations in beluga is useful for determining the dietary exposure of these metals to Inuit populations consuming large amounts of this traditional food. An example of this use is presented in chapter 3, using Hg in beluga teeth and soft tissues.

Chapter 3 presents estimates of dietary exposure to Hg from the consumption of beluga whale in modern and historic (pre-Euroamerican contact) Inuit in the Mackenzie Delta. These exposure estimates were constructed, for the historic population, using teeth collected from archaeological sites in the Delta, and by applying the known tooth Hg: soft tissue Hg ratios developed by Outridge et al (2000).

Chapter 3

Reconstructing historical mercury exposure from beluga whale consumption among Inuit in the Mackenzie delta

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3.1 Abstract:

Modern increases in mercury levels in Arctic marine mammals have important implications for people who consume these resources as part of a traditional diet. This paper establishes estimates of dietary exposure to mercury from the consumption of beluga whales (*Delphinapterus leucas*) for the historic (pre-Euroamerican contact) and modern Mackenzie Delta Inuit populations. For the historic population, beluga intake was estimated from reconstruction of the pre-Euroamerican traditional diet, using archaeological and ethnographic data, while mercury levels in consumed flesh were estimated from beluga teeth recovered from archaeological sites. For modern populations, beluga consumption was estimated from recent dietary surveys, while mercury levels in consumed flesh were measured directly in harvested whales. Despite higher mercury levels in modern beluga, the estimated average exposure to mercury from the consumption of beluga is 15-73 µg Hg/person/day for historic Inuit populations compared to only 2-12 µg Hg/person/day for modern Inuit populations. This result is due to the higher average daily consumption of beluga whale flesh among historic Inuit populations, and may have placed these populations at greater risk for toxic mercury exposure than modern populations. Nevertheless, as a result of the recent increases in environmental mercury levels, mercury exposure remains a significant health issue for modern community members with diets particularly rich in marine mammals.

Key words : mercury, beluga whale, Inuit, traditional foods, temporal changes, exposure assessment.

3.2 Introduction:

Mercury (Hg) contamination in the Canadian Arctic has, over the past several decades, become a problem of significant concern, especially for those who regularly consume marine mammals (Woshner et al. 2001). Beluga whales (*Delphinapterus leucas*), like many marine mammals, often exhibit high levels of Hg. This occurs for two main reasons: 1) within the Arctic food web, Hg levels have biomagnified through several trophic levels before ingestion by beluga; 2) beluga are slow-growing and long-living organisms, causing contaminants to bioaccumulate over a long period of time (Muir et al. 1999). Moreover, soft tissue analysis of the meat and organs of marine mammals, such as beluga, indicate an increasing trend of Hg contamination, which may be attributed to increasing global Hg pollution (Wagemann et al. 1996; Muir et al. 1999).

Inuit living in the Mackenzie Delta, in the Northwest Territories of Canada, have consumed beluga for many centuries as part of their traditional subsistence lifestyle (Betts and Friesen 2004). Beluga consumption has been identified as a major contributor to Hg exposure among the Inuit (Chan et al. 1995). Increase in Hg concentrations in beluga will increase Hg exposure and have potentially significant health effects (Kuhnlein et al. 2000).

The prehistoric Inuit of the Mackenzie Delta were one of the largest groups in the Canadian Arctic, with an estimated pre-contact population of 2,500-4,000 individuals (Usher 1971; McGhee 1974). They called themselves 'Siglit' and covered a territory that ranged from Barter Island to Cape Bathurst. The Mackenzie Delta is a highly productive area, which is likely why it was able to sustain such a large population (Morrison 1997). The territory along the east channel of the Delta is particularly suited to hunting beluga. Large numbers of beluga enter the shallow waters of Kugmallit Bay every summer, near the Siglit settlements of Gupuk and Kittigazuit, which were occupied in both summer and winter. During this season, Inuit congregated at summer hunting camps situated to take advantage of the beluga in the Bay. Beluga were hunted cooperatively, using a drive-style technique (Whittaker 1937; Nuligak 1966). As many as forty kayaks would surround a pod and drive whales towards shore, where they became trapped in shallow waters and were more easily harpooned. Archaeological work in the Delta has shown that the economy of the Siglit was highly specialized towards the harvest of beluga whales (McGhee 1974; Friesen and Arnold 1995a; Betts and Friesen 2004). After several decades of economic and cultural exchange with Euro-American whalers, fur-traders and missionaries, the Siglit were decimated by two major measles epidemics, in 1900 and 1902 (Usher 1971). The few Siglit who remained were integrated into the new Alaskan Inupiat communities in the Delta. The current Inuit people in the Mackenzie Delta are known as the Inuvialuit, and are generally of mixed descent (Morrison 1997).

Today, the modern Inuvialuit population is composed of around 5,000 people, most of whom live in towns such as Aklavik, Holman, Inuvik, Paulatuk, Sachs Harbour and Tuktoyaktuk. Although most people work in the communities, many still go out onto the land to hunt and fish (Usher 2002). In communities where economic and social upheaval are common problems, the harvesting of traditional foods promotes self-sufficiency, transmission of knowledge, and spiritual satisfaction (Wheatley and Wheatley 2000). Unfortunately, harvesting traditional foods has become associated with health risks, since high levels of organic contaminants and heavy metals, such as Hg, have been measured in many traditional food species (Wagemann et al. 1996; Muir et al. 1999; Braune et al. 2005).

Hg accumulates at different concentrations and in different forms in the tissues of beluga. The beluga tissues with the highest concentrations of Hg are the liver and kidney, but these tissues are not eaten by the Inuit, and most of the Hg in these tissues is in the inorganic form (Woshner et al. 2001). The tissues that are commonly eaten, the muscle and muktuk (epidermis and blubber), have lower Hg concentrations, but the Hg is nearly all in the toxic organic form, methylmercury

(MeHg) (Woshner et al. 2001). Organic Hg has a higher toxicity than the inorganic form, so the lower total Hg concentrations of the muscle and muktuk may nonetheless represent a greater physiological and neurological danger to the consumer. MeHg damages cells in the cerebellum and the cortex, and thus toxicity manifests itself in neurological abnormalities. Abnormal gait, ataxia, constriction of the visual field, emotional instability and dysathria (difficulties with speech articulation) are the main symptoms of MeHg toxicity (Watanabe and Satoh 1996; Clarkson et al. 2003).

Because Hg has always been present in the Inuit diet, there is an interest in characterizing the risk of Hg exposure in the historical context. The objectives of this study are two-fold: 1) to calculate the importance of beluga whales in the pre-contact diet of the Inuit population in the Mackenzie Delta, and to estimate the corresponding dietary exposure to Hg from this important food source; and 2) to compare these results to modern Inuit beluga consumption and Hg exposure from beluga sources.

3.3 Methods:

Estimates of dietary exposure to mercury are calculated by the following equation:

Hg intake (ug/day) = Concentration of Hg in tissues (ug/g) * Consumption rate of the tissue (g/day)

The edible beluga tissues considered in this analysis are muscle and muktuk. These are commonly consumed tissues that can accumulate high levels of Hg. Muscle and muktuk are considered separately in the construction of Hg exposure estimates since these tissues are eaten in differing quantities and do not have the same Hg accumulation pattern.

Concentration of Hg in historic beluga tissues.— Hg concentrations were measured in beluga teeth recovered from archaeological excavation at Gupuk site, Northwest Territories, Canada (Outridge et al. 2002). These teeth have been dated to 1450-1650 AD. This time period is before Hg was commonly used as a catalyst in industrial processes and before coal burning became a major contributor of Hg to the atmosphere (Outridge et al. 2002).

Tooth Hg levels in beluga are significantly linearly correlated with the corresponding Hg levels in the edible soft tissues, muscle and muktuk (Outridge et al. 2000). Using calculated conversion equations presented in Outridge et al. (2000), the Hg concentrations analyzed from the beluga teeth (in ng/g dry weight) were converted to approximate edible soft tissue Hg concentrations (in μg/g wet weight) (Table 3.1). The mean, minimum, maximum Hg concentrations from the data set are presented.

Historic beluga consumption.— The reconstruction of the Siglit Inuit diet is based on three factors: the total amount of animal meat consumed per day; the consumption of beluga muscle and muktuk as a percentage of the total meat diet; and the relative proportion of consumed beluga flesh that is muscle or muktuk. This information is combined to produce an estimate of average daily consumption levels for each tissue type (in g/person/day).

Faunal analysis has indicated that beluga whales may have made up to a quarter of the total grams of meat consumed in the yearly diet of the Siglit population (McGhee 1974; Friesen and Arnold 1995b). Ethnographic reports observed a heavy reliance on beluga in the summer and winter months (Stefansson 1914; Whittaker 1937; Nuligak 1966; Friesen 2004). Three different intake scenarios were constructed to reflect seasonal consumption rates:

Scenario I. A low intake category was constructed assuming 10% of the total meat consumption in the pre-contact Siglit diet was from beluga. This scenario may best reflect spring and fall consumption, when the diet was most diverse. Commonly harvested resources include fish, caribou (*Rangifer tarandus*), ptarmigan (*Lagopus muta)*, goose (family *Anatidae*), rabbit (family *Leporidae*), moose (*Alces alces*) and ringed seal (*Phoca hispida*) and bearded seal (*Erignathus barbatus*) (Stefansson 1914; Friesen 2004)

Scenario II. A medium intake of 25% of the total meat consumption is assumed to represent summer beluga consumption. The months of July and August were peak beluga hunting season, so fresh meat, muktuk and blubber would have been eaten in abundance (Whittaker 1937). However, other resources, such as fish and caribou, were harvested (and consumed) concurrently (Stefansson 1914).

Scenario III. The season of highest intake was probably winter, when the Siglit relied exclusively on stored supplies of beluga and fish (Stefansson 1914; Friesen and Arnold 1995b). This scenario assumes a high intake level of 50% of total meat consumption from beluga sources.

In this analysis, it is assumed that each individual (men and women) ate an average of 500 g of meat per day. Gender equity is assumed in regards to access to staple foods, such as beluga (Stefansson 1914).

In the historic context, the consumption of muscle and muktuk is consistent with the availability of these tissue types on the typical beluga carcass. The relative proportion of consumed muscle and muktuk is therefore based on a meat utility index derived for Odontocetes (toothed whales). Although this utility index was constructed from the processing of a harbour porpoise (*Phocoena phocoena*), it is considered to be an accurate representation of beluga whale body composition (Savelle and Friesen 1996). The index shows that the sculp (skin and blubber, called muktuk when eaten) makes up more than a third of the total weight of the body, and meat represents a quarter of total body weight (Table 3.2).

For this analysis, muktuk is considered to be composed of equal parts skin and blubber (Kuhnlein et al. 2000). Blubber does not build up significant concentrations of Hg, and is not a relevant source of Hg in the diet. Accordingly, the reconstructions of beluga consumption presented here include blubber intake, but the corresponding dietary Hg exposure estimates only consider the 50% of muktuk that is Hg-accumulating epidermis.

Concentration of Hg in modern beluga tissues.— Hg concentrations in muscle and muktuk from beluga harvested in 1993 by Inuit hunters in the Mackenzie Delta are used (Outridge et al. 2002) (Table 3.3). For muktuk, only Hg concentrations from the mercury-accumulating epidermis layer are included. The mean, minimum, maximum Hg concentrations from the data set are presented.

Modern beluga consumption.— Modern Inuit consumption of traditional foods, including beluga muscle and muktuk, is reported in a dietary survey of Canadian Inuit traditional food consumption completed in 1998 (Kuhnlein et al. 2000). In the Mackenzie Delta region, the towns of Aklavik, Tuktoyuktuk, and Paulatuk participated in the study. A random sample of 10% of the community population was invited to participate. Interviews included a food frequency questionnaire of traditional food use and a 24-hour total food recall. The interviews were conducted twice, during different seasons, to capture seasonal variability in traditional food consumption. The consumption of beluga meat and muktuk (skin only) in the Inuvialuit region was drawn from these results, and organized by age and gender. The data were averaged over the two seasons, giving a mean yearly intake level. This intake data represents mean community consumption, and includes data from many participants who rarely consume beluga. However, in these communities, there are still people who eat beluga on a regular basis. To capture potential risk for high frequency consumers, a high intake scenario was constructed based on 7-day food records and 24-hour recall collected from Aklavik and from an Inuit summer fishing camp (Kuhnlein et al. 2000). This intake scenario uses the average serving size of beluga muktuk (skin only) and assumes that this food is consumed every third day.

3.4 Results:

Historic beluga consumption.— The calculated consumption of beluga muscle and muktuk (skin and blubber) for each intake scenario is presented in Table 3.4. Consumption of muktuk is higher than muscle consumption for all intake scenarios.

Historic Hg exposure.— Daily intake of Hg from muscle and muktuk (skin only) are summed to produce the total daily Hg intake for the historic Inuit population (Table 3.5). For each intake scenario, the mean, minimum and maximum Hg intakes are presented based on the calculated soft tissue Hg concentrations, while the consumption rate is assumed constant. Because the Hg in beluga muscle and muktuk has been shown to be mainly in the organic form (Woshner et al. 2001), it is assumed that the Hg intake from the diet was methylated.

Modern beluga consumption.— Mean yearly beluga consumption is relatively low (Figure 3.1). Generally, men eat more beluga than women, as a result of greater energy requirements and, as the primary harvesters, greater access to traditional animal foods. A trend can also be observed indicating increasing beluga consumption with increasing age. An aberrance occurs in men in the 41-60 age category. This demographic reported a very low consumption of beluga muktuk, which is unusual considering this cohort is probably composed of the greatest number of active harvesters.

Modern Hg exposure.— Mean Hg intake from beluga consumption is summarized in Table 3.6. Because they eat more beluga, men are exposed to higher concentrations of Hg than women, but this gender difference is not large. Men and women in the older age categories have a greater Hg intake from beluga.

High Intake Scenario. Within the high intake scenario, the estimate of daily muktuk (skin only) consumption is 25.6 g/day. This estimate does not account for gender or age differences, but it is likely that the population segment consuming this quantity of beluga is predominantly older and/or male. The Hg intake estimates corresponding to minimum, mean, and maximum tissue Hg concentrations are 7.2 μg Hg/person/day, 30.5 μg Hg/person/day, and 68.6 μg Hg/person/day, respectively.

3.5 Discussion:

The estimated historic Hg concentration in beluga tissues range from 0.45 μg/g to 0.60 μg/g in muscle and 0.31 μg/g to 0.41 μg/g in muktuk. This is lower than the range in the modern beluga samples used in this study, which ranged from 0.56 μg/g to 1.75 μg/g in muscle and 0.28 μg/g to 1.19 μg/g in muktuk (Outridge et al. 2002). Beluga sampled from the same region between 1981 and 2002 show a similar range of means, from 1.12-1.95 μg/g for muscle and 0.57-1.15 μg/g for muktuk (Lockhart et al. 2005).

The dietary Hg exposure estimates presented here show that the historic population may have ingested, on average, higher quantities of MeHg from beluga consumption than the modern population currently does. This result is somewhat counter-intuitive, since Hg levels in modern Beaufort Sea beluga are significantly higher than concentrations measured in the corresponding pre-industrial beluga population (Outridge et al. 2002). Despite the well-documented increase in beluga Hg concentrations since the pre-industrial era, the historic Inuit population shows a higher intake of Hg from beluga simply because they were consuming such large quantities of beluga flesh.

The dietary Hg intake estimates suggest that the historic population had a mean daily MeHg intake level from beluga flesh ranging from 15 to 73 ug Hg/day. The Provisional Tolerable Daily Intake (PTDI) is 0.47 μg MeHg/kg/day, or 35 μg MeHg/day for an average 70 kg individual. During seasons of low beluga consumption (scenario I) the historic MeHg intake was below this PTDI, but during seasons when beluga was more frequently eaten (scenarios II and III), the average intake of MeHg is estimated to have been above the current recommended guideline level. The modern population, with a mean Hg intake range of 2.13 μg Hg/day to 10.93 μg Hg/day for women and 4.36 μg Hg/day to 11.59 μg Hg/day for men, is below the PTDI. However, frequent consumers of beluga in modern populations (represented by the high intake scenario) may have daily MeHg intake from beluga above the PTDI, particularly when consuming beluga with high tissue Hg concentrations.

Since MeHg inhibits neuronal cell division and migration, children are most sensitive to the effects of MeHg, as are developing fetuses. (Clarkson et al. 2003). Fetal exposure occurs by maternal consumption of fish and marine mammals. This study examines dietary Hg exposure only in adults. Children were not considered because their caloric needs are highly variable, and because dietary information is largely unavailable and is difficult to estimate. The potential for developmental delays means children and women of child-bearing age are considered to be within a high risk group. Accordingly, Health Canada states a PTDI for children and women of child-bearing age of 0.2 μg MeHg/kg body weight/day (Health Canada 1998). For a 70 kg adult woman, this represents a daily MeHg intake of 14 μg. Historic estimates show that women consuming low levels of beluga flesh (scenario I) have mean MeHg intake levels around this PTDI. For medium and high beluga intake levels (scenarios II and III) the mean total MeHg intake from beluga calculated for Siglit women is many times this guideline limit, indicating that developmental delays resulting from high exposure could have been a concern in this population. In the modern population, women consuming beluga with the minimum and mean tissue Hg level have Hg intake levels from beluga consumption below the PTDI. Women in the 41-60 and 61+ age category, consuming beluga with tissue Hg levels near the maximum reported here, have Hg intake levels from beluga consumption above the recommended PTDI. However, these women are generally beyond child-bearing age, so high Hg intakes among individuals of this age group do not carry risk for developing fetuses. For the high intake scenario, women consuming beluga with mean or maximum Hg concentrations are well above the PTDI, indicating that high consumption of beluga could result in the risk of developmental delays in the modern population. However, since the most frequent consumers of beluga and other traditional foods tend to be older and male, it is likely that very few women of child-bearing age consume the quantity of beluga represented by the high intake scenario.

A fundamental limitation to this study involves the difficulties inherent in accurately recreating the historic diet. Archaeological investigations from the Mackenzie Delta area give information only on winter occupation. The dependence on beluga during the spring, fall, and summer is therefore uncertain (Friesen and Arnold 1995b). Although it is likely that the Siglit ate beluga flesh in abundance during the summer hunting season, it is difficult to quantitatively estimate dietary reliance on this resource without any archaeological evidence. Ethnographic reports indicate that beluga was frequently eaten (both in the summer and winter), but they do not contain any quantitative nutritional data. These ethnographic sources, consequently, are only able to support consumption estimates drawn from archaeological research, but do not supply any independent evidence of historical beluga consumption patterns.

Friesen and Arnold's (1995a) estimate of the dietary reliance on beluga is based on a technique developed by White (1953) to calculate the available meat at hunter-gatherer archaeological sites. For every animal species present at the site, the MNI (minimum number of individuals) is multiplied by the average body weight of an individual of that species, and by the percent of available meat and fat on an average carcass. Each species is then considered as a percentage of the total meat available at the site, which indicates the relative importance of each potential food source. There are certain potential errors inherent in White's method of calculating dietary importance: the edible tissue estimate is based on modern butchering techniques, which may differ in efficiency from the Indigenous technique; the calculation is based on average weight for the species, but weight can vary significantly with age, sex, and season; finally, there is a strong tendency for small sample size to exaggerate MNI calculations (Sergeant and Brodie 1969; Stewart and Stahl 1977; Grayson 1978; Friesen and Arnold 1995b). Friesen and Arnold (1995b) adjusted for potential error by using a conservative average weight for beluga, and noted that the sample size of beluga bones at the Gupuk site is sufficiently large to avoid an exaggerated MNI calculation for this species. In addition, large marine mammals, such as beluga, tend to be under-represented in the bone assemblages of residential sites, because the large heavy bones of these animals are more likely to be left on the beach after the useful body parts have been removed, rather than carried and stored at the winter site (Whitridge 1992; Savelle 1995). In this case, bone frequencies may not be a true representation of available meat at the site, and may under-represent the amount of meat that was consumed from large animals. Finally, the authors noted that even if there is error in their dietary reliance estimate, the calculated importance of beluga in the diet is so far above the next most important species, that even if available beluga meat has been over- or under- estimated, beluga remain far more important in the Siglit diet than all other available food sources. In this study, a range of intake scenarios were used to calculate beluga consumption and the daily Hg intake in order to reflect the potential variation and uncertainty surrounding the use of beluga in the pre-contact population.

The dietary exposure estimates calculated in this study only take into account MeHg intake from beluga as a food source. Many other species in the traditional diet are potential sources of MeHg. By not including these sources in our analysis, the estimate of dietary MeHg exposure from the traditional diet is incomplete. For historic exposure estimates, it is, unfortunately, difficult to take other sources into account, because no Hg values have been measured in other faunal remains from the Gupuk site. However, beluga bones so strongly dominate the faunal array at Gupuk, that other food sources may be relatively less important in contributing to MeHg intake. Beluga bones make up 66% of the available meat at the site, while the next most available species are ringed and harbour seals, which together represent only 7.1% of the total available meat (Friesen and Arnold 1995a). Although seals do accumulate Hg in their edible tissues, modern data show that seal tissue has lower Hg concentrations than beluga tissue (AMAP 2001). The contribution of dietary Hg from seals would be less than 10% of what beluga consumption contributed to the dietary Hg intake of the Gupuk site inhabitants. Beluga consumption probably provided most of the Hg exposure in the historic population. This is not the case in modern populations, where consumption of beluga is relatively infrequent. Caribou, which is highly consumed, contributes a small amount but a significant proportion of MeHg exposure (Van Oostdam et al. 2005). Caribou meat represents 69.8% by weight of the Inuvialuit traditional diet, and contributes 51.7% of total Hg from traditional food sources (Kuhnlein et al. 2000). Other high Hg contributors, such as beluga muktuk, lake trout (*Salvelinus namaycush*) flesh, caribou liver, and duck (family *Anatidae*) flesh, are not frequently eaten (Table 3.7). Seals, walrus (*Odobenus rosmarus*) and other marine mammals, commonly associated with Inuit diet and with contaminant risk in other Arctic regions, are not frequently eaten by the Inuvialuit, and therefore are not large contributors to daily Hg intake.

Changes in beluga-hunting techniques also have implications for dietary Hg exposure. The drive-hunting method of the Siglit resulted in “catastrophic” kills, where beluga of all ages and both sexes were taken and eaten. The Siglit would have therefore been exposed to the approximate population mean level of Hg through consumption of beluga soft tissues. Conversely, in the modern hunt each motor boat (which may hold several hunters) acts as an independent unit, and hunters tend to preferentially harvest the biggest animals (Hunt 1979; Fraker 1980). This penchant for larger whales (which provide greater food yields) means there is an unintentional selection for older, more contaminated animals. Accordingly, many beluga consumers in the modern population may have daily MeHg intakes closer to the 'maximum' exposure estimate listed in Table 6. This selection of potentially more contaminated animals does not, however, seem to place the modern population at great risk of Hg exposure from beluga consumption, simply because most people, with the exception of high frequency consumers, do not eat many of these highly contaminated beluga.

Estimates of dietary exposure to environmental contaminants are often validated by corresponding biomarkers. In the case of Hg, human hair and blood Hg levels are appropriate biomarkers frequently used to substantiate exposure models and provide indications of error or bias (Ponce et al. 1998). Hair is considered a particularly powerful indicator of Hg body burden, because it grows in steady increments and can therefore be used to measure Hg absorption over the long-term. Unfortunately, this study was not able to use biomarkers to validate the historic dietary exposure estimates presented here, because no human remains are found at the Gupuk site. Hair Hg concentrations can be estimated from dietary intake using pharmacokinetic factors such as: half life of Hg in the body; Hg body absorption rate; Hg accumulation in liver and brain; ratio of hair to blood Hg levels; and rate of hair growth. However, these parameters are known to vary between individuals and groups (Bartlett et al. 2000; Canuel et al. 2006). The models commonly used by epidemiologists and policy makers to construct guideline intake levels may not accurately apply to all ethnic groups (Canuel et al. 2006). The calculated dietary Hg intake to hair Hg ratios from two Inuit populations in Nunavik and Nunavut (Arctic Canada) give an average ratio of 4.0 (Solomon 2005; Canuel et al. 2006). Using this ratio, which may be most appropriate for the Inuit population in this study, the adult Siglit mean hair Hg levels from beluga consumption are estimated at 0.9 μg/g Hg, 2.1 μg/g Hg, and 4.2 μg/g Hg for low, medium and high intake scenarios, respectively (Table 3.8). Since beluga was probably the main source of Hg in the pre-contact Siglit diet, the mean total hair Hg levels from all food sources may not have been much higher than the hair Hg estimates presented here. These estimated hair Hg levels for the historic population are similar to hair Hg concentrations measured in preserved human hair from three other concurrent Arctic sites. Hansen (1981) reports a mean total Hg concentration of 3.82 μg/g in four Greenland mummies from the Umanak region (Western Greenland); Egeland et al (1999) found a mean total Hg concentration of 1.33 μg/g from 16 Alaskan mummies (Kodiak Archipelago); and Wheatley and Wheatley (1988) reported a mean MeHg concentration of 1.7 μg/g from eight mummies from the Eastern Canadian Arctic (near Pond Inlet, Nunavut). The variation in mean hair Hg concentrations between these populations could be explained by dietary differences, regional geological differences which impact the cycling of Hg in the ecosystem, and potential degradation of preserved mummy hair samples. The World Health Organization (WHO) recommends that maternal hair Hg concentrations not exceed 10-20μg/g Hg, a level which has been associated with a 5% risk of neonatal developmental disorders (WHO 1990). The estimated hair Hg levels for the historic population are below this level, as are the Hg concentrations measured in the preserved human hair from other Arctic sites. The preserved hair samples are generally lower than current (1972-1989) human hair Hg levels in similar geographic regions (Van Oostdam et al. 1999). Recent maternal hair Hg measurements in the Inuvaliut region are below the WHO guideline level (geometric mean of 0.49 μg Hg/g in Inuvik and 2.29 μg Hg/g in Tuktoyuktuk; maximum measurement of 11.5 μg Hg/g in both communities) (Snider and Gill 2001, as cited in Van Oostdam et al, 2005). Measurements taken from Inuit communities across Canada indicate a wide range of hair Hg concentrations (Wheatley and Paradis 1995). Inter- and intra-community variability in hair Hg levels is generally indicative of the wide range in traditional food consumption. Geographic variability of Hg exposure may also stem from the different array of harvested animal foods in different regions of the arctic, as well as spatial variation in environmental Hg concentrations.

Despite the well-documented increase in beluga Hg concentrations since the pre-industrial era, the historic Inuit population shows a higher intake of Hg from beluga simply because they were consuming such large quantities of beluga flesh. Traditional food consumption among Canada's Inuit has decreased significantly in the past fifty years. A century ago, traditional foods accounted for nearly all of the diet; currently, the Inuvialuit consume 12.9% of energy in the winter and 24.6% of energy in the summer from traditional food sources (Kuhnlein et al. 2000). The gradual shift from reliance on fish-eating marine mammals to market foods represents a decline in trophic position of modern Inuit populations relative to their ancestors. Thus, despite exploiting a marine environment characterized by low natural mercury levels, prehistoric Inuit populations appear to have occupied a sufficiently high trophic position to be characterized by mercury exposure exceeding modern health guidelines for children and women of child-bearing age. On the other hand, for many modern Inuit communities, the reduction in trophic position associated with increased reliance on market foods has likely served to counteract potential increases in Hg exposure resulting from elevated contaminant levels in post-industrial marine food webs. However, given the high within and among community variation in traditional food consumption (Kuhnlein et al. 2000), the persistence of elevated mercury levels in modern, marine food webs remains an important potential health risk associated with heavy reliance on traditional diets.

3.6 Conclusions:

The Inuit living in the Mackenzie Delta, prior to Euroamerican contact, were highly dependent on beluga as a key food resource. Despite overall low levels of mercury in pre-industrial environments, this heavy reliance on a top predator in a marine food web would have led to levels of Hg dietary exposure exceeding modern health guidelines. Estimates of historic hair Hg concentrations, based on the calculated Hg intakes for this population, are similar to measured hair Hg concentrations in mummy samples from other Arctic regions. Our results suggest that Inuit populations in the Mackenzie Delta have had a long history of mercury health risks and emphasize the importance of considering both the contaminant levels in traditional foods and the consumption rates of these foods when evaluating contaminant risk assessments.

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3.8 Tables and Figures:

Table 3.1 Measured and calculated mercury concentrations in tooth (ng/g dry weight), muscle and muktuk (μg/g wet weight) of historic beluga expressed per unit dry weight (dw) or wet weight (ww). Mean, minimum, and maximum Hg estimates are based on Hg concentrations measured in beluga teeth dated from 1450 to 1650 AD (Outridge et al. 2002).

tissue Hg	tooth Hg	muscle Hg	muktuk Hg
mean	5.00	0.46	0.32
minimum	1.61	0.45	0.31
maximum	19.7	0.60	0.41

Table 3.2 Proportional contribution of sculp (skin and blubber) and meat to beluga body weight, based on the standard odontecete body composition calculated by Savelle and Friesen (1996).

body part	weight (g)	% of total body weight	% of total sculp and meat weight
sculp (skin/blubber)	9 781	35.9	**58.3**
meat (easily rem.)	6 986	25.7	**41.7**
sculp and meat	16 768	61.7	100
total animal	27 200	100	--

Table 3.3 Measured mean, minimum and maximum mercury concentrations (μg/g) in muscle and muktuk of modern beluga, harvested from the Mackenzie Delta, expressed per unit wet weight (ww), as presented by Outridge et al. (2002).

Hg measurement	muscle (μg Hg/g ww)	muktuk (skin only) (μg Hg/g ww)
minimum	0.56	0.28
maximum	3.52	2.68
mean	**1.75**	**1.19**

Table 3.4 Estimated consumption of meat and muktuk in the historic Inuit population, in g/person/day. Estimates are expressed for low, medium and high levels of intake, in which beluga make up 10%, 25%, and 50% of total weight of meat consumed per day, respectively.

tissue type	1. low intake	2. medium intake	3. high intake
meat	21	52	104
muktuk (skin and blubber)	30	73	146
total	**51**	**125**	**250**

Table 3.5 Estimates of total mercury intake in the historic Inuit population, in μg Hg/person/day. Estimates are expressed for low, medium and high levels of intake, in which beluga make up 10%, 25%, and 50% of total weight of meat consumed per day, respectively. Mean, minimum, and maximum Hg estimates are based on Hg concentrations measured in beluga teeth dated from 1450 to 1650 AD (Outridge et al. 2002).

tissue Hg level	1. low intake	2. medium intake	3. high intake
minimum	14	34	69
maximum	19	46	93
mean	**15**	**37**	**73**

Table 3.6 Estimates of mean modern mercury intake from beluga consumption, in g/person/day, presented by gender and age. Mean, minimum, and maximum tissue Hg concentrations were measured in meat and muktuk of modern beluga samples, as presented by Outridge et al. (2002).

gender	tissue Hg level	age 15-19	age 20-40	age 41-60	age 61+	total
men	min	1.05	1.83	0.52	3.00	1.55
	max	9.72	15.33	5.01	25.29	13.26
	mean	**4.36**	**7.04**	**2.23**	**11.59**	**6.06**
women	min	0.50	0.78	2.54	3.06	1.39
	max	4.79	6.11	24.01	23.22	11.91
	mean	**2.13**	**2.85**	**10.73**	**10.93**	**5.43**

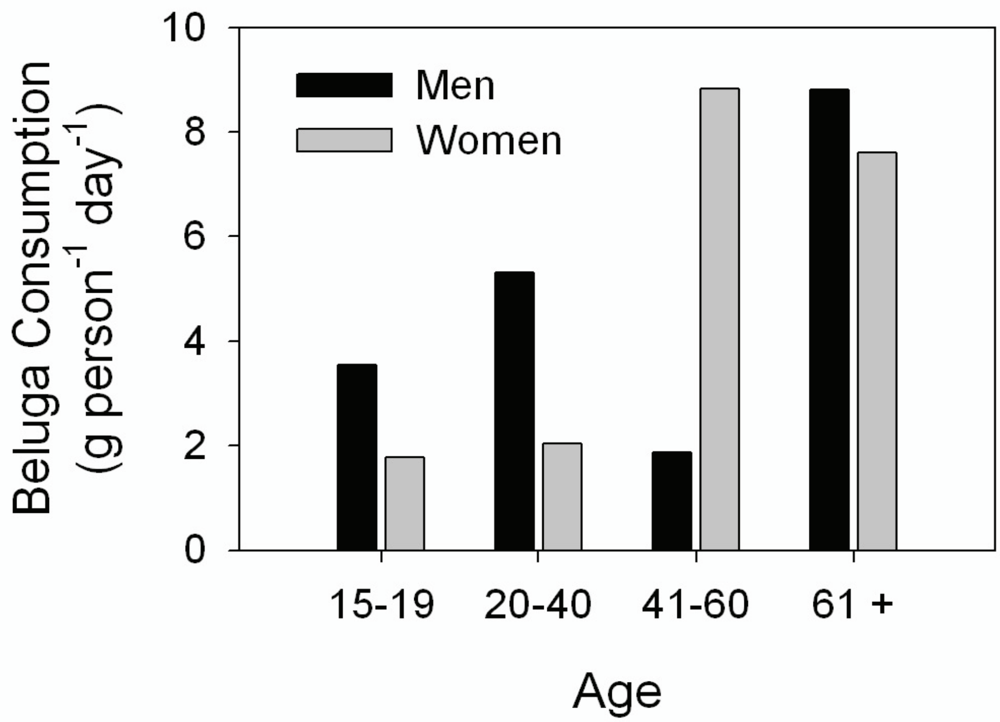
Table 3.7 Estimated contribution of various Traditional Foods (TF) to total mercury intake in the modern Inuit diet, based on information presented by Kuhnlein et al. (2000). Mean measured Hg concentrations are expressed per unit wet weight (ww).

species	% of total TF consumption	% total Hg in diet	mean [Hg]
caribou flesh	69.8	51.7	0.06
beluga muktuk	1.5	11.4	0.73
lake trout flesh	1.1	11.2	0.85
caribou liver	0.5	4.1	0.62
duck flesh	0.4	3.6	0.80

Table 3.8 Estimates of historic hair mercury levels resulting from three levels of beluga intake and a range of tissue Hg levels, expressed in µg/g Hg. Mean, minimum, and maximum Hg estimates are based on Hg concentrations measured in beluga teeth dated from 1450 to 1650 AD (Outridge et al. 2002). Estimates are expressed for low, medium and high levels of intake, in which beluga make up 10%, 25%, and 50% of total weight of meat consumed per day, respectively.

tissue Hg level	1. low intake	2. medium intake	3. high intake
minimum	0.8	0.9	1.1
maximum	3.9	4.2	5.3
mean	**0.9**	**2.1**	**4.2**

Figure 3.1 Mean beluga consumption (g/person/day) in the modern Inuit population, presented by age and gender. Modified from Kuhnlein et al. (2000).



Chapter 4:

4.1 Conclusion

This thesis examined the use of Hg and Se measurements in beluga teeth to assess the accumulation of these contaminants in soft tissues, and subsequent dietary exposure to Inuit populations consuming this traditional food resource. The role that Se plays in detoxifying Hg in the organs of marine mammals has implications for understanding how both elements accumulate and are distributed within the mammalian body, and thus how the consumption of different traditional foods affects dietary exposure to contaminants. Teeth are a useful tool for assessing the accumulation of trace elements because they grow incrementally and are not remodeled during the lifetime of an animal. Preserved teeth can therefore be used to examine temporal trends in contaminant levels in marine mammals, and to assess the implications of potential changes over time on the dietary exposure to contaminants from the traditional food systems of the Inuit people.

This study is the first to measure Se in the teeth of a marine mammal species. Tooth Se was found to be effective for use as a biomonitor of Se in the liver and muscle of beluga whales. Additionally, this study is the first to use heavy metal concentrations in beluga teeth (Hg in this case) to examine how dietary exposure to contaminants in this important traditional food source has changed over time. This information contributes to the assessment of risk involved in the consumption of traditional foods in the modern and historic context.

Specifically, HG-AFS is found to be an effective technique for determining Se in beluga teeth. Tooth Se is moderately effective as a predictor of liver and muscle Se, though these relationships are not as strong as those of Hg in teeth and soft tissues. Despite the weaker correlation coefficients between tooth Se and liver and muscle Se, relative to Hg in these tissues, this study provides important information about the storage of Se in beluga whales. The large variation in Se concentrations between tissues indicates that Se is stored in the organs with high concentrations of this element (i.e. liver and kidney), on the other hand, tissues with lower concentrations of Se (tooth and muscle) represent body compartments where Se is metabolically active or where accumulation is reflective of the level of Se circulating in the blood. Increased knowledge about the distribution of Se in the bodies of marine mammals contributes to a deeper understanding of the role of Se in protecting against the toxicity of Hg and other heavy metals.

Future research in this area should examine the relationship between tooth Se and soft tissue Se in beluga whales in other geographical regions of the Arctic. Additionally, the storage of Se in beluga hard and soft tissues should be examined in the context of trophic positioning, which can be determined using C and N stable isotope measurements. Tooth Se measurements should also be taken from other high trophic marine mammal species, such as dolphins and seals, which also exhibit a strong protective mechanism operating between Hg and Se. Finally, the accumulation of Se in the teeth of beluga and other marine mammals should be examined in the context of traditional food consumption and dietary exposure assessment, similar to the use of tooth Hg to assess dietary Hg exposure in historical Inuit populations in chapter 3 of this study.

The dietary exposure assessment constructed in chapter 3 show that the Inuit living in the Mackenzie Delta prior to Euroamerican contact were highly dependent on beluga as a key food resource. Despite overall low levels of mercury in pre-industrial environments, this heavy reliance on a top predator in a marine food web would have led to levels of Hg dietary exposure exceeding modern health guidelines. This dietary exposure estimates for this population show Hg intake from this single food source may have been above the intake levels currently considered as ‘high risk’. These results suggest that Inuit populations in this area of the Arctic have had a long history of mercury health risks. In addition, these results emphasize the importance of considering both the contaminant levels in traditional foods and the consumption rates of these foods when evaluating contaminant risk assessments.

This study provides an important initial examination of changing exposure to contaminants in the Inuit traditional diet; however, a complete assessment of dietary exposure to Hg must consider the intake of this contaminant from other food sources. Although the historic population may have received the majority of their exposure from the consumption of beluga, which was a focal resource for these people, the modern population has a much more diverse diet, which includes many different traditional and market food sources. A complete assessment of dietary exposure to Hg in the modern population must consider the contaminant levels in all of these other food sources. As such, this study should not be considered as representative of the general dietary Hg exposure in this population. The modern population is exposed to increasing Hg levels in a wide variety of food sources which were not examined by this study. Consequently, dietary exposure to Hg remains a significant health issue for modern community members, particularly for those with diets rich in marine mammals. In addition, the presence of industrial pollutants (organic contaminants) which were not present in the environment prior to the industrial era, contribute to the uncertainty regarding the safety of traditional food consumption in the Arctic.

In general, the results from this thesis indicate that teeth can be used to effectively track long-term changes in trace element and contaminant exposure over time. This is useful not only for assessing changes in dietary exposure levels, as was examined in chapter 3 of this thesis, but also can contribute to increased knowledge about how the accumulation of trace elements in the Arctic food web is influenced by changes in environmental conditions. For example, environmental Hg levels are influenced by climate, as well as by tectonic activity and local geology (Petit 1977; Wagemann et al. 1996; Macdonald et al. 2003), and changes in these levels will be incorporated into the food web and reflected in the accumulation of Hg in the preserved calcified tissue of higher trophic marine mammals. Examining temporal trends in Se accumulation in marine mammals would also be valuable, since Se and Hg are strongly related in modern marine mammals. It is likely that natural fluctuations in Hg levels over time would be reflected in a reconstruction of concurrent Se levels. The use of teeth in the reconstruction of temporal contaminant levels trends in the Arctic is also useful for assessing the potential impacts of anthropogenic sources of contamination. This work has already begun for Hg (Outridge et al. 2002), lead (Outridge et al. 1997), and cadmium (Outridge et al. 2005). Since anthropogenic input of contaminants into the Arctic food web could have a significant impact on the Inuit traditional food system, this work also has important public health considerations.

Overall, the results of this study give new insights into the safety of traditional food consumption in the historic and modern context, and into the use of marine mammal teeth as biomonitors of contaminant levels in soft tissues.

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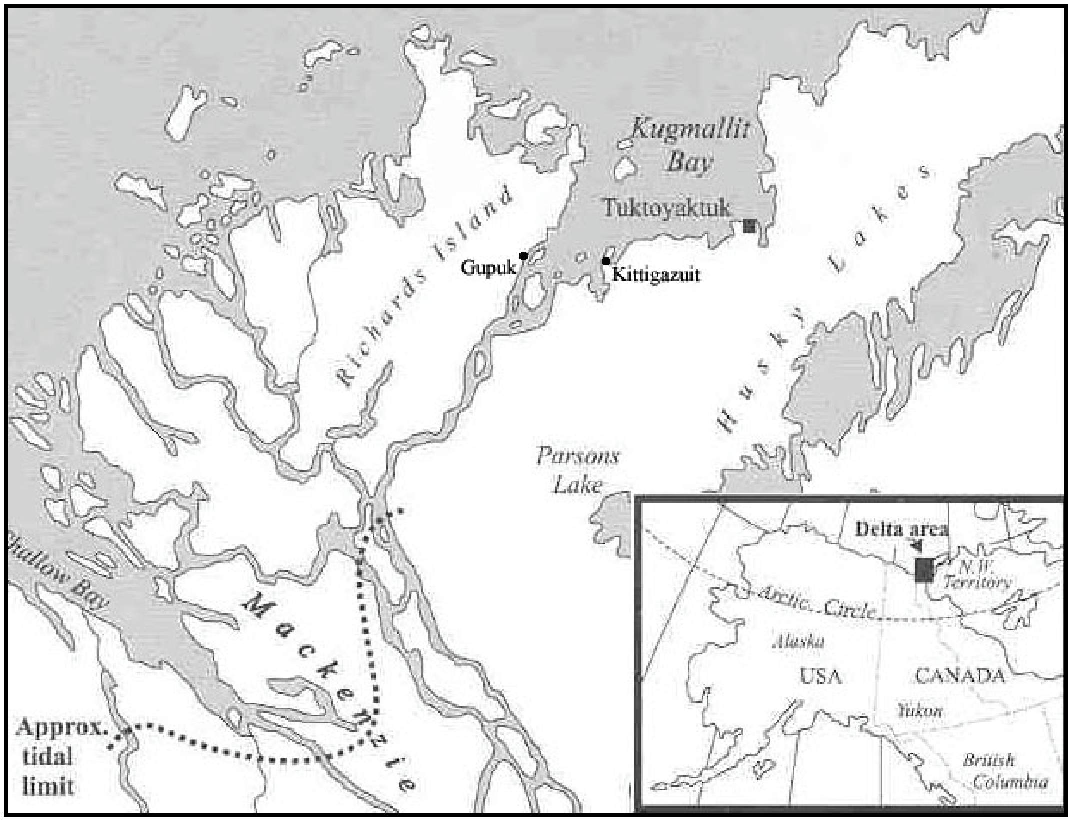
Appendix I : Map



Appendix II : Co-author waivers

July 4th, 2006

To Whom It May Concern:

This letter confirms that the co-author (Laurie H.M. Chan) agrees that the candidate (April Kinghorn) includes in her thesis the manuscript entitled *Teeth as biomonitors of selenium concentrations in tissues of beluga whales (Delphinapterus leucas)***.**

.

The candidate's role in the study was to develop the research methods, measure Se in all tooth samples, and to manage, analyze and interpret the data. The candidate wrote the manuscript with the guidance of the co-authors, and modified it in response to their feedback.

April Kinghorn

I, the co-author, allow the candidate, April Kinghorn, to include the manuscript entitled *Teeth as biomonitors of selenium concentrations in tissues of beluga whales (Delphinapterus leucas)* in her thesis.

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Laurie H.M. Chan

July 4th, 2006

To Whom It May Concern:

This letter confirms that the co-author (Laurie H.M. Chan) agrees that the candidate (April Kinghorn) includes in her thesis the manuscript entitled *Reconstructing Historical Mercury Exposure from Beluga Whale Consumption among Inuit in the Mackenzie Delta*.

.

The candidate's role in the study was to develop the research methods, and to manage, analyze and interpret the data. The candidate wrote the manuscript with the guidance of the co-authors, and modified it in response to their feedback.

April Kinghorn

I, the co-author, allow the candidate, April Kinghorn, to include the manuscript entitled *Reconstructing Historical Mercury Exposure from Beluga Whale Consumption among Inuit in the Mackenzie Delta* in her thesis.

____

Laurie H.M. Chan

July 6th, 2006

To Whom It May Concern:

This letter confirms that the co-author (Murray Humphries) agrees that the candidate (April Kinghorn) includes in her thesis the manuscript entitled *Teeth as biomonitors of selenium concentrations in tissues of beluga whales (Delphinapterus leucas)*.

The candidate's role in the study was to develop the research methods, measure Se in all tooth samples, and to manage, analyze and interpret the data. The candidate wrote the manuscript with the guidance of the co-authors, and modified it in response to their feedback.

April Kinghorn

I, the co-author, allow the candidate, April Kinghorn, to include the manuscript entitled *Teeth as biomonitors of selenium concentrations in tissues of beluga whales (Delphinapterus leucas)* in her thesis.

____

Murray Humphries

July 6th, 2006

To Whom It May Concern:

This letter confirms that the co-author (Murray Humphries) agrees that the candidate (April Kinghorn) includes in her thesis the manuscript entitled *Reconstructing Historical Mercury Exposure from Beluga Whale Consumption among Inuit in the Mackenzie Delta*.

The candidate's role in the study was to develop the research methods, and to manage, analyze and interpret the data. The candidate wrote the manuscript with the guidance of the co-authors, and modified it in response to their feedback.

April Kinghorn

I, the co-author, allow the candidate, April Kinghorn, to include the manuscript entitled *Reconstructing Historical Mercury Exposure from Beluga Whale Consumption among Inuit in the Mackenzie Delta* in her thesis.

____

Murray Humphries

June 19, 2006

To Whom It May Concern:

This letter confirms that the co-author (Peter Outridge) agrees that the candidate (April Kinghorn) includes in her thesis the manuscript entitled *Selenium in the teeth and soft tissues of beluga whales (Delphinapterus leucas)*.

The candidate's role in the study was to develop the research methods, measure Se in all tooth samples, and to manage, analyze and interpret the data. The candidate wrote the manuscript with the guidance of the co-authors, and modified it in response to their feedback.

April Kinghorn

I, the co-author, allow the candidate, April Kinghorn, to include the manuscript entitled *Selenium in the teeth and soft tissues of beluga whales (Delphinapterus leucas)* in her thesis.

Peter Outridge

June 15, 2006

To Whom It May Concern:

This letter confirms that the co-author (Peter Outridge) agrees that the candidate (April Kinghorn) includes in her thesis the manuscript entitled *Reconstructing Historical Mercury Exposure from Beluga Whale Consumption among Inuit in the Mackenzie Delta*.

The candidate's role in the study was to develop the research methods, and to manage, analyze and interpret the data. The candidate wrote the manuscript with the guidance of the co-authors, and modified it in response to their feedback.

April Kinghorn

I, the co-author, allow the candidate, April Kinghorn, to include the manuscript entitled *Reconstructing Historical Mercury Exposure from Beluga Whale Consumption among Inuit in the Mackenzie Delta* in her thesis.

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Peter Outridge