BONE AS A BIOMARKER OF MERCURY EXPOSURE IN PREHISTORIC ARCTIC HUMAN POPULATIONS: INITIAL METHOD VALIDATION USING ANIMAL MODELS

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ABSTRACT

Marine mammals are dietary staples among many indigenous peoples of the Arctic, but these foods sometimes contain high levels of mercury, a toxic heavy metal that can cause nerve and brain damage. Because mercury can be released into the environment by both industrial and natural processes, prehistoric marine mammal consumers may have been exposed to this toxicant, but little is known about preindustrial mercury levels. This research examined the potential for using the mercury concentration of archaeological bone as a biomarker of mercury exposure. Two requirements of valid biomarkers of exposure were explored: 1) measurement accuracy (trueness and precision) and 2) correspondence with the extent of exposure. Measurement accuracy was evaluated using repeated determinations of mercury concentration in a sample of modern seal bones. Correspondence with exposure was examined by comparing bone mercury concentration to controlled exposure level in laboratory rats, and to the stable nitrogen isotope ratio ($\delta^{15}N$) (a proxy measure of exposure) in prehistoric ringed seals from Thule-period archaeological sites in Alaska. Results show that mercury measurements have acceptable accuracy and that bone mercury is strongly related to exposure. These promising results suggest that, with further validation on human subjects, bone mercury may provide a reliable archive of mercury exposure in preindustrial archaeological populations.

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1. INTRODUCTION

Marine mammals are dietary staples among many coastal populations of the world, but these traditional foods often contain high levels of mercury, a toxic heavy metal that can cause nerve and brain damage (EPA, 1997c:11; National Research Council, 2000; WHO, 1990). People who consume these traditional resources can accumulate mercury in their tissues to levels that are considered unsafe (Burkow and Weber, 2003; EPA, 1997a; Van Oostdam and Tremblay, 2003). Today, some of the highest documented tissue mercury levels occur in the coastal Inuit of arctic North America and Greenland, where diets are rich in marine foods (AMAP, 2003a; Deutch, 2003). Mercury-related decreases in cognitive function may have grave societal repercussions, including economic costs associated with diminished productivity (Grandjean and Perez, 2008; Trasande et al., 2006)

The ultimate source of mercury in marine foods is unclear, because mercury can be released into the environment by both industrial activities, such as coal burning, and by natural processes, such as volcanic eruptions (AMAP, 2002; Pyle and Mather, 2003). After mercury is released into the atmosphere, it can travel thousand of kilometers through the hemisphere and deposit onto the surface far from where it originated. Some of the deposited mercury settles in marine sediments, where it can be taken up by organisms and enter the marine food chain (Clarkson and Magos, 2006). Once in the food web, mercury biomagnifies, so that animals at the apex of long food chains, such as marine mammals, have mercury levels many times over those at the base.

The same pathway that operates today to cause high mercury levels in arctic marine animals and in the people who consume them likely operated in the preindustrial past, since mercury can be emitted to the atmosphere through natural processes. In this way, preindustrial arctic Eskimo and Inuit who relied heavily on marine foods may have been exposed to relatively high levels of mercury from their diet, just as their modern counterparts are today. Naturally-released mercury is indistinguishable from industrially-released mercury (Wiener et al., 2003) and therefore may have the same toxic properties

While preindustrial levels of mercury in the atmosphere have been studied through sediment and ice core deposits (Biester et al., 2007; Givelet et al., 2004; Schuster et al., 2002), little information is available on preindustrial mercury levels in food webs. A potential archive of such information is the skeletal remains of animals and humans recovered from archaeological sites. Levels of mercury in preserved bone could serve as an index of preindustrial exposure, if bone can be shown to be a valid biomarker of mercury exposure.

Biomarkers of exposure characterize exposure to a substance based on its concentration in a biological tissue (National Research Council, 2006). For mercury, typical biomarkers of exposure in living humans and mammals include blood and hair, for which the relationships between mercury dose and tissue concentrations have been well established (National Research Council, 2000). Ancient human and animal hair samples have been analyzed for mercury content, but preserved hair is rare in

archaeological sites (Aufderheide, 2003). Bones are more commonly preserved, and mercury has been detected in human and animal bone, including archaeological bone, with published mean total mercury concentrations ranging from the parts per billion to parts per million range (Baranowski et al., 2002; Yamada et al., 1995). However, bone concentrations of an element do not always reflect dietary intake levels, because elements ingested by humans and animals have complex pathways of absorption, movement, incorporation into tissues, and excretion (Ezzo, 1994). Bone mercury concentration must be validated as a biomarker of exposure before it can be applied to human skeletal samples to reconstruct mercury exposure in the past.

This dissertation research investigated the potential for using mercury concentration in archaeologically-recovered bone as a biomarker of mercury exposure in preindustrial animals and humans. The ultimate research goal is to find a convenient, reliable indicator of past mercury exposure in order to establish the natural baseline level of human exposure, to track changes in exposure levels through time, and to reconstruct the health consequences of mercury exposure in past populations. While mercury has been measured in bone, including prehistoric bone, the mere presence of a substance in a tissue is not enough to ensure that its concentration reflects exposure level. A candidate biomarker must be validated before being used to assess exposure in individuals and populations, be it in modern or prehistoric populations. For a biomarker of exposure to be valid, it must meet two criteria: 1) the analytical method used to measure the biomarker must produce accurate results (analytical validity) and 2) the biomarker must correspond with the extent of exposure (intrinsic validity) (Lee et al., 2006; WHO,

2001). Since mercury exposure in humans and animals is almost entirely through diet, a biomarker of mercury exposure must reflect dietary mercury intake. Given the requirements for a valid biomarker of mercury exposure, this dissertation was guided by two central research questions:

1. Can bone mercury be accurately measured?

2. Does bone mercury level reflect dietary intake level?

Bone mercury measurement accuracy was assessed in terms of its two components: trueness, which refers to how close measurement results are to accepted values, and precision, which refers to how close repeated measurements are to each other (Thompson et al., 2002). A series of bone samples was analyzed for mercury concentration using Cold Vapor Atomic Fluorescence Spectrometry (CVAFS), which is a well established method for measuring mercury in many biological tissues (Jones et al., 1995), but which had not been tested on bone. Trueness and precision were calculated from the resulting bone mercury concentration measurements, and these were compared to internationally recognized standards for chemical measurement accuracy. The sample used to assess measurement accuracy included bones of modern marine mammals from western Alaska that had been collected by a state game management agency.

The intrinsic validity of a biomarker, or the correspondence of the biomarker to the extent of exposure, was examined in two ways. First, bone mercury concentrations were compared to known, controlled exposure levels in mercury-dosed laboratory rats. If

bone mercury reflects dietary mercury intake, then bone mercury concentration should increase as daily exposure increases. Second, mercury level in a sample of prehistoric marine mammal bone was compared to the stable nitrogen isotope ratio, used here as a proxy measure of mercury exposure, since it tends to increase with trophic position. If bone mercury concentration faithfully tracks dietary intake level, then bone mercury concentration should increase as the stable nitrogen isotope ratio increases.

Research area

As a method validation study, much of this dissertation research transcended any particular geographic area. Of necessity, it relied heavily on controlled experiments using laboratory animals. At the same time, this study extended beyond the laboratory and into natural populations, and here the focus was on the Arctic. The coastal areas of arctic North America and Greenland offer an obvious location for the study of mercury exposure, since indigenous populations here, the Yupik and Inupiaq Eskimo of Alaska and the Inuit of Canada and Greenland (Figure 1.1), have some of the highest exposure levels in the world due to reliance on sea mammals in the diet.

While the Arctic may be defined in many ways, it will here be defined as that area of the circumpolar north that is beyond tree line (see Figure 1.2). This treeless tundra landscape is remarkable for its low productivity, with a vegetation consisting largely of a mat of mosses, lichens, and low bushes that supports few large terrestrial mammals (Moran, 1982; Weddell, 2002). The productivity of the oceans, however, is much greater (Weddell, 2002). Though ice-covered for much of the year, the sea supports an

abundance of large-bodied and easily-storable mammals, including a variety of seals, walrus, and whales (Freeman, 1988). Not surprisingly, marine mammals have been dietary mainstays of the Eskimo and Inuit from prehistoric through modern times. Archaeological sites throughout arctic North America and Greenland are rich with the remains of these animals, and occasionally of the humans who hunted them, affording a unique opportunity to study mercury exposure through time. In this initial method validation study, the focus is on the archaeologically-derived remains of animals that formed the diet of the prehistoric coastal Eskimo of Alaska.

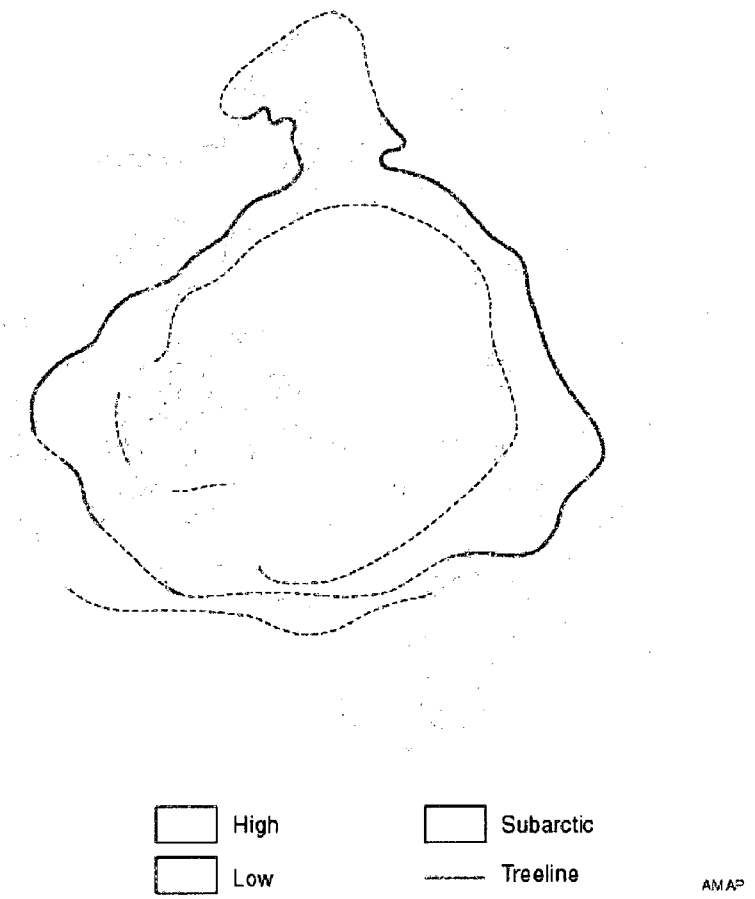

Figure 1.1. Arctic boundary as defined by treeline. Reprinted from AMAP (1998).

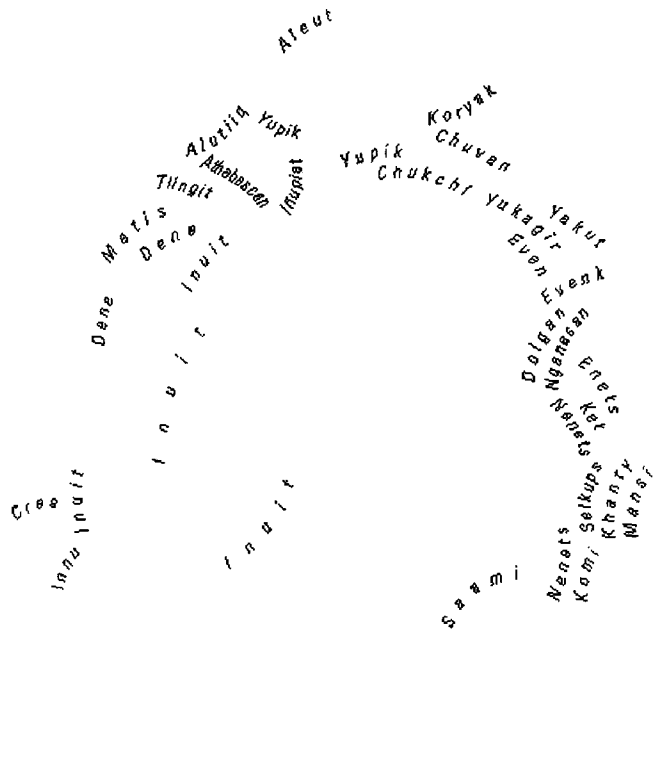

Figure 1.2. Cultural map of the Arctic showing the area inhabited by the Alaskan Eskimo (Yupik and Inupiat) and the Canadian and Greenlandic Inuit. Reprinted from AMAP (1998).

Organization of Dissertation

The remainder of this dissertation is organized as follows. Chapter Two, the Research Background, includes a review of the literature on diet and mercury exposure in modern Eskimo and Inuit populations and in their prehistoric ancestors, the Thule. This is followed by a discussion of previous attempts to reconstruct prehistoric mercury exposure using preserved hair as a biomarker, and the potential advantages and limitations of using archaeological bone as a biomarker of prehistoric exposure. The bioarchaeological applications of analyzing trace elements and toxic metals in human skeletal remains are also summarized. Finally, methods of validating candidate biomarkers are examined.

Chapter Three, Research Materials and Methods, describes the research design, specific research questions addressed, and materials used in the study. The data collection and data analysis procedures are also detailed, including the statistical tests used and the formulation of specific statistical hypotheses.

Chapter Four, Results, presents descriptive and inferential statistics for the variables studied in each of the samples. The outcomes of specific statistical hypotheses tested are also explained.

Chapter Five, Discussion, compares the results of this study to previous findings and to expected results. Alternative explanations for the findings are considered.

Chapter Six, Summary and Conclusions, explores the results of this study in the context of the major research aims and questions. It discusses the broader implications of the research and makes suggestions for future studies on bone as a biomarker of ancient mercury exposure.

Appendix A includes a list of abbreviations used in this dissertation.

2. RESEARCH BACKGROUND

Mercury toxicity

Mercury is a heavy metal that exists naturally in many forms, including elemental, inorganic, and organic mercury (ATSDR, 1999b). All forms of mercury are toxic, but the organic form methylmercury (MeHg) is of the greatest health concern because it is easily taken up by organisms, including humans, through foodstuffs (EPA, 1997a). The primary target for methylmercury damage is the central nervous system (Clarkson and Magos, 2006).

Adults exposed to high levels of methylmercury in accidental large-scale poisoning incidents in Iraq and Japan in the 1960s and 1970s suffered a number of central nervous system effects depending on degree of exposure, from parasthesia (numbness in the fingers and toes), to loss of motor coordination, deafness, blindness, and finally, coma and death (Clarkson and Magos, 2006; FDA, 1994). Analysis of these accidental poisonings suggests that the threshold for the mildest clinical symptoms in adults (parasthesia) occurs at a hair total mercury concentration of around 100 ppm, which corresponds to a blood total mercury concentration of around 400 ppb (Clarkson and Magos, 2006; WHO, 1990:section 10.3). Blood and hair mercury concentrations in this range can be achieved with a daily ingestion of around 10 micrograms (μg) of methylmercury per kilogram of body weight (WHO, 1990:section 10.2.1). Death occurs at blood levels over 2000 ppb (WHO, 1976:section 6.6; WHO, 1990:section 9.4.1.2).

Methylmercury is especially toxic prenatally, when the brain is developing rapidly (Myers and Davidson, 2000). Infants born to mothers who accidentally ingested methylmercury showed central nervous system impairments, including delays in speech and motor development, mental retardation, cerebral palsy, reflex abnormalities, and seizures (EPA, 1997c; Myers and Davidson, 2000). Estimates of the threshold for delayed walking in infants exposed in utero are as low as 10 ppm maternal hair total mercury (40 ppb blood total mercury) (Clarkson and Magos, 2006).

Detrimental effects have also been seen in children who were exposed prenatally to methylmercury through maternal consumption of fish and marine mammals. Major longitudinal studies on the effects of fetal methylmercury exposure have been conducted in two seafood-eating populations, the inhabitants of the Faroe Islands (located in the North Atlantic between Scotland and Iceland) and the Seychelle Islands (located in the Indian Ocean northeast of Madagascar), but their conclusions are inconsistent. The Faroe Islands study found an adverse association between prenatal methylmercury exposure and performance on neurodevelopmental tests, especially on tests of attention, fine-motor function, language, visual-spatial abilities, and verbal memory (Myers and Davidson, 2000; National Research Council, 2000). However, in the Seychelles study, which used a different battery of tests, no neurodevelopmental deficits were observed (Myers and Davidson, 2000; National Research Council, 2000).

Based on the Faroe Islands study, the U.S. Environmental Protection Agency (EPA) (2001c) concluded that the methylmercury benchmark dose—the dose at which there is

moderate increase in the frequency of adverse effects over the background rate—is 85 ppb maternal blood mercury. The lower 95% confidence limit on this dose, the "benchmark dose lower limit" or BMDL, is 58 ppb maternal blood mercury. The benchmark dose lower limit is assumed to represent a dose level at which no adverse effects are seen (Clarkson and Magos, 2006). A 58 ppb blood mercury concentration can be achieved with a steady daily ingestion of around 1 microgram of methylmercury per kilogram of body weight. The EPA applied an uncertainty factor of 10 to this dose to arrive at its "reference dose" (RfD)—a dose that is "likely to be without an appreciable risk of deleterious health effects during a lifetime"—of 0.10 μg/kg bw/day for methylmercury (EPA, 2001c:4-1).

As seen in Table 2.1, the World Health Organization's recommended tolerable intake is more liberal, at 0.23 μg/kg bw/day for methylmercury (WHO, 2004). Variations between the EPA and WHO in acceptable levels of methylmercury exposure are due to reliance on different studies, different points of departure, and different levels of uncertainty (Myers and Davidson, 2000; National Research Council, 2000). Table 2.1 also shows the blood and hair mercury levels that correspond to the intake levels. Note that the allowable intake refers specifically to *methylmercury*, while the tissue concentrations are measured as *total* mercury.

Table 2.1. Guidelines from EPA and WHO for tolerable levels of methylmercury intake and corresponding blood and hair levels

Agency	Guideline	Allowable MeHg intake (μg/kg bw/day)	Associated blood THg (ppb)	Associated hair THg (ppm)	Reference
EPA[a]	Reference Dose	0.10	5.80	1.34	(EPA, 2001c)
WHO[b]	Provisional Tolerable Daily Intake	0.23	8.76	2.19	(WHO, 2004)

[a]EPA associated blood THg is the EPA benchmark dose lower limit (58 ppb blood THg), divided by the EPA uncertainty factor of 10 (EPA, 2001c:4-61). EPA associated hair THg was converted from blood level using a hair-to-blood ratio of 250:1 (EPA, 2001c:4-77).

[b]WHO daily intake guideline has been converted from the Provisional Tolerable Weekly Intake (WHO, 2000; WHO, 2004). WHO associated hair THg is the WHO benchmark dose lower limit (14 ppm hair THg) divided by the WHO uncertainty factor of 6.4 (WHO, 2004:sections 4.3 and 5). WHO associated blood THg was converted from the hair level assuming a hair-to-blood ratio of 250:1 (WHO, 2004:section 4.3).

Pathways of methylmercury exposure

Today, human exposure to methylmercury is almost exclusively through consumption of fish and marine mammals, and individuals who rely on these foods can accumulate mercury in their tissues to levels that may be unsafe (Burkow and Weber, 2003; Clarkson and Magos, 2006). Methylmercury occurs in high levels in marine mammals and predatory fish due to a complex pathway of transport, transformation, uptake, and magnification. It begins when inorganic mercury is released into the atmosphere by either industrial activities, such as coal burning, or by natural processes, such as volcanic eruptions (AMAP, 2002; Pyle and Mather, 2003). Mercury in the atmosphere can travel thousands of kilometers throughout the hemisphere. Some of the inorganic mercury in the atmosphere is deposited onto the surface, settles in marine sediments, and is converted to methylmercury by microorganisms (Clarkson and Magos, 2006).

Once methylated, mercury enters the marine food chain and becomes concentrated (biomagnifies) as it moves up the chain. Because ocean food chains are often long, animals at the apex have mercury levels many times over those at the base, and their tissue mercury levels can exceed those considered safe for humans to eat (AMAP, 2002; FDA, 1994). A similar mercury pathway occurs in freshwater systems, where predatory fish can accumulate high levels of mercury (Power et al., 2002). However, freshwater systems will not be emphasized in this dissertation, because of the dearth of fish remains from prehistoric archaeological sites on the arctic tundra (as reviewed by Whitridge,

2001). The same pathway that operates today to cause high mercury levels in marine animals likely operated in the preindustrial past, since mercury can be emitted to the atmosphere through natural processes. In this way, preindustrial seafood consumers may well have been exposed to relatively high levels of mercury from their diet, just as their modern counterparts are today.

Mercury exposure in modern populations

Some of the highest documented exposures to methylmercury occur in the coastal Inuit populations of arctic Greenland and Canada, where diets are rich in marine foods (AMAP, 2003a; Deutch, 2003). Dietary surveys suggest that the Inuit of Baffin Island have an average total mercury intake of 1.03 μg/kg bw/day (Kuhnlein et al., 2000), which is several times higher than the EPA and WHO limits for methylmercury intake of 0.10 and 0.23 μg/kg bw/day (see Table 2.1). In this population, ringed seals are the greatest contributor to mercury intake. In adult Inuit of arctic Quebec, blood total mercury levels average 21.3–23.7 ppb (Dewailly et al., 2001), far exceeding the WHO and EPA safety guidelines of 5.8 ppb and 8.7 ppb, respectively. Here, blood mercury concentration is directly correlated with frequency of seal and beluga whale consumption. Similar high exposures are seen for Inuit adults in southwestern Greenland, where dietary surveys suggest an average total mercury consumption of 1.33 μg/kg bw/day, with seal, especially ringed seal, and whales contributing most to the intake (Deutch, 2003). Average blood total mercury concentrations for Inuit men in three regions of Greenland are extremely high, ranging from 14.2 ppb in the central

eastern region to 52.0 in the central western region, and total blood mercury appears to be associated with the frequency of seal consumption (Deutch, 2003).

Studies that assess mercury exposure from traditional foods are lacking for Alaska. However, dietary surveys and subsistence harvest data suggest that the coastal Eskimo of Alaska consume substantial amounts of marine mammals, including seals and whales (Ballew et al., 2006; Ballew et al., 2004; Conger and Magdanz, 1990). Preliminary results from a study by the Alaska Native Tribal Health Consortium show mean blood total mercury concentrations of 1.5 ppb for a sample of 29 women in Barrow (presumably Inupiaq Eskimo) and 6.5 ppb for 52 women from Bethel (presumably Yupik Eskimo) (as reported in Arnold and Middaugh, 2004). The latter concentration exceeds the EPA guideline for "safe" mercury intake. No corresponding dietary information is available for these subjects.

Ringed seal consumption and mercury exposure

In modern Inuit populations, ringed seal (*Phoca hispida*), is a common source of dietary mercury exposure, as detailed in previous sections. This small ice seal has a circumpolar distribution and is dependent on sea ice for feeding, resting, and breeding (Jefferson, 1993; Quakenbush and Sheffield, 2006). An adult ringed seal typically weighs between 50 to 110 kg (110–240 lb) (Jefferson, 1993). As a fish-eater, the ringed seal occupies a position near the top of the marine food chain, and the relatively high levels of mercury in its tissue reflect this. Table 2.2 shows reported levels of total mercury in muscle of ringed seals from Alaska, Canada, and Greenland.

Ringed seal do not have the highest levels of tissue mercury among arctic marine mammals, as even higher levels are found in the toothed whales (e.g., beluga whale and narwhal), which often have average muscle mercury levels above 1.0 ppm (AMAP, 2005). But, in many areas of the Arctic, ringed seal is the most frequently consumed marine mammal in coastal populations. To put ringed seal mercury levels into perspective, Table 2.3 shows the mean mercury levels for selected traditional foods for the Inuit of west Greenland. Here, plants and terrestrial animals have low mercury concentrations (<0.015 ppm), as do marine invertebrates. Most marine fish have levels below 0.10 ppm, while the marine mammals listed here—all fish-eating species—have the highest levels.

Table 2.2. Mean total mercury concentration in muscle of ringed seal from Alaska, Canada, and Greenland

Location	Mean muscle THg µg/g (ppm) (wet weight)	n	Reference
Barrow Alaska USA	0.10	59	Dehn et al. (2005)
Ungava Bay Nunavut Canada	0.25	13	NCP (2003)
Hudson Strait Nunavut Canada	0.18	22	NCP (2003)
Nain/Makkovik Labrador Canada	0.33	28	NCP (2003)
West Greenland	0.22	>20	NCP (2003)

Table 2.3. Mean total mercury levels in selected traditional Inuit foods in west Greenland

Food Item	Scientific name	Tissue	n	Mean THg µg/g (ppm)[a]
Terrestrial				
Plants				
Blueberry	*Vaccinium uliginosum*	Berry	5	<0.001
Mammals				
Caribou	*Rangifer tarandus*	Muscle	>100	0.014
Musk ox	*Ovibos moschatus*	Muscle	9	0.002
Marine				
Invertebrates				
Blue mussel	*Mytilus edulis*	Soft parts	15	0.001
Fish				
Arctic char	*Salvelinus alpinus*	Muscle	72	0.043
Atlantic salmon	*Salmo salar*	Muscle	20	0.040
Atlantic cod	*Gadus morhua*	Muscle	9	0.014
Halibut	*Reinhardtius hippoglossoides*	Muscle	>5	0.154
Mammals				
Ringed seal	*Phoca hispida*	Muscle	>20	0.221
Harp seal	*Pagophilus groenlandicus*	Muscle	>10	0.210
Beluga whale	*Delphinapterus leucas*	Muscle	>10	0.790

[a] All values are from Johansen et al. (2003).

The amount of ringed seal muscle that would have to be consumed to exceed the various safety and health outcome thresholds can be calculated for each person based on body weight. For example, to meet the EPA (2001c) allowable mercury intake (the RfD) of 0.1 μg/kg bw/day, a 60 kg person would have to ingest mercury at a level of 6.0 μg/day, which could be accomplished by eating 6 g/day of a food item with a mercury concentration of 1.0 μg/g. For thresholds that are expressed in terms of blood mercury levels, the corresponding daily dose must be calculated using the EPA (2001c:4-77) dose conversion formula:

Daily methylmercury intake = (blood THg *x* b *x* V)/ (A *x* f *x* bw)

where:

daily methylmercury intake is expressed as μg MeHg/kg bw/day

blood THg is expressed as μg/L (ppb)

b = the elimination constant (= 0.014 $days^{-1}$)

V = the volume of blood in the body (= 5 L for a 65 kg woman)

A = the absorption factor (= 0.95, unitless)

f = the fraction of daily intake taken up by blood (= 0.059)

bw = body weight in kg

Table 2.4 shows the intake of ringed seal muscle required for a 60 kg (132 lb) person to meet selected health thresholds, assuming that the seal has an average mercury concentration of 0.2 ppm. For foods with this concentration of mercury, a person weighing 60 kg will exceed the EPA Reference Dose by ingesting only 30 g (1.0 oz) of the food per day. Daily consumption of 480 g (17 oz) of such a food will exceed the EPA benchmark dose, the dose that corresponds to a 5% increase over background rates in the incidence of adverse effects in prenatally exposed children. The intake amount required to meet the threshold for observable clinical symptoms in adults is 3000 g (106 oz) per day.

Table 2.4. Calculated daily intake of a food with 0.2 ppm mercury required to meet selected threshold doses associated with health outcomes

Threshold Description	Threshold daily dose (µg/kg bw/day)	Corresponding mercury intake (µg/day) for 60 kg person	Corresponding food intake (g/day) for a food with 0.2 µg/g mercury
EPA Reference Dose (EPA, 2001c)	0.1	6	30 g (1 oz)
EPA Benchmark Dose[a] (EPA, 2001c)	1.6	96	480 g (17 oz)
Parasthesia in adults (WHO, 1990:section 10.2.1)	10	600	3000 g (106 oz)

[a] EPA benchmark "dose" is expressed as a blood mercury level of 85 ppb; it was converted to a daily mercury dose using the EPA (2001c:4-77) dose conversion formula (see text for further explanation).

Ringed seal in the historic Eskimo and Inuit diet

Written accounts of Eskimo and Inuit life during the historic period often contain general descriptions of hunting activities and the major animal resources used, but rarely provide quantitative data on food consumption. Nevertheless, these early accounts reveal that marine mammals, particularly seals, were key to the Eskimo and Inuit diet. For instance, John Murdoch (1892:61), naturalist for the International Polar Expedition to Point Barrow (1881-1883) wrote of the Eskimo diet that "the staple article of food is the flesh of the [ringed] seal, of which they obtain more than of any other meat." In this passage from Boas (1888:419) describing the Central Inuit of Canada (i.e., the Caribou, Copper, Netsilik, Iglulik, Baffin Island, Labrador, and east Hudson Bay Inuit) during the late 19th century, seals are seen as critical to survival:

> As the inhospitable country does not produce vegetation to an extent sufficient to sustain life in its human inhabitants, they are forced to depend entirely upon animal food. In Arctic America the abundance of seals found in all parts of the sea enables man to withstand the inclemency of the weather and the sterility of the soil.

Writing of the Greenlandic Inuit in the late 19th century, Dr. H. J. Rink, a geographer-naturalist and later Royal Inspector of southern Greenland for the Danish government, observed that "the sustenance of the Eskimo is entirely derived form the capture of seals and cetaceous animals" (Rink, 1875:6). Rink provides the only quantitative information on the historic period Inuit diet. He conducted an annual food

consumption survey among the Inuit of southwestern Greenland in 1855. Based on Rink's survey, Sinclair (1953) estimated that the typical daily diet of an adult Inuit included 3359 calories and contained 63% protein (nearly all animal protein), 27% fat, and 10% carbohydrate by weight. In terms of specific foodstuffs, the diet was predominantly seal flesh, both in terms of weight (860 g/2175 g = 40%) and calories (1686 Cal. /3359 Cal. = 50%) (Table 2.5). The next most important food item was capelin (a small salmonid fish), with other fish and mammals making up most of the remainder.

Some researchers have used interviews with 20th century Eskimo elders, supplemented by historical documents, to reconstruct the traditional diet. Using this method, Ray (1964) determined that there were three traditional subsistence patterns among the 19th century Inuit around Bering Strait: 1) the whaling pattern, focused on bowhead whales, walrus, and seals; 2) the caribou hunting pattern, focused on caribou, fish, and some sea mammals; and 3) the small sea mammals pattern, focused on seals and beluga. At the same time, Ray (1964:61) noted that "all of the inhabitants of the area, however, depended on seals and fish for basic foods." Based on interviews with Inupiaq elders in the Point Hope region in the late 1950s, Foote (1965:286) estimated that in the mid-nineteenth century, the Point Hope Eskimo derived most of their winter food from seals (40%), followed by bowhead whale (35%), and walrus (10%).

Table 2.5. Southwestern Greenlandic Inuit daily diet ca. 1855 AD as reconstructed by Sinclair (1953)

Food Item	Amount consumed (g)	Weight %	Calories consumed	Calorie %
Seal flesh	860	40	1686	50
Capelin (salmonid)	620	29	645	19
Other fish	370	17	444	13
Other flesh	225	10	441	13
Berries	50	2	14	1
Eggs	5	<1	8	<1
Imported foods	45	2	121	4
Total	2175	100%	3359	100%

Ringed seal in the prehistoric Eskimo/Inuit (Thule) diet

Archaeologists widely agree that the modern Eskimo and Inuit of Alaska, Canada and Greenland are direct biological and cultural descendents of the prehistoric Thule (Ackerman, 2001; Dumond, 1987a; Giddings and Anderson, 1986; Hayes et al., 2005; Maxwell, 1980; Maxwell, 1985; Morrison, 1994; Morrison, 1999). This archaeological culture emerged around the beginning of the Christian era in the islands of Bering Strait, and was established on mainland Alaska by around 500 AD (Dumond, 1987a; Dumond, 1987b). For decades, conventional wisdom held that the Alaskan Thule began to spread east around 1000 AD, reaching Greenland within two or three hundred (Maxwell, 1980:171). Newer analyses of radiocarbon dates suggest that the initial migration may have begun centuries later, around 1200 AD (Friesen and Arnold, 2008; McGhee, 2000), although Morrison (2001:82) suggests that this view is "too extreme," and that the Thule reached the eastern Canadian Arctic "within a generation or two" of 1000 AD. In terms of cultural remains, the Thule Tradition is characterized by large coastal settlements with multi-roomed houses, kayaks and umiaks, ground-slate tools, grit-tempered pottery, toggling harpoon heads, and seal oil lamps (Ackerman, 2001; Dumond, 1987a; Giddings and Anderson, 1986; Mason, 1992; Maxwell, 1980; Maxwell, 1985; Morrison, 1994; Morrison, 1999).

Faunal remains from Thule sites suggest that a variety of sea mammals were harvested, including seals, walrus, beluga whales, and bowhead whales, in addition to terrestrial mammals, such as caribou and musk ox (Morrison, 1994; Whitridge, 2001;

Yorga, 1979). Whitridge (2001) analyzed faunal data from 43 prehistoric Thule sites across Canada and estimated the dietary contribution of various categories of vertebrates (sea mammals, land mammals, fish, and birds) based on the number of individual specimens (NISP) combined with usable meat weights of each species. Whitridge's calculations suggest that sea mammals made up an extraordinary 90% or more of the diet at nearly half of these sites, and between 60% and 90% of the diet at an additional one-quarter of the sites.

Whitridge (2001) does not specify which species made up the marine mammal portion of the Thule diet. Some archaeologists attribute the fluorescence of Thule to a new subsistence focus on whale hunting, especially the large bowhead whale (Harritt, 1994). Indeed, whaling has been considered synonymous with Thule since the earliest description of this prehistoric culture by Mathiassen (1927). The spread of Thule eastward coincides with a period of climatic warming (the Medieval Warm Period, ca. 900–1300 AD) (Mann, 2002) that may have extended the range of bowhead whales (Dumond, 1987a; Harritt, 1994; Morrison, 1999). Others argue that whales have been overplayed as central to Thule subsistence (Freeman, 1979).

Park (1999:82) notes that while Thule in some areas hunted the enormous bowhead whale or the smaller beluga whale and narwhal, "seal remains represent the most abundant finds by far in the faunal assemblages." Furthermore, he contends that of the three seal species routinely hunted—ringed, bearded, and harp—the ringed seal was the most important because their use of breathing holes in the winter sea ice made them

available year-round. The importance of ringed seals to the prehistoric inhabitants of the Arctic is echoed by Murray (2008:S50), who notes that along the coastal areas of arctic Alaska, Canada, and Greenland, "ringed seal remains are ubiquitous in archaeological deposits, regardless of cultural affiliation, region, or time period."

The importance of seals to the Thule diet also finds support from stable isotope studies of skeletal remains from Thule sites. Coltrain et al. (2004b) measured carbon and nitrogen stable isotope ratios on both human and animal remains from three archaeological sites in the Hudson Bay region. Using simple linear mixing models, they calculated that seals made up between 47% and 87% of the prehistoric Thule diet in this area. It should be noted, however, that this analysis was constrained to consider only three food items (the researchers selected seals, caribou, and whales), and that modeled values (versus actual measured values) were used for caribou nitrogen isotope ratios.

Mercury levels in humans and animals during Thule times

Mercury concentrations have been measured in small samples of archaeologically preserved human and animal hair from Thule sites in Alaska, Canada, and Greenland (Table 2.6). The mean values for the Alaskan and Greenlandic Thule hair are both around 3.0 ppm total mercury. Modern hair mercury levels are not available for the same regions of Alaska and Greenland, but blood total mercury levels have been published, and they can be converted to hair levels using the hair-to-blood ratio of 250 (EPA, 2001c). Conversion of the mean blood mercury concentration for a sample of 29 modern Barrow Inupiaq Eskimo women reported in Arnold and Middaugh (2004) gives a calculated mean hair total mercury concentration of 0.3 ppm, which is *lower* than the Barrow Thule average. In contrast, the calculated mean hair total mercury level is 13.0 ppm for a group of 48 adult Inuit men from the Uummanaq region of Greenland (based on blood levels from Deutch, 2003), which is much higher than the Thule-period sample from the same region. The Canadian Thule hair sample has an average methylmercury concentration of 1.3 ppm, while the mean for modern Inuit in Nunavut/Northwest Territories is 8.0 ppm (Wheatley and Paradis, 1998; Wheatley and Wheatley, 1988).

Animal hair from Thule sites has also been analyzed for mercury (Table 2.7). Caribou hair from the Deering site in northwest Alaska has a mean total mercury level of less than 0.1 ppm, which is similar to modern caribou hair from the same region (Duffy et al., 2005; Gerlach et al., 2006). Polar bear hair from the Nunguvik site on Baffin

Island, Canada, has a mean methylmercury concentration of 3.0 ppm, compared to 6.6 ppm for modern polar bears (Wheatley and Wheatley, 1988).

Mercury concentrations have been measured in tooth cementum from beluga whale and walrus recovered from Thule sites in arctic Canada (Table 2.8). Cementum levels are lower than hair levels and are expressed in the parts per billion (ppb) range. The ancient beluga show a lower mean total mercury concentration (5.0 ppb) in tooth cementum compared to a modern sample (98.4 ppb), but the Thule period and modern walrus samples have nearly identical mean total mercury concentrations (around 1.0 ppb) (Outridge, 2005; Outridge et al., 2002).

Table 2.6. Mercury concentrations in preserved human hair from Thule-period archaeological sites

Site/Region	Date	Mean THg µg/g (ppm)	Mean MeHg µg/g (ppm)	n	Reference
Utqiagvik Alaska USA	ca. 1500–1850 AD	3.0	NA	2	(Newell, 1984; Toribara and Muhs, 1984)
Nunguvik Baffin Island CAN	ca. 1150 AD	NA	1.3	5	(Wheatley and Wheatley, 1988)
Qilakitsoq Greenland	ca. 1401–1500 AD	3.2	NA	6	(Hansen et al., 1989)

Table 2.7. Mercury concentrations in preserved animal hair from Thule-period archaeological sites

Species	Site/Region	Date	Mean THg µg/g (ppm)	Mean MeHg µg/g (ppm)	n	Reference
Polar Bear	Nunguvik Baffin Island CAN	ca 1000-1500 AD	NA	3.0	3	(Wheatley and Wheatley, 1988)
Caribou	Deering Alaska USA	ca. 1035-1270 AD	<0.1	NA	37	(Gerlach et al., 2006)

Table 2.8. Total mercury concentrations in Thule period marine mammal tooth cementum.

Species	Site/Region	Date	Mean THg ng/g (ppb)	n	Reference
Beluga	Gupuk NW Territories CAN	ca. 1450-1650 AD	5.0	28	(Outridge et al., 2002)
Walrus	Igloolik, Nunavut CAN	ca. 1200-1500 AD	1.0	11	(Outridge, 2005)

Bone as a potential biomarker of mercury exposure in prehistory

While hair is a well-validated and commonly used biomarker of mercury exposure (National Research Council, 2000), it is rarely preserved in archaeological sites (Aufderheide, 2003). In contrast, the bones of animals and humans are more commonly preserved, and usually they can be securely dated (Reitz and Wing, 1999). The use of ancient bone as a biomarker of prehistoric mercury exposure would allow the tracking of exposure levels in animals and humans at a fairly fine-grained scale through space and time. However, before bone mercury can be employed to answer questions about preindustrial exposure, it must be validated as a biomarker of exposure.

In the field of toxicology, biomarkers of exposure characterize exposure to a substance based on its concentration in a biological tissue, such as blood or hair (National Research Council, 2006). Validating a biomarker of exposure involves demonstrating that a relationship exists between the biomarker and external exposure (WHO, 2001). Validity refers to "the accuracy with which a biomarker reflects the true exposure level" (WHO, 2001:Section II.3.3). For methylmercury, validated biomarkers of exposure in living humans and mammals include blood or hair, for which the relationships between methylmercury dose and tissue concentrations have been well established (National Research Council, 2000).

The National Research Council (2006) recognizes a progression in the validation of biomarkers in humans. The earliest step is observing a substance in a tissue (Group I biomarker), followed by demonstrating that sampling and analytical methods yield

reproducible results (Group II). Subsequent validation steps include demonstrating a relationship between external dose and concentration of the biomarker in laboratory animals (Group III) and demonstrating a relationship between external dose and concentration of the biomarker in humans (Group IV). External dose is here defined as "the amount of chemical that is inhaled, is ingested, or comes in dermal contact and is available for systemic absorption" (National Research Council, 2000:75).

Bone mercury has met only the lowest level of validation; that is, mercury has been detected in bone. Reports of mercury concentrations in either modern or ancient bone are sporadic, and none have focused on addressing the relationship between mercury concentration and exposure. Table 2.9 provides published mean mercury levels in modern human, while tables 2.10 and 2.11 show values for modern terrestrial and marine mammal bone, respectively. Table 2.12 shows values for ancient human bone. For both the modern and the prehistoric human samples, published means for bone total mercury vary widely. Among the modern human samples, mean values range from a low of 10 ng/g in Polish neonates to a high of 2300 ng/g in Korean adults. In modern terrestrial mammal samples, reported means for bone total mercury range from only 10 ng/g in gray squirrels to 680 ng/g in the white-toothed shrew. Marine mammals have reported mean bone mercury levels of between 38 ng/g in Pacific harbor seal pups from California to 7900 ng/g in a single bottlenose dolphin from the French Mediterranean. A sample of five caribou bones from a historic Inupiaq site in Alaska produced an average total mercury concentration of 103 ppb (Duffy et al., 2003) (not shown on table). Total mercury has also been measured on bird bone, with reported mean concentrations

varying from less than 1 ppb to over 400 ppb in Alaskan waterfowl (Rothschild and Duffy, 2005).

It is unclear whether these values reflect actual variation in bone mercury levels, or whether variation is due to differences in the bone element sampled (often unspecified), rigorousness of bone cleaning (e.g., removing traces of muscle tissue and blood), or method of tissue digestion and mercury detection. Methods of mercury detection can vary by orders of magnitude in their minimum detection limits, such that some methods may detect mercury in the ultratrace range (parts per trillion) while others detect mercury only if it is above several parts per million (Clevenger et al., 1997). This may lead to inflation in reporting means when a significant proportion of the sampled individuals have "not detected" mercury levels, and only those with unusually high levels are used in computing means. The wide variation in bone mercury values highlights the need to determine the causes of this variation and to investigate whether bone mercury accurately reflects exposure levels.

Table 2.9. Total mercury concentration in bones of modern humans

Region and country	Age	Bone	n	Mean THg ng/g (ppb)	Reference
Minnesota USA	Adult	NA	30	180	(Bush et al., 1995)
Skelleftehamn Sweden	Adult	Femur	7	45[a]	(Lindh et al., 1980)
Glasgow Scotland	Adult	NA	16	450	(Liebscher and Smith, 1968)
Bohemia Czech Republic	Adult	Parietal	70	70[a]	(Beneš et al., 2000)
Upper Silesian Poland	Neonate	Frontal	77	10	(Baranowski et al., 2002)
Seoul Korea	Adult	NA	161	2300	(Yoo et al., 2002)

[a] Median value.

Table 2.10. Total mercury concentration in bones of modern terrestrial mammals

Species	Region and country	Age	Bone	n	Mean THg ng/g (ppb)	Reference
Cottontail rabbit (*Sylvilagus floridanus*)	Ohio USA	NA	Femur	30	140	(Lynch, 1973)
Gray and Fox Squirrel (*Sciurus sp.*)	Ohio USA	NA	Femur	20	10	(Lynch, 1973)
White-tailed deer (*Odocoileus virginianus*)	Ohio USA	NA	Metacarpal	29	21	(Lynch, 1973)
White-toothed shrew (*Crocidura russala*)	Medas Islands Spain	Adult	NA	5	680	(Sánchez-Chardi et al., 2007)

Table 2.11. Total mercury concentration in bones of modern marine mammals

Species	Region and country	Age	Bone	n	Mean THg ng/g (ppb)	Reference
Pacific harbor seal (*Phoca vitulina richardii*)	California USA	<1 yr	Femur & Rib	26	38	(Brookens et al., 2008)
Bottlenose dolphin (*Tursiops truncates*)	Mediterranean France	3.5 yr	NA	1	7900	(Frodello et al., 2000)
Common dolphin (*Delphinus delphis*)	Mediterranean France	2 yr	NA	1	3400	(Frodello et al., 2000)
Pilot whale (*Globicephala melas*)	Mediterranean France	6 yr	NA	1	2300	(Frodello et al., 2000)
Striped dolphin (*Stenella coeruleoalba*)	Mediterranean France	>7 yr	NA	1	2100	(Frodello et al., 2000)
	Kii Peninsula Japan	20.5	Combined elements	1	2060	(Honda et al., 1984)

Table 2.12. Total mercury concentrations in archaeological human bone

Site and Country	Date	Maturity	Bone	n	Mean THg ng/g (ppm)	Reference
Odense Franciscan Friary (Denmark)	ca. 1400 – 1800 AD	adult	femur	17	67[a]	(Rasmussen et al., 2008)
Svendborg Franciscan Friary (Denmark)	ca. 1400 –1650 AD	adult	femur	16	49[a]	(Rasmussen et al., 2008)
Øm Cistercian Abbey (Denmark)	ca. 1170 – 1530 AD	adult	femur	27	89[a]	(Rasmussen et al., 2008)
Tokushima and Matsuyama, (Japan)	ca. 1100 – 1700 AD	adult	NA	11	8200	(Yamada et al., 1995)
PUM II mummy of unknown provenance (Egypt)	ca. 170 B.C.	adult	vertebra	1	430	(Cockburn et al., 1975)
PUM I mummy of unknown provenance (Egypt)	ancient	adult	long bone	1	100	(Cockburn et al., 1975)

[a]Mean and standard deviation recalculated from data presented in Rasmussen et al. (2008) to include only adult, non-diseased femora.

Validation of bone trace elements as biomarkers of nutritional exposure

Though the term "biomarker" is not used in paleodietary studies, the concentration of a trace element in a prehistoric bone is treated as a biomarker when it is used to reconstruct dietary intake of ("exposure to") that element. In fact, the term "biomarker of nutritional exposure" is now used in modern diet studies to mean a marker that reflects intake of a nutrient or element (Marshall, 2003). In modern dietary studies, these markers are typically hair, blood, or nails, but a wide variety of trace elements, including many metals, accumulate in the skeleton. These elements occur as impurities in the bone mineral, where they are incorporated into the hydroxyapatite crystals or adsorbed onto the surface of the crystals (Ezzo, 1994). Trace elements can also be attached to the protein phase of bone, which is predominately collagen (Ezzo, 1994; Tuross, 2003). The concentrations of trace elements in bone can vary from population to population, and from person to person, based on the elements contained in the foods eaten, the water consumed, the air breathed, and even the substances touched. This variation is of interest to bioarchaeologists, who study prehistoric human and animal skeletal remains to answer questions about past diet and health (Burton and Price, 2000; Larsen, 1997; Sanford and Weaver, 2000).

As with any biomarker, paleodietary indicators must be validated. Bone concentrations of an element do not always reflect dietary intake levels due to complex pathways of absorption, movement, incorporation into tissues, and excretion (Ezzo, 1994). Unfortunately, bioarchaeologists have sometimes been too hasty in using bone

trace element concentrations to reconstruct aspects of past diet without proper validation (see reviews in Burton and Price, 2000; Ezzo, 1994). The use of unproven bone trace elements to support far-reaching and sometimes fantastical conclusions about past diet has brought wholesale discredit to the trace element approach. The misuse of bone element data is unfortunate, because more careful validation studies have demonstrated that some bone trace elements can provide accurate signatures of past diet, and, just as importantly, that others are not valid indicators of past diet and should be abandoned.

Validation of candidate bone paleodietary indicators should proceed in the same fashion as for biomarkers of toxic exposure. Yet, as noted by Sanford and Weaver (2000:334), "the effects of dietary intakes . . . on skeletal element concentrations remain among the least explored areas of anthropological trace element research." There are a few exceptions to this characterization. An extensive bone paleodietary indicator validation study was conducted by Lambert and Weydert-Homeyer (1993), who used a complex controlled feeding experiment in rats to determine the relationship between diet and bone concentrations of ten elements (phosphorous, calcium, magnesium, strontium, barium, potassium, sodium, zinc, iron, and aluminum). The results of this study indicate that only iron and potassium have a strong positive relationship between diet levels and bone levels. Bone levels of some of the other elements do not have a simple relationship with diet, but can be explained in terms of interactions between elements. For example, when strontium and barium are considered as ratios to calcium (Sr/Ca and Ba/Ca), the correlations between diet and bone are high ($r=0.83$ and $r=0.42$, respectively) (Lambert and Weydert-Homeyer, 1993).

Other controlled feeding experiments conducted by bioarchaeologists include a study of strontium in rats (Price et al., 1986), which showed that bone strontium levels reflect dietary intake levels when other dietary factors are held constant. Klepinger (1990) fed high and low levels of magnesium to pigs and found that bone magnesium levels do *not* reflect ingestion levels. Bioarchaeological researches have also relied on studies conducted by researchers in other disciplines to validate biomarkers of nutritional exposure. In this manner, Klepinger (1993) synthesized results from several controlled feeding studies of zinc in rats to conclude that bone zinc is responsive to dietary zinc.

A few validation studies of bone trace elements as paleodietary indictors have built on laboratory controlled feeding experiments and examined the behavior of the candidate indicator in natural samples, both modern and prehistoric. Burton et al. (1999) measured bone Sr/Ca and Ba/Ca ratios in several modern mammal and plant species and confirmed that the bone ratios faithfully reflect the dietary ratios. A similar investigation was conducted in South Africa, where bones from modern marine and terrestrial animals confirmed the theoretically expected pattern of reduced Ba/Sr and Ba/Ca ratios in marine animals (Gilbert et al., 1994). This validation was carried further by comparing Ba/Sr and Ba/Ca ratios to stable carbon isotope ratios, an established indicator of marine food intake, in prehistoric skeletal material. Burton and Price (1990) field validated the use of the Ba/Sr ratio as a paleodietary indicator of marine diets by comparing the ratios measured in prehistoric human bone from coastal sites to those from inland sites.

Bone heavy metal concentrations as biomarkers of toxic exposure

In addition to studying paleodietary indicators in bone, bioarchaeologists have studied indicators of toxic metal exposure in bone, especially exposure to the heavy metal lead (Arnay-de-la-Rosa et al., 2003; Aufderheide et al., 1992; Aufderheide et al., 1988; Budd et al., 2004; Drasch, 1982; Ericson et al., 1979; Ericson et al., 1991; Farrer, 1993; Flegal and Smith, 1992; González-Reimers et al., 2005; González-Reimers et al., 2003; Grandjean et al., 1979; Handler et al., 1986; Hisanaga et al., 1988; Keenleyside et al., 1996; Kowal et al., 1991; Kowal et al., 1989; Patterson et al., 1991; Steinbock, 1979; Waldron, 1982; Waldron et al., 1976; Waldron and Wells, 1979; Wittmers et al., 2002; Woolley, 1984). Lead exposure in archaeological populations varied tremendously, depending on the use of lead in technology (Aufderheide, 1989). While modern populations are exposed to lead largely through inhaling polluted air from industrial discharge and lead fuel additives, in antiquity, exposure was largely due to consumption of lead-contaminated foods and water or through inhalation of lead dust and fumes produced from mining and processing ores (Aufderheide, 1989; Budd et al., 2004).

Toxicological studies of living humans and animals have demonstrated that lead concentration in bone is a reliable indicator of exposure to the metal (ATSDR, 1999a). Because bone has a slow turnover rate, lead levels in bone are generally thought to represent cumulative, lifetime exposure to the metal (Aufderheide and Rodríguez-Martin, 1998). Bone lead can be reliably measured using both non-destructive X-ray

methods and direct measurement methods, and bone lead concentrations are usually in the parts per million range (Aufderheide, 1989).

Studies of lead concentrations in ancient human remains have been conducted to establish baseline levels of lead exposure in pre-metallurgical societies, to assess the health consequences of exposure, and even to determine the socioeconomic status of individuals (Aufderheide, 1989). Lead determinations on human skeletal remains from pre-metallurgical societies show a clear pattern of lead concentrations below 1.0 ppm, compared to around 5.0 ppm in present-day populations with no unusual lead exposure (Drasch, 1982; Grandjean et al., 1979; Patterson et al., 1991).

A particularly fascinating line of inquiry has been lead poisoning among the ancient Romans, who mined, processed, and utilized lead in a number of ways (Aufderheide, 1989). The population at large was exposed to this toxic metal through the use of lead drinking water pipes, foodware made of pewter (a lead-tin alloy), and the widespread use of "sugar of lead" or lead acetate to sweeten wine and other foods (Aufderheide, 1989; Nriagu, 1983a; Waldron and Wells, 1979; Woolley, 1984). Analyses of Roman-age skeletal remains confirm high lead exposure throughout the Roman Empire, with bone lead concentrations generally above 40.0 ppm (Aufderheide et al., 1992; Bisel and Bisel, 2002; Grattan et al., 2002; Wittmers et al., 2002). Based on historical descriptions of food preparation, storage, and consumption, Nriagu (1983b) estimated that lead intake among Roman aristocrats was high enough to have caused lead poisoning, and may even have contributed to the fall of the Empire (Gilfillan, 1990).

The health effects of ancient lead exposure can be reconstructed by translating bone lead levels into blood levels, which are correlated with health outcomes (Aufderheide and Rodríguez-Martin, 1998). This procedure has been used to assess the health status of historic rum-plantation slaves in Barbados, who consumed lead-contaminated rum. By estimating blood lead from lead measurements made on the skeletal remains of Barbadian slaves, researchers concluded that a significant fraction of the slaves would have experienced mild to moderate lead poisoning symptoms (e.g., stomach cramping to peripheral nerve defects), and that a small number with bone lead concentrations over 200 ppm experienced severe poisoning and likely died of lead-related brain toxicity (Handler et al., 1986).

Skeletal remains from members of the ill-fated Sir John Franklin expedition of 1845–1848, in which 129 crewmen and officers died in search of the Northwest passage, have also been analyzed for lead content (Keenleyside et al., 1996; Kowal et al., 1990). These individuals were likely exposed to lead by the solder used to seal the food tins that made up the bulk of their provisions (Kowal et al., 1990; Kowal et al., 1991). Translating bone lead levels into blood levels and related health effects, Kowal et al. (1991:194) concluded that most of the crew members suffered from lead poisoning and that "the physiological and neurological effects of lead intoxication could have played a major role in the loss of the expedition."

Validating biomarkers of mercury exposure

Researchers must be confident that biomarker measurements are accurate in order to make statements about individual or group differences in exposure or health risks. But measurements of chemical concentration are never exact, as the very act of measurement causes variation in the analytical result due to imperfect performance of instruments and observers (Schuster and Powers, 2005; Vineis et al., 1993). Analytical results should thus be viewed not as concentrations, but as "error-prone estimates of concentrations" (AOAC, 2002). This error—the difference between the true value and the analytical result—may be random, which causes imprecision, or nonrandom, which causes bias (Taverniers et al., 2004; Vineis et al., 1993). The accuracy of measurement results is usually expressed in terms of both trueness (or bias) and precision (or imprecision) of the results (Hauck et al., 2008).

Accuracy is here defined as the "closeness of agreement between a quantity value obtained by measurement and the true value" (Menditto et al., 2007:45). Under this definition, accuracy reflects both the degree of trueness (lack of bias) and precision (lack of imprecision) of a measurement result. Measurement accuracy is not a quantity and should not be given a value, but "a measurement is said to be more accurate when it offers a smaller measurement error" (Hauck et al., 2008:841).

Trueness is defined as "the closeness of agreement between a test result and the accepted reference value" (Thompson et al., 2002:847). Trueness is expressed quantitatively, often as the percentage of recovery of a known amount of analyte in a

sample. Precision is "the closeness of agreement between test results" (Thompson et al., 2002:848). It too is expressed quantitatively, usually in terms of imprecision, as the standard deviation or relative standard deviation of sample replicates. The term "repeatability precision" refers to within-laboratory variation from repeated sample measurements made in a single laboratory on the same day or different days. In the latter case, the term "intermediate within-laboratory precision" is sometimes used (AOAC, 2002:21).

According to the above definitions, trueness is inversely related to systematic measurement error (bias), while precision is inversely related to random measurement error (Hauck et al., 2008). Note that the terms trueness and precision can be used to characterize measurement values as well as to characterize the measurement method used to derive the values (AOAC, 2002; Hauck et al., 2008).

There is a great deal of inconsistency in the use of the terms accuracy and trueness in the measurement methods literature. As defined above, accuracy (or inaccuracy) is the deviation of a result from the true value due to both random and systematic effects (Hauck et al., 2008). This definition, in which accuracy encompasses both trueness and precision, is used by the International Organization for Standardization (ISO) and the International Union of Pure and Applied Chemistry (IUPAC) and appears in the *International Vocabulary of Metrology* (*VIM*) (as summarized in Hauck et al., 2008). This usage has recently been adopted by the U.S. Environmental Protection Agency in its guidelines for validation of methods of chemical analysis (Mishalanie et al., 2005).

However, the term accuracy is sometimes used instead of trueness to refer to the degree of systematic error only. This usage is still in effect for most of the U.S. pharmaceutical industry as well as in much of the atomic spectrometry literature (Hauck et al., 2008; Menditto et al., 2007).

Several international organizations concerned with the quality of analytical measurement results have offered guidelines for assessing the accuracy and other performance characteristics of methods of chemical measurement, including the IUPAC, ISO, and AOAC International (formerly the Association of Official Analytical Chemists). These organizations have collaborated to standardize or "harmonize" analytical validation procedures, and have jointly published protocols such as the "Harmonized guidelines for single-laboratory validation of methods of analysis" (Thompson et al., 2002) and the "Guidelines for single-laboratory validation of analytical methods for trace-level concentrations of organic chemicals" (Alder et al., 2000). These guidelines include procedures for evaluating measurement accuracy (trueness and precision). Specific criteria for acceptable levels of precision (as the repeatability relative standard deviation) and trueness (as percent recovery) are provided by AOAC International (AOAC, 2002).

Trueness of a chemical measurement is often expressed as the percent recovery of a known amount in a certified reference material. However, certified reference materials do not exist for every matrix of interest, so trueness can also be expressed in terms percent recovery of a known amount of analyte that has been added to a sample (a

"spike") (Thompson et al., 1995). Recovery tends to vary as a function of analyte concentration, with better recovery at high concentrations (AOAC, 2002). Thus, the acceptable recovery limits for an analyte with a concentration around 100 ppm are between 85–100%, while the limits for concentrations around 1 ppm are between 75–120% (see Table 3.3 for a complete list of acceptable recoveries).

The repeatability relative standard deviation (RSD_r), sometimes referred to as the coefficient of variation, is calculated from repeated measures on the same sample or samples and provides a measure of the magnitude of measurement imprecision, expressed as a percent (see calculation in Chapter 3). A higher RSD_r indicates greater imprecision among repeated measurements. The precision of concentration measurements tends to decrease as concentration of the analyte decreases. Given this relationship, acceptable RSD_r values vary as a function of concentration (AOAC, 2002). For example, the within-laboratory RSD_r for analyte concentrations around 100 ppm is expected to be around 4%, while the RSD_r for analyte concentrations around 100 ppb is expected to be around 11% (see Table 3.2 for complete list of expected RSD_r values by concentration).

Published guidelines for validating biomarkers of exposure stress the importance of ensuring measurement accuracy, including both measurement trueness and precision. Measurement trueness (as recovery) and precision (as the repeatability relative standard deviation) have often been explicitly addressed in the literature when researchers have proposed a novel or improved method of mercury measurement (e.g., mercury detection

by inductively-coupled plasma-atomic emission spectroscopy). In such cases, precision and accuracy are typically assessed using repeated analysis of a certified biological tissue, such as whole animal blood or human hair (Barbosa et al., 2004; Gill et al., 2004; Sandborgh-Englund et al., 1998).

Few researchers have explicitly considered measurement accuracy when introducing a novel biomarker of mercury exposure. For example, both Rees et al. (2007) and Alfthan (1997) reported on human toenail mercury as a possible biomarker of exposure, but neither study addressed the accuracy of toenail mercury determinations (by Cold Vapor High Resolution Inductively Coupled Mass Spectrometry and Automated Cold Vapor Atomic Absorption Spectrometry, respectively). One study compared the precision of several mercury biomarkers of exposure (whole blood, red blood cells, plasma, hair, and urine) based on repeated measurements of certified reference materials. They found the greatest precision in hair and the lowest precision in blood and plasma (Berglund et al., 2005).

Reporting of analytical error in biomarker measurement is also inconsistent and incomplete in studies applying mercury biomarkers to assess exposure levels and health outcomes, including the well-known longitudinal studies conducted in the Faroe and Seychelle islands. The National Research Council (2000:127-128) criticizes the reporting in both of these studies for either a complete lack of measurement bias and imprecision data or for characterizing these data in vague terms such as "within the acceptable range," or within the "target value," without defining the acceptance criteria.

Another early step in the process of validating a potential biomarker of exposure is to demonstrate a relationship between external dose and concentration of the biomarker through controlled dosing experiments. For mercury, such studies have been conducted on a variety of animals, including small rodents, rabbits, cats, pigs, and monkeys, and these have provided information on the quantitative relationship between exposure level and blood and organ concentrations (Gyrd-Hansen, 1981; Magos and Butler, 1976; Thomas et al., 1988; WHO, 2000). These studies have not been geared specifically towards validating biomarkers of methylmercury exposure, but towards modeling the biokinetics of methylmercury. Biokinetics, also referred to as toxicokinetics or pharmacokinetics, is the study of the absorption, tissue distribution, metabolism, and excretion of chemical substances in the body (Clewell and Clewell, 2008).

Biokinetic studies of mercury involve tracking the fate of mercury in the body over time after one dose or a series of doses. This allows the determination of various biokinetic parameters, such as the absorption rate, the elimination rate, and distribution rate (fraction of the dose that is distributed to the tissue of interest) (Gyrd-Hansen, 1981). These biokinetic parameters can then be used to formulate biokinetic equations to predict tissue mercury concentration from dose, and, by extension, to back calculate dose from tissue concentration.

The most commonly used biomarker of mercury exposure in humans, blood mercury concentration, has been shown to be related to exposure through controlled or semi-controlled biokinetic studies of methylmercury administered to human volunteers via

injection or via consumption of methylmercury-containing fish (Kershaw et al., 1980; Miettinen et al., 1971; Sherlock et al., 1984; Smith et al., 1994). These investigations have allowed the development of biokinetic models that link mercury dose to blood concentration (EPA, 2001c; Stern, 1997; WHO, 1990).

Some models linking methylmercury dose to blood mercury level are simple linear equations, such as that used by the United Nations Environment Programme (UNEP, 2008) in which Dose (μg/kg bw/day) = blood THg (μg/L) X 0.02. However, probably the most widely accepted model for converting human blood mercury concentration to daily dietary intake is the more complicated one-compartment biokinetic model used by the World Health Organization and the U.S. Environmental Protection Agency (EPA, 2001c; WHO, 1990). It is called "one compartment" because in this model, all of the body compartments are compressed to one: blood. The dose conversion equation, which was introduced in a previous section, includes several physiological and metabolic parameters, such as the absorption rate, the elimination rate, the fraction of absorbed mercury present in blood, etc. The one-compartment dose conversion model has been shown to have a reasonably good fit to observed blood mercury concentrations in humans exposed to controlled levels of methylmercury in fish (Ginsberg and Toal, 2000). However, the dose conversion model has not been rigorously tested under natural conditions, and where studies have been done, there is some disagreement between observed and predicted methylmercury intake.

A recent study of 385 seafood consumers living in French coastal areas compared estimated methylmercury intake calculated from a food frequency survey to estimated methylmercury intake calculated from blood mercury concentrations using the one-compartment dose conversion formula (Sirot et al., 2008). While the two estimates were highly and significantly correlated, the intake estimates from the food surveys (mean = 0.23 μg MeHg/kg bw/day) were much higher than the intake estimates from blood mercury (mean = 0.09 μg MeHg/kg bw/day). The authors conclude that the food surveys overestimated methylmercury intake, rather than that the dose conversion equation underestimated intake, but the evidence supporting their conclusion is not clearly stated (Sirot et al., 2008:37).

While the relationship between dietary methylmercury level and blood concentration can be readily examined through controlled feeding experiments, further validation of the biomarker in natural populations is more difficult, since dietary mercury intake is unknown. Such field validation studies usually rely on proxy measures of mercury intake. Proxy measures of exposure are "substitutes for direct measurements" that approximate exposure levels (Friis, 2007:40). For humans, proxy measures of mercury exposure have ranged from the crude, such as distance of residence from a point source of mercury pollution, to the more refined, such as self-reported frequency of fish meals (Barany et al., 2003; Björnberg et al., 2005; Hodgson et al., 2007; Hsu et al., 2006; Khoury et al., 1993; Rees et al., 2007). More refined still is the "duplicate diet" method of estimating mercury exposure, in which identical portions of foods actually consumed are analyzed for mercury concentration (National Research Council, 2000). Biomarkers

of exposure such as blood or hair are themselves surrogate measures of exposure (Khoury et al., 1993). Numerous studies have found a positive correlation between blood mercury and the self-reported frequency of fish or sea mammal consumption as an indicator of exposure level in humans (Barany et al., 2003; Berglund et al., 2005; Björnberg et al., 2005; Chang et al., 1992; Dewailly et al., 2001; Gundacker et al., 2006; Hightower and Moore, 2003; Hsu et al., 2006; Ip et al., 2004; Mahaffey et al., 2004).

Human hair is a widely accepted biomarker of mercury exposure. Indeed, hair mercury is used interchangeably with blood mercury in exposure estimates based on the one-compartment dose conversion equation by simply using the hair-to-blood mercury ratio of 250:1 (EPA, 2001c; National Research Council, 2000; Stern, 1997; WHO, 1990). The relationship between human hair mercury and exposure does not seem to have been studied through controlled dosing experiments, although information was gained through comparisons of hair concentrations to the level of methylmercury accidentally ingested in the widespread Iraqi poisoning incident (Bakir et al., 1973). Hair has been further validated as a biomarker of mercury exposure through field studies that have shown a correlation between hair mercury and indicators of exposure, such as blood mercury (WHO, 2000:3.2) or and the frequency of fish and sea mammal consumption (Batista et al., 1996; Berglund et al., 2005; Björnberg et al., 2005; Ip et al., 2004; Pesch et al., 2002; Rojas et al., 2007).

Few studies have considered biomarkers of mercury exposure other than human blood or hair. Two separate studies attempted to validate human toenail mercury

concentration as a biomarker of exposure by examining the relationship between toenail mercury and indicators of exposure, including hair and blood mercury and the frequency of fish consumption (Alfthan, 1997; Rees et al., 2007). The Alfthan (1997) study found high correlations between toenail mercury and hair and blood mercury as well as with the frequency of fish consumption, while the Rees et al. (2007) study found only a moderate correlation with fish consumption. A recent study investigated rat nails as a biomarker of mercury exposure through a controlled dosing experiment and found a high correlation between dose and nail mercury level (Brockman et al., 2008). Another study evaluated urine as a potential biomarker of methylmercury exposure by comparing urine mercury concentration to the frequency of fish consumption, but found that the two variables were unrelated (Pesch et al., 2002).

Biomarkers of mercury exposure in wildlife have been evaluated through controlled feeding studies in captive birds and mammals, as well as through field studies (Casini et al., 2003; Fournier et al., 2002; Wiener et al., 2003; Wobeser et al., 1976). As mentioned previously, field validation studies must rely on proxies of mercury intake when evaluating biomarkers. In one study, which investigated blood mercury concentration as a potential biomarker of exposure in loons, daily mercury intake was estimated by combining information on mercury levels in dietary items with their consumption rates (Merrill et al., 2005). Another field validation method is to compare mercury levels in potential biomarkers, such as bird feathers or mammalian hair, to levels in a previously validated biomarker, such as blood or kidney (Evans et al., 1998; Evers et al., 2001; Ikemoto et al., 2004; Klenavic et al., 2008; Mierle et al., 2000). The

average mercury level in prey in an animal's feeding area was used as a surrogate measure of mercury exposure in an evaluation of fur as a biomarker in mink and otter (Wren et al., 1986).

In animal studies, mercury exposure has been approximated using the trophic level at which an animal feeds, based on the observation that mercury tends to biomagnify up the food chain (EPA, 1997b; EPA, 2006; Nichols et al., 1999). A potential proxy measure of mercury exposure is the stable nitrogen isotope ratio, which increases with an animal's trophic position, and thus may serve as an indirect measure of mercury exposure. The stable nitrogen isotope ratio is now widely used as a continuous measure of relative trophic position (Fry, 2006; Kelly, 2000; Vander Zanden and Rasmussen, 1996), which, if high, suggests that high mercury levels may also be found. Potential biomarkers of exposure have been compared to stable isotope ratios as a proxy for mercury intake, as was done in evaluations of bird feathers (Sanpera et al., 2007b; Thompson et al., 1998) and turtle blood (Bergeron et al., 2007). Stable isotope ratios are of special interest here, because they can be measured on bone collagen, including prehistoric bone collagen. Thus, stable isotope ratios could be used to help validate bone as a biomarker of mercury exposure using skeletal remains from archaeological sites.

Stable isotope ratios and mercury levels

Studies indicate that elevated mercury levels correlate with elevated stable nitrogen isotope values in animal tissues (Atwell et al., 1998; Cabana and Rasmussen, 1994). The latter is determined by measuring via mass spectrometry the ratio of ^{15}N to ^{14}N

($^{15}N/^{14}N$) in an organic sample. The resulting value is conventionally expressed in delta (δ) notation in parts per thousand (‰ or "per mil") relative to an international standard. The stable nitrogen isotope ratio, or $\delta^{15}N$, has been shown to systematically increases by about 3‰ from diet to consumer tissues, such that carnivores typically have higher values than herbivores, which have higher values than plants (Ambrose, 2000; Kelly, 2000). The basis for the trophic increase in $\delta^{15}N$ is fractionation, or the change in isotope ratios between product and substrate (Fry, 2006). Isotopes of an element have small mass differences, which causes them to behave differently during chemical reactions and in physical processes, such that the lighter isotope reacts more quickly and forms weaker bonds than the heavier isotope (Gannes et al., 1998). Differences in fractionation among biological materials leads to distinctive isotopic compositions or "signatures" in biological materials (Gannes et al., 1998).

In marine ecosystems, the nitrogen isotope composition of phytoplankton at the base of the foodweb depends largely on the $\delta^{15}N$ of the nitrogen source (mostly dissolved nitrate), the isotopic fractionation during assimilation, and on the fraction of the nitrogen supply consumed (Schell et al., 1998; Schulz and Zabel, 2000). In laboratory studies, there is a small isotopic fractionation during nitrogen assimilation, such that phytoplankton are depleted in ^{15}N relative to nitrate by about 5‰, although this varies between taxa, with growth conditions, and on the supply of nitrogen (Peterson and Fry, 1987; Schulz and Zabel, 2000). In field studies, the $\delta^{15}N$ of phytoplankton (~ 6‰ in the North Pacific) tends to resemble that of upwelled nitrate (~ 5 to 7‰) (Schell et al., 1998). In general, the nitrogen isotope composition of marine organisms is not useful

for identifying sources of nitrogen, with the exception of reefs and mangrove ecosystems in which nitrogen-fixing blue-green algae contribute substantially to the nitrogen pool and estuarine systems with significant inputs of terrestrial nitrogen (Keegan and DeNiro, 1988; Michener and Schell, 2007). Instead, the utility of the stable nitrogen isotope ratio is in tracing trophic level, due to the fractionation that occurs between diet and consumer. During nitrogen assimilation by animals, the lighter isotope (^{14}N) is preferentially excreted in urea, leading to $\delta^{15}N$ values that are around 3‰ higher in consumer tissues relative to diet (Gannes et al., 1998; Kelly, 2000; Peterson and Fry, 1987). Fry (2006:56) describes this regular $\delta^{15}N$ increase as a nitrogen isotope "trophometer." The nitrogen stable isotope ratio is now widely used as a continuous measure of relative trophic position (Fry, 2006; Kelly, 2000).

Stable nitrogen isotope ratios have been used to assess degree of carnivory versus herbivory in both terrestrial and aquatic food webs and in both modern and archaeological populations (Ambrose, 1993; Gannes et al., 1998). Stable nitrogen isotope composition can also assess the relative contributions of marine vs. terrestrial foods to the diet, because marine plants and animals tend to be enriched in ^{15}N (have higher $\delta\ ^{15}N$ values) compared to terrestrial plants and animals (Gannes et al., 1998; Larsen, 1997; Schoeninger and DeNiro, 1984). For bone collagen, typical $\delta^{15}N$ values are around 5‰ for terrestrial herbivores and 8‰ for terrestrial carnivores (Schoeninger and DeNiro, 1984). Marine mammal bone collagen shows a similar trophic level distinction, with $\delta^{15}N$ values of around 14‰ for invertebrate and plankton consumers and around 17‰ for fish eaters (Schoeninger and DeNiro, 1984). The bone collagen

$\delta^{15}N$ values of humans whose diet was mostly from marine sources cluster around 14.0‰ to 20.0‰, compared to around 6.0‰ to 12.0‰ for agriculturalists (Richards and Hedges, 1999; Schoeninger et al., 1983). Because of its slow turnover rate, bone collagen is thought to represent an individual's diet over a period of 10 years or more (Hedges et al., 2007).

Since both mercury and $\delta^{15}N$ tend to increase with trophic level or reliance on marine foods, the two variables should be positively correlated in animal tissues. The stable nitrogen isotope ratio is now a well established correlate of mercury level in animal tissues across a broad range of species and environments, including entire marine and freshwater foodwebs (Atwell et al., 1998; Campbell et al., 2005; Dehn et al., 2006b; Jarman et al., 1996; McIntyre and Beauchamp, 2007; Rigét et al., 2007a; Rigét et al., 2007b), in marine mammals (Capelli et al., 2008; Dehn et al., 2006a; Dietz et al., 2004; Hobson et al., 2004), in marine fish (Bank et al., 2007; Cai et al., 2007), in fish-eating birds (Elliott, 2005; Ricca et al., 2008; Tavares et al., 2007) and in freshwater fish and reptiles (Bergeron et al., 2007; Burgess and Hobson, 2006; Cabana and Rasmussen, 1994; Campbell et al., 2008; Campbell et al., 2004; McIntyre and Beauchamp, 2007; Muir et al., 2005; Power et al., 2002).

No studies could be found that compared human mercury concentrations to $\delta^{15}N$ in human tissues. Humans are among the few mammals that may routinely feed from both the aquatic and the terrestrial food chains. Others include mink (*Mustela vison*) (Braune et al., 1999), brown bear (*Ursus arctos*) (Felicetti et al., 2004), arctic fox (*Alopex*

lagopus) (Hoekstra et al., 2003), and raccoon (*Procyon lotor*) (Lord et al., 2002). Unfortunately, the relationship between mercury levels and $\delta^{15}N$ has not been studied extensively in these species either. One exception is a study of raccoons, which found a positive relationship between muscle mercury concentration and $\delta^{15}N$ (Gaines et al., 2002).

Possible confounding factors that may affect the relationship between mercury concentration and $\delta^{15}N$ include fluctuations in tissue values due to diet shifts, variation in tissue protein content, and variation in mercury levels of different feeding zones (Balshaw et al., 2008; Sanpera et al., 2007b; Thompson et al., 1998; Tremblay et al., 1996). Some have suggested that it may be useful if mercury concentration were normalized in terms of protein matter or sulfhydryl content (Woshner et al., 2008).

Stable carbon isotope ratios ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) have also been studied in relation to mercury levels in tissue, but the expected relationship between the two variables is not clear-cut. Stable carbon isotope ratios are not good indicators of trophic position; rather, they can distinguish among food chains (horizontal food web position) because there is variation in carbon isotope composition at the base of different food chains (Gannes et al., 1998; Kelly, 2000). In aquatic animals, differences in $\delta^{13}C$ largely reflect feeding zone, such as inshore versus offshore or benthic versus pelagic (Clementz and Koch, 2001; Hobson et al., 1997).

Marine primary producers include microalgae (phytoplankton), macroalgae (seaweed) and seagrass (Dawes, 1998), which exhibit large ranges of $\delta^{13}C$ values:

typically around −30 to −18‰ for phytoplankton, -27 to -8 for macroalgae and −15 to −3‰ for seagrasses (Fry and Sherr, 1984). Factors controlling this variation are complex, and include differences in the isotopic composition of source carbon, differences among species in fractionation during photosynthesis, and microenvironmental factors, such as turbulence, light intensity, and temperature (France, 1995; Goericke et al., 2007). The source of carbon for primary producers at the base of marine food webs is dissolved inorganic carbon, mostly bicarbonate and carbon dioxide, which typically have $\delta^{13}C$ values of around 0‰ and −9‰, respectively (Goericke et al., 2007; Hemminga and Mateo, 1996; Michener and Schell, 2007). During photosynthesis, fractionation of carbon isotopes results in preferential incorporation of the lighter isotope (^{12}C) in marine primary producers. The differences in isotopic composition among marine primary producers are passed on to consumers, with little further fractionation between diet and the consumer's whole body, although there is variation in isotopic composition among tissue types (Gannes et al., 1998; Kelly, 2000).

Stable carbon isotope ratios are often enriched for nearshore feeders compared to offshore feeders, and for benthic (bottom) feeders compared to pelagic (open-water) feeders (Burton et al., 2001; Clementz and Koch, 2001; France, 1995; McConnaughey and McRoy, 1979; Walker et al., 1999). Mercury levels sometimes vary in the opposite way, with higher levels in open-water and offshore feeders compared to bottom and nearshore feeders (Chen et al., 2008; Embury, 2000; Goodale et al., 2006; Lacerda et al., 2007; Lasora and Allen-Gil, 1995; Ricca et al., 2008; Rumbold et al., 2003) although this pattern has not been fully investigated. Some studies have found a negative

relationship between mercury concentration and stable carbon isotope ratios, including in fish-eating birds (Bearhop et al., 2000; Ricca et al., 2008) and freshwater fish (Ethier et al., 2008; McIntyre and Beauchamp, 2007; Power et al., 2002). However, other studies comparing mercury concentration and $\delta^{13}C$ in animal tissues have found no relationship (Hobson et al., 2004; McIntyre and Beauchamp, 2007; Rigét et al., 2007a; Thompson et al., 1998) or a positive relationship between the two variables(Dehn et al., 2006a; Dietz et al., 2004; McIntyre and Beauchamp, 2007). Possible confounding factors that may affect the distribution of mercury by feeding location include depth of the water column, freshwater inputs, and point sources of mercury pollution (Al-Majed and Preston, 2000; Chumcal et al., 2008; Sanpera et al., 2007a).

Summary

Marine mammals are dietary staples among the arctic Eskimo and Inuit, but these traditional foods often contain high concentrations of mercury, a toxic heavy metal (ATSDR, 1999b; EPA, 1997c:11; National Research Council, 2000; WHO, 1990). People who consume these traditional resources are exposed to mercury, which can accumulate in their tissues to levels that are considered unsafe (Burkow and Weber, 2003; EPA, 1997a; Van Oostdam and Tremblay, 2003). A key contributor to mercury exposure today is the ringed seal, which tends to have high mercury concentrations in its edible tissues and is frequently consumed by indigenous peoples of the Arctic.

Because mercury can be released into the environment through natural as well as industrial processes, the same pathway that operates today to cause high mercury

concentrations in arctic marine animals and their consumers likely operated in the preindustrial past. In this way, prehistoric Eskimo and Inuit (Thule) who relied heavily on marine foods, including the ringed seal, may well have been exposed to relatively high levels of mercury, just as their modern counterparts are today.

Little information is available on preindustrial mercury levels in the food chain. One potential archive of past mercury levels is the skeletal remains of animals and humans from archaeological sites. Mercury concentrations in preserved bones could indicate the degree of preindustrial exposure, if bone can be shown to be a valid biomarker of mercury intake. Biomarkers of exposure can be validated through controlled feeding experiments and through field studies that compare the candidate biomarker to estimates or proxies of mercury intake. One such proxy is the stable nitrogen isotope ratio ($\delta^{15}N$) which increases with an animal's trophic position, and thus may serve as an indicator of mercury exposure. Many studies have found that mercury level and $\delta^{15}N$ are correlated in animal tissues. Because $\delta^{15}N$ can be measured on bone collagen, this chemical measurement offers a means to validate bone as a biomarker of mercury exposure using skeletal material preserved in archaeological sites.

3. RESEARCH MATERIALS AND METHODS

The aim of this dissertation research was to investigate the potential for using archaeological bone mercury concentration as a biomarker of mercury exposure in preindustrial animals and humans. While mercury has been measured in bone, including prehistoric bone, the mere presence of a substance in a tissue is not enough to ensure that its concentration reflects exposure. A candidate biomarker must be validated before being used to assess exposure in individuals and populations, whether those individuals and populations are modern or prehistoric. For a biomarker of exposure to be valid, it must meet two criteria: 1) the analytical method used to measure the biomarker must produce accurate results (analytical validity) and 2) the biomarker must correspond with the extent of exposure (intrinsic validity). The value of a biomarker depends on the quality of its measurement; if measurements differ markedly from one occasion to another, then the biomarker is of little value. By the same token, a biomarker that can be accurately measured but that cannot be linked to exposure is also of limited value. In the case of methylmercury, since exposure in humans and animals is almost entirely through diet, a candidate biomarker of mercury exposure should reflect dietary mercury intake.

Given the requirements for a valid biomarker of mercury exposure, this dissertation was guided by two central research questions:

1. Can bone mercury be accurately measured?

2. Does bone mercury level reflect dietary intake level?

Measurement accuracy was evaluated in terms of the trueness and precision of a series of mercury concentration measurements made on bone from modern western Alaska ringed seals. Trueness was calculated based on the percent recovery of a known amount of mercury added to a select number of samples, while precision was calculated based on the variation in repeated measurements of mercury concentration.

The association between bone mercury and exposure was examined by first comparing bone mercury concentrations to controlled exposure levels in mercury-dosed laboratory rats. To further explore the association, bone mercury was compared to a proxy measure of mercury exposure, the stable nitrogen isotope ratio, in a sample of prehistoric archaeofaunal remains. The remainder of this chapter details the methods and samples used to address each of the biomarker validity criteria.

Bone mercury in methylmercury-dosed laboratory rats

A valid biomarker of exposure must correspond to actual exposure level. When exposure is mainly through diet, as for methylmercury, laboratory biomarker validation is typically accomplished using controlled oral dosing, either through food or water. Potential biomarkers of methylmercury exposure have been studied in this way in laboratory rodents by comparing the mercury concentration in the candidate biomarker to the known exposure level (Nielsen et al., 1994; Woods, 1996). The mercury concentration in the biomarker may also be compared to the concentration in an internal organ as a measure of "internal dose" (WHO, 2001). In the present study, laboratory rats were dosed with known levels of methylmercury in their drinking water, and bone

mercury concentration was compared to both known exposure level and to kidney mercury concentration. The kidney is often the organ of greatest mercury accumulation in the rat and other mammals (Gyrd-Hansen, 1981; Magos and Butler, 1976), and is here used as an independent measure of internal dose, which refers to the amount of a chemical found in a biological medium (National Research Council, 2006:289). In addition, to shed light on other factors contributing to bone mercury variation, mercury concentrations were compared in three different bone elements (cranium, humerus, and femur), and the proportion of organic mercury to inorganic mercury in bone was measured.

Subjects (methylmercury-dosed rats)

Subjects were 15 Long-Evans adult female rats, some of which were exposed to methylmercury chloride as part of an unrelated project conducted at Auburn University under the direction of Professor Christopher Newland. The original study was limited to female rats because one of the treatment categories involved breeding the rats to examine gestational exposure to methylmercury; however, the specimens used in the present study were not breeders.

Exposure details can be found in Newland et al. (2006). Briefly, approximately 16 week-old Long-Evans rats were purchased from Harlan (Indianapolis, IN), pair-housed in conventional shoebox type cages, and fed a casein-based semipurified diet (AIN-93 formulation for rodents). Methylmercury exposure began four weeks after arriving at the Auburn colony, when the rats were about 20 weeks of age (Newland et al., 2006).

Exposure was via drinking water that contained 0, 0.5 or 5.0 ppm of mercury as methylmercuric chloride (CH_3ClHg). This produces approximately 0, 40, and 400 μg/kg bw/day of methylmercury exposure (Newland and Reile, 1999). The present study used four rats that consumed 0.5 ppm Hg in drinking water and eight that consumed 5.0 ppm in water for 96 days before being sacrificed. Three control subjects were treated in the same manner as exposed rats, but did not receive mercury in their water. As part of the original experiment, subjects were assigned to a high- or low-selenium diet. This treatment did not influence blood or brain concentrations at six months of exposure (Newland et al., 2006), and no selenium diet effect was detected in the bone samples, so diet groups were combined for the present analyses.

Tissue sampling and pretreatment (methylmercury-dosed rats)

Rat carcasses were transferred to the University of Alaska Fairbanks, where they were kept frozen until preparation for soft tissue removal by dermestid beetles. All instruments and labware used in preparing bone samples were acid-cleaned prior to use and in between subjects. Skin, organs, and major muscle masses were removed with a scalpel prior to placing specimens into the beetle colony. The right kidney was retained and frozen until further preparation. Soft tissue remaining after the dermestid treatment was removed by gently scraping with a scalpel, followed by rinsing in ultrapure water (18.0 megohm/cm resistivity), and air drying overnight. Cleaned and dried bones were kept frozen until further preparation.

Three types of bone were isolated for further analysis: humeral shaft cortical bone, femoral shaft cortical bone, and combined cranial bones. For long bones, bone marrow and trabecular bone were removed after detaching the proximal and distal epiphyses. The cortical bone portion of long bone shafts was chosen for analysis because this dense bone is often preserved in archaeological contexts and is though to be less susceptible to post-depositional mineral uptake than the more porous trabecular bone (Kohn and Cerling, 2002; Reitz and Wing, 1999). Femoral shafts were defatted by soaking for 24 hours in a 3:2 hexane:isopropanol solution; however, this step was not performed on the other bones because of concerns that the solvents could extract methylmercury. To isolate cranial bones, mandibles were removed, and maxillary teeth were removed after soaking skulls in ultrapure water until the teeth were loose enough for pulling (approximately four hours). Crania were selected for both total mercury and methylmercury determinations because of their higher sample mass (~2 g) compared to the combined femora and humeri (~ 0.3-0.5 g).

Cleaned humeri, femora, and crania were freeze dried for 24 hours, pulverized in a ball mill to less than 0.3 mm, and stored in plastic containers at room temperature until further analysis. Whole kidneys were rinsed in ultrapure water, blotted with a delicate task wipe, and kept frozen until further analysis.

Mercury determination (methylmercury-dosed rats)

Pulverized bone samples, weighing approximately 0.3 g each for humeri (combined right and left shafts, cortical bone only), 0.5 g for femora (combined right and left shafts,

cortical bone only) and 2.0 g each for crania (combined bones of the cranium, both cortical and trabecular bone), and whole frozen kidneys weighing approximately 0.7 g were shipped to Frontier Geosciences, Inc. (Seattle, Washington) for total mercury analysis (all bone types and kidneys) and methylmercury analysis (crania only) by Cold Vapor Atomic Fluorescence Spectrometry. Frontier Geosciences was selected as the analytical laboratory because it is a recognized leader in the development and refinement of methods for mercury analysis, and was one of the chief architects of the methods employed by the U.S. Environmental Agency for mercury determination in water, solids, and biological tissues (EPA, 2001a; EPA, 2001b; EPA, 2002).

Frontier Geosciences determines total mercury in tissue using a modified version of EPA Method 1631 (EPA, 2001a) (C. Molder, Frontier Geosciences, personal communication 2008). Mercury may exist in tissues in several forms, so treatment of tissue prior to spectroscopic analysis involves a series of steps designed to release all of the mercury from the matrix and then convert it to its elemental form, Hg^0. Briefly, the tissue is digested by hot refluxing in a 70:30 mixture of concentrated nitric acid (HNO_3) and sulfuric acid (H_2SO_4), followed by bromine monochloride (BrCl) oxidation (EPA, 2001a). The BrCl addition both destroys remaining organic matter and oxidizes all mercury present to Hg^{2+} (mercuric mercury). The oxidized digestate is then reduced with stannous (tin) chloride (SnCl) to convert all Hg^{2+} to elemental mercury, Hg^0. The Hg^0 is separated from solution by purging with nitrogen or argon gas, collected onto a gold trap, and finally, desorbed from the trap in a gas stream that carries the Hg into the cold-vapor atomic fluorescence spectrometer for detection.

For methylmercury detection, samples are first digested in a 25% potassium hydroxide and methanol solution to release methylmercury from its host matrix (EPA, 2001b; Puckett and Buuren, 2000). The methylmercury in solution is then distilled and ethylated with sodium tetraethyl borate. The ethylated methylmercury is purged from solution with nitrogen gas, collected on a carbon trap, and heated in a pyrolytic decomposition chamber to convert the organic mercury to its elemental form, Hg^0. Finally, the elemental mercury is carried into the cold-vapor atomic fluorescence spectrometer for detection. This method detects all forms of methylmercury including CH_3Hg^+, CH_3HgCl, CH_3HgOH, and CH_3HgS-R (Puckett and Buuren, 2000).

Using the methods described above, the method detection limit, defined as the lowest concentration at which a substance can be detected, is around 0.24 to 0.48 ng/g for mercury (EPA, 2001a). The minimum level of quantitation, defined as the lowest concentration in a sample that can be measured with a known level of confidence, is 1.0 ng/g for mercury in solids (EPA, 2001a). Frontier's standard quality control measures per analytical batch of 20 samples include mercury analysis of the following (C. Molder, Frontier Geosciences, personal communication, 2008):

1. Method blanks (3). Method blanks include all sample processing steps and chemicals without any sample material. Blank THg must be less than 50 ng/L (parts per trillion).

2. Sample duplicate (1). The relative percent difference between the two measurements must be 25% or less. The relative percent difference is the

absolute difference between the sample and the sample duplicate, divided by the mean of the two measurements, expressed on a percent basis (EPA, 2000:8-37).

3. Spiked sample (1, in duplicate). Percent recovery must be between 75-125%.

4. Certified reference material (1, in duplicate). Percent recovery must be between 75-125%.

Statistical analysis (methylmercury-dosed rats)

All statistical analyses were conducted using SPSS version 15.0. Initial inspection of the mercury concentration data indicated that variances were not equal across exposure groups, so to equalize variance, all bone and kidney THg concentrations were transformed to their common logarithms ($\log_{10}$) following Zar (1996). For all statistical hypothesis testing, the null hypothesis was rejected if the p-value was less than the significance level (α) of 0.05.

One-way analysis of variance (ANOVA) was conducted to identify differences in mean bone THg ($\log_{10}$ ng/g) among the three exposure groups: control (0 μg/kg bw/day methylmercury), low exposure (40 μg/kg bw/day methylmercury) and high exposure (400 μg/kg bw/day methylmercury). The ANOVA tested the following null (H_0) and alternative (H_A) hypotheses:

H_0: The true means for bone total mercury are equal among the three exposure groups.

H_A: At least two group means are unequal.

Note that the ANOVA on log-transformed variables is a comparison of geometric means (White and Thompson, 2003). If significant differences were found, a multiple comparisons test (Least Significant Difference) was conducted to identify which pairs of means differ. The Least Significant Difference (LSD) procedure is appropriate when the number of comparisons is small (Cabral, 2008), as is the case in this study with only three pairs of groups.

Least-squares linear regression analysis was performed to determine the form, strength, and significance of the relationship between bone mercury and daily mercury exposure level. Linear regression analysis describes the relationship between a dependent variable, x, and an independent variable, y, in the form of an equation in which y is a linear function of x, as follows: $y = a + bx$. In this equation, a is the y-intercept and b is the slope, which describes the change in y for a one-unit increase in x (Agresti and Finlay, 1997). If there is no linear relationship between the two variables, then y is equally likely to increase or decrease with a unit increase in x, and the slope of the regression line will be zero (Agresti and Finlay, 1997). Significance testing in regression analysis thus examines the possibility that the slope of the regression line is equal to zero In the present research, linear regression analysis tested the following null and alternative hypotheses (following Sokal and Rohlf, 1969):

H_0: The true slope of the regression line relating log-transformed bone total mercury to daily methylmercury exposure level is equal to zero.

H_A: The true slope of the regression line is not equal to zero.

In other words, the null states that bone mercury does not tend to increase or decrease with a unit increase in exposure level. The regression analysis was conducted for each of the three bone types (cranium, humerus, and femur).

Linear regression analysis was also conducted to determine if bone mercury is related to kidney mercury as a measure of internal mercury dose. As before, this analysis tested the following null and alternative hypotheses:

H_0: The true slope of the regression line relating log-transformed bone total mercury to log-transformed kidney total mercury is equal to zero.

H_A: The true slope of the regression line is not equal to zero.

The adequacy of each model to describe the relationship between the dependent and independent variables was assessed by examining the model's slope significance level, the coefficient of determination (r^2), and the prediction residuals.

To explore differences in mean mercury concentration among bone types (cranium, humerus, and femur), a repeated measures ANOVA was conducted. The repeated measures ANOVA tested the following null and alternative hypotheses:

H_0: The true means for log-transformed bone total mercury are equal for the three bone groups.

H_A: At least two group means are unequal.

As mentioned previously, the ANOVA on log-transformed variables is a comparison of geometric means. If significant differences were found, a multiple comparisons test (Least Significant Difference) was conducted to identify which pairs of means differ.

Relationships between pairs of bone mercury variables were explored using the Pearson correlation coefficient (r), which measures the strength of the linear association between variables and varies from −1 (perfect negative linear relationship) to +1 (perfect positive linear relationship) (Norušis, 2004). An r value of 0 indicates the two variables do not covary in a linear fashion. Correlation coefficients were calculated for three pairs of variables: 1) cranium and humerus THg ($\log_{10}$ ng/g), 2) cranium and femur THg ($\log_{10}$ ng/g), and 3) humerus and femur THg ($\log_{10}$ ng/g). In terms of significance testing, correlation analysis tested the following null and alternative hypotheses for each bone combination (following Sokal and Rohlf, 1969):

> H_0: The true correlation coefficient between log-transformed bone total mercury in any two bone elements is equal to zero.
>
> H_A: The true correlation coefficient is not equal to zero.

In other words, the null hypothesis states that there is no linear association between the two variables (e.g., between log-transformed cranium THg and log-transformed humerus THg).

The relationship between cranial bone THg (log_{10} ng/g) and MeHg (log_{10} ng/g) was also explored through correlation analysis, which tested the following null and alternative hypotheses:

> H_0: The true correlation coefficient between log-transformed cranium total mercury and log-transformed cranium methylmercury is equal to zero.
>
> H_A: The true correlation coefficient is not equal to zero.

Bone mercury and indicators of exposure in archaeofauna

Testing the correspondence between a candidate biomarker and exposure levels in natural populations is difficult because actual exposure level is unknown. Thus, investigators must estimate exposure or use proxy measures of exposure. Because tissue mercury concentration tends to increase with trophic position (Atwell et al., 1998; Cabana and Rasmussen, 1994), and $\delta^{15}N$ is a measure an individual's relative trophic position (Ambrose, 2000; Kelly, 2000), $\delta^{15}N$ can be considered to be a proxy measure of mercury exposure. If bone mercury concentration reflects dietary mercury level, then bone mercury should increase as $\delta^{15}N$ increases, assuming that both variables are in equilibrium with the diet (i.e., when tissue values have stabilized and are not fluctuating due to dietary changes). In the present study, bone mercury was compared to bone collagen $\delta^{15}N$ in a sample of prehistoric ringed seals derived from a Thule-period archaeological site in arctic Alaska. To shed light on additional factors that might affect mercury deposition in bone, bone mercury concentration was compared to the stable

carbon isotope ratio ($\delta^{13}C$) measured on bone collagen, as well as to other elemental concentrations measured on whole bone, including total nitrogen (%N), carbon (%C), and sulfur (%S). These variables are related to factors that may confound the relationship between tissue mercury and $\delta^{15}N$, including variation in feeding zone (as evidenced by $\delta^{13}C$) and variation in tissue protein content (as evidenced by %N, %C, and %S) (Chen et al., 2008; Tremblay et al., 1996). Finally, in an exploratory test, two bone samples were demineralized prior to mercury analysis to determine if mercury can be measured on isolated bone protein.

Materials (prehistoric ringed seals)

A total of 23 archaeologically-derived ringed seal mandibles were selected for analysis of total mercury and $\delta^{15}N$. Ringed seal was selected as an appropriate species to analyze for mercury content because this top marine predator has relatively high tissue mercury levels in the present and likely had high levels, relative to other species, in the past. Also, this species was a primary food source for both prehistoric and historic Eskimo and Inuit in the coastal areas of arctic Alaska, Canada, and Greenland, and today is a primary contributor to mercury exposure in these populations. Finally, because ringed seal has a circumpolar distribution, it is used as a biomonitoring species, allowing geographic comparison of mercury levels (Riget et al., 2005), and could potentially be used to compare regions in prehistory. Mandibles were chosen as the bone element to study because this dense bone is often preserved in archaeological contexts. Also, government wildlife management agencies often collect mandibles from modern

subsistence-hunted seals for the purpose of determining age of the animal using growth rings that occur on tooth cementum. Thus, bone mercury levels could potentially be compared between past and present.

The ringed seal mandibles originate from three sites, KTZ-087, KTZ-088, and KTZ-101 that are part of a cluster of Western Thule period sites on Cape Espenberg, Alaska. The cape, which forms the northernmost portion of the Seward Peninsula, lies just above the Arctic Circle, jutting into the Chukchi Sea at the entrance to Kotzebue Sound (see map in Figure 3.1). The sites were excavated by National Park Service archaeologists in the 1980s and are described in a detailed monograph by Harritt (1994). The Cape Espenberg sites were selected because they were known to contain ringed seal remains, had been radiocarbon dated, and their faunal remains had already been studied and described in the literature (Saleeby, 1994).

Sites KTZ-087, KTZ-088, and KTZ-101 are located on adjacent coastal beach ridges approximately 1000 m northwest of the terminus (southeastern end) of the cape (Harritt, 1994:98). The sites contain the remains of multi-roomed houses, cache pits, harpoon heads, ulus, sled runners, and pottery, among other items. They have been interpreted as representing small winter villages where seals and some caribou were hunted. Whaling, however, does not seem to have been important, based on the paucity of whale bones and whaling tools, such as large harpoon heads (Harritt, 1994).

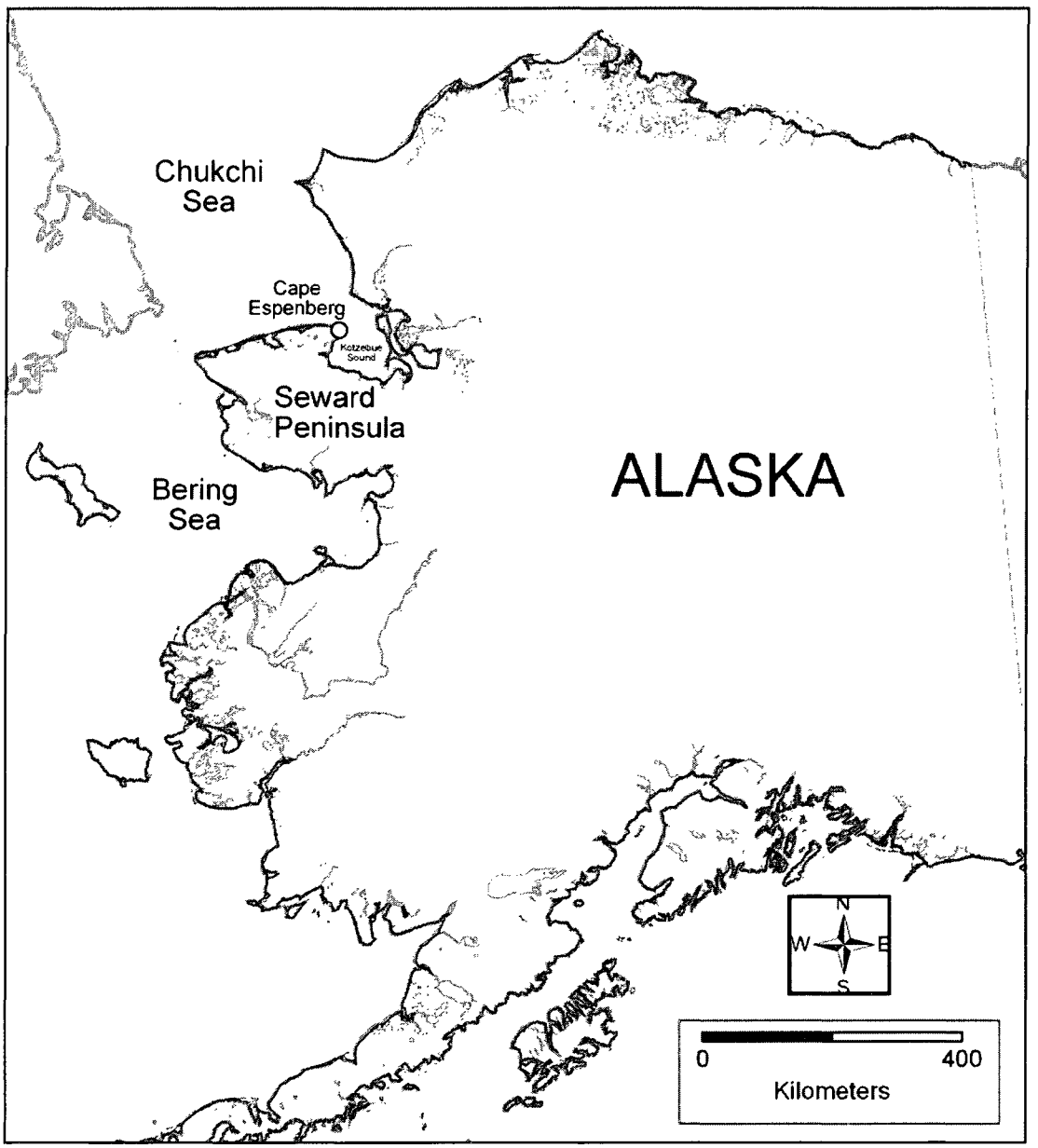

Figure 3.1. Map of Alaska showing location of Cape Espenberg.

The seal mandibles analyzed in this project derive from various features at the two sites that date to the Western Thule period on the Seward Peninsula (ca. 950 AD to the 19th century) (Harritt, 1994:250). Table 3.1 shows the site and feature affiliation of each of the specimens studied. All of the dates for site KTZ-087 cluster around 1270–1280 cal AD (calibrated radiocarbon age intercept). Dates for KTZ-088 include one in this same range (1272 cal AD) and one younger date (1636 cal AD), while dates for KTZ-101 fall around 1640 AD (all dates are from Harritt, 1994).

The bones selected for analysis here had been previously identified as ringed seal mandibles by trained faunal analysts (as described in Saleeby, 1994). The National Park Service Western Arctic National Parklands office in Anchorage (Alaska) provided computer printouts from an electronic database that described the faunal analysis findings for each of the specimens from KTZ-088 and KTZ-101, and provided copies of the original "Faunal Analysis Record" sheets for KTZ-087. These described the taxon, bone element, and completeness for each specimen. Based on these lists, a request was made to the National Park Service for sampling of all of the identified ringed seal mandibles. Permission was granted under BELA Loan Out L.2008.002 dated April 23, 2008 and Loan Out L.2008.003 dated April 28, 2008.

All ringed seal mandibles identified in the faunal records were inspected for inclusion in the study. Specimens were selected for further analysis if they were at least 1.5 g in weight (as estimated without teeth), included the entire portion of the mandible

under the post-canine tooth row, and were not obviously burned or otherwise severely degraded. This resulted in a final sample of 23 mandibles.

The sex and age for individual ringed seal specimens were not described in the National Park Service faunal remains database or record sheets, likely because these two variables are difficult to determine from morphological features of the mandible. While age can be accurately determined from microscopic examination of tooth cementum annuli (Stewart et al., 1996), most of the Cape Espenberg ringed seal specimens used in this study lacked teeth, so this method could not be used. However, as indicators of body size, the length of the post-canine tooth row and the maximum thickness (bucco-lingual) inferior to post-canine 5 were measured, following Woollett et al. (2000). No size effect on bone total mercury concentration was detected, so all Cape Espenberg ringed seal specimens were combined for further analysis.

Table 3.1. Ringed seal specimens from prehistoric Thule levels at sites KTZ-087, KTZ-088, and KTZ-101 (Cape Espenberg, Alaska) used in this study

ID #	NPS Catalog #	Feature[a]	Level	Feature calibrated radiocarbon dates (intercept and one-sigma range)[b]	Material dated	Lab ID
2001	KTZ087.10 F013	10	2	1279 (1262-1375) cal AD	charcoal	Beta 28006
2002	KTZ087.10 F013	10	2			
2004	KTZ087.10 F015	10	2			
2005	KTZ087.10 F015	10	2			
2006	KTZ087.10 F015	10	2			
2008	KTZ087.10 F023	10	2			
2009	KTZ087.10 F089	10	2			
2010	KTZ087.10 F001	10	2			
2011	KTZ087.10 F109	NA	NA			
2016	KTZ087.10 F202	30	8	1272 (1225-1295) cal AD	wood/charcoal	Beta 28011
2017	KTZ087.10 F222	30	9			
2018	KTZ087.10 F234	30	NA			
2019	KTZ087.10 F242	30	7			
2020	KTZ087.10 F253	30	4			
2022	KTZ088.10 F136	24	9	1272 (1221-1374) cal AD	wood	Beta 28013
2026	KTZ088.10 F196	24	3	1636 (1505-1651) cal AD	wood	Beta 28195
2027	KTZ088.10 F211	24	9			
2028	KTZ088.10 F230	24	9			
2029	KTZ088.10 F237	24	9			
2031	KTZ101.10 F032	NA	NA			
2032	KTZ101.10 F099	NA	NA			
2033	KTZ101.10 F189	2	NA	1648 (1528-1663) cal AD	wood	Beta 28019
2034	KTZ101.10 F268	15	6	1639 (1474-1952) cal AD	wood	Beta 28021
				1654 (1528-1955) cal AD	wood/charcoal	Beta 28022

[a] Specimens listed here as Feature 30 are associated with site KTZ-087 on the original labeled collection bags and Appendix I of Harritt (1994), but with KTZ-088 in the main text of Harritt (1994) and the faunal analysis by Saleeby (1994).

[b] Dates are calibrated radiocarbon age intercepts and 1 sigma range from Harritt (1994:82,141, 299-301). .

Bone sampling and pretreatment (prehistoric ringed seals)

Samples weighing at least 1.0 g were removed from the prehistoric ringed seal mandibles using a diamond cutting wheel attached to a Dremel tool. For the larger and more complete specimens, the sampling area included the inferior (bottom) portion of the horizontal ramus in the region posterior to post-canine 5 and anterior to the mandibular foramen (Figure 3.2). When necessary because of small size or incompleteness, sections were detached that included the superior portion of the horizontal ramus, or that included sections anterior to post-canine 5 or posterior to the mandibular foramen. The detached bone chunks were lightly abraded with a sanding drum to remove sediment and other contaminants at the surface of the bone, after which they were ultrasonicated in ultrapure water for 15 minutes and air dried overnight. Cleaned samples were freeze-dried for 24 hours, pulverized in a ball mill to less than 0.3 mm. Samples of bone powder were separated into portions intended for total mercury analysis (~800 mg), total nitrogen and carbon analysis (~2 mg), total sulfur analysis (~40 mg), and collagen extraction followed by $\delta^{15}N$ and $\delta^{13}C$ analysis (~50 mg). They were stored in plastic containers at room temperature until further analysis.

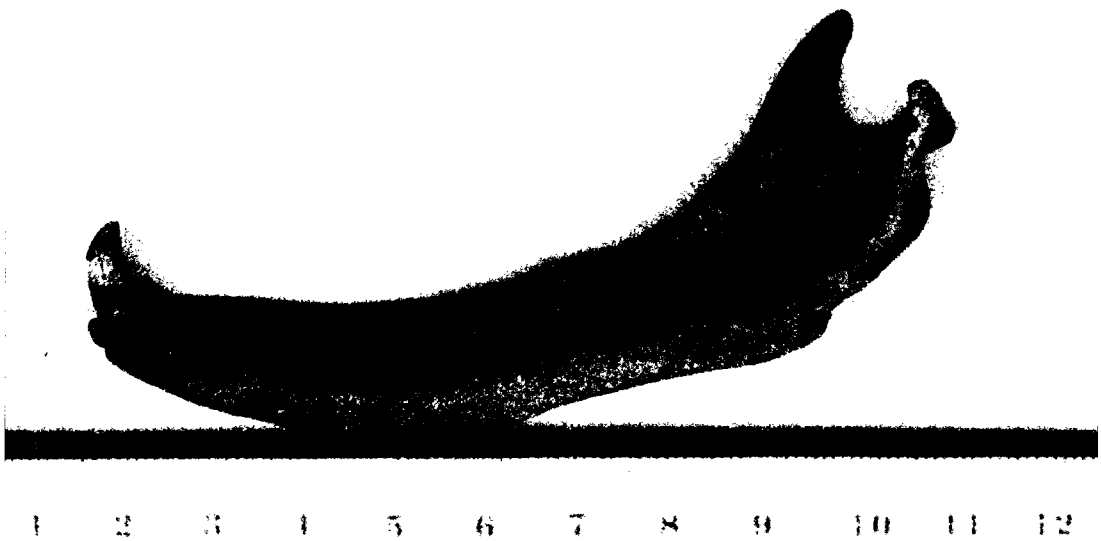

Figure 3.2. Ringed seal mandible recovered from prehistoric Thule site KTZ-087, Cape Espenberg, Alaska, ca. 1275–1640 AD. Scale is centimeters.

Two bone samples prepared in the above fashion were split, and one portion was further treated to remove bone mineral. The samples were demineralized as follows, using a modified version of the method described by Bell et al. (2001):

1. Approximately 0.8 to 1.0 g of cleaned, freeze-dried bone powder was weighed into a 45.0 ml plastic centrifuge tube.

2. Approximately 20 ml of 0.5 M HCl was added to the tube, vigorously mixed with the Touchmixer, and allowed to stand for 30 minutes, with vigorous mixing at 10 minute intervals.

3. Samples were centrifuged at 3000 rpm (relative centrifugal force = 1811 x g) for 2 minutes, and the supernatant was poured off.

4. Steps 2 and 3 were repeated two more times for a total of 90 minutes in the acid solution.

5. Samples were rinsed three times in ultrapure water, with centrifuging and pouring off the supernatant in between each rinse.

6. After freezing overnight, the samples were freeze-dried for 24 hours.

Note that modifications to the referenced method include a larger starting mass and a stronger acid concentration (0.5 M versus 0.1 M), as discussed in more detail in a later section.

Total mercury, nitrogen, carbon, and sulfur (prehistoric ringed seals)

Approximately 0.25–1.0 g of cleaned, freeze-dried prehistoric ringed seal bone powder was weighed off and shipped to Frontier Geosciences Inc. (Seattle, Washington) for total mercury analysis by Cold Vapor Atomic Fluorescence Spectrometry. Methods used by Frontier were described previously for methylmercury-dosed laboratory rat bone.

For total nitrogen and carbon determinations, samples of bone powder weighing approximately 0.5 mg were submitted to the Alaska Stable Isotope Facility at the University of Alaska Fairbanks. This facility determined percent nitrogen and carbon using the Costech Elemental Analyzer (ECS 4010), with peptone from animal tissue as a standard.

Because the Alaska Stable Isotope Facility does not conduct total sulfur determinations, samples were sent to the Washington State University Stable Isotope Core Laboratory for this analysis. This facility was selected for its rigorous quality control measures, including using multipoint normalizations and "blind" quality control samples in each analytical run (Benjamin Harlow, personal communication, 2009). This laboratory calculated percent sulfur with the ECS 4010 Elemental Analyzer using sulfanilamide as a standard.

Nitrogen and carbon stable isotope ratios (prehistoric ringed seals)

Nitrogen and carbon stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) were determined on bone collagen, so whole bone first had to be pretreated to remove mineral and contaminating substances. Bone powder was first defatted, since lipids are typically depleted in ^{13}C relative to protein (Liden et al., 1995). Lipid extraction followed the method of Radin (1981), as described below:

1. Approximately 0.05 g of cleaned, freeze-dried bone powder was weighed into a 2.0 ml microcentrifuge tube.

2. Approximately 1.5 ml of a 3:2 mixture of hexane:isopropanol was added to the bone powder, mixed vigorously with a vortex mixer and left to stand for one minute.

3. The sample was centrifuged for 15 seconds at 5000 rpm (relative centrifugal force = 1677 x g) and the supernatant poured off.

4. An additional 1.5 ml of defatting solution was added to the vial, mixed, left to stand for two minutes, centrifuged, and poured off.

5. Step 4 was repeated once.

6. The sample was left with the cap open under the fume hood overnight to evaporate the remaining hexane:isopropanol solution.

Next, the bone powder was demineralized following Bell et al. (2001), (with modifications, discussed below) as follows:

1. Approximately 1.5 ml of 0.5 M HCl was added to the vial containing the defatted bone powder, vigorously mixed with a vortex mixer, and allowed to stand for 30 minutes, with vigorous mixing at 10 minute intervals.

2. The sample was centrifuged for 15 seconds at 5000 rpm (relative centrifugal force = 1677 x g), and the supernatant poured off.

3. Steps 1 and 2 were repeated two more times for a total of 90 minutes in the acid solution.

4. The sample was rinsed three times in ultrapure water, with centrifuging and pouring off the supernatant in between each rinse.

One minor modification from the Bell et al. (2001) method is that the present study used a larger starting sample mass for ease in handling and because it was not constrained by the small sample masses resulting from bone density fractionation as in the referenced study. The amount of treatment solution was likewise increased to accommodate the increased sample amount. Note also that the acid concentration is a departure from Bell et al. (2001), who used a weaker acid strength (0.1 M HCl). The concentration was changed in the present study after initial informal laboratory trials indicated that the weaker acid strength did not fully demineralize the bone samples, as judged from the percent yield. The percent yield is the post-demineralization mass

expressed as a percent of the initial sample mass. Since collagen makes up approximately 20–30% of modern whole bone (Kennedy, 1988), percent yields above this indicate incomplete demineralization, and the laboratory trials using the 0.1 M HCl produced yields that were all over 50%. An HCl concentration of 0.5 M was selected because it is the concentration used by many leading bone collagen stable isotope researchers (Bronk Ramsey et al., 2004; Richards et al., 2006; Richards and Hedges, 1999; Sealy, 1997), and initial laboratory trials showed that the post demineralization percent yields were within the acceptable range (<30%).

To remove humic acid and other base-soluble contaminants, the samples were treated with an alkali solution, following Bell et al. (2001), as follows:

1. Approximately 1.5 ml of a 0.1 M NaOH solution was added to the sample vial containing the defatted and demineralized bone powder, vigorously mixed with a vortex mixer, and left to stand for 10 minutes.

2. The sample was centrifuged at 5000 rpm (relative centrifugal force = 1677 x g) for 15 seconds and the supernatant poured off.

3. The sample was rinsed three times in ultrapure water, with centrifuging and pouring off the supernatant in between each rinse.

4. The sample was frozen for at least one hour, followed by freeze-drying for 24 hours.

In paleodietary studies, the product remaining after bone demineralization and alkali treatment is typically referred to as "collagen" (sometimes in quotation marks), but it likely contains non-collagenous proteins as well. Technically, the product is weak-acid-insoluble bone protein (Bell et al., 2001).

The origin of the bone collagen extraction method used here is not explicitly described in Bell et al. (2001); however, based on author affiliation, the procedure appears to have derived from the method employed by the so-called "Cape Town group" (van der Merwe et al., 2003:249). As described by Sealy and van der Merwe (1986) with updates provided by van der Merwe et al. (2003), the collagen extraction method employed by the Cape Town group is noted for its use of bone chunks, rather than bone powder, and for excluding the collagen gelatinization treatment (dissolving the protein in hot dilute acid) introduced by Longin (1971). The Bell et al. (2001) method thus deviates from the typical Cape Town procedure by using bone powder, which is also used by many isotope researchers (Ambrose et al., 1997; Bocherens et al., 1991; Bronk Ramsey et al., 2000; Brown et al., 1988).

Nitrogen and carbon isotopic analyses of bone collagen were conducted at the Alaska Stable Isotope Facility housed at the University of Alaska Fairbanks. Bone collagen samples of approximately 0.3 mg were loaded into tin cups for stable isotopic analysis on the Thermo Finnigan Delta-Plus XP continuous flow isotope ratio mass spectrometer, coupled to a Costech Elemental Analyzer (ECS 4010). Typically, instrument precision for this lab is <0.2‰ based on repeated analysis of an in-house

standard (animal meat peptone) (T. Howe, Alaska Stable Isotope Facility, personal communication, 2008). A sample of modern ringed seal bone collagen from the present study was run a total of 12 times on the same and different days, and results yielded a standard deviation of 0.1‰ for both $\delta^{15}N$ and $\delta^{13}C$ (data not shown).

The ratios of nitrogen stable isotopes ($^{15}N/^{14}N$) and carbon stable isotopes ($^{13}C/^{12}C$) are conventionally expressed in delta (δ) notation in parts per thousand (per mil; ‰) relative to a standard:

$$\delta\ (‰) = ((R_{sample} - R_{standard})/R_{standard}) \times 1000 \qquad \text{[Eq. 1]}$$

where R_{sample} and $R_{standard}$ are the ratios of the heavier to the lighter isotope in the sample and the standard, respectively (Ambrose, 1993; Kelly, 2000). The delta value is a ratio of a ratio and is thus dimensionless. However, it has a "pseudo-dimensional unit," the "per mil" (Speakman, 1997:256). The delta value is the difference between the sample isotope ratio and the standard isotope ratio, expressed in thousandths of the standard isotope ratio (Speakman, 1997). The international standard for nitrogen is atmospheric nitrogen (AIR), while that for carbon is Peedee belemnite (PDB) (Ambrose, 1993). A delta value of 0‰ means there is no difference in the ratio of the heavy to light isotope between the sample and the standard. A positive δ value indicates that the sample has more of the heavy isotope than the standard (i.e., is enriched), while a negative value indicates that the sample has less of the heavy isotope than the standard (i.e., is depleted).

Statistical analysis (prehistoric ringed seals)

Initial inspection of the mercury concentration data indicated that variances were not equal across values of the independent variables, so bone THg concentrations were transformed to their common logarithms ($\log_{10}$) following Zar (1996). For all statistical analyses, specimens from all sites (KTZ-087, KTZ-088, and KTZ-101) were combined, since exploratory data analysis showed no significant differences among sites for all variables. As stated for the statistical analysis of methylmercury-dosed rats, for statistical hypothesis testing, the null hypothesis was rejected if the p-value was less than the significance level (α) of 0.05.

Separate least-squares linear regression analyses were performed to determine the strength, form, and significance of the relationship between log-transformed bone total mercury concentration (the dependent variable) and several independent variables: bone collagen $\delta^{15}N$ and $\delta^{13}C$, as well as whole bone %N, %C, and %S. For each analysis, the following null and alternative hypotheses were tested (following Sokal and Rohlf, 1969):

> H_0: The true slope of the regression line relating log-transformed bone total mercury to the independent variable is equal to zero.
>
> H_A: The true slope of the regression line is not equal to zero.

In other words, the null states that log-transformed bone THg does not tend to go up or down with a unit increase in the independent variable.

As discussed previously for the rat bone data set, the adequacy of each regression model to describe the relationship between log-transformed bone mercury concentration and the independent variables was assessed by examining the model's slope significance level, coefficient of determination (r^2), and prediction residuals.

Bone mercury measurement accuracy

Accuracy is here defined as the "closeness of agreement between a quantity value obtained by measurement and the true value" (Menditto et al., 2007:45), and is expressed in terms of both trueness and precision of the results (Hauck et al., 2008). Trueness is defined as "the closeness of agreement between a test result and the accepted reference value" (Thompson et al., 2002:847). Trueness is usually expressed as the percentage of recovery of a known amount of analyte in a sample. Precision is "the closeness of agreement between test results" (Thompson et al., 2002:848), and is usually expressed in terms of imprecision, as the standard deviation or relative standard deviation of sample replicates.

In the present study, bones of modern ringed seal from western Alaska were analyzed for mercury concentration using CVAFS. Trueness and precision were calculated from the resulting bone mercury concentration measurements, and these were compared to internationally recognized standards for chemical measurement accuracy. Specific criteria for acceptable levels of precision (as the repeatability relative standard deviation) and trueness (as percent recovery) followed those provided by AOAC International (AOAC, 2002).

Materials (modern ringed seals)

The sample used to assess bone mercury measurement accuracy included modern ringed seal mandibles from western Alaska. A total of 20 modern ringed seal dentaries (i.e., the right or left half of a mandible) were sampled and analyzed for total mercury concentration. These mandibles had been subjected to varied methods of preparation for museum storage, which may have included treatments that removed inorganic or organic mercury from the bone, including submersion in maceration tanks and/or soaking in ammonium hydroxide. The very low concentrations of mercury found in these mandibles suggest that some storage treatment or treatments did extract mercury. Because of the probable leaching of mercury from these bone samples, they could not be used to compare bone to soft tissue mercury concentrations, as originally intended. However, these specimens are still useful for determining the bone mercury measurement quality. Additionally, four of the methylmercury-dosed rat bone samples were included in the trueness (percent recovery) analysis.

Bone sampling and pretreatment (modern ringed seals)

Samples weighing at least 1 g were removed from the mandible in the same manner as described previously for prehistoric ringed seals. These were stored in plastic vials at room temperature until further analysis.

Total mercury determinations (modern ringed seals)

Approximately 0.75 g of cleaned, freeze-dried modern seal bone powder was weighed off and shipped to Frontier Geosciences Inc. (Seattle, Washington) for total mercury analysis by cold vapor atomic fluorescence spectrometry, as described in previous sections. At Frontier, samples were split prior to sample digestion and prepared and analyzed in separate runs approximately two weeks apart. This approach allows calculation of between-run (different-day) analytical error. Also, four samples were run in duplicate within the same run, allowing measurement of within-run (same-day) error. To assess measurement trueness, four modern seal bone samples were spiked with a known quantity of mercury (to equal approximately 400 ppb) and percent of mercury recovered was calculated. In addition, four methylmercury-dosed rat bones were spiked with a similar quantity of mercury to allow calculation of percent recovery. The amount of the spike added should be high enough to overwhelm the contribution from the native mercury already present in the sample (Lee et al., 2006), although the analytical method validation literature offers little detail as to appropriate spiking levels. Since little information was available on the expected range of mercury concentrations in bone (from any species), the Laboratory's default spike level of 400 ppb was used (C. Molder, Frontier Geosciences Inc., personal communication, 2008).

Statistical analysis (modern ringed seals)

The differences between the duplicate THg measurements were calculated and inspected for outliers, following Fraser (2004). Any absolute difference measurement

that was greater than three standard deviations above or below the mean absolute difference was classified as an outlier (following Stevens, 1999) and eliminated from consideration. While eliminating deviant cases from a data set intended to measure variation may at first seem counterintuitive, Fraser (2004:21) points out that outliers must be removed because "even a single unusual observation, perhaps resulting from an analytical mistake or simple misdirection of a sample, can greatly influence the estimate of the components of variance."

Precision of bone mercury determinations was estimated using the repeatability standard deviation and the relative repeatability standard deviation. The repeatability standard deviation (SD_r) was calculated as follows, for repeats that are *n* sets of duplicate measurements (AOAC, 2006: D8; Synek, 2008):

$$\text{Repeatability Standard Deviation } (SD_r) = \sqrt{(sum\ of\ d^2)/2n} \qquad \text{[Eq. 2]}$$

where *d* is the difference between the duplicates and *n* is the number of pairs. This provides a measure of imprecision in units of the original data (e.g., ng/g).

The repeatability relative standard deviation provides a measure of the magnitude of the method imprecision and is expressed as a percent. The repeatability relative standard deviation was calculated as follows (AOAC, 2006:D9):

$$\text{Repeatability Relative Standard Deviation } (RSD_r) = 100(SD_r/\bar{x}) \qquad \text{[Eq. 3]}$$

where SD_r is the repeatability standard deviation, and $\bar{x}$ is the mean of all of the measurements. The repeatability relative standard deviation is sometimes referred to as

the "relative standard deviation" or the "coefficient of variation" (EPA, 2000:8-37; Willetts and Wood, 1999:288). The RSD_r was calculated for the 20 samples measured in duplicate and the result was compared to the acceptance criteria in Table 3.2. The expected within-laboratory $RSD_r = C^{-0.15}$, where C is the concentration of the analyte expressed as a mass fraction. Note that the formula for between-laboratory expected RSD_r is $2C^{-0.15}$ (AOAC, 2002:21; AOAC, 2006). Acceptable limits are between 0.5 and 2 times the expected RSDr (AOAC, 2002:22), up to a maximum of 30% (Horwitz and Albert, 2006). Values below the lower limit are not undesirable (i.e., they show better precision) but fall outside the range of historically expected precision, which could indicate unreported averaging or previous knowledge of the analyte concentration (Horwitz and Albert, 2006). If the RSDr value for duplicate THg determinations on modern seal bone was within the recommended range, it was deemed acceptable.

Trueness was expressed as the percent recovery of a spiked sample. The percent recovery was calculated as follows (AOAC, 2006:D8):

$$\text{Percent Recovery} = 100 * ((C_f - C_u)/C_A) \qquad \text{[Eq. 4]}$$

Where C_f is the measured concentration of the fortified (spiked) sample; C_u is the measured concentration of the unfortified sample (before spiking); and C_A is the amount of analyte added to the sample. The mean percent recovery was calculated for the modern ringed seal sample and the mercury-dosed rat sample, and the results were compared to the acceptance criteria as outlined in Table 3.3. If the mean percent recoveries were within the recommended range, they were deemed acceptable.

Table 3.2. Acceptable within-laboratory precision limits for concentration measures as a function of concentration

Analyte Mass Fraction	Unit	Expected RSD_r (%)[a]	Allowable RSD_r (%)[b]
1	100%	1	0.5–2
0.1	10%	1.5	0.7–3
0.01	1%	2	1–4
0.001	0.10%	3	1.5–6
0.0001	100 ppm	4	2–8
0.00001	10 ppm	6	3–12
0.000001	1 ppm	8	4–16
0.0000001	100 ppb	11	5.5–22
0.00000001	10 ppb	16	8–30
0.000000001	1 ppb	22	11–30

[a] The expected within-laboratory $RSD_r = C^{-0.15}$, where C is the concentration of the analyte expressed as a mass fraction. (AOAC, 2002:21; AOAC, 2006).

[b] Acceptable limits are between 0.5 and 2 times the expected RSDr (AOAC, 2002:22), up to a maximum of 30% (Horwitz and Albert, 2006).

Table 3.3. Acceptable recovery limits (%) as a function of analyte concentration

Analyte Mass Fraction	Unit	Acceptable Recovery Limits (%)[a]
1	100%	98–101
0.1	10%	95–102
0.01	1%	92–105
0.001	0.10%	90–108
0.0001	100 ppm	85–110
0.00001	10 ppm	80–115
0.000001	1 ppm	75–120
0.0000001	100 ppb	na
0.00000001	10 ppb	70–125
0.000000001	1 ppb	na

[a] From AOAC (2002:19)

4. RESULTS

Bone mercury in methylmercury-dosed laboratory rats

Bone mercury versus daily exposure (methylmercury-dosed rats)

Some mercury was detected in all bone samples from methylmercury-dosed rats, including those from the control (and nominally unexposed) group (Table 4.1). All quality control measures for mercury detection fell within the acceptance criteria of the analytical laboratory (Frontier Geosciences), (as described in the Methods section. Table 4.2 shows arithmetic and geometric mean total mercury (THg) concentrations in cranial bone, humeral cortical bone, and femoral cortical bone, for each exposure group. The one-way ANOVAs on log-transformed cranium, humerus, and femur THg identified significant differences in means ($p < 0.001$) among the exposure groups for all bone types (Table 4.3). The null hypothesis of equal means among exposure groups was thus rejected for all bone types. Subsequent multiple comparisons tests (Least Significant Difference) showed that differences are significant among all possible pairs of means for all bone types (Table 4.4).

Table 4.1. Bone and kidney total mercury concentrations in methylmercury-dosed rats by individual

ID	MeHg Exposure (μg/kg bw/day)	Cranium THg (ng/g)	Humerus THg (ng/g)	Femur THg (ng/g)	Kidney THg (ng/g)
100	0	5.1	3.2	1.8	14.7
150	0	5.0	3.3	2.8	14.5
200	0	3.9	3.9	2.0	15.3
250	40	16.6	11.6	10.8	2990.0
300	40	9.2	6.8	5.3	1810.0
350	40	9.9	8.2	5.6	945.0
400	40	5.9	6.4	5.2	1190.0
450	400	907.0	850.0	907.0	81300.0
500	400	720.0	894.0	523.0	43800.0
550	400	1130.0	1150.0	1020.0	79600.0
600	400	778.0	381.0	493.0	53300.0
650	400	2640.0	1060.0	721.0	69100.0
700	400	1500.0	928.0	699.0	67300.0
750	400	1820.0	672.0	597.0	NA
800	400	1740.0	793.0	570.0	53400.0

Table 4.2. Means and standard deviations for bone and kidney total mercury by exposure group in methylmercury-dosed rats

Tissue	Exposure Group[a]	n	Arithmetic Mean THg (ng/g)	Standard deviation	Geometric mean THg (ng/g)
Cranium (dry weight)	Control	3	4.67	0.64	4.64
	Low	4	10.39	4.49	9.71
	High	8	1404.37	655.16	1279.87
	Total	15	752.70	857.11	113.17
Humerus (dry weight)	Control	3	3.47	0.37	3.45
	Low	4	8.25	2.36	8.02
	High	8	841.00	237.96	803.96
	Total	15	451.43	462.77	79.11
Femur (dry weight)	Control	3	2.20	0.53	2.16
	Low	4	6.73	2.72	6.39
	High	8	691.25	187.76	670.62
	Total	15	370.90	378.54	61.54
Kidney (wet weight)	Control	3	14.83	0.42	14.83
	Low	4	1733.75	913.19	1570.66
	High	7	63971.43	14226.94	62563.34
	Total	14	32484.25	34083.83	3649.91

[a] Low exposure = 40 μg/kg bw/day methylmercury x 96 days; High exposure = 400 μg/kg bw/day methylmercury x 96 days

Table 4.3. Results of one-way ANOVA comparing mean bone total mercury (log_{10} ng/g) among three exposure categories in methylmercury-dosed rats

Bone	df between groups, df within groups	F	p
Cranium	2, 12	296.4	<0.001
Humerus	2, 12	525.1	<0.001
Femur	2, 12	632.3	<0.001

Note: exposure categories include: 1) Control (no exposure); 2) Low exposure (40 μg/kg bw/day methylmercury x 96 days); and 3) High exposure (400 μg/kg bw/day methylmercury x 96 days)

Table 4.4. Multiple pairwise comparisons (Least Significant Difference) for mean bone total mercury (log_{10} ng/g) by exposure categories in methylmercury-dosed rats

Exposure Group Comparison	Mean Difference bone THg (log_{10} ng/g)	p
Cranium		
Control–Low	−0.32	0.038
Control–High	−2.44	<0.001
Low–High	−2.12	<0.001
Humerus		
Control–Low	−0.37	0.003
Control–High	−2.37	<0.001
Low–High	−2.00	<0.001
Femur		
Control–Low	−0.47	<0.001
Control–High	−2.02	<0.001
Low–High	−2.02	<0.001

Note: exposure categories include: 1) control (no exposure); 2) Low exposure (40 μg/kg bw/day methylmercury x 96 days); and 3) High exposure (400 μg/kg bw/day methylmercury x 96 days)

Linear regression plots relating log-transformed bone THg concentration (log_{10} ng/g) to exposure level (μg/kg bw/day methylmercury) in methylmercury-dosed rats are shown in figures 4.1–4.3 for each bone type. The plots indicate that each of the log-transformed bone total mercury variables has a positive linear relationship with raw exposure level. Linear regression equations relating log-transformed bone THg to daily exposure are found in Table 4.5. The slopes for the regression lines relating log-transformed cranium THg, humerus THg, and femur THg to exposure are similar, rounding to 0.006, and all differ significantly from zero ($p<0.001$). The null hypothesis that the true slope is equal to zero for the regression line relating log-transformed bone THg to exposure level was thus rejected for all three bone types.

The coefficients of determination (r^2) are high (0.98–0.99) for the linear regression models for all three bone types, indicating that between 98% and 99% of the variation in bone THg may be accounted for by variation in exposure level. Visual examination of the linear regression plots (figures 4.1–4.3) shows that the models slightly overestimate mercury concentration for the control group for all bone types, but that the predicted values for the other exposure groups appear to be unbiased.

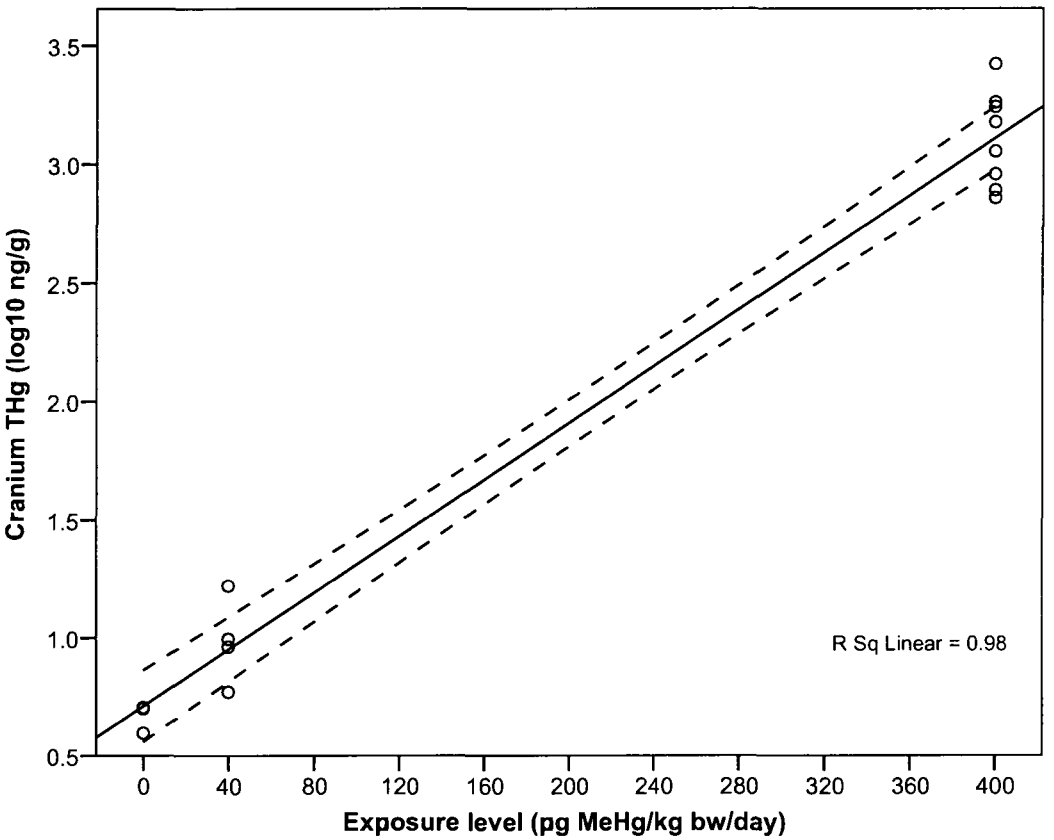

Figure 4.1. Cranium total mercury vs. exposure level in methylmercury-dosed rats, showing least-squares regression line (solid) and 95% confidence belt for the predicted mean (dashed lines).

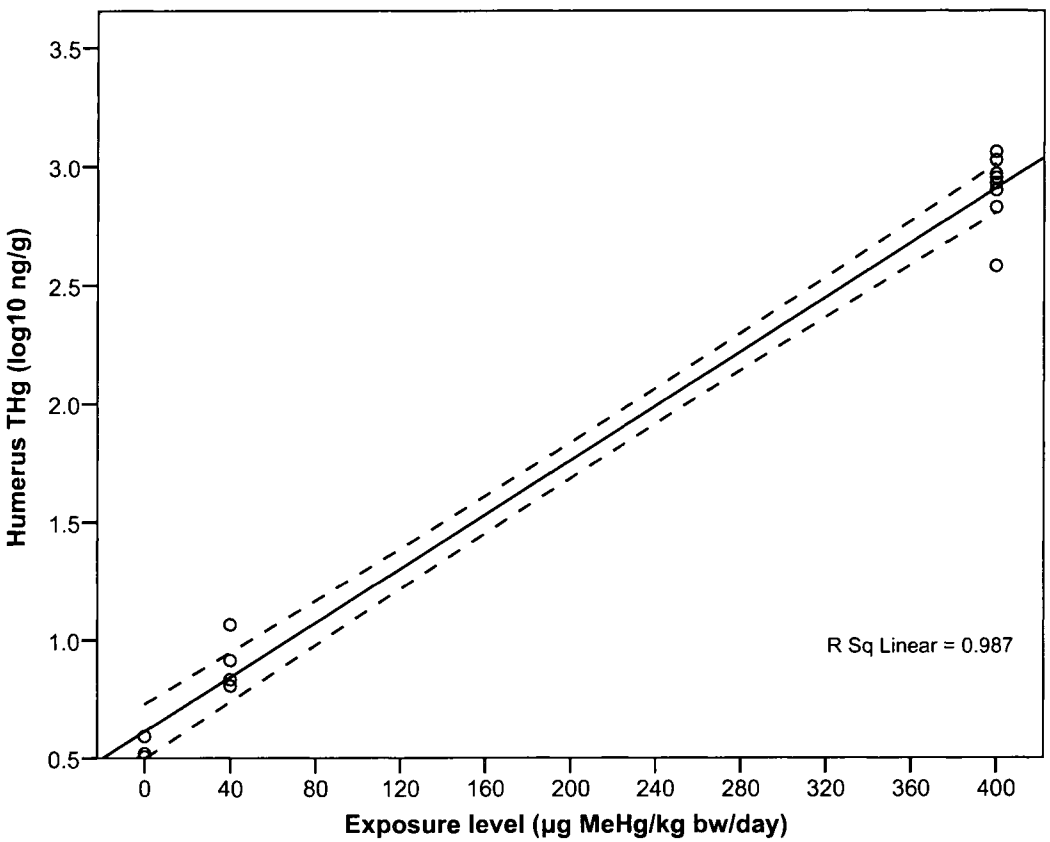

Figure 4.2. Humerus total mercury vs. exposure level in methylmercury-dosed rats, showing least-squares regression line (solid) and 95% confidence belt for the predicted mean (dashed lines).

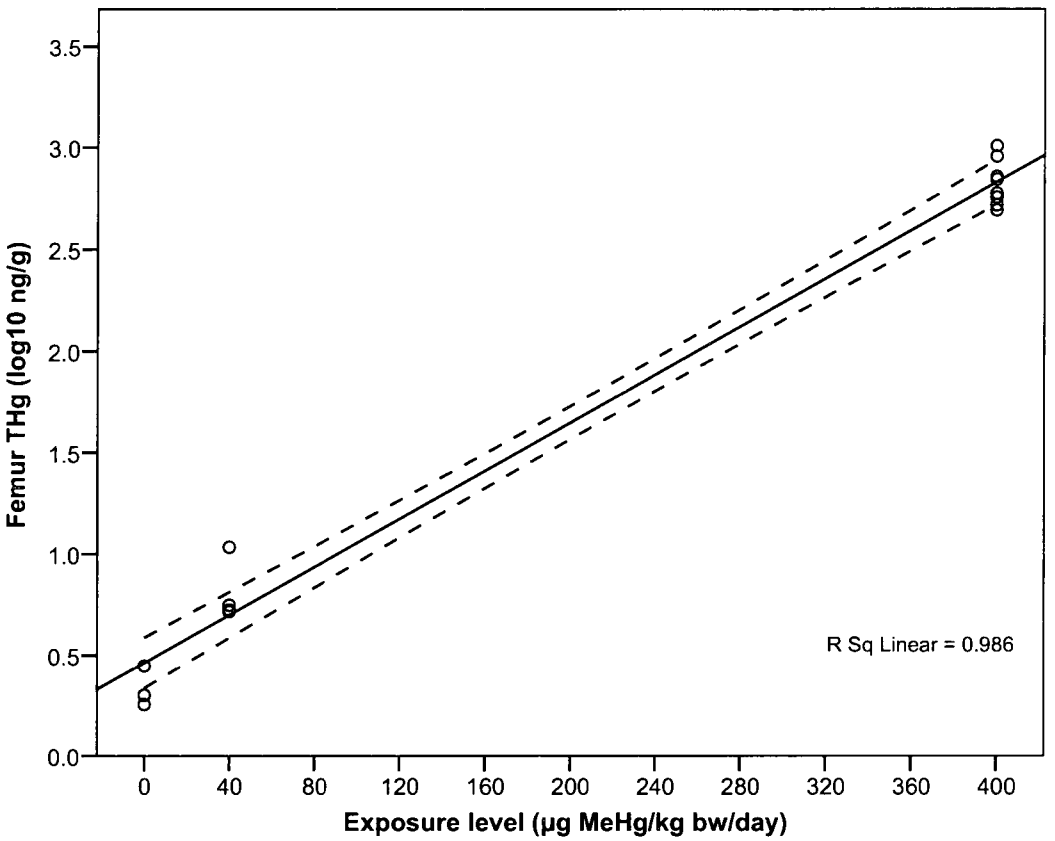

Figure 4.3. Femur total mercury vs. exposure level in methylmercury-dosed rats, showing least-squares regression line (solid) and 95% confidence belt for the predicted mean (dashed lines).

Table 4.5. Regression equations relating bone THg to exposure level in methylmercury-dosed rats

Predictor variable (x)	Response variable (y)	r^2	Standard error of estimate	Regression equation	p
MeHg exposure (μg/kg bw/day)	cranium THg ($\log_{10}$ ng/g)	0.98	0.175	$y = 0.0060x + 0.711$	<0.001
	humerus THg ($\log_{10}$ ng/g)	0.99	0.134	$y = 0.0057x + 0.613$	<0.001
	femur THg ($\log_{10}$ ng/g)	0.99	0.145	$y = 0.0059x + 0.462$	<0.001

Bone mercury versus kidney mercury (methylmercury-dosed rats)

Mercury was detected in all rat kidneys (Table 4.1), with mean concentrations varying by several orders of magnitude across exposure groups (Table 4.2). Linear regression plots relating log-transformed bone mercury concentrations to log-transformed kidney mercury concentrations for all exposure groups combined are shown in figures 4.4 through 4.6. Visual inspection of the regression plots shows that the linear function provides a poor fit to the data for all bone types, despite the fact that the models have high significance ($p<0.01$) and high r^2 values (>0.80). The plots clearly show systematic underprediction of bone THg values for the control and high dose groups and large overprediction of bone THg values for the individuals in the low-dose group. Predicted bone mercury values for this last group were all overestimated by a factor of two to six (data not shown).

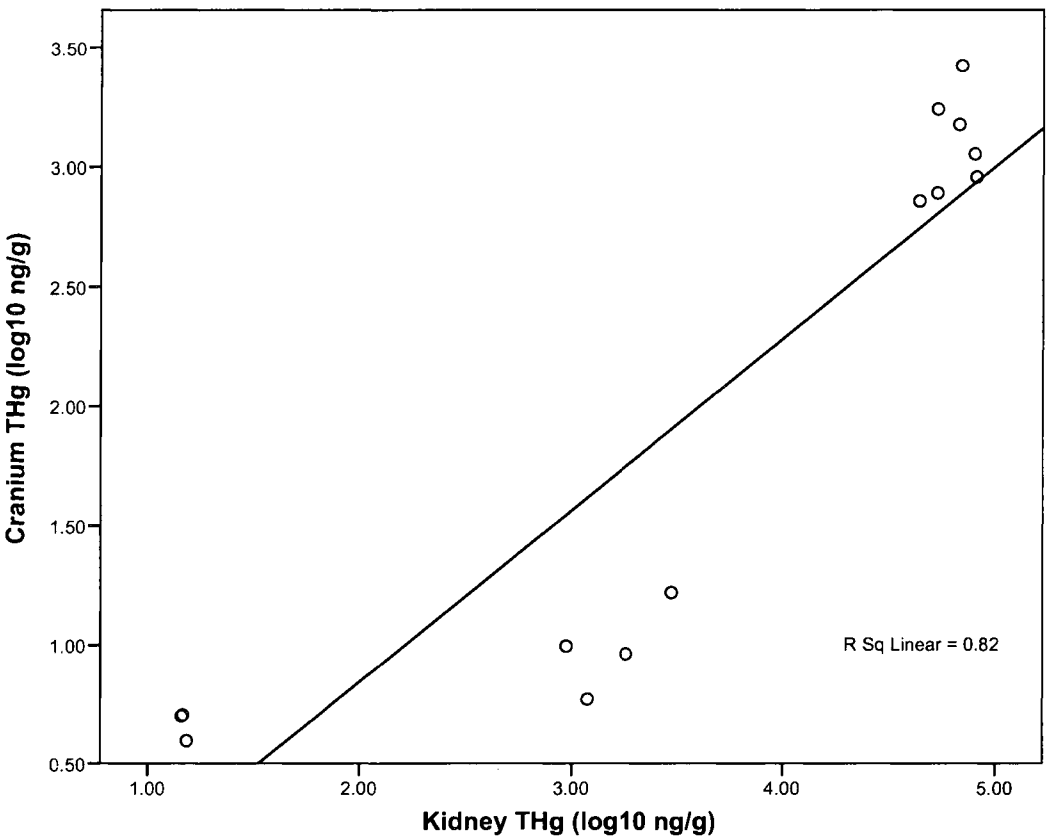

Figure 4.4. Cranium THg (log_{10} ng/g) versus kidney THg (log_{10} ng/g) in methylmercury-dosed rats (control group included). Note the poor fit between the prediction line and the data points, despite the high r^2 value.

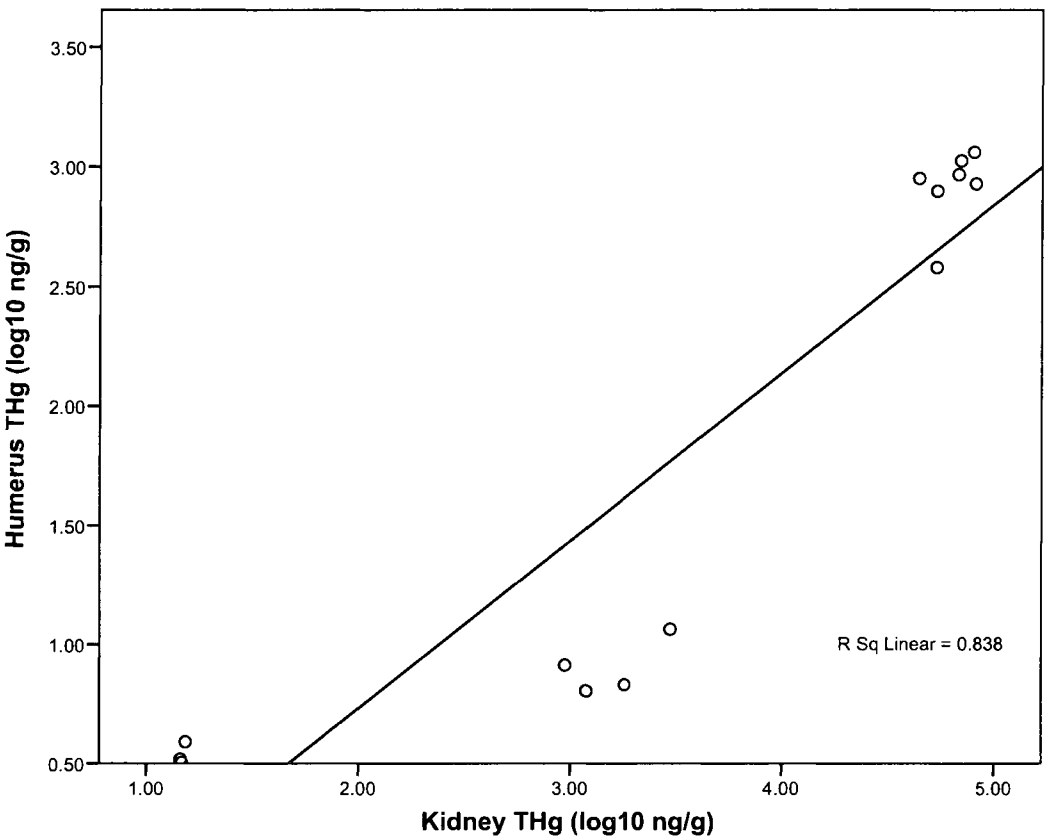

Figure 4.5. Humerus THg (log_{10} ng/g) versus kidney THg (log_{10} ng/g) in methylmercury-dosed rats (control group included). Note the poor fit between the prediction line and the data points, despite the high r^2 value.

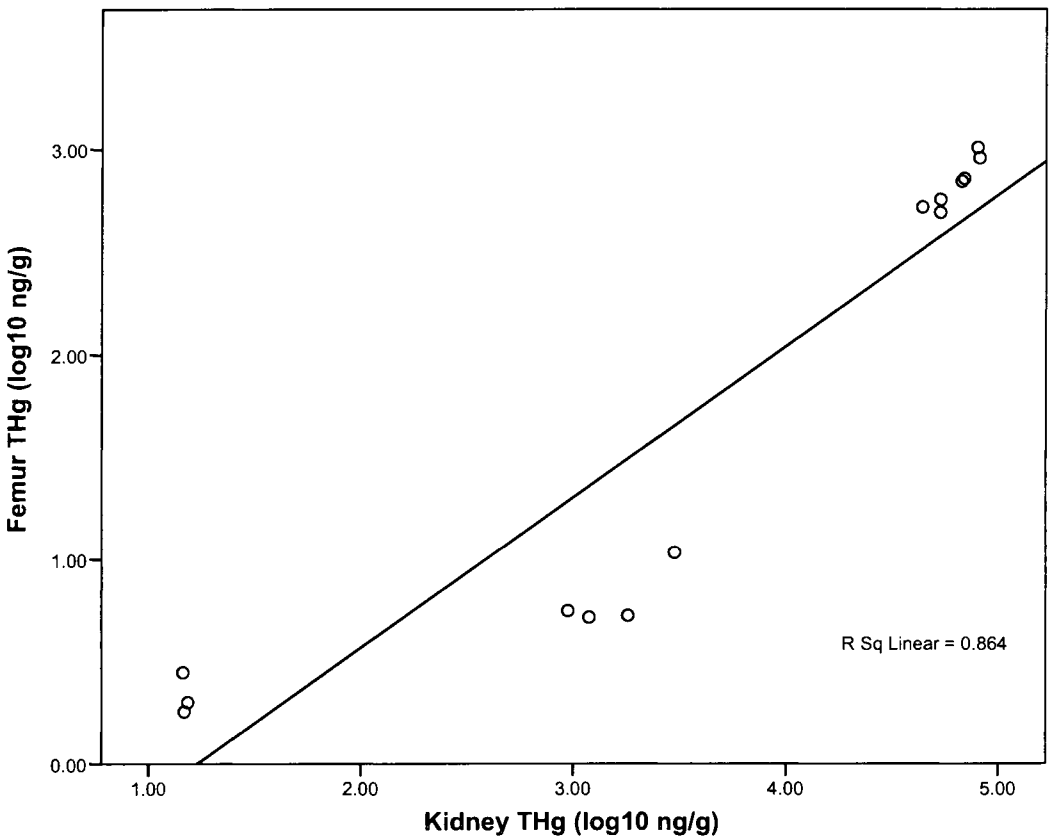

Figure 4.6. Femur THg (log_{10} ng/g) versus kidney THg (log_{10} ng/g) in methylmercury-dosed rats (control group included). Note the poor fit between the prediction line and the data points, despite the high r^2 value.

When only the exposed rats are considered, however, the linear regression models relating $\log_{10}$ bone THg to $\log_{10}$ kidney THg appear to provide a provide a better fit, as seen in figures 4.7–4.9. Table 4.6 provides the regression equations relating bone THg to kidney level with the control group excluded. For all three bone types, the slopes of the regression lines are positive and significantly different from zero ($p<0.001$). Thus, the null hypothesis that the true slope is equal to zero for the regression line relating log-transformed bone THg to log-transformed kidney THg was rejected for all three bone types. The models have high coefficients of determination for all three bone types (Table 4.3), indicating that over 96% of the variance in bone total mercury may be explained by variation in kidney total mercury. Visual inspection of the regression models indicates that there is no systematic over- or under-prediction of bone THg from kidney THg.

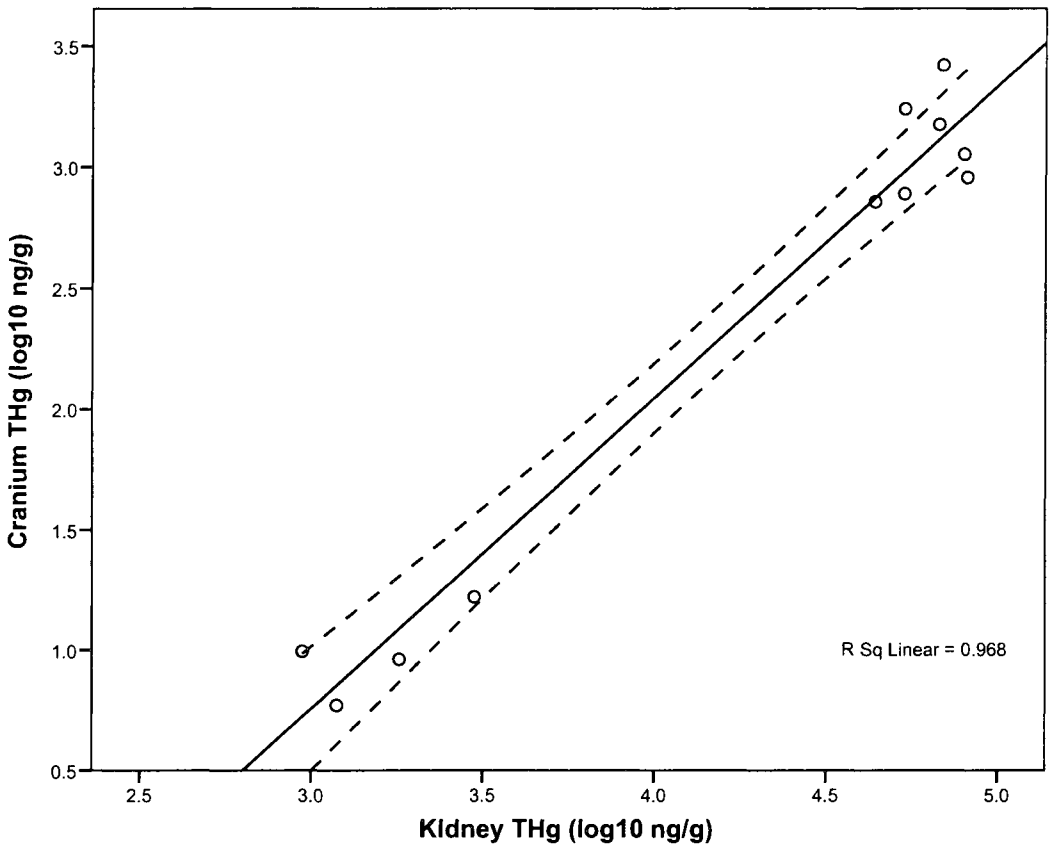

Figure 4.7. Cranium THg ($\log_{10}$ ng/g) versus kidney THg ($\log_{10}$ ng/g) in methylmercury-dosed rats (control group excluded), showing least-squares regression line (solid) and 95% confidence belt for predicted means (dashed lines).

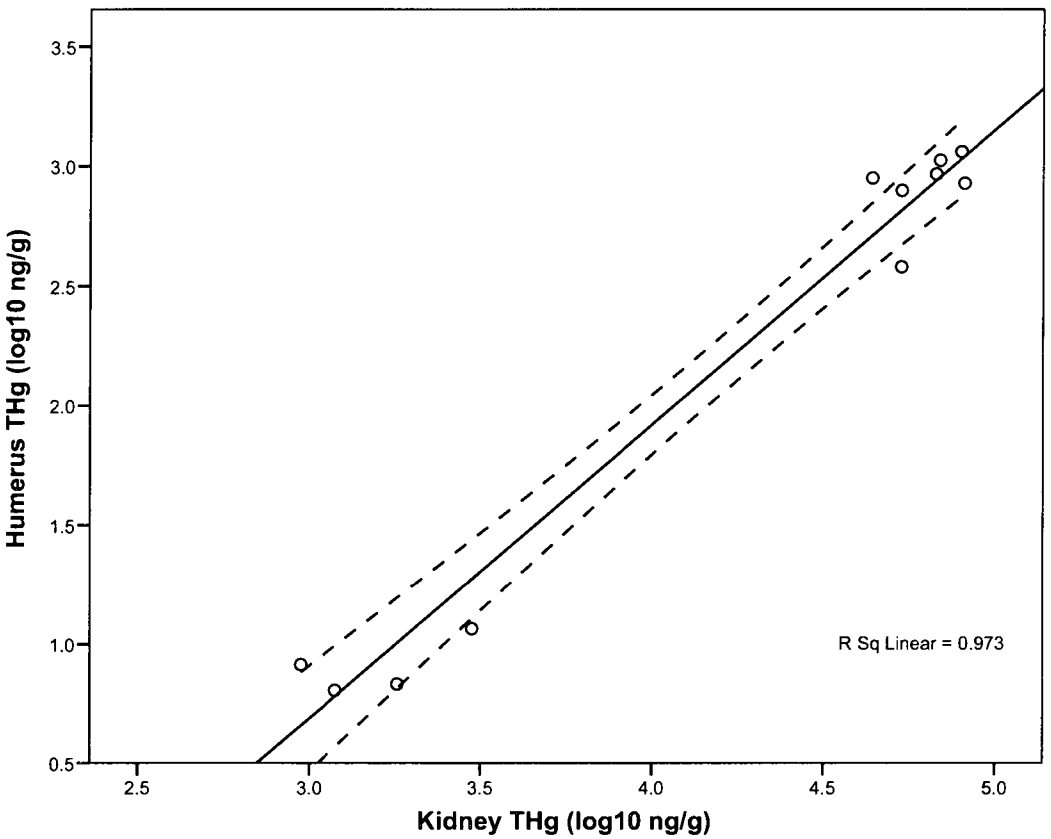

Figure 4.8. Humerus THg ($\log_{10}$ ng/g) versus kidney THg ($\log_{10}$ ng/g) in methylmercury-dosed rats (control group excluded), showing least squares regression line (solid) and 95% confidence belt for predicted means (dashed lines).

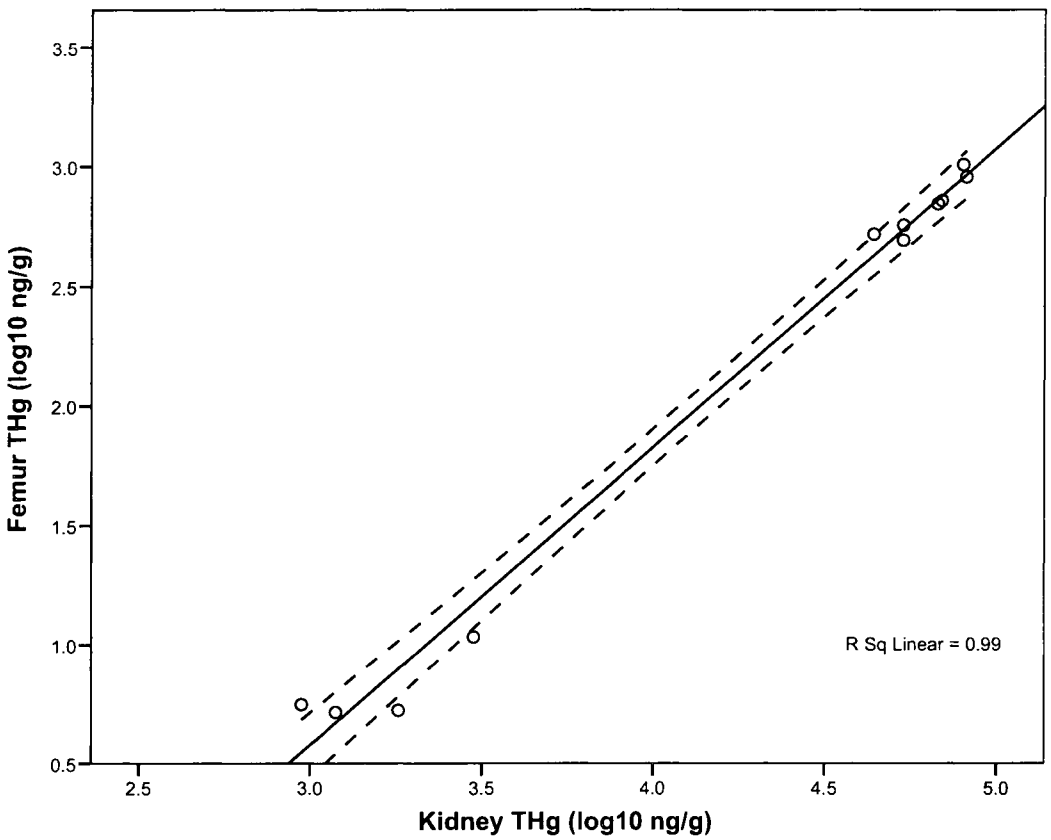

Figure 4.9. Femur THg (log_{10} ng/g) versus kidney THg (log_{10} ng/g) in methylmercury-dosed rats (control group excluded), showing least squares regression line (solid) and 95% confidence belt for predicted means (dashed lines).

Table 4.6. Linear regression equations for log-transformed bone THg against log-transformed kidney THg in methylmercury-dosed rats (control group excluded)

Predictor variable (x)	Response variable (y)	r^2	Standard error of estimate	Regression equation	p
Kidney THg ($\log_{10}$ ng/g)	Cranium THg ($\log_{10}$ ng/g)	0.97	0.20	$y = 1.29x - 3.11$	<0.001
	Humerus THg ($\log_{10}$ ng/g)	0.97	0.18	$y = 1.23x - 3.01$	<0.001
	Femur THg ($\log_{10}$ ng/g)	0.99	0.09	$y = 1.25x - 3.18$	<0.001

Bone mercury in cranium, humerus, and femur (methylmercury-dosed rats)

Differences in geometric mean THg concentration among methylmercury-dosed rat cranial bone, humeral bone, and femoral bone were examined using the repeated-measures ANOVA on log-transformed total mercury concentrations. The results show a significant difference in geometric mean THg concentration among the three types of bone ($F_{2,28}$=23.63; $p<0.001$), in which the order of means is cranial THg > humerus THg > femur THg. Thus, the null hypothesis that there is no difference in mean bone THg ($\log_{10}$ ng/g) among the three bone types (cranium, humerus, and femur) was rejected. Subsequent pairwise comparisons (Least Significant Difference) showed that all possible mean pairs are significantly different (Table 4.7).

Figures 4.10–4.12 show scatterplots of pairs of log-transformed bone total mercury concentrations (cranium vs. humerus, cranium vs. femur, and humerus vs. femur). These plots show clear clusters of points, which correspond to the three exposure groups, and all pairs of variables have high and significant Pearson correlation coefficients ($r>0.98$, two-tailed $p<0.001$). Thus, for each bone combination, the null hypothesis that the true correlation coefficient between the two bone total mercury concentrations is zero was rejected.

Table 4.7. Multiple pairwise comparisons (Least Significant Difference) for mean bone total mercury (log_{10} ng/g) by bone type (cranium, humerus, femur) in methylmercury-dosed rats

Bone Type Comparison	Mean Difference bone THg (log_{10} ng/g)[a]	p
Cranium–Humerus	0.155	0.002
Cranium–Femur	0.265	<0.001
Humerus–Femur	0.109	0.001

[a] Note that the difference measure is in log_{10} units

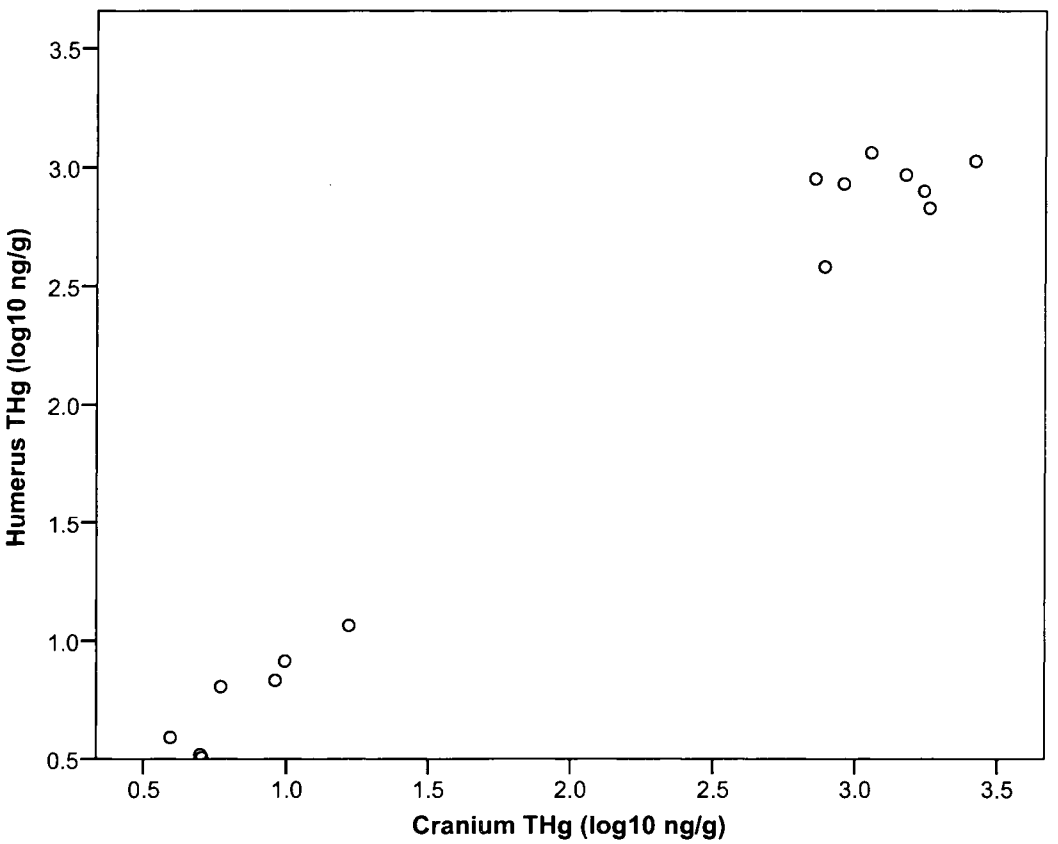

Figure 4.10. Bivariate scatterplot of cranium THg (log_{10} ng/g) and humerus (log_{10} ng/g) in methylmercury-dosed rats.

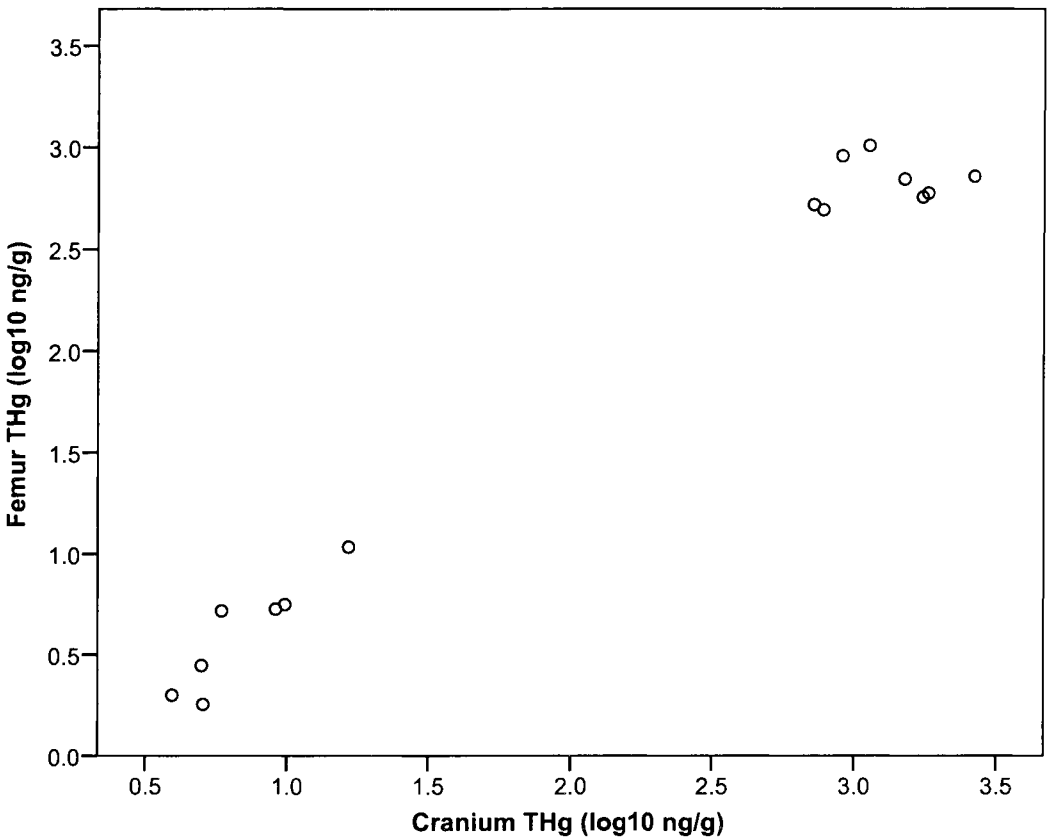

Figure 4.11 Bivariate scatterplot of cranium THg ($\log_{10}$ ng/g) and femur THg ($\log_{10}$ ng/g) in methylmercury-dosed rats.

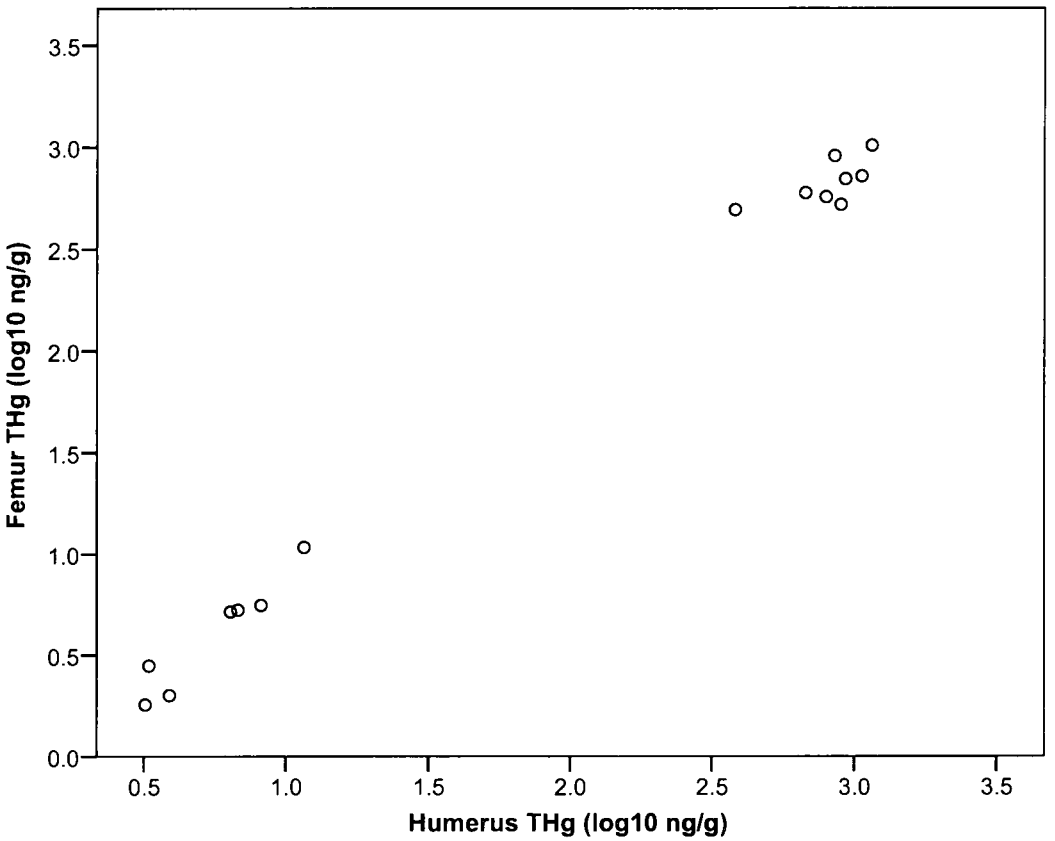

Figure 4.12. Bivariate scatterplot of femur THg (log_{10} ng/g) and humerus THg (log_{10} ng/g) in methylmercury-dosed rats.

Organic and inorganic mercury in bone (methylmercury-dosed rats)

Table 4.8 presents results for methylmercury (MeHg) determinations compared to total mercury (THg) for crania in methylmercury-dosed rats. Methylmercury was detected at or above the minimum reporting limit (4.0 ng/g) in all of the high exposure individuals, two of the low exposure samples, and none of the control group samples. When detected, methylmercury makes up approximately 67% to 94% of total mercury in the cranial bone samples. A scatterplot of $\log_{10}$ cranium THg against $\log_{10}$ cranium MeHg appears in Figure 4.13. The two variables have a significant and nearly perfect correlation (r=0.999; two-tailed $p<0.001$), so the null hypothesis that the true correlation coefficient between $\log_{10}$ cranium THg and $\log_{10}$ cranium MeHg is zero was rejected.

Table 4.8. Cranium THg compared to MeHg in methylmercury-dosed rats

Exposure Group	ID #	THg (ng/g)	MeHg (ng/g)	%MeHg
Low	250	16.6	11.8	71
	350	9.9	6.6	67
High	450	907.0	708.0	78
	500	720.0	610.0	85
	550	1130.0	782.0	69
	600	778.0	599.0	77
	650	2640.0	2480.0	94
	700	1500.0	1220.0	81
	750	1820.0	1490.0	82
	800	1740.0	1630.0	94

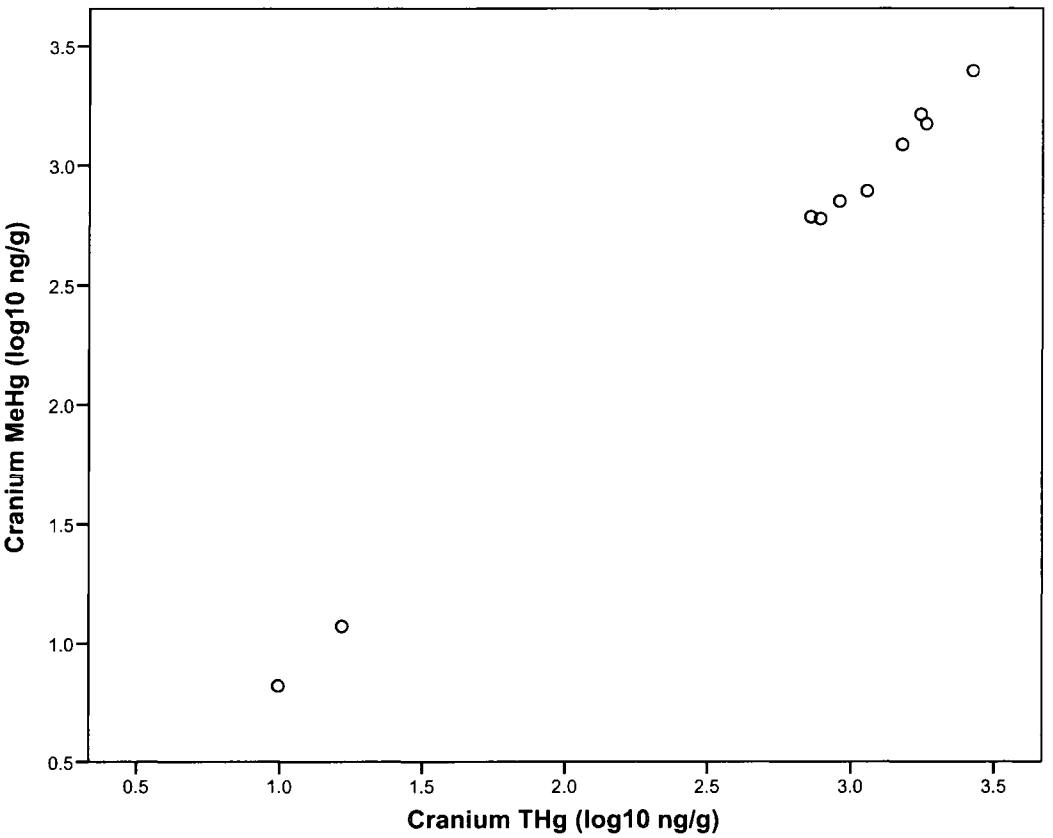

Figure 4.13. Bivariate scatterplot of cranium methylmercury (log_{10} ng/g) and total mercury (log_{10} ng/g) for methylmercury-dosed rats.

Bone mercury and indicators of mercury exposure in archaeofauna

Bone mercury, nitrogen, carbon, and sulfur (prehistoric ringed seals)

Frontier Geosciences reported that all quality control measures were within the established control limits for total mercury determinations (C. Molder, personal communication, 2008). Mercury is present in all of the prehistoric ringed seal mandibles, as shown in Table 4.9. One case (2022) with a very high concentration of mercury (55.1 ng/g) was eliminated as an outlier as defined by Stevens (1999), since it was over 3.5 standard deviations from the mean of all THg values.

A second case (2022) was eliminated as a regression outlier, which is a case with an observed value that differs appreciably from the predicted value under a regression model. Following Heiberger and Holland (2004), cases with studentized deleted residual values greater than 2 should be considered outliers in small (<30) samples, and case 2022 had values of over 2.6 to 4.0 for regressions of log-transformed bone THg versus $\delta^{15}N$, %N, %C, and %S. The values for the outlier cases are shown in the data tables (tables 4.9 and 4.10) but are excluded from the calculation of summary statistics and from all further statistical analyses. The outlier cases may represent a single individual, as they are right and left dentaries and were recovered from adjacent excavation units at site KTZ-088 (as indicated on original sample collection bag). They have similar values for all metrics, including size measurements, although their total mercury values, while both high, are disparate.

For the remaining 21 cases, concentrations of total mercury range from 4.36 ng/g to 32.80 ng/g, with a mean of 13.10 ± 6.5 ng/g . Table 4.9 also shows the percent nitrogen, carbon, and sulfur on whole bone. Total nitrogen ranges from 3.30% to 4.40% (mean=3.90 ± .3%), while total carbon ranges from 9.82% to 13.74% (mean=11.76 ± 1.2). Total sulfur concentrations are lower, ranging from 0.086% to 0.123%, with a mean of 0.109 ± .01%.

Nitrogen and carbon isotope ratios on bone collagen (prehistoric ringed seals)

Table 4.10 shows the $\delta^{15}N$ value for each sample, along with the collagen quality indicators. For the non-outlier cases only (n=21), the $\delta^{15}N$ values range from 17.1‰ to 18.7‰, with a mean of 17.8 ± 0.4‰, while $\delta^{13}C$ values range from −14.9‰ to −12.5‰, with a mean of −13.6‰ ± 0.6‰. All quality indicators are within accepted limits for well preserved bone collagen, including %N above 5% and %C above 13% (Ambrose, 1990), atomic C:N ratios between 2.9 and 3.6 (DeNiro, 1985), and collagen yields above 1% (van Klinken, 1999).

Table 4.9. Whole bone THg, %N, %C, and %S for prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska

Specimen ID	THg (ng/g)	%N	%C	%S
2001	14.20	4.08	13.08	0.1154
2002	16.70	4.26	13.74	0.1226
2004	11.80	4.17	13.22	0.1220
2005	12.40	4.24	13.22	0.1099
2006	9.56	3.82	11.85	0.1185
2008	14.00	4.28	13.14	0.1207
2009	13.10	3.40	10.50	0.0993
2010	8.72	4.40	12.44	0.0979
2011	6.63	3.85	11.34	0.1014
2016	6.74	3.36	10.01	0.1026
2017	10.20	4.06	11.73	0.1116
2018	15.10	4.12	13.07	0.1172
2019	32.80	4.38	12.63	0.1200
2020	4.36	3.30	9.82	0.0864
2022*	55.10	3.84	11.55	0.1205
2026	18.70	3.89	11.42	0.1108
2027*	32.00	3.84	11.05	0.1042
2028	19.50	4.04	11.63	0.1198
2029	16.80	3.85	11.11	0.0996
2031	21.00	4.03	11.79	0.1097
2032	6.20	3.58	10.90	0.1065
2033	10.30	3.52	10.12	0.0926
2034	6.49	3.64	10.29	0.1002
Mean**	13.10	3.91	11.76	0.1088
Std. Dev.**	6.54	0.34	1.20	0.0105
Geometric mean**	11.72	--	--	--

* Outlier case excluded from all summary statistics and analyses.

** Summary statistics exclude outlier cases.

Table 4.10. Bone collagen $\delta^{15}N$ and $\delta^{13}C$ and quality indicators for prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska

Sample ID	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰	%N	%C	Atomic C:N[a]	Yield (%)
2001	18.0	-13.5	16.8	46.3	3.2	19.9
2002	18.0	-14.2	16.1	46.3	3.4	20.4
2004	17.6	-13.9	16.3	47.4	3.4	21.2
2005	17.9	-14.2	16.4	47.8	3.4	22.4
2006	17.2	-13.3	17.3	48.3	3.3	19.5
2008	18.4	-14.9	16.1	47.3	3.4	22.7
2009	18.0	-13.8	16.6	47.3	3.3	20.2
2010	18.0	-13.6	18.0	47.9	3.1	19.2
2011	17.1	-12.8	17.5	48.0	3.2	21.5
2016	17.8	-13.1	17.9	47.5	3.1	21.6
2017	17.5	-13.4	16.8	46.4	3.2	20.8
2018	17.9	-13.8	16.1	45.3	3.3	22.6
2019	18.7	-14.1	18.0	47.7	3.1	20.4
2020	17.3	-13.3	17.4	48.0	3.2	15.8
2022*	17.7	-12.9	16.2	45.1	3.3	17.6
2026	18.3	-13.3	16.5	46.6	3.3	21.9
2027*	17.3	-12.5	16.4	43.5	3.1	19.7
2028	17.9	-13.1	16.4	45.7	3.2	19.5
2029	17.4	-12.5	17.4	46.8	3.1	19.8
2031	18.1	-14.8	16.7	46.7	3.3	21.3
2032	17.4	-13.0	17.0	46.1	3.2	18.0
2033	18.0	-13.3	16.6	45.3	3.2	18.7
2034	17.2	-13.1	15.9	45.7	3.4	18.3
Mean**	17.8	-13.6	16.8	46.9	3.3	20.3
Std. deviation**	0.4	0.6	0.7	1.2	0.1	1.7

[a] Atomic C:N ratio = (%C/%N) * 1.167 (Kennedy, 1988:54)

* Outlier case excluded from all summary statistics and analyses.

** Summary statistics exclude outlier cases.

Bone mercury versus bone collagen $\delta^{15}N$ (prehistoric ringed seals)

Figure 4.14 shows log-transformed bone total mercury concentration ($\log_{10}$ ng/g) regressed against bone collagen $\delta^{15}N$ for prehistoric ringed seals, while Table 4.11 shows the linear regression equation relating the two variables. The slope of the regression line is positive (b=0.37) and differs significantly from zero ($p < 0.001$), so the null hypothesis that the true slope is equal to zero for the regression line relating log-transformed bone THg to bone collagen $\delta^{15}N$ was rejected. The regression coefficient of determination (r^2) for the linear regression equation is high (0.55), suggesting that 55% of the variation in bone mercury is accounted for by variation in $\delta^{15}N$. Furthermore, visual inspection of the regression plot shows no systematic bias in prediction of log-transformed bone THg from bone collagen $\delta^{15}N$.

Table 4.11. Linear regression equations relating bone THg (log_{10} ng/g) to $\delta^{15}N$, $\delta^{13}C$, %N, %C and %S for prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska

Response variable (y)	Predictor variable (x)	r^2	Std. error of the estimate	Regression equation	p
Seal mandible THg (log_{10} ng/g)	Bone collagen $\delta^{15}N$ (‰)	0.55	0.15	$y = 0.373x - 5.567$	<0.001
	Bone collagen $\delta^{13}C$ (‰)	0.22	0.19	$y = -0.163x - 1.141$	0.034
	Bone %N	0.38	0.17	$y = 0.384x - 0.437$	0.003
	Bone %C	0.30	0.18	$y = 0.095x - 0.052$	0.011
	Bone %S	0.39	0.17	$y = 12.609x - 0.303$	0.003

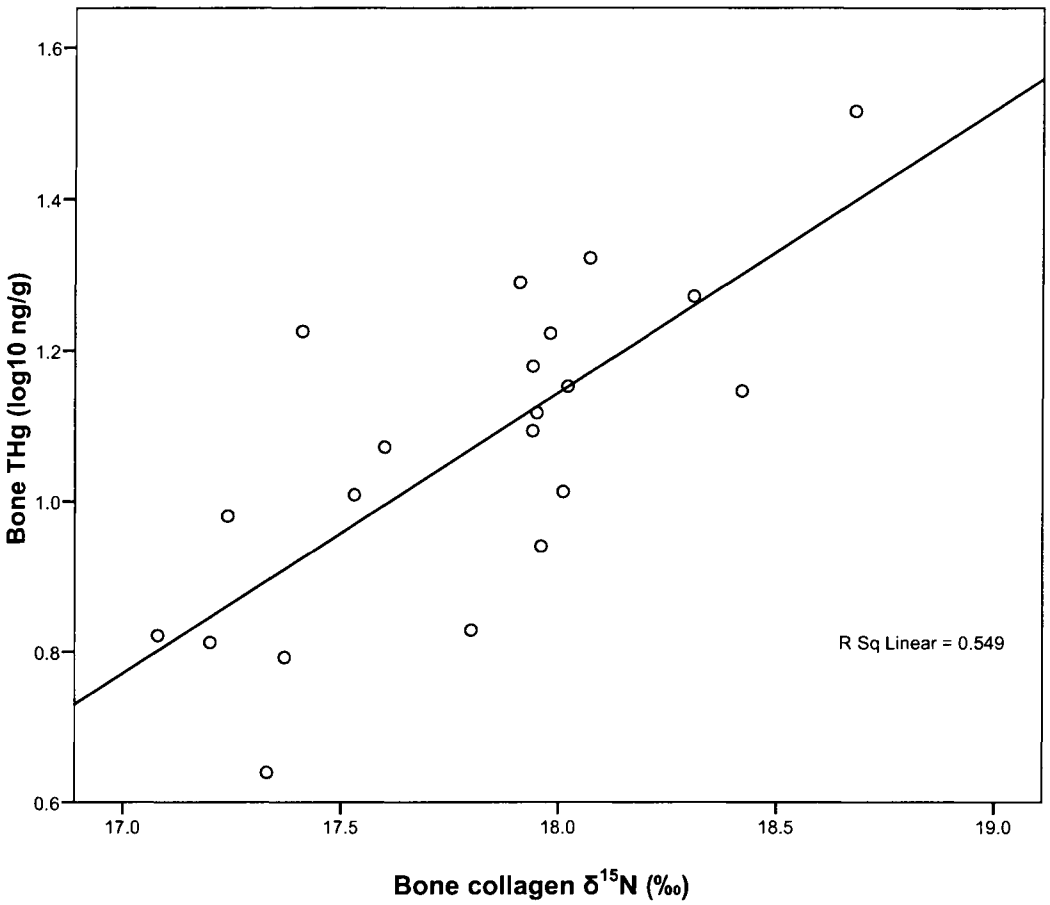

Figure 4.14. Bone THg (log_{10} ng/g) versus bone collagen $\delta^{15}N$ (‰) for prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska.

Bone mercury versus bone collagen $\delta^{13}C$ (prehistoric ringed seals)

Figure 4.15 shows log-transformed bone total mercury concentration ($\log_{10}$ ng/g) regressed against bone collagen $\delta^{13}C$ for prehistoric ringed seal mandibles, while Table 4.10 shows the linear regression equation relating the two variables. The slope of the regression line is negative, and differs significantly from zero, so the null hypothesis that the true slope is equal to zero for the regression line relating bone THg ($\log_{10}$ ng/g) to bone collagen $\delta^{13}C$ was rejected. The regression coefficient of determination (r^2) for log-transformed bone THg versus bone collagen $\delta^{13}C$ is moderate (0.22), suggesting that only 22% of the variation in bone mercury is associated with the variation in $\delta^{13}C$.

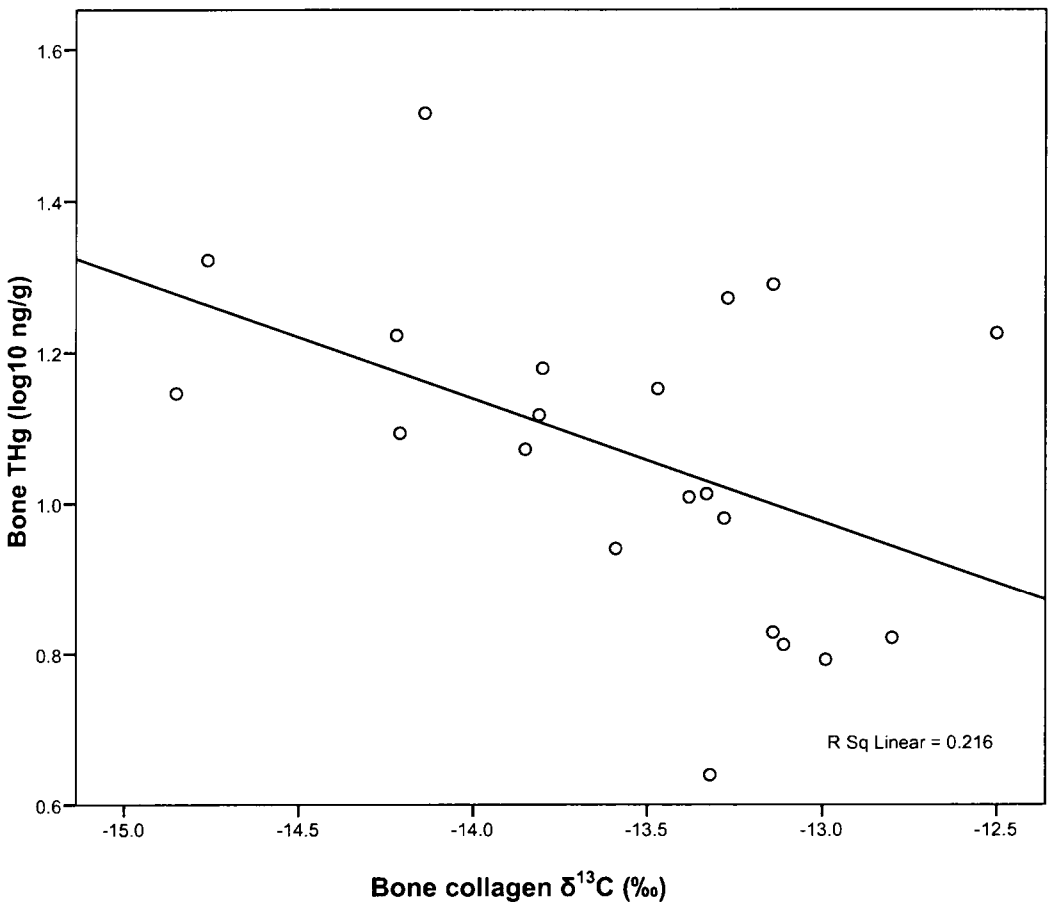

Figure 4.15. Bone THg (log_{10} ng/g) versus bone collagen $\delta^{13}C$ (‰) for prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska.

Bone mercury versus %N, %C, and %S (prehistoric ringed seals)

Figures 4.16 through 4.18 show log-transformed bone total mercury concentration ($\log_{10}$ ng/g) regressed against percent nitrogen, percent carbon, and percent sulfur, respectively, for the prehistoric seal mandibles. Linear regression equations relating log-transformed bone THg to the %N, %C and %S can be found in Table 4.10. All of the slopes are positive and differ significantly from zero ($p \leq 0.011$), so the null hypothesis that the true slope of the regression line relating bone THg ($\log_{10}$ ng/g) to the independent variable is equal to zero was rejected for the three independent variables (%N, %C, and %S).

The regression coefficient of determination (r^2) for log-transformed bone THg versus bone percent nitrogen is high (0.37), suggesting that 37% of the variation in bone THg may be accounted for by variation in bone nitrogen content. The r^2 value for bone mercury versus bone sulfur is likewise high (0.39), while that for carbon is somewhat lower (0.30), suggesting that less of the variation in bone mercury may be accounted for by variation in the concentration of carbon.

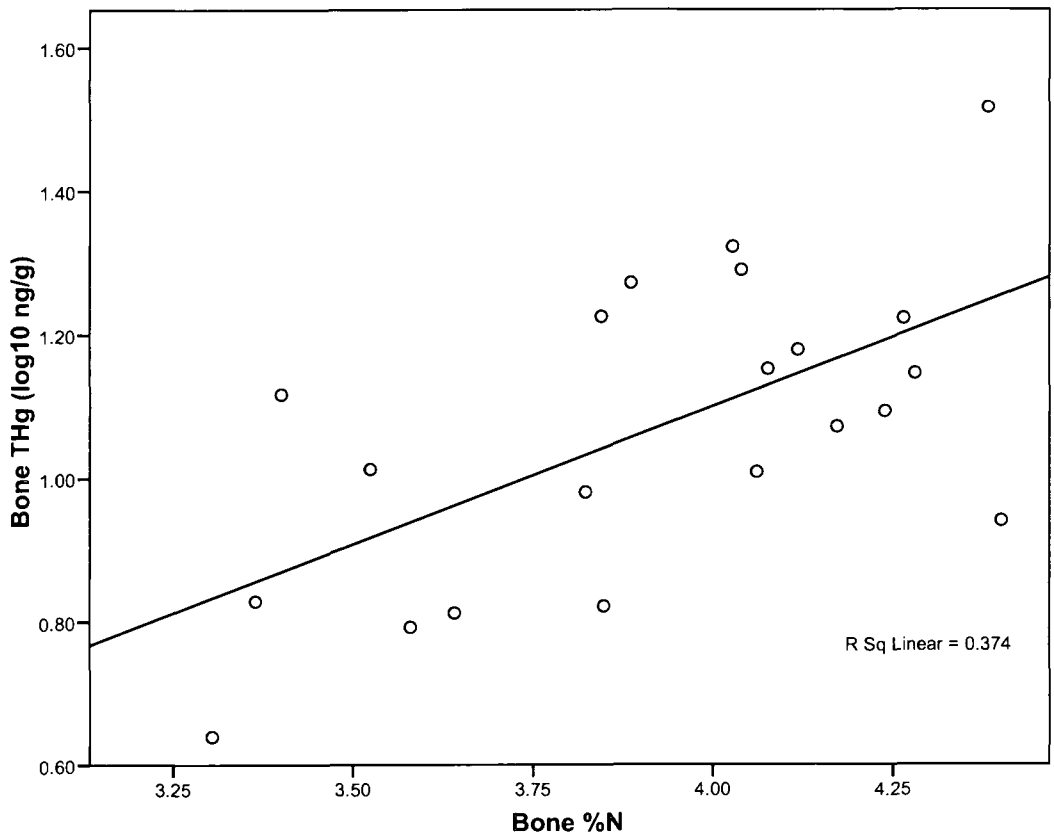

Figure 4.16. Bone total mercury (log_{10} ng/g) versus bone percent nitrogen for prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska.

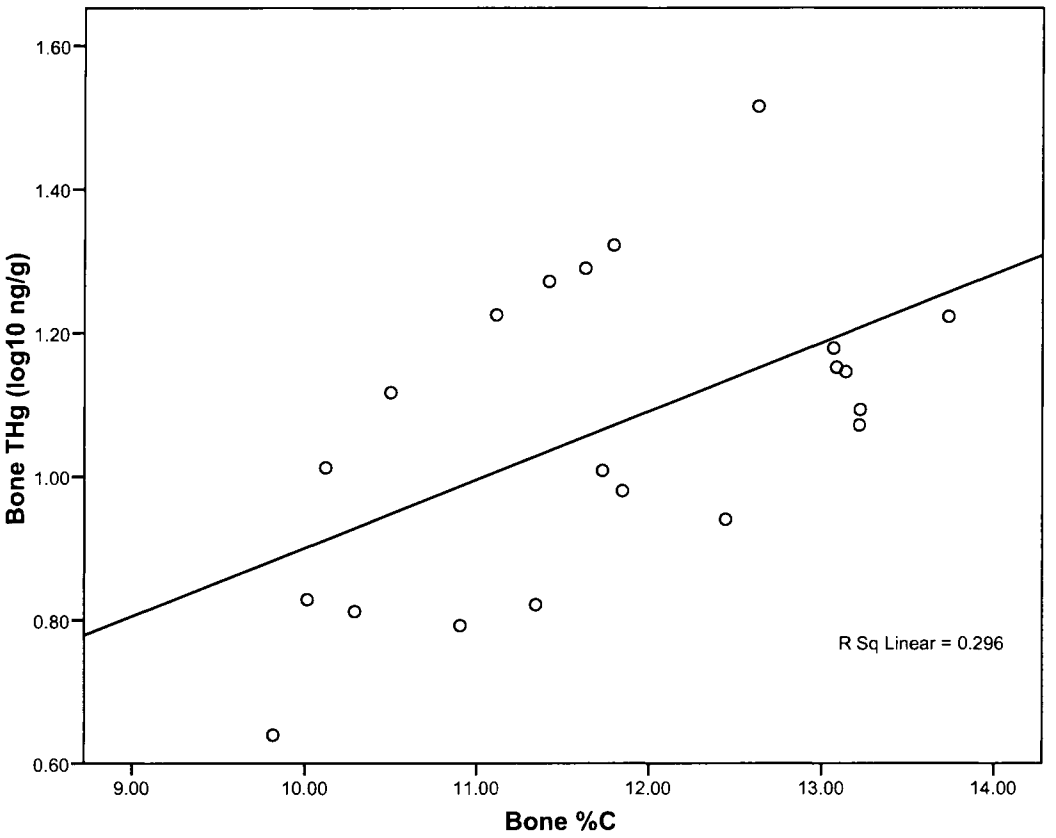

Figure 4.17. Bone total mercury (log_{10} ng/g) versus bone percent carbon for prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska.

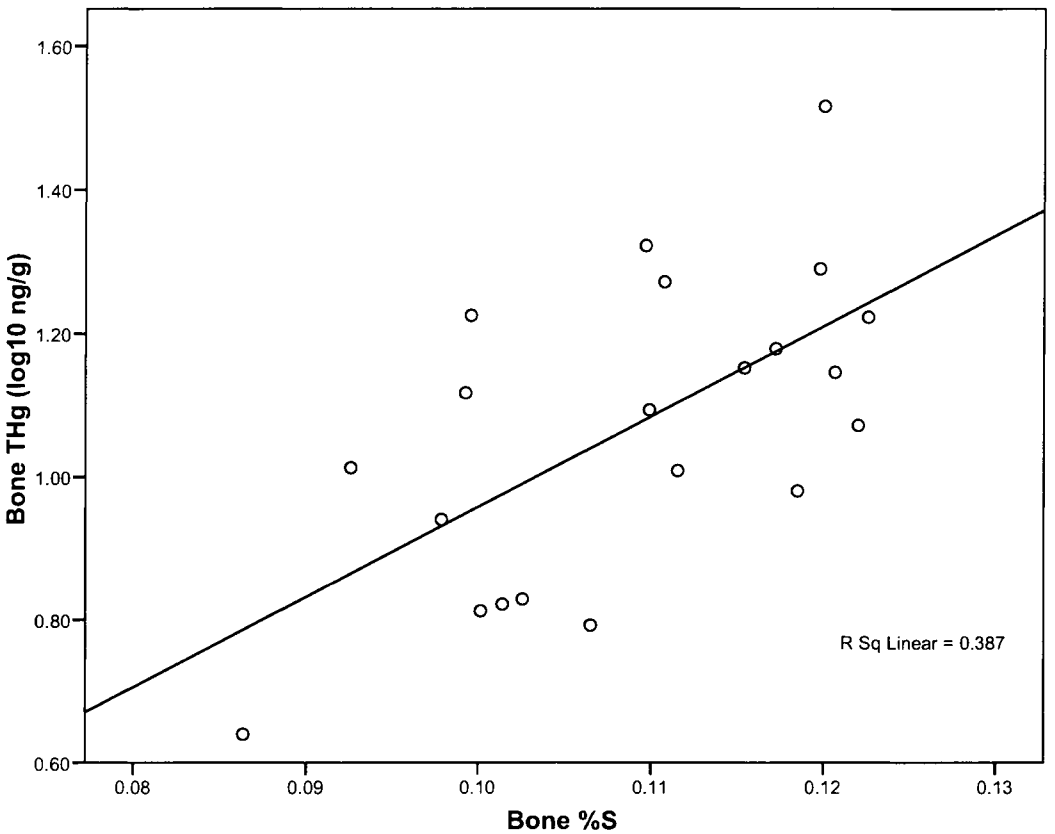

Figure 4.18. Bone total mercury (log_{10} ng/g) versus bone percent sulfur for prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska.

Whole-bone vs. demineralized bone mercury (prehistoric ringed seals)

Mercury was detected in the two experimental bone samples that were demineralized to extract bone protein prior to mercury analysis. The bone protein samples have mercury concentrations that are 3.0–3.5 times greater than their untreated mineralized counterparts as shown in Table 4.12.

The increase in concentration is not due solely to loss of bone mineral. That is, the protein yields of 19% and 22% (from whole bone starting weight) should produce mercury concentrations that are around 4.5 times to 5.2 times higher than the whole bone mercury concentrations if no mercury was removed through the demineralization process. For example, sample 2010 has 8.72 ng/g THg on whole bone; if the same amount of mercury was present in 0.22 g of tissue (the protein yield), the THg concentration would be 39.6 ng/g (i.e., 8.72 ng/0.22 g). Since the actual increase in mercury concentration is less than this, some mercury must have been removed during the demineralization treatment. For both of the experimental samples, the calculated amount of mercury that was lost through demineralization is around 33–34%. The dissolved mercury may have been the portion associated with the bone mineral.

Table 4.12. Whole bone THg compared to demineralized bone THg in experimental samples of prehistoric ringed seal mandibles from Thule archaeological sites at Cape Espenberg, Alaska

Sample ID	Whole bone THg (ng/g)	Demineralized bone THg (ng/g)	Protein yield[a]	Expected demineralized bone THg (ng/g)[b]	Calculated THg loss from demineralization[c]
2010	8.72	26.1	0.22	39.6	34.1%
2026	18.7	65.5	0.19	98.4	33.4%

[a] Protein yield = (Sample weight after demineralization)/(Starting weight)

[b] The expected THg concentration in demineralized bone if the increase is due solely to loss of bone mass through demineralization (with no loss of mercury) is: (whole bone THg)/(protein yield)

[c] Calculated loss through demineralization = ((Expected demineralized bone THg) − (Observed demineralized bone THg))/ (Expected demineralized bone THg), expressed as a percent.

Bone mercury measurement accuracy

Bone mercury measurement precision (modern ringed seals)

Results for duplicate total mercury determinations on modern ringed seal mandibles are shown in Table 4.13. Overall, mercury concentrations are quite low, ranging from 1.59 to 9.54 ng/g. Concerning within-run measurement imprecision, four sets of same-day duplicate measurements produced a grand mean of 3.18 ng/g and a repeatability standard deviation of 0.22 ng/g. The within-run imprecision, given by the relative repeatability standard deviation (RSD_r), is 6.9%.

The estimate of between-run imprecision is based on total mercury determinations for 20 modern seal mandibles run in duplicate on separate days. As shown in Table 4.12, the absolute differences between the duplicates range from 0.03 to 5.42 ng/g. The high difference value (5.42 ng/g from case 1003) is clearly an outlier, falling more than four standard deviations above the mean absolute difference, so this case was excluded from further analysis, as recommended by Fraser (2004). Figure 4.19 shows a plot of the duplicate mercury determinations, excluding the outlier.

The grand mean for the remaining 19 duplicate measurements is 3.47 ng/g, and the repeatability standard deviation (SD_r) is 0.42 ng/g. The between-run imprecision, given by the repeatability relative standard deviation (RSD_r), is 12.1% based on the 19 sets of duplicate measurements.

Table 4.13. Within-run and between-run duplicate bone THg determinations on modern ringed seal mandibles

Sample ID[a]	Original THg (ng/g)	Duplicate THg (ng/g)	Difference
Within-run duplicates			
1001 (UAM89338)	2.27	2.35	-.08
1012 (UAM100109)	3.37	3.19	.18
1013 (UAM100110)	3.34	3.33	.01
1013 (UAM100110)	3.51	4.09	-.58
SD_r			0.22 ng/g
RSD_r			6.9%
Between-run duplicates			
1001 (UAM89338)	2.27	2.99	0.72
1002 (UAM89339)	2.53	2.98	0.45
1003 (UAM97739)*	7.49	2.07	-5.42
1004 (UAM97838)	2.73	2.93	0.20
1005 (UAM97912)	2.39	2.20	-0.19
1006 (UAM97914)	1.90	2.16	0.26
1007 (UAM98148)	2.53	2.31	-0.22
1008 (UAM98260)	5.77	5.47	-0.30
1009 (UAM98261)	6.71	7.05	0.34
1010 (UAM98284)	2.91	2.62	-0.29
1011 (UAM100108)	2.90	2.87	-0.03
1012 (UAM100109)	4.05	3.19	-0.86
1013 (UAM100110)	3.51	3.34	-0.17
1014 (UAM100111)	2.80	2.43	-0.37
1015 (UAM100112)	3.92	3.37	-0.55
1016 (UAM100115)	3.49	2.64	-0.85
1017 (UAM100116)	1.76	1.59	-0.17
1018 (UAM100119)	9.54	8.51	-1.03
1019 (UAM100120)	4.67	3.21	-1.46
1020 (UAM100203)	1.73	1.80	.07
SD_r **			0.42 ng/g
RSD_r **			12.1%

a Internal sample ID number with UA Museum of the North catalog numbers

* Outlier case excluded from all summary statistics and analyses.

** Summary statistics exclude outlier cases.

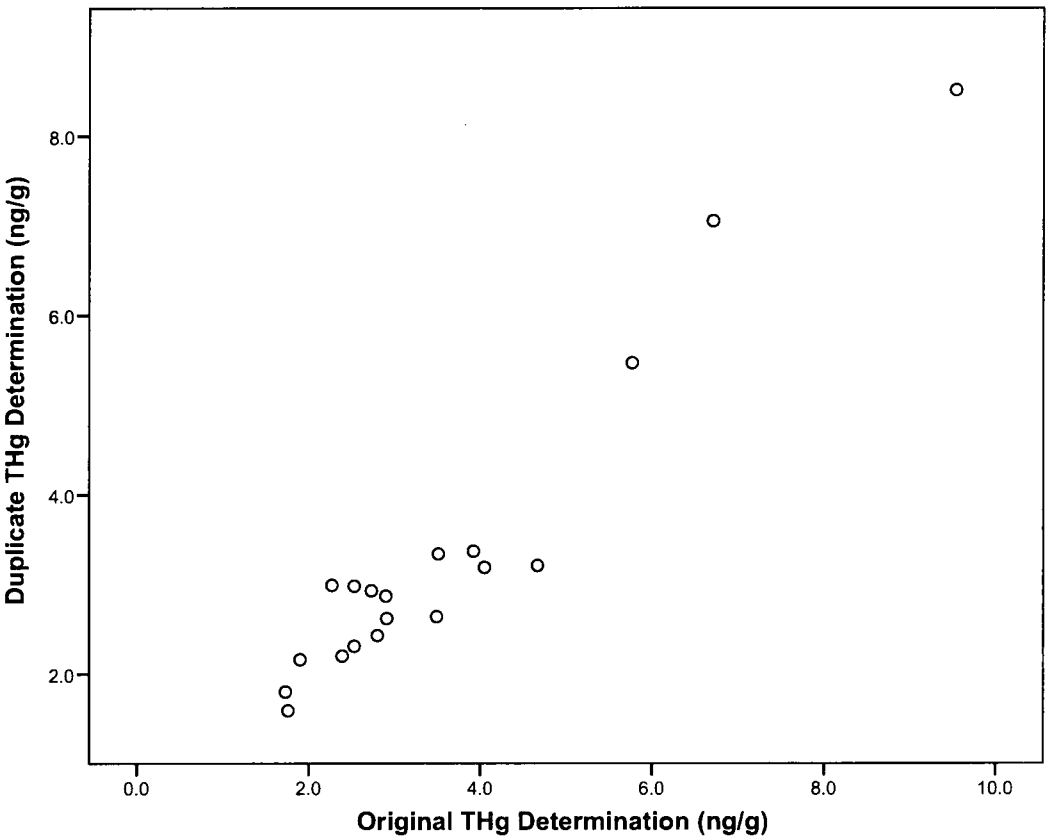

Figure 4.19. Duplicate mercury determinations made on modern seal bone on different days.

As expected, the within-run imprecision is lower than the between-run imprecision. Both the within-run and between-run RSDr values are better (lower) than the expected RSD_r value of 18% for samples with analyte concentrations of around 4 ppb (calculated as $0.000000004^{-0.15}$) and are well within the acceptable limits of 9–30% (see Table 3.2) (AOAC, 2002; Horwitz and Albert, 2006).

Bone mercury measurement trueness (spike recovery) (modern ringed seals)

A total of four modern ringed seal mandible samples were spiked with a known quantity of mercury to equal approximately 400 ppb. Trueness, as measured by percent recovery of spiked samples, ranges from 87.7% to 93.0%, with a mean of 90.5 ± 2.6 % for four spiked samples (Table 4.14). This is well within the AOAC (2002) recovery limits of 75-120% for analyte concentrations no greater than 1.0 ppm.

Four of the methylmercury-dosed rat bone samples were spiked with a similar quantity of mercury (Table 4.15). The average percent recovery for these samples is nearly identical to that for the modern ringed seal bone, at 91.0%, and is well within the AOAC recovery limits. The range of recovery for the mercury-spiked rat bones is slightly larger than that for the modern seal bones, with a low value of 82.7% and a high value of 97.2%. Together, the spike recovery information from the modern seal bone samples and the methylmercury-dosed rat samples shows that recovery is within acceptable limits, but that recovery is less than 100% in all cases.

Table 4.14. Recovery of mercury spikes on modern ringed seal bone

Sample	Sample THg (ng/g)	Spike THg (ng/g)	Measured THg (ng/g)	Recovery (%)
1001	2.27	396.83	368.50	92.3
1001	2.27	398.41	372.70	93.0
1001	2.99	400.00	353.70	87.7
1002	2.98	403.23	361.8	89.0
Mean				90.5
Std. dev.				2.6

Table 4.15. Recovery of mercury spikes on bone from methylmercury-dosed rats

Sample	Element	Sample THg (ng/g)	Spike THg (ng/g)	Measured THg (ng/g)	Recovery (%)
200	humerus	2.0	410.0	341.4	82.7
350	cranium	9.9	404.9	385.6	92.8
350	cranium	9.9	395.3	371.1	91.4
400	femur	5.2	398.0	392.1	97.2
Mean					91.0
Std. dev.					6.1

5. DISCUSSION

Bone mercury in methylmercury-dosed laboratory rats

Bone mercury and daily exposure

Rat bone mercury concentration clearly increased in an exposure-related fashion in the controlled dosing experiment. For all of the bone types studied (cranium, humerus, and femur), mean bone total mercury is lowest in the control group and highest in the high exposure group. Furthermore, the linear regressions of log-transformed bone THg against daily mercury exposure show strong positive relationships, and indicate that most of the variation (98-99%) in bone total mercury can be explained by variation in exposure level. A controlled dosing experiment, such as the one employed in the present study, is one condition under which causation may be attributed to the independent variable (Sokal and Rohlf, 1969). The significant regressions found here, therefore, suggest that a large proportion of the variation in bone mercury level is caused by changes in exposure level.

At the same time, the results of the rat dosing experiment show a large degree of individual variation in the relationship between dose and bone THg, with the latter varying by a factor of three or more within the same dose group. Two factors may be contributing to the variation in bone mercury within a dose group: inter-individual differences in the actual methylmercury dose and inter-individual differences in the biokinetic parameters controlling mercury tissue distribution (Berglund et al., 2005;

Stern, 2005). Some variation in actual dose may have arisen due to differences in water intake among subjects, since methylmercury was delivered via drinking water. A more important contributor to differences within the same exposure group is likely individual differences in metabolism of mercury, including, for example, differences in the absorption rate, the fraction of absorbed dose circulating in the blood, and the half-life in blood. These parameters have been found to vary widely among human subjects exposed to methylmercury (Canuel et al., 2006; Stern, 2005). In a controlled study of 20 humans exposed to methylmercury through fish, Sherlock et al. (1984) found large variations in the half-life of mercury in blood, which ranged from 42 to 70 days.

The strength of the bone mercury-exposure relationship found here is similar to that found for soft tissue and exposure in another study of methylmercury-dosed rats. In that study, which used rats from the same colony and the same dosing levels as in the present study, regression analysis showed that daily exposure level explained much of the variation (95–98%) in brain and blood mercury (Newland and Reile, 1999).

The strong dependence of rat bone mercury concentration on daily exposure level means that bone mercury may be predicted from daily exposure. The inverse prediction—estimating mercury exposure level from bone mercury concentration—is also possible, by simple algebraic rearrangement of the linear regression equations:

$$Exposure = (cranium\ THg - 0.711)/0.0060 \quad \text{[Eq. 5]}$$

$$Exposure = (humerus\ THg - 0.613)/0.0057 \quad \text{[Eq. 6]}$$

$$Exposure = (femur\ THg - 0.462)/0.0059 \quad \text{[Eq. 7]}$$

where *Exposure* is measured in μg/kg bw/day methylmercury, and *THg* is expressed in $\log_{10}$ ng/g. To illustrate, a rat humerus with a THg concentration of 10.0 ng/g (i.e., 1.0 $\log_{10}$ ng/g) suggests an exposure level of around 68 μg/kg bw/day methylmercury.

Such inverse prediction equations are of interest to bioarchaeologists, who wish to reconstruct exposure levels or dietary intake levels of substances from prehistoric bone element concentrations. However, using the equations developed here for mercury-exposed rats may not be appropriate for other species, especially large species. Typically, large mammals have higher mercury tissue concentrations than small mammals at identical doses, because large mammals have slower elimination rates (WHO, 2000). Given the broad similarities among mammalian species in the physiological mechanisms governing mercury metabolism (Young et al., 2001), it is reasonable to expect that other mammals, including humans, will exhibit a relationship between bone mercury and exposure. But, the exact form of the prediction equations relating rat bone mercury to daily mercury intake should not be extrapolated directly to other mammals until the correct allometric adjustments can be made.

Direct extrapolation of the rat bone model to estimate human mercury intake from bone concentrations clearly overestimates intake. As a case in point, published median human bone mercury concentrations from autopsy subjects in Sweden (femur) (Lindh et al., 1980) and the Czech Republic (parietal) (Beneš et al., 2000) are 40 ng/g and 70 ng/g, respectively. Simple extrapolation would have these populations consuming around 190 μg/kg bw/day methylmercury, which exceeds the estimated daily intake that would lead

to severe clinical effects and death in humans (WHO, 1976:section 6.6; WHO, 1990:section 9.4.1.2). Worldwide, estimates of typical methylmercury intake are less than 0.5 μg/kg bw/day for most populations, although in some communities individual intakes may exceed 10–20 μg/kg bw/day (UNEP, 2002; WHO, 1990).

Bone mercury and internal dose (methylmercury-dosed rats)

The linear regressions of log-transformed bone THg against log-transformed kidney THg, an indictor of internal mercury dose, show strong positive relationships for methylmercury-exposed rats, but not when the control group is included. The lack of fit for the model that included all subjects suggests that the form of the function relating bone mercury to kidney mercury differs at the very low exposure levels experienced by the control group. Additional samples at very low exposure levels would be informative here.

The linear regression equations for predicting rat bone mercury from kidney mercury (control group excluded) may be rearranged to predict kidney mercury from bone mercury as follows:

$$\textit{Kidney THg} = (\textit{cranium THg} + 3.11)/1.29 \quad \text{[Eq. 8]}$$

$$\textit{Kidney THg} = (\textit{humerus THg} + 3.01)/1.23 \quad \text{[Eq. 9]}$$

$$\textit{Kidney THg} = (\textit{femur THg} + 1.25)/1.25 \quad \text{[Eq. 10]}$$

where *Kidney THg* and *Bone THg* are expressed in $\log_{10}$ ng/g. Thus, for example, a rat humerus with a THg concentration of 10.0 ng/g (i.e., 1.0 $\log_{10}$ ng/g) suggests a kidney THg level of around 1820 ng/g (3.26 $\log_{10}$ ng/g). As discussed previously for the bone-to-exposure relationship, the bone-to-tissue relationship found here for rats may not directly translate to other species. Thus, the equations developed for rats should not be directly extrapolated to other animals until the relationship between bone mercury and soft tissue mercury can be further explored

A recent study by Brookens et al. (2008) compared total mercury concentrations among a number of tissues, including bone (combined rib and femur) and kidney, in a sample of 26 modern free-ranging and captive Pacific harbor seal pups (*Phoca vitulina richardii*). They found that while bone mercury *concentration* is not correlated with kidney mercury concentration, bone mercury *burden* (tissue concentration multiplied by total tissue mass) is significantly correlated with mercury burden in liver, kidney, pelt, muscle, heart, and brain, although the correlations are weak ($r \leq 0.3$) (TJ Brookens, personal communication 2008). One complicating factor in the seal pup study is that some subjects had consumed a natural diet throughout life, while others had consumed a rehabilitation diet while in captivity for up to six weeks. The switch to a captive diet may have blurred the natural mercury exposure signal in some of the seal tissues.

Between-bone variation in mercury level (methylmercury-dosed rats)

The three rat bone types investigated in the present study (cranium, humerus, and femur) differ in mean total mercury concentration, with crania having the highest

mercury levels and femora the lowest levels. These differences may have a physiological basis, such as differences in blood flow to cranial versus long bones, or they may be due to variations in the pretreatment methods of the bones, such as the inclusion of trabecular bone in the cranial samples but not the long bone samples and the defatting of the femora but not of the crania or humeri.

Concentrations of lead have also been shown to vary by bone element, but the pattern appears to depend on age. In a study of bone lead in 134 human autopsy subjects with "normal" exposure to lead, Wittmers et al. (1988) found that in the adult subjects, lead concentration was higher in the tibia than in the cranium, but for the juvenile subjects, the pattern was reversed. Among the 21-35-year-olds, lead distribution was found to be nearly uniform between the bone elements. Wittmers et al. (1988) suggest that over the long term, bones with a higher proportion of cortical bone, such as the tibia, have higher lead concentrations than bones with a high proportion of trabecular bone, such as the cranium. While additional controlled studies must be conducted in order to isolate the causes of inter-bone mercury variation, the findings from the present study, as well as those from bone lead studies, suggest that application of the bone mercury method to prehistoric skeletal samples will need to control for bone element.

Organic and inorganic mercury in bone (methylmercury-dosed rats)

While total mercury was detected in all of the methylmercury-dosed rat cranial bone samples, methylmercury was below the reporting limit of 4.0 ng for all of the control group samples and two of the low-exposure group samples, suggesting that some

fraction of the mercury in these bones is inorganic. In the samples for which methylmercury was detected, it makes up around 67% to 94% of total mercury. In other studies of methylmercury-dosed rats, the fraction of organic mercury found in soft tissues depended on the duration of exposure and the length of time after cessation of exposure. In rats exposed daily for 64 days and sacrificed immediately, organic mercury made up over 90% of the total mercury in brain, blood, liver, and fur, but only 54% in kidney (Magos and Butler, 1976). However, in another study in which rats were sacrificed a month after dosing stopped, the percent organic mercury was much lower than 90% in most tissues, as follows: 91% in fur, 83% in blood, 60% in brain, 38% in liver, and only 8% in kidney (Thomas et al., 1988). In humans exposed to lethal levels of methylmercury, the fraction of organic to total mercury was around 93% for blood, 80% for brain and hair, and 15% for kidney (National Research Council, 2000). The trend of increasing inorganic mercury over time demonstrates that organic mercury is demethylated to inorganic mercury in the body; this conversion may occur in the intestinal tract as well as within specific organs, especially the kidneys and brain (Clarkson and Magos, 2006). Based on experiments with monkeys (*Macaca fascicularis*), inorganic mercury in the brain appears to be virtually immobile, with a half-life on the order of hundreds of days (Vahter et al., 1995). It is unknown whether the inorganic mercury detected in bone in the present study was formed through demethylation in the bone itself or whether it was demethylated elsewhere in the body and transported to bone.

The finding that a large fraction of the mercury in bone is organic mercury offers insight into the question of *where* this metal is deposited in bone. Some heavy metals, such as lead, are known to deposit in the bone mineral, where the metal, usually as a divalent cation (e.g., Pb^{2+}), replaces the divalent calcium ion (Ca^{2+}) in the hydroxyapatite crystal (O'Flaherty, 1991; Pan and Fleet, 2002). Organic mercury (CH_3Hg^+) is thus likely in the wrong form to be deposited in the apatite crystal structure, although it may be contained within the hydrated shell surrounding the crystal, which can entrap organic complexes (Priest, 1990). Theoretically, inorganic mercury, as a divalent cation (Hg^{2+}), could substitute for calcium in the bone mineral crystal. However, a study of ion exchange of Ca^{2+} in synthetic hydroxyapatite with Hg^{2+} found that the two ions did not easily exchange, possibly because of the strong bonds between Hg^{2+} and anions in solution (Miyake et al., 1990).

Both organic and inorganic mercury may bind to proteins (WHO, 1990; WHO, 1991). Mercury has a high affinity for the sulfhydryl group (-SH), which occurs in the amino acid cysteine (Harris et al., 2003; Rabenstein and Fairhurst, 1975). Cysteine is found in procollagen and many of the noncollagenous proteins of the bone matrix, although it is not present in mature bone collagen (Ayad et al., 1998).

Limitations of the methylmercury-dosed rat data set

The mercury-dosed rat data set has some limitations. One concern is the small sample size, since a small sample may not be representative of the population from which it was drawn (in this case, the population of 20-week-old female Long-Evans

rats). In terms of statistical hypothesis testing, the greatest drawback of a small sample size is the reduction in statistical power; that is, as sample size decreases the null hypothesis is less likely to be rejected even when it is false (Sokal and Rohlf, 1969). Also, if the underlying population distribution of a variable is not normal, then the sampling distribution of a statistic (e.g., mean, regression slope etc.) may not be normal for small samples. Depending on the shape of the population distribution, the minimum sample size needed to approximate a normal sampling distribution is as small as 5, but might also be 20, 30, 100 or more (Agresti and Finlay, 1997; Wilcox, 2001).

Another issue with this data set concerns the mercury dosing schedule. While the range of methylmercury exposures used in this study (0, 40, and 400 μg/kg bw/day) is considered low to moderate for rats (Newland et al., 2006), this type of discrete dosing for around 100 days may not adequately approximate the chronic, continuous exposure levels experienced by humans and free-ranging animals. Also, as already discussed, rats may eliminate mercury more rapidly than larger bodied animals, so the exact form of the relationship found here between exposure and bone mercury may not apply to other species.

Bone mercury and indicators of exposure in archaeofauna

Prehistoric ringed seal bone mercury and Thule mercury exposure

Mercury was found in all of the ringed seal bones from the prehistoric Thule sites at Cape Espenberg (sites KTZ-087, KTZ-088, and KTZ-101). While no published studies

of mercury in prehistoric seal or other marine mammal bone could be found, a study by Brookens et al. (2008) measured total mercury in bone (combined rib and femur) for 26 modern Pacific harbor seal pups (*Phoca vitulina richardii*) from California. They found a mean total mercury concentration of 38.0 ng/g (range: 17–63 ng/g), which is nearly three times higher than the mean of 13.1 ng/g found in the present study.

The presence of mercury in the ancient ringed seal bones demonstrates that these seals were exposed to mercury and, by extension, so were their human consumers. The degree of exposure experienced by the prehistoric Thule population at Cape Espenberg cannot be precisely calculated at this time, because the level of mercury in bone must be translated into levels in edible tissues, such as muscle. The study by Brookens et al. (2008) did not find a correlation between mercury concentration in bone and that in muscle in Pacific harbor seal pups, but the mercury concentration in bone was consistently lower than that in all of the other tissues, except blubber. Therefore, it can be assumed that muscle mercury in the prehistoric Cape Espenberg seals was higher than bone mercury. If prehistoric Cape Espenberg Thule folk consumed an average of 860 g of ringed seal per day—the amount of seal consumed by Greenlandic Inuit according to a dietary survey in 1855 AD (see Table 5.1)—and ringed seal muscle was at least as high as that measured in their preserved bones (i.e., around 13 ng/g or 0.013 μg/g), then the typical adult consumed at least 11.2 μg of mercury per day. For a person weighing 65 kg (143 lb), that is equivalent to around 0.2 μg/kg bw/day, which exceeds the EPA safety level of 0.1 μg/kg bw/day (EPA, 2001c). Since prehistoric ringed seal muscle was likely

much higher in mercury than the bones measured in the present study, this is a minimum estimate.

Faunal remains from the three Cape Espenberg sites studied here, KTZ-087, KTZ-088, and KTZ-101 suggest that the prehistoric Thule inhabitants may have consumed varying amounts of ringed seal during these different occupations. At all Cape Espenberg sites, fish remains are virtually nonexistent, and bird and shell remains are present but infrequent (Saleeby, 1994). While poor preservation of these more delicate bones cannot be ruled out, it will be assumed that these food categories contributed little to the prehistoric diet. Instead, all of the sites are rich in marine mammals remains, with some caribou. Table 5.1 shows the abundance of each mammal identified to species from the sites. This table excludes probable non-dietary species (canids, mustelids, small rodents), which are not abundant at the sites in any case.

Based on number of identified specimens (NISP), ringed seal clearly dominates all of the assemblages, forming over 80% of the total specimens. However, NISP does not take into account the differences in weight among the animals. To get an idea of the relative dietary importance of each faunal taxon, meat yields were calculated for each species by multiplying their NISP by their edible meat weight, following Whitridge (2001) and White (1953). For the Thule at KTZ-087 (ca. 1275 AD), ringed seal appears to have made up 46% of the meat diet by weight, but for KTZ-088 it was 87%, and for KTZ-101 it was 57% (Table 5.1).

To estimate the intake of each species in grams per day, the percent contribution of each species was multiplied by 2000 g, the daily meat/fish intake observed for 19th century Greenlandic Inuit adults (Sinclair, 1953). Using this formula, the estimated daily intake of ringed seal for the prehistoric Thule at Cape Espenberg is 920 g for KTZ-087, 1740 g for KTZ-088, and 1140 g for KTZ-101. Consumption of these amounts of ringed seal daily would have delivered at least 12, 22, and 15 μg of mercury, respectively, if ringed seal muscle mercury concentration was at least as high as that measured on bone (i.e., $\geq$ 0.013 μg/g). For a 65 kg-person, the calculated daily mercury intakes are all above the EPA safety levels of 0.10 μg/kg bw/day, and for KTZ-088, the intake is over three times the "safe" level (EPA, 2001c). Obviously, the foregoing analysis makes many assumptions: that NISP captures faunal abundance in the past; that faunal remains represent animals that were consumed by humans; that calculated edible meat weights provide an accurate ranking of the dietary importance of each species; and that the amount of meat consumed daily by Greenlandic Inuit in 1855 AD is similar to that of prehistoric Thule in Alaska ca. 1275–1640 AD.

Table 5.1. Frequencies and estimated dietary importance of mammalian archaeofauna from prehistoric Thule sites (KTZ-087 , KTZ-088, & KTZ-101), Cape Espenberg, Alaska ca. 1275–1640 AD

Species	Meat yield per animal (kg)[b]	NISP[a] KTZ-087	KTZ-088	KTZ-101
Ringed seal (*Phoca hispida*)	63.7	467	700	273
Ribbon seal (*Phoca fasciata*)	47.6	14	5	1
Bearded seal (*Erignathus barbatus*)	196.0	11	0	19
Walrus (*Odobenus rosmarus*)	730.1	42	7	10
Caribou (*Rangifer tarandus*)	47.7	31	21	40
Grizzly bear (*Ursus arctos*)	231.7	0	2	1
Total NISP		565	735	344
Total meat yield (kg)		64,712	51,403	30,601
Ringed seal meat yield (% of total)		46%	87%	57%
Estimated ringed seal daily intake by adults (g)[c]		920	1740	1140

[a] NISP (number of individual specimens) from Saleeby (1994); includes only specimens identified to species level. Feature 30 specimens were listed under KTZ-088 in the Saleeby report but are here classified as KTZ-087 following the information on the original faunal analysis sheets and collection bags.

[b] Meat yield = (mean adult animal weight) x (edible fraction). Weights for ringed seal and grizzly bear are from Banfield (1974), bearded seal and caribou are from Friesen and Arnold (1995), and ribbon seal and walrus are from Wynne (1997). Edible fractions follow Friesen and Arnold (1995).

[c] Assumes 2000 g of meat consumed per day, following Greenlandic Inuit diet ca. 1855 AD (Sinclair, 1953)

Bone total nitrogen, carbon, and sulfur (prehistoric ringed seals)

The whole bone nitrogen content for nearly all of the prehistoric Cape Espenberg ringed seal mandibles is in the range of modern bone (4.5-3.5%) (Stafford et al., 1988), suggesting excellent bone protein preservation. According to the bone preservation classification scheme presented by Stafford et al. (1988), all of the Cape Espenberg ringed seal remains fall into either the Class I (Modern) or Class II (Very Well Preserved) preservation categories based on whole-bone nitrogen content. While nitrogen content is not routinely measured on archaeological bones, reported values for well-preserved bones are generally in the 1.5–3.0% range (Bocherens et al., 1997; Coltrain et al., 2004a; Petchey and Higham, 2000; Schutkowski et al., 1999; Stafford et al., 1988). It is unlikely that the nitrogen in the Cape Espenberg seal bones derives from any soil organic contaminant, such as nitric or fulvic acid, since these compounds tend to be predominately carbon by weight (>50%) with a low nitrogen content (<3.5%) (Tan, 2003), and their presence would tend to lower the nitrogen content of bone.

The carbon content of the Cape Espenberg Thule-period seal bones varies from 9.8% to 13.7%, which is similar to the range of 10.5% to 16.0% observed in a small sample of modern terrestrial herbivore bone (Bocherens et al., 2005). The carbon content of whole archaeological bone is rarely measured, so comparisons are limited. Bocherens et al. (2005) measured percent carbon on whole bone from herbivores, boars, dogs, and humans dating to the prehistoric period in Iran and found values ranging form around 2% to 11%, with most clustering around 8%. Medieval human skeletal remains from

Germany have reported whole bone carbon concentrations from as low as 3.6% to 13.8% (and one anomalous reading of 35.2%), with means of 7.1% to 10.6% (Schutkowski et al., 1999). Pleistocene cave bear remains from Grotte Chauvet, France produced whole bone carbon in the range of <1.0% to 5.5% (Bocherens et al., 2008).

Most organic contaminants of archaeological bone are carbon-rich, so their presence in bone tends to skew the carbon-to-nitrogen ratio. One way to assess the origin of carbon in archaeological bone is to calculate the expected carbon content from the nitrogen content. Bocherens et al. (2005) developed the following formula, based on the empirical relationship found between carbon and nitrogen content in modern mammal bone: Expected %C = (%N x 2.7) + 1.4. The first part of this equation (%N x 2.7) describes the fraction of whole bone carbon associated with bone protein, while the last term (1.4) is the percent of whole bone carbon found in bone mineral. When observed carbon content is higher than predicted, the presence of exogenous carbon is indicated. Applying this formula to the prehistoric ringed seal remains produced expected whole bone carbon values that are within 1.0 percentage point above or below the actual carbon values. This suggests that bone carbon has not been increased by exogenous carbon, although it is unknown if modern sea mammal bone follows the same empirical relationship between nitrogen and carbon observed in terrestrial mammal bone.

Few reports of total sulfur content of whole bone are available, either for modern or archaeological specimens. The Cape Espenberg prehistoric seal bones in the present study have an average total sulfur content of around 0.11%, which is lower than that

reported for three modern human femora (0.19–0.24%) and for a single modern herbivore long bone (0.16%) (Reiche et al., 2003; Shinomiya et al., 1998). Two archaeological long bone specimens from a French Neolithic site were reported to have a total sulfur content of around 0.51% (Reiche et al., 2003).

Stable isotopes in tissues of prehistoric and modern ringed seals

There are few reports of the stable nitrogen isotope composition of ringed seal bone collagen. Coltrain et al. (2004b) analyzed a small sample of prehistoric Thule-age ringed seal remains from the Hudson Bay area of Canada and found a mean $\delta^{15}N$ value of 17.2‰ (table 5.2), which is similar to the mean of 17.8‰ found here. Published mean $\delta^{15}N$ values for modern ringed seal muscle from Alaska and Canada are all lower than the mean value found in this study for ancient ringed seal bone collagen, but stable isotope ratios may not be directly comparable across tissue types. Results of controlled feeding studies differ as to the spacing between muscle and bone collagen $\delta^{15}N$ values, with one study of mice finding no difference between the two tissues (DeNiro and Epstein, 1981), and another of rats finding that bone collagen $\delta^{15}N$ values are around 1.4‰ higher than those in muscle in mature individuals (Ambrose, 2000). Using these two extremes, Table 5.2 shows the estimated bone collagen $\delta^{15}N$ for modern ringed seal samples based on measured muscle values. The mean $\delta^{15}N$ value found in this study for ancient seal bone collagen fits within the estimated bone collagen $\delta^{15}N$ for most modern ringed seal samples, including the sample from Barrow, Alaska.

Table 5.2. $\delta^{15}N$ in modern and Thule-period ringed seal tissues from the Arctic

Time Period	Location	Tissue	$\delta^{15}N$ mean & standard deviation (‰)	Estimated Bone collagen $\delta^{15}N$ mean (‰)[a]	Reference[b]
Prehistoric	Cape Espenberg AK USA	Bone collagen	17.8 +/- 0.4		This study
	Silumiut (Hud. Bay) NU Canada	Bone collagen	17.2 +/- 1.0		(Coltrain et al., 2004b)
Modern	Barrow AK USA	Muscle	16.9 +/- 0.6	16.9 to 18.3	(Dehn et al., 2006b)
	Sachs Harbor NT Canada	Muscle	17.3 +/- 0.5	17.3 to 18.7	(Butt et al., 2008)
	Holman NT Canada	Muscle	17.2 +/- 0.7	17.2 to 18.6	(Dehn et al., 2006b)
	Gjoa Haven NU Canada	Muscle	17.9 +/- 0.7	17.9 to 19.3	(Butt et al., 2008)
	Barrow Strait NU Canada	Muscle	17.3 +/- 1.1	17.3 to 18.7	(Hobson and Welch, 1992)
	Resolute Bay NU Canada	Muscle	17.5 +/- 0.3	17.5 to 18.9	(Butt et al., 2008)
	Arviat NU Canada	Muscle	16.6 +/- 0.4	16.6 to 18.0	(Butt et al., 2008)

[a] Estimated bone collagen $\delta^{15}N$ = muscle $\delta^{15}N$ (‰)+ 0–1.4‰, which is the range of spacing reported from controlled feeding studies (Ambrose, 2000; DeNiro and Epstein, 1981)

[b] References are for stable isotope ratios on the original tissue, not estimated bone collagen stable isotope ratios.

Table 5.3. $\delta^{13}C$ in modern and Thule-period ringed seal tissues from the Arctic

Time Period	Location	Tissue	$\delta^{13}C$ mean & standard deviation (‰)	Estimated Bone collagen $\delta^{13}C$ mean (‰)[a]	Reference[b]
Prehistoric	Cape Espenberg AK USA	Bone collagen	–13.6 +/– 0.6		This study
	Silumiut (Hud. Bay) NU Canada	Bone collagen	–13.9 +/– 0.7		(Coltrain et al., 2004b)
Modern	Barrow AK USA	Muscle	–18.5 +/– 0.8	–16.5 to –14.5	(Dehn et al., 2006b)
	Sachs Harbor NT Canada	Muscle	–20.3 +/– 0 5	–18.3 to –16.3	(Butt et al., 2008)
	Holman NT Canada	Muscle	–20.4 +/– 0.4	–18.4 to –16.4	(Dehn et al., 2006b)
	Gjoa Haven NU Canada	Muscle	–22.9 +/– 0.2	–20.9 to –18.9	(Butt et al., 2008)
	Barrow Strait NU Canada	Muscle	–17.3 +/– 0.7	–15.3 to –13.3	(Hobson and Welch, 1992)
	Resolute Bay NU Canada	Muscle	–18.9 +/– 0.5	–16.9 to –14.9	(Butt et al., 2008)
	Arviat NU Canada	Muscle	–19.1 +/– 0.2	–17.1 to –15.1	(Butt et al., 2008)

[a] Estimated bone collagen $\delta^{13}C$ = muscle $\delta^{13}C$ (‰)+ 2–4‰ following Loken et al. (1992).

[b] References are for stable isotope ratios on the original tissue, not estimated bone collagen stable isotope ratios.

The mean bone collagen $\delta^{13}C$ found in the present study for ringed seal mandibles from prehistoric Thule-period archaeological sites in Alaska (mean $\delta^{13}C = -13.6‰$) is similar to that found by Coltrain et al. (2004b) for ringed seal remains from Thule-period sites in the Hudson Bay area of Canada (mean $\delta^{13}C = -13.9‰$) (Table 5.3). Published mean carbon stable isotope ratios for modern ringed seal *muscle* from Alaska and Canada are all lower by around 4‰ or more than the mean values found in this study for ancient ringed seal collagen (see Table 5.3), but $\delta^{13}C$ values cannot be directly compared between tissue types. Mammal bone collagen is generally more enriched in heavy carbon by about 2-4‰ than the corresponding muscle (DeNiro and Epstein, 1978; Hare et al., 1991; Loken et al., 1992). Table 5.3 shows the reported mean muscle carbon stable isotope ratio for modern ringed seals and the estimated bone collagen equivalents. An additional correction must be made when comparing bone collagen $\delta^{13}C$ values between prehistoric and modern samples because of the effects of recent fossil fuel inputs to the marine carbon reservoir, by subtracting 1‰ from preindustrial samples (Burton et al., 2001). When this correction is made (not shown in Table), the mean bone collagen $\delta^{13}C$ for Cape Espenberg Thule-period seals falls within the upper end of the estimated bone collagen values for modern ringed seals from Barrow and many of the other arctic locations.

Bone mercury vs. $\delta^{15}N$, $\delta^{13}C$, %N, %C, and %S (prehistoric ringed seals)

Linear regression analysis suggests that seal bone mercury increases in relation to $\delta^{15}N$, which is a measure of trophic level. Since mercury concentrations in animal

tissues tend to increase with trophic level, (Atwell et al., 1998; Cabana and Rasmussen, 1994), $\delta^{15}N$ can be considered to be a proxy measure of mercury exposure. The two variables, mercury concentration and $\delta^{15}N$, should be positively related in animal tissues if the tissue is faithfully tracking mercury exposure, and if the two variables are in equilibrium with the diet. The significant positive relationship found in this study agrees with several studies that compared mercury concentrations to $\delta^{15}N$ values on soft tissues of modern seals, including ringed seals from western Canada and northwest Greenland (Dehn et al., 2005; Rigét et al., 2007a). However, some modern seal samples have shown no significant correlation between the two variables, including a sample of ringed seals from northern Alaska (Dehn et al., 2005) and grey seals from the northeast Atlantic (Das et al., 2003). A positive correlation between THg and $\delta^{15}N$ was also found in tooth cementum, a hard tissue, in a historical (late 19th century) sample of beluga whales from Central Canada, but not in a comparative modern sample (Outridge et al., 2005).

The slope of the regression line for $\log_{10}$ bone THg versus bone collagen $\delta^{15}N$ in the prehistoric ringed seal sample (b=0.37) is similar to that found by Capelli et al. (2008) for marine mammals in the Mediterranean. However, it is higher than the slopes reported for entire arctic marine food webs (i.e., from phytoplankton or zooplankton to top marine mammals), which are in the range of 0.10 to 0.26 (Atwell et al., 1998; Campbell et al., 2005; Loseto et al., 2008; Rigét et al., 2007b).

Bone total mercury appears to vary negatively with bone collagen $\delta^{13}C$ (see Figure 4.15), although the relationship is only moderate. For aquatic animals, $\delta^{13}C$ is an

indicator of feeding location, and values are usually higher in nearshore and bottom-feeding animals compared to offshore and open-water feeding animals (Burton et al., 2001; Clementz and Koch, 2001; France, 1995; McConnaughey and McRoy, 1979; Walker et al., 1999), while mercury levels have been shown to sometimes vary in the opposite direction, although this pattern has not been fully investigated (Chen et al., 2008; Embury, 2000; Goodale et al., 2006; Lacerda et al., 2007; Lasora and Allen-Gil, 1995; Ricca et al., 2008; Rumbold et al., 2003). The negative relationship found here for prehistoric ringed seals suggests that nearshore and bottom-feeding individuals may have been less exposed to mercury than their pelagic-feeding counterparts. Other studies have found a negative relationship between mercury concentration and stable carbon isotope ratios, including in fish-eating birds (Bearhop et al., 2000; Ricca et al., 2008) and freshwater fish (Ethier et al., 2008; McIntyre and Beauchamp, 2007; Power et al., 2002).

No studies could be found that reported a negative relationship between total mercury concentration and $\delta^{13}C$ in marine mammals, although several studies reported a lack of association between these two variables (Hobson et al., 2004; McIntyre and Beauchamp, 2007; Rigét et al., 2007a; Thompson et al., 1998). In a study by Dehn et al. (2005) the correlation between liver THg and muscle $\delta^{13}C$ was not significant for ringed seal from Holman Canada, but was significant and *positive* for ringed, bearded, and spotted seal from Barrow Alaska. The different outcomes among studies suggest that mercury levels do not vary in a consistent fashion across food chains, such as benthic versus pelagic, or nearshore versus offshore.

Linear regression analysis revealed a strong positive relationship between total mercury and total nitrogen measured on whole bone of prehistoric ringed seals. This finding agrees with a study by Honda et al. (1984), who found a significant and strong positive relationship ($r^2 = 0.88$, $p < 0.001$) between bone mercury and bone protein (calculated from nitrogen content) in modern striped dolphin (*Stenella coeruleoalba*). This association offers insight into where mercury is deposited in bone. Nitrogen does not occur in the bone hydroxyapatite, but is a key building block of the amino acids making up all proteins. Thus, a relationship between total mercury and total nitrogen suggests that mercury in bone is associated with the protein phase, rather than the mineral phase.

Additional evidence for bone mercury being located in the protein phase of bone is provided by the two experimental samples that were demineralized prior to mercury analysis. The mercury concentrations in the two demineralized bone samples were roughly three times higher than in the paired untreated samples. This might indicate that the majority of bone mercury is in the bone protein, although the possibility that the mineral-associated mercury was not dissolved along with the bone mineral cannot be ruled out. Some of the bone mercury appears to have been dissolved by the weak-acid demineralizing treatment, since the increase in mercury concentration in the demineralized bone samples is not as large as expected if the increase were due solely to a loss of bone mass, with no concomitant loss of mercury.

Both organic and inorganic mercury bind to proteins in non-bone tissues, including the protein albumin in blood plasma and milk, hemoglobin in red blood cells, and keratin in hair (Cernichiari et al., 2007; Sundberg et al., 1999; WHO, 1991). While the exact nature of the binding has not been elucidated, the mercury in these proteins is thought to be bound to the amino acid cysteine, which contains a sulfhydryl group (–SH, also called a thiol group) (Clarkson, 2002). The affinity of methylmercury for the thiol group is so high that "it is assumed that methylmercury binds exclusively to thiol-containing molecules" (Cernichiari et al., 2007:1016-1017). In bone, the amino acid cysteine occurs in procollagen (but not mature collagen), in osteonectin, and in most of the other noncollagenous bone proteins (Ayad et al., 1998).

The supposition that mercury is associated with sulfur-containing complexes in bone receives further support from the finding that bone mercury concentration is related to bone sulfur content. Sulfur is present in the protein phase of bone, although not exclusively so. It may also exist in the bone mineral, as sulfate (SO_4), which may occasionally replace the phosphate group in the hydroxyapatite crystal interior or may exist as sulfate ions or calcium sulfate ($CaSO_4$) at the crystal surface (Monteil-Rivera and Fedoroff, 2004; Pan and Fleet, 2002; Richards et al., 2003). Inorganic mercury could potentially bind to sulfate to form mercuric sulfate ($HgSO_4$).

Whole bone carbon content is also moderately associated with bone mercury content in the prehistoric ringed seal sample. Like sulfur, carbon may occur in both the bone proteins and the bone mineral. In the hydroxyapatite crystal, there is extensive

replacement of the phosphate group (PO_4) by carbonate (CO_3), such that carbonate makes up around 3% or more of bone mineral by weight, and the mineral carbon makes up around 1.4% of whole bone carbon (the balance being found in the protein) (Bocherens et al., 2005; Driessens and Verbeeck, 1990; Pan and Fleet, 2002). In archaeologically-derived bones, carbon may also be present as a contaminant. However, the prehistoric ringed seal bones studied here do not appear to contain exogenous carbon based on the calculation of expected carbon content using the Bocherens et al. (2005) formula (discussed previously).

Limitations of the prehistoric seal bone data set and analysis

Some limitations of the prehistoric seal bone data set must be discussed. One concern is the modest sample size, as previously discussed for the methylmercury-dosed rat data set. A greater concern for the prehistoric seal bone data is that diagenesis may have affected bone chemical composition, including mercury and other elemental concentrations and stable isotope ratios. Diagenesis refers to changes in the chemical constituents of tissues after deposition in sediments, and this is always a concern in trace element and stable isotope analysis of archaeological bone (Sanford and Weaver, 2000).

There are no studies on the diagenesis of mercury in buried bone. This is a difficult issue because while there are widely-used tests to assess the state of bone alteration in general (e.g., increased porosity or loss of organic matter) (Hedges, 2002), there are no widely agreed-upon tests for measuring diagenetic change on an element-by-element basis Tests for diagenetic changes in metal concentrations in bones generally fall into

two categories, as described by Edward and Benfer (1993): 1) comparisons of metal levels in bone with those of the surrounding soil, or 2) comparisons of metal levels in archaeological bone with those in presumed unaltered bone. The latter category includes several types of "unaltered" bone, such as modern (or less ancient) bone, interior bone (versus surface bone), or certain bone elements (e.g., tibia) thought to be more resistant to change.

All of these methods are imperfect. For example, Aufderheide et al. (1992) tested for lead diagenesis in bones of ancient Romans by comparing lead concentrations in more ancient specimens to those in more modern specimens, assuming that if lead had been transferred into bone postmortem, the older bones would have higher lead concentrations. However, decreasing metal levels in bone through time could also reflect actual decreasing exposure through time due to changes in environmental or cultural conditions. Another test of diagenetic metal uptake has been to compare right and left bones of the same element; if the metal levels are different, then diagenesis is assumed to have taken place (Drasch, 1982). The assumption in this case is also somewhat flawed, in that similar metal levels for both sides could simply mean a similar degree of diagenetic alteration. A widely-used test for metal uptake by buried bone is to compare concentrations at bone surfaces to those in deeper layers of bone; if higher levels are found at the surface, post-depositional uptake is indicated (Ericson et al., 1991). However, studies of metal distribution in the bones of living humans and animals indicate that a higher concentration at the bone surface is the natural physiological pattern for some metals (Aufderheide, 1989; Priest, 1990; Wittmers et al., 1988).

A common test for diagenetic change in bone metal concentration is to compare the metal levels in bone to those in the burial soil. If the two variables are correlated, postmortem metal uptake by bone is presumed to have occurred (Aufderheide et al., 1992; Vuorinen et al., 1996; Waldron, 1982). This test is flawed, however, because the extent of bone diagenetic uptake of a particular metal depends largely on the concentration of the metal in the surrounding soil pore water, not the whole soil, as well as the propensity of bone to sorb the metal (Hedges and Millard, 1995; Pike and Richards, 2002). The metal must move from the soil to the soil pore water, then from the soil pore water to the water held within the bone's pores, and finally it must be fixed in the bone (Pike and Richards, 2002). Thus, the concentration of a metal in soil may not be directly correlated with that in bone, since it is the metal in soil pore water that interacts with bone (Pike and Richards, 2002).

The concentration of mercury in soil pore water has been reported for several locations worldwide. As seen in Table 5.4, the mercury concentrations in soil pore water are very low, in the parts per trillion (ng/l) range. Mean (or median) values are usually less than 50 ng/l (0.05 ppb), with reported ranges of between 1.8 ng/l to 188.9 ng/l.

The extremely low mercury concentrations in soil pore water as shown in Table 5.4 suggest that the potential for diagenetic uptake of mercury by bone is low. There are no measurements of soil pore water mercury from the Cape Espenberg archaeological sites or its environs, so the relative likelihood of post depositional mercury uptake by the prehistoric seal bones used in this study cannot be addressed.

Little information could be found on the propensity of bone to sorb mercury. One study investigated the immobilization of toxic metal cations onto synthetic hydroxyapatite, and found that mercury (as Hg^{2+}) has a very low affinity for apatite compared to lead, cadmium, and zinc (Monteil-Rivera and Fedoroff, 2004). The reaction of Hg^{2+} with the synthetic apatite attained steady state quickly, and the uptake of mercury ions was smaller than for all of the other ions.

Two findings from the present study indirectly suggest that the prehistoric Cape Espenberg ringed seal specimens have retained their biogenic mercury signal. First is the high concentration of nitrogen found in whole bone. As previously mentioned, the levels are similar to modern bone and suggest excellent overall preservation (Stafford et al., 1988). Second is the finding of a relationship between bone mercury concentration and $\delta^{15}N$, which is the expected biological pattern. Diagenetic mercury uptake would likely blur this relationship, unless mercury uptake was accompanied by a concomitant shift in $\delta^{15}N$ values.

Table 5.4. Total and organic mercury concentrations in soil pore water worldwide

Location	Environment	Soil type	Pore water THg ng/l (ppt)	Statistic	Pore water MeHg ng/l (ppt)	Statistic	Reference
Svartberget Sweden	Boreal forest	Peat & podzol	15.5	Median	0.22–2.11	Min/Max	(Skyllberg et al., 2003)
Gammtratten Sweden	Boreal forest	Organic podzol	21.8	Mean	NA	NA	(Åkerblom et al., 2008)
Tiveden Sweden	Boreal forest	Podzol	6.8–15.1	Means	NA	NA	(Aastrup et al., 1991)
Lake Gårdsjön Sweden	Boreal forest	Podzol	1.8–23.0	Min/Max	0.01–0.08	Min/Max	(Lee et al., 1994)
Aneboda Sweden	Boreal forest	Organic podzol	32.2–47.0	Means	NA	NA	(Åkerblom et al., 2008)
Bavaria Germany	Spruce forest	Cambisol & podzol	<15.0–31.0	Min/Max	<0.2–1.1	Means	(Schwesig et al., 1999)
New York USA	Conifer & decid. forest	NA	2.7–188.9	Min/Max	NA	NA	(Kalicin et al., 2001)
Ontario Canada	Lake bank	Lacustrine sand	<10.0	Mean	<1.0	Mean	(He et al., 2007)
Wisconsin USA	Lake bank	Lacustrine sand	3.4	Median	NA	NA	(Krabbenhoft et al., 1998)

Bone mercury measurement accuracy

The present study found that bone mercury measurements made on modern ringed seal bone had acceptable precision and trueness, where acceptability was determined by comparison to internationally recognized standards. The between-run imprecision for bone THg as given by the repeatability relative standard deviation(RSD_r) is 12.1% based on 19 sets of duplicate measurements on modern seal bone, and this is well within the AOAC limit (9–30%) for analyte concentrations of around 4 ppb (AOAC, 2002; Horwitz and Albert, 2006). The between-run imprecision estimate also compares favorably to imprecision estimates found for mercury determinations on other biological tissues, though it is slightly higher than most. The RSD_r value for a certified biological reference material obtained by the same laboratory used in the present study (Frontier Geosciences, Inc.) and using the same method of mercury determination is 11% for mussel tissue (certified value=61 ppb (EPA, 2001a).

Similar imprecision estimates have been found for mercury in blood, which is the most commonly used biomarker of mercury exposure, and which usually has mercury concentrations in the parts per billion range. Based on duplicate analysis of samples, Sandborgh-Englund et al. (1998:158) calculated a between-run imprecision ("coefficient of variation") of 6% to 12% for animal and human blood total mercury determined by cold vapor atomic fluorescence spectrometry. Barbosa et al. (2004:1005) calculated a between-run imprecision ("intermediate precision") of around 10% for repeated analysis of a single sample of prepared animal blood (THg=5.2 ppb) by cold vapor atomic

absorption spectrometry. Repeated ultraviolet absorption spectrometry analysis of a control animal blood sample with a THg concentration of around 10 ppb produced an imprecision estimate of 14% (Grandjean et al., 1992). Using inductively-coupled plasma mass spectrometry, Palmer et al. (2006) found RSD_r levels of between 3% and 9% for prepared animal blood with target THg concentrations of between 1.3 ppb and 11.8 ppb.

A single study was found for mercury in hard tissue. Saber-Tehrani et al. (2007) determined mercury on permanent human teeth (type not specified) via CVAAS and found an RSD_r of 17% for repeated determinations on a single sample; this is quite high, if their reported tooth Hg concentrations of between 490 ppb and 9220 ppb are correct. Specific causes of analytical error may include weighing errors, heterogeneity of the sample, and variation of the chemical treatments, as well conditions associated with runs on different days, such as change of analyst, different batches of reagents, instrument recalibration, and changes in the laboratory ambient environment (temperature, humidity, etc.) (Thompson et al., 2002).

Results from the mercury analysis of modern seal bone also indicate that bone mercury measurements have an acceptable level of trueness, as measured by percent spike recovery. The four bone samples spiked with around 400 ppb mercury had an average recovery of 90.5%, which is well within the AOAC (2002) recovery limits of 75-120% for substances in the concentration range of 1.0 ppm or less. An additional four samples of bone from methylmercury-dosed rats were spiked at the same level and

showed a nearly identical spike recovery (91.0%). While the percent recovery values are within the AOAC limits, all of the values are below 100% (87.7–93.0% for modern seal bone and 82.7–97.2% for methylmercury-dosed rat bone). This suggests that there is a consistent, though small, negative bias. Such a bias is usually due to some type of matrix interference; for example, the presence of one or more elements in bone may interfere with the detection of mercury in the sample (Mishalanie et al., 2005). For bone, the high level of dissolved salts in the solution of digested samples may suppress detection of the analyte of interest (Lambert and Weydert-Homeyer, 1993).

Recovery estimates for mercury in blood and hair are usually performed on certified reference materials, rather than on materials prepared in-house by spiking. Reported recoveries for mercury on a certified bovine blood reference material (NIST Standard Reference Material 966: THg=31.4 ppb) are around 93% for determinations by ICP-MS (Palmer et al., 2006) and 89–97% for determinations by CVAAS (Barbosa et al., 2004; Ertas and Tezel, 2004). For a human hair reference sample (IAEA-086: THg=573 ppb), reported average recoveries are from 96–101% (Berglund et al., 2005; Gill et al., 2004). Recovery levels for mercury in teeth were around 85–92% for mercury spikes of 25 ppb to 100 ppb (Saber-Tehrani et al., 2007).

Analytical chemists disagree as to whether recovery analysis should be used to "correct" measurement results. Arguments in favor of correction emphasize that the goal of a measurement method is to obtain "the true concentration of the native analyte," which can be estimated only if "low recoveries of an analyte are corrected" (Thompson

et al., 1995:8). Others argue against correction, noting that corrected results may still be biased, and that estimated correction factors have high uncertainly and vary for different concentrations of the analyte (Thompson et al., 1995). The "Harmonised guidelines for the use of recovery information in analytical measurement" states that IUPAC and the ISO embrace the policy that results be corrected for recovery, while AOAC "does not agree that analytical results should be corrected for recovery as a general policy" (Thompson et al., 1995:12). Mercury determinations in the present study were not corrected for recovery, since so little is known about the range of recovery over different concentrations and in different bones.

The levels of precision and trueness found here for bone total mercury measurements serve as estimates of expected measurement quality for new bone samples treated and analyzed in a similar manner. In this study, sample preparation prior to submission to the analytical laboratory included cleaning by lightly abrading with a sanding drum and soaking in an ultrasonic bath with ultrapure water, freeze drying, and grinding to a fineness of less than 300 micrometers. At the analytical laboratory, mercury determination was by acid digestion and BrCl oxidation, followed by cold vapor atomic fluorescence spectrometry, following EPA Method 1631 (EPA, 2001a).

Limitations of the modern ringed seal bone data set and analysis

The modern ringed seal bone samples used in the measurement accuracy analysis have one important shortcoming, and that is the lack of information on chemical and other washing treatments prior to their preparation for mercury analysis. As described

previously, an unknown number of specimens were soaked in ammonium hydroxide, which may have leached out mercury present in the bone. Since measurement trueness and precision tend to decrease with decreasing mercury concentration, the modern ringed seal data set may present a "worst case" for assessing measurement accuracy. That is, bone samples with higher concentrations of mercury may produce better measurement trueness and precision than these low-mercury samples.

In terms of assessing measurement trueness, the spike recovery method has some recognized limitations. These include differences in the chemical form and behavior of the native analyte compared to the added analyte (Mishalanie et al., 2005; Willetts and Wood, 1999). Also, it is difficult to check all "relevant" concentration ranges (Willetts and Wood, 1999). Because the relevant range of mercury concentrations in bone was unknown prior to the present study, the suitable spike amount was unknown, and the laboratory default level of 400 ppb was used. In light of the range of mercury concentrations seen for natural samples in the present study, which range from around 4 ppb to 40 ppb, lower spiking levels may be more appropriate. The EPA suggests spiking levels from 2 to 5 times the expected range (i.e., 8 ppb to 200 ppb for the range observed here) (EPA, 2001a).

Some other important sources of mercury measurement error were not assessed in the present study. Thompson (2000:2020) describes that an analytical result arises from a "ladder of errors": result = true value + method bias + laboratory bias + between-run error + within-run error. The present study examined only the last two of these, the error

within a single laboratory. The other sources of analytical variability, including between-laboratory differences and between-method differences (i.e., using another method of mercury determination, such as inductively-coupled plasma mass spectrometry) have not been addressed.

6. SUMMARY AND CONCLUSIONS

The aim of this dissertation research was to investigate the potential for using archaeological bone mercury concentration as a biomarker of mercury exposure in preindustrial animals and humans. Mercury is a toxic heavy metal that can occur in high levels in marine mammals and fish and the in the people who consume them. Today, some of the highest tissue mercury concentrations are found among the coastal populations of arctic North America and Greenland, where diets are rich in marine foods (Burkow and Weber, 2003; EPA, 1997a; Van Oostdam and Tremblay, 2003). Because mercury can be released into the environment through natural as well as industrial processes, the same pathway that operates today to cause high mercury concentrations in arctic marine animals and humans likely operated in the preindustrial past. In this way, prehistoric Eskimo and Inuit who relied heavily on marine mammals may well have been exposed to relatively high levels of mercury from their diet, just as their modern counterparts are today.

Little information is available on preindustrial mercury levels in the environment, and archives that have been studied, such as sediment and ice cores, provide no information on mercury in the preindustrial food chain. A potential archive of past mercury levels is animal and human bone recovered from archaeological sites. Prior to this dissertation research, it had been established that mercury could be detected in modern and prehistoric bone, but there was no information on whether bone was a valid biomarker of past mercury exposure. For a biomarker of exposure to be valid, the

analytical method used to measure the biomarker must produce accurate results, and the biomarker must correspond to the extent of exposure (Lee et al., 2006; WHO, 2001). This dissertation was thus guided by two central research questions: Can bone mercury be accurately measured? and Does bone mercury level reflect dietary intake level?

Can bone mercury be accurately measured?

Results of the mercury analysis on modern Alaskan ringed seal bone indicate that bone mercury measurements meet accepted standards of measurement accuracy (precision and trueness). Accepted standards are those recognized by several international organizations concerned with the quality of analytical measurement results, including IUPAC, ISO, and AOAC, as detailed in Alder et al. (2000) and AOAC (2002).

Duplicate measurements of total mercury concentration by Cold Vapor Atomic Fluorescence Spectrometry in modern Alaskan ringed seal bones show low imprecision (RSD_r = 12.1%), which is well within the acceptable limits of 9–30% for materials with analyte concentrations around 4 ppb (AOAC, 2002; Horwitz and Albert, 2006). This level of imprecision is comparable to estimates obtained for whole blood mercury, the most commonly employed biomarker of mercury exposure in animals and humans (Barbosa et al., 2004; Palmer et al., 2006; Sandborgh-Englund et al., 1998). Trueness of mercury determinations, as measured by recovery of known amounts of mercury added to bone samples, is also acceptable. Recovery of mercury from spiked samples of modern ringed seal bone, as well as methylmercury-dosed rat bone, was around 91%, which is well within the guidelines of 75-125% (AOAC, 2002). While a 91% recovery

rate indicates a low level of measurement bias, all recovery measurements were below 100%, suggesting that there is some consistent matrix interference that is slightly suppressing the mercury signal.

Similar levels of precision and trueness can be expected for new bone samples that are pretreated in a manner similar to that in the present study and analyzed by CVAFS. A key consideration for choosing an analytical laboratory to measure bone total mercury is the laboratory's minimum detection limit. Since bone total mercury concentrations in the present study were as low as 1.8 ng/g (ppb), the minimum detection limit must be no greater than 1 ppb.

Protocols for preparing archaeological bone for mercury analysis

As an initial biomarker validation study, this research did not formally compare competing protocols for preparing archaeological bone for mercury analysis. However, some insights have been gained as to which aspects of preanalytical treatment may be important in ensuring that bone mercury measurements are unbiased and precise. A chief consideration, discussed in a later section, is whether bone should be demineralized prior to mercury analysis. Regardless of which matrix, whole bone or demineralized bone, proves to be more reliable, the extent of sample cleaning prior to analysis will undoubtedly prove to be important in obtaining reliable bone mercury measurements. Protocols for trace element and stable isotope analysis of prehistoric bone almost always include cleaning steps designed to remove possible contaminants (Lambert et al., 1990). These protocols vary from mild to rigorous and can include rinsing in water, abrading

with sanding discs, and soaking in weak acids. Here, archaeological bone samples were both abraded and washed in ultrapure water prior to further sample preparation. The application of strong acids or alkalis is not recommended for any biological tissue sample intended for mercury analysis, since these are used to *extract* inorganic and organic mercury for spectroscopic determination (EPA, 2001a; Puckett and Buuren, 2000).

Homogenization of tissue samples plays an important role in measurement reliability of trace element determinations by spectroscopic analysis (Krejčová et al., 2008). Here, bone samples were ground to a fineness of <0.3 mm, but the optimum size of the grind is unknown. To eliminate variation in mercury concentration due to differences in water content, samples were freeze dried before powdering.

Does bone mercury level reflect dietary intake level?

Establishing a relationship between dietary exposure and bone mercury is a fundamental step in validating the use of archaeological bone as a biomarker of mercury exposure. Several converging lines of evidence in this study suggest that bone mercury is sensitive to mercury exposure level. The analysis of methylmercury-exposed laboratory rats indicates that bone mercury concentration increases in an exposure-related fashion. Mean bone mercury concentration differs significantly among rat exposure groups, and bone mercury concentration has a strong and positive linear relationship with daily exposure level. Furthermore, for the exposed individuals, bone mercury level is positively related to internal dose, as measured by kidney mercury level.

These findings are consistent across all of the bone types measured in rats, including cranium, humerus, and femur.

Further evidence linking bone mercury to dietary mercury is provided by the stable isotope analysis of ringed seal mandibles from prehistoric Thule sites in northwestern Alaska, dating to approximately 1270–1640 AD. Because dietary mercury level could not be measured directly in this sample, the ratio of stable nitrogen isotopes ($\delta^{15}N$) was used as a proxy measure of mercury exposure. Since $\delta^{15}N$ is a measure of trophic level, and mercury exposure increases with tropic level, the two variables should covary in bone, if bone mercury reflects dietary inputs. Results from the prehistoric seal sample suggest that bone mercury concentration tends to increase with $\delta^{15}N$ and, by extension, with mercury exposure. This finding is consistent with many studies of modern aquatic animals, which have found that tissue mercury concentration increases with trophic level as measured by $\delta^{15}N$. While not invariable, this relationship has been found over entire foodwebs, in segments of a foodweb, and within single species in both marine and freshwater ecosystems (Atwell et al., 1998; Bergeron et al., 2007; Burgess and Hobson, 2006; Cabana and Rasmussen, 1994; Campbell et al., 2008; Capelli et al., 2008; Dehn et al., 2006a; Dehn et al., 2006b; Dietz et al., 2004; McIntyre and Beauchamp, 2007; Power et al., 2002). The relationship between tissue mercury and $\delta^{15}N$ has been surprisingly little studied in terrestrial ecosystems.

Based on analysis of the prehistoric ringed seal mandibles, bone mercury tends to decrease as bone collagen $\delta^{13}C$ increases, although the relationship is only moderate.

For aquatic animals, $\delta^{13}C$ is an indicator of feeding location, and values are usually higher in nearshore and bottom-feeding animals compared to offshore and open-water feeding animals (Burton et al., 2001; Clementz and Koch, 2001; France, 1995; McConnaughey and McRoy, 1979; Walker et al., 1999). Mercury concentrations do not seem to be consistently patterned in a similar way. Thus, studies comparing mercury concentration to $\delta^{13}C$ in modern animal tissues have variously found no significant relationship (Hobson et al., 2004; McIntyre and Beauchamp, 2007; Rigét et al., 2007a; Thompson et al., 1998), a positive relationship (Dehn et al., 2006a; Dietz et al., 2004; McIntyre and Beauchamp, 2007), or a negative relationship (Bearhop et al., 2000; Ethier et al., 2008; McIntyre and Beauchamp, 2007; Power et al., 2002; Ricca et al., 2008).

Additional chemical analyses of the Thule-period ringed seal sample suggest that factors other than exposure may influence the concentration of mercury in whole bone, including the nitrogen content of bone. Because nitrogen is found only in the protein phase of bone, this finding in turn implies that mercury may be physically associated with bone protein, rather than bone mineral. This argument receives some support from the experimental measurement of mercury on two demineralized bone samples. The concentration of mercury in these two bone protein samples was much higher than in their untreated counterparts.

The possible association of mercury with the protein portion of bone is also supported by the finding that bone mercury is significantly related to the carbon and sulfur content of bone. Carbon is mostly associated with the protein phase of bone,

although it can also be found in the bone mineral or as a post-depositional contaminant. Mercury is known to have a high affinity for the sulfur-containing amino acid cysteine (Clarkson et al., 2007), which occurs in many of the bone proteins, including procollagen (though not collagen) and the non-collagenous proteins.

One limitation of the present research is the lack of information about the susceptibility of bone mercury to diagenesis, the chemical changes that occur to bone after burial. Worldwide estimates of mercury concentrations in soil pore water, one of the chief factors controlling the uptake of metals by bone (Hedges and Millard, 1995; Pike and Richards, 2002), are extremely low (in the parts per trillion range) (Aastrup et al., 1991; Åkerblom et al., 2008; He et al., 2007; Kalicin et al., 2001; Krabbenhoft et al., 1998; Lee et al., 1994; Mitchell et al., 2008; Schwesig et al., 1999; Skyllberg et al., 2003). The low soil water mercury levels suggest that the potential for diagenetic uptake of mercury by bone is also low. In terms of the diagenetic alteration of the prehistoric Thule-period ringed seal bones used in this study, the excellent state of preservation of the bones, as measured by the whole bone nitrogen content, argues against major diagenetic change.

Recommendations for future studies

The possibility that bone mercury is associated with bone protein opens some fascinating avenues for future research. Chief among these is determining whether mercury measured on bone protein would be a better indicator of mercury exposure than whole bone mercury. The amount of protein can vary among different bones within a

single individual (e.g., higher in long bones than in flat or irregular bones) and even within a single bone (e.g., increasing from the outer cortex towards the medullary cavity) (Rogers et al., 1952). Thus, variation in whole bone mercury measurements may derive partially from variation in the ratio of protein to mineral in whole bone. If mercury is mostly associated with bone protein, it may be more stable over time in buried contexts than are metals associated with the bone mineral. Bone proteins, including collagen and the non-collagenous proteins, appear to be protected against post-burial chemical deterioration or biodegradation by the bone mineral itself (Collings et al., 2002; Grupe et al., 2000).

Determining in which bone fraction mercury resides—the organic or the mineral—may be difficult. The chemical processes used to isolate bone protein may also remove some of the mercury bound to it; likewise, procedures used to isolate bone mineral may retain some of the mercury bound to collagen (Spadaro, 1969). The challenge will be to find a method that selectively destroys the unwanted matrix, without attacking the metal-bearing complex. The results from the two highly experimental bone samples that were demineralized prior to mercury analysis show that a weak-acid treatment will not remove all of the mercury present in bone, but does not prove that only the mineral-bound mercury was removed or that all of the mineral-bound mercury was removed. Still, a logical line of inquiry would be to analyze paired samples of whole bone and demineralized bone for mercury content and to compare their concentrations to either known exposure (e.g., from controlled feeding experiments) or indicators of exposure, such as $\delta^{15}N$, soft tissue mercury, or whole-body mercury burden. This will help to

determine which matrix—whole bone or bone protein—is the better indicator of mercury exposure.

To further validate archaeological bone as a biomarker of past mercury exposure, additional laboratory and field studies are necessary to examine the relationships among mercury exposure, bone mercury, and soft tissue mercury in other mammals, particularly in humans, as well as to assess the stability of mercury in buried bone through time. Relating human bone mercury concentration to daily exposure level would allow an assessment of the possible health consequences of mercury exposure in preindustrial times, since the correlations between daily exposure and health outcomes have been established in modern humans. Establishing the relationship between human mercury exposure and bone mercury can stem, for example, from analyses of modern human bone biopsy samples paired with blood mercury concentration, since the blood mercury–dose conversion equation has already been formulated. Similarly, human bone mercury could be compared to hair mercury in prehistoric remains where hair is preserved, since this tissue, too, has already been related to daily dose. Finally, studies comparing prehistoric human bone mercury to $\delta^{15}N$ could help to establish that a relationship exists between exposure and bone mercury, though it would not allow a reconstruction of the absolute daily intake rate.

There appears to be little published information on the relationship between mercury levels and $\delta^{15}N$ in humans or in other terrestrial mammals. This is surprising, since there are scores of such studies for aquatic animals, as described previously. For humans and

other terrestrial mammals, tissue stable $\delta^{15}N$ values tend to increase with the extent of reliance on aquatic resources. Since mercury levels tend to increase in the same manner, the two variables should covary, and future studies should test this prediction. Human hair is an obvious choice of matrix for such a study, since it is a validated mercury exposure biomarker and is used in stable isotope studies (O'Connell and Hedges, 1999). Furthermore, hair is often the biomarker of choice in mercury biomonitoring studies conducted by public health agencies (Arnold and Middaugh, 2004; CDC, 2001), so the possibility exists for collaborating with large-scale, ongoing studies.

Studies focused on elucidating the relationship between bone mercury and soft tissue mercury would also be informative. Currently there is little information on bone mercury in modern mammals and its relationship to mercury in other tissues, except for a single study of modern Pacific harbor seal pups (Brookens et al., 2008). The seal pup study did not find significant correlations between mercury concentration in bone and that in most other tissues, but it did find significant correlations between the mercury *burden* in bone and that in other tissues. Possibly, mercury concentrations measured on bone protein would be more strongly associated with mercury concentrations measured on protein-rich soft tissues.

Relating bone mercury (or bone protein mercury) to soft tissue mercury may open another avenue for estimating prehistoric human exposure. If the mercury concentrations in archaeological faunal remains can be translated into mercury

concentrations in dietary tissues such as muscle, then it may be possible to reconstruct human exposure from diet mercury levels.

An example of the dietary reconstruction approach to estimating prehistoric mercury exposure was presented in this dissertation based on mercury concentrations found for prehistoric Thule-period ringed seals from archaeological sites at Cape Espenberg, western Alaska. Assuming that the mercury level in seal muscle tissue was at least as high as that in bone (mean THg = 13 ng/g), and further assuming that the Cape Espenberg Thule consumed at least 860 g of ringed seal per day, it was estimated that the typical adult consumed at least 11.2 μg of mercury per day. For a person weighing 65 kg (143 lb), that is equivalent to around 0.2 μg/kg bw/day, which exceeds the EPA safety level of 0.1 μg/kg bw/day (EPA, 2001c).

Possible data sets for studying the relationship between mercury in bone and that in soft tissue include the carcasses of hunted wildlife. These could be obtained with the permission of the hunter, or possibly from government game wildlife management agencies, which often require hunters to submit body parts of killed animals for regulatory purposes.

A major research gap that must be addressed in order to use bone mercury as a biomarker of exposure in prehistory is the lack of knowledge about bone mercury diagenesis over time. This is also a challenging avenue of study, because it is impossible to recreate the circumstances impacting bone chemical change over hundreds or thousands of years or more. All of the current methods for assessing the degree of

diagenetic change in single elements within bone are imperfect. Nevertheless, experimental studies to assess the propensity for bone to sorb mercury would help to address this issue.

This initial validation study has shown that bone has the *potential* to serve as a reliable biomarker of mercury exposure in preindustrial human populations. Continued efforts to validate and apply bone mercury as a biomarker of exposure may help to establish natural baseline levels of human mercury exposure, as well as to track changing levels of human mercury exposure and health effects through time.

Exposure to mercury is an important contemporary health concern, since human industrial activities release this metal into the environment. Documenting the toxic metal levels in humans from the recent and distant past can provide a rich source of information and a record of change that will add to our understanding of the cultural, ecological, and biological determinants of toxic metal exposure.

Beyond the field of bioarchaeology, historical information on mercury exposure should be of interest to medical anthropologists, who seek to identify the cultural practices that lead to heavy metal exposure and to overcome the cultural barriers to reducing exposure (Riley et al., 2001; Trotter, 1990). Nutritional anthropologists, too, should find value in historical information about diet and toxic metal exposure, since they are concerned with the health and mortality consequences of dietary patterns (Goodman et al., 2000). Finally, a historical record of mercury exposure promises to help public health officials and policymakers in their efforts to develop the best

strategies for reducing the risk of human exposure to this toxic heavy metal, while preserving the health and cultural benefits of many marine foods.

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APPENDIX A. ABBREVIATIONS

AOAC	Formerly Association of Official Analytical Chemists
CVAAS	Cold Vapor Atomic Absorption Spectrometry
CVAFS	Cold Vapor Atomic Fluorescence Spectrometry
EPA	United States Environmental Protection Agency
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ISO	International Standards Organization
IUPAC	International Union of Pure and Applied Chemists
MeHg	methylmercury
ng/g	nanograms per gram (equivalent to ppb)
ppb	parts per billion (equivalent to ng/g)
ppm	parts per million (equivalent to μg/g)
ppt	Parts per trillion (equivalent to ng/L)
RfD	Reference Dose; term used by EPA (2001c) in human health risk assessment to describe a dose of a toxicant that is likely to be without appreciable risk of deleterious health effects

RSD_r	Repeatability Relative Standard Deviation
SD_r	Repeatability Standard Deviation
THg	total mercury
WHO	World Health Organization
µg/g	micrograms per gram (equivalent to ppm)
µg/kg bw/day	micrograms per kilogram of body weight per day