CONTAMINANT EXPOSURE AND ASSOCIATED BIOLOGICAL RESPONSES IN SOUTHERN BEAUFORT SEA POLAR BEARS

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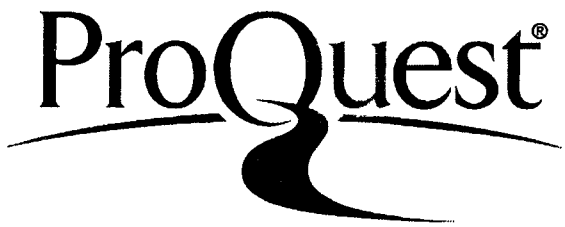

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ABSTRACT

Concentrations of mercury (Hg) and polychlorinated biphenyls (PCBs) were examined in polar bear (*Ursus maritimus*) to assess variations among sex and age cohorts, and evaluate possible adverse impacts of combined toxicant exposures. Biomarkers of selenium (Se) status (whole blood and serum Se concentrations, glutathione peroxidase activity), and thyroid status (total and free concentrations of thyroxine and tri-iodothyronine) were examined in Southern Beaufort Sea (SBS) polar bears. Both Hg and PCBs tended to be greater in female than in male polar bears and likely related to the type and proportion of marine-based prey in their overall diet. Significant positive relationships between circulating concentrations of PCBs, specific blood lipids (e.g., triglycerides and free fatty acids) and reduced body condition scores suggest combined contaminant-environmental stressors for SBS polar bears. Polar bear milk contained detectable concentrations of both Hg and PCBs. Estimated tolerable daily intake levels for PCBs through milk consumption by cubs of the year (< 6 months of age) exceeded available toxicity thresholds and could indicate possible adverse consequences of contaminant exposure during critical stages of neonatal development. Significantly positive and negative associations between contaminants and biomarkers indicated a possible oxidative stress response and thyroid disruption in SBS polar bears. Definitive relationships between contaminants and these physiologically-based biomarkers, however, could not exclude natural variations and equally possible impacts of nutritional stress and changes in physiological status. Female and young polar bears are the cohorts of concern for chronic low-level exposure to chemical mixtures. These data provide a better understanding of the physiological interactions underlying toxicity, and the multiple environment-toxicant stressors projected for arctic species with changes in climate.

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INTRODUCTION

Climate changes affecting sea-ice dynamics, sea currents, and ocean warming will alter prey availability (e.g., abundance, spatial and temporal distribution patterns) in marine environments and force arctic species to modify their habitat use patterns, transition to food sources that may be less nutritious, and rely for longer periods of time on body stores of lipid and protein (Corell 2006; Derocher et al. 2004). Marine mammals such as ringed seals (*Pusa hispida)*, bearded seals (*Erignathus barbatus*), and polar bears (*Ursus maritimus*), whose survival is wholly or in part influenced by stable sea-ice conditions during part of the year, are suspected to be most impacted by direct and indirect effects to their feeding and health (Burek et al. 2008). Direct effects include loss of sea-ice platforms for hunting, scavenging, or breeding, and indirect effects include changes in the transmission of pathogens and toxicant pathways. For example, recent studies reported that female polar bears with dependent young had lower leukocyte counts than other cohorts suggesting that that this cohort may be less resilient to the stress of ecosystem changes (Kirk et al. 2010). Declines in sea-ice due to climate warming and the potential effects on wildlife are well documented (Bluhm and Gradinger 2008; Durner et al. 2009; Jenssen 2006; Laidre et al. 2008; Moore and Huntington 2008; Ragen et al. 2008; Walsh 2008). Ice dependent species will adapt or be resilient to the reduction of sea-ice for their continued survival. Those animals unable to respond to the changing arctic conditions will likely become extinct or be highly endangered as limited refuge is predicted for large species. Assessing the potential health impacts of contaminants depends on appropriate monitoring of an organism's present physiological condition and examination of potential eco-physiological drivers in the changing arctic environment (**Figure 1**). Studies are needed to provide a better understanding of the

potential causes and effects of contaminants in wildlife, and identify critical cohorts that may be most at risk from both a toxicological risk and population dynamics perspective.

Arctic contaminants

Many contaminants reach the Arctic through long-range transport from lower latitudes via air, water, and biota (AMAP 2004a, b, 2009, 2011). Environmental contaminants include heavy metals such as mercury (Hg) and cadmium (Cd), and persistent organic pollutants (POPs) such as the organohalogen (chlorinated, brominated and fluorinated, including polychlorinated biphenyls, PCBs) compounds (Klassen 2001; O'Shea 1999). Of this diverse group of contaminants, Hg and PCBs have received the most attention in the circumpolar Arctic (e.g., land masses and waters within the political boundaries of Canada, Greenland, Norway, Sweden, Finland, the Russian Federation and Alaska (United States of America)) because of the relatively high concentrations in apex predators and concern for the health of arctic humans and wildlife (AMAP 2004a, b, 2009, 2011; Fisk et al. 2005; Sonne 2010; Letcher et al. 2010). Hg enters the environment through both natural (e.g., weathering of the earth's crust, volcanoes, forest fires) and anthropogenic sources (e.g., mining, coal burning; AMAP 2011; O'Shea 1999; Swain et al. 2007). The increase of industrial activities in Asia and worldwide has led to increased concentrations of Hg in arctic species and their environment (AMAP 2011, Gaden et al. 2009; Loseto et al. 2008). PCBs include mixtures of 209 congeners designed for their chemical stability and used as dielectric fluids in industrial transformers, heat transfer systems, lubricants, and hydraulics (AMAP 2004b; O'Shea 1999). During the 1970s, PCBs were banned due to worldwide distribution in the abiotic and biotic environment, and potential for adverse health effects in wildlife and humans (O'Shea 1999). The concentrations of these contaminants in the abiotic and biotic arctic environment have been reported to be in decline (AMAP 2009), although

other studies estimate that over 95% of the PCBs produced are still in landfills and circulating in the environment (Buckley 1982).

Food chain transfer and bioaccumulation of contaminants

Dietary intake is the main route of contaminant exposure for arctic species. The oceans are a sink for Hg and PCBs and both compounds adsorb to organic molecules (O'Shea 1999). PCBs are taken up by plankton, and sulfate-reducing bacteria convert inorganic mercury to more bioavailable methylmercury. Both Hg and PCBs bioaccumulate and biomagnify resulting in the highest concentrations in animals at the top of the food chain (Atwell et al. 1998; Hoekstra et al. 2003; Newman 2010). Arctic species are especially vulnerable to the accumulation of PCBs and other lipophilic contaminants because of the high-lipid content transferred through marine food webs, and the long life spans of many arctic species (AMAP 2004b; Lee et al. 1971). Many ecotoxicologists assume that Hg and PCBs will accumulate in parallel despite differences in compound structure and basic chemistry. The concurrent bioaccumulation of Hg and PCBs, however, is rarely examined. Understanding the toxicodynamics of combined toxicant exposures are critical when assessing the potential health impacts that may result in additive or synergistic impacts to neurologic, reproductive and immune systems (Haschek et al. 2002; Klassen 2001). Climate changes are expected to affect the environmental fate and transfer of both Hg and PCBs by altering chemical, physical and biological drivers of partitioning in the abiotic and biotic environment (Hung et al. 2010; Macdonald et al. 2005). The rates at which these changes will take place and the impact on arctic species remain unknown. Apex predators are expected to be the first to show adverse physiological responses to these combined contaminant – environment stressors.

Toxicodistribution, biotransformation, and elimination

The systemic biological impact of contaminants after oral intake depends on the bioavailability of the compound to the circulation and target sites for toxicity (AMAP 2004b; Newman 2010; Smith et al. 2007; **Figure 2**). Although dietary exposure may occur in parallel for Hg and PCBs, tissular distribution varies between these compounds. After the consumption of lipophilic compounds they tend to accumulate in adipose and fatty tissues (i.e., brain, liver), whereas heavy metals accumulate primarily in the liver and kidneys (Clarkson et al. 2007; Diamond and Zalups 1998; Haschek et al. 2002; Kelly et al. 2004; Klassen 2001). Mercury concentrations in tissues are comprised of both organic and inorganic forms that have a relatively short half-life before excretion (30-40 days), whereas the half- life for PCBs can be up to 5 years depending on the congener and tissue type examined (Dietz et al. 2004; Kelly et al. 2004; Klassen 2001; Mathews and Detrick 1984). Toxicodistribution also depends on the type of tissue and form of the contaminant consumed (i.e., inorganic or methylated Hg form), the bacterial flora in the gastrointestinal system, and the animals' nutritional status (Smith et al. 2007). Damage to organ systems via toxicosis, nutritional deficiency, or infectious disease can also modify the tissular distribution and concentration of contaminants (Haschek et al. 2002; Wobeser 2006). Excretion routes for Hg and PCBs include hair, feces, and urine, as well as the maternal transfer of toxicants to offspring during gestation and lactation (AMAP 2011; Clarkson et al. 2007; Kelly et al. 2004).

Biomarkers of health and effects of toxicant exposure

Biomarkers in toxicology are used to assess the potential responses of biological systems to contaminant exposure. Biomarkers have been categorized as biomarkers of exposure, biomarkers of effect, or biomarkers of susceptibility in comparisons between exposed and unexposed

individuals, cohorts, or populations (Newman 2010). The lack of unexposed or control (reference) animals in studies of free-ranging species makes these comparisons difficult, and researchers have relied on the use of correlative studies, a weight of evidence based approach, clinical diagnostics, or comparisons to threshold effect levels of health risks established for unrelated species (Letcher et al. 2010; Sonne 2010). Interpretation of biomarkers in contaminant effects assessments must include information on the organism's life history, behavioral traits, general condition, pathogen infection, and feeding ecology, as well as knowledge of relevant toxicant-receptor interactions (Smith et al. 2007; Letcher et al. 2010).

Mercury toxicity is based on what form is absorbed, its distribution through the body, and its interaction with target cells. The main target of toxicity for methylmercury (MeHg) is the central nervous system (including fetal), whereas inorganic Hg (IHg) targets the kidney, liver and gastrointestinal tract (Diamond and Zalups 1998; Haschek et al. 2002). Toxicity is based on the oxidative damage that occurs when Hg is converted to different forms (i.e., reduction of MeHg to IHg), accumulation and binding of IHg to proteins, the resultant inflammatory response, and the overwhelming of Se-dependent protein activities (Berry and Ralston 2008; Haschek et al. 2002). Hg concentrations have been related to the inhibition of the re-uptake mechanisms of neurotransmitters resulting in prolonged stimulation and neurological impairment (e.g., effects on memory and learning, neuromuscular defects, neuroendocrine defects), as well as endocrine disruption in birds, mammals and humans (Basu et al. 2009; Scheuhammer et al. 2008; Rolland 2000).

The biological effects of polychlorinated biphenyls (PCBs) are largely focused on endocrine disruption. Many PCBs act as hormone mimics, bind hormone receptors, and activate or inhibit

hormone processes (O'Shea 1999). Disruption of hormone processes has been associated with direct mortality, reproductive impairments, and increased susceptibility to disease (O'Shea 1999; Sonne 2010). The main targets for several PCB congeners include cortisol, thyroid, and reproductive hormone systems. Cytochrome P450 enzymes in the liver catalyze oxidative metabolism of xenobiotics and promote their excretion (O'Shea 1999; Sonne 2010). The induction of cytochrome P450 enzymes and production of PCB metabolites can disrupt hormone production and biliary excretion (Brouwer et al. 1998; McNabb and Fox 2003). Binding of PCBs to the Ah (aryl hydrocarbon) receptor and induction of cytochrome P450 enzymes, therefore, has been related to cell toxicity associated with disruption of normal endocrine function, growth retardation, learning deficits, impaired immunity, and neoplasia in human and animal studies (Kannan et al. 2000; Lindstrom et al. 1995).

Polar bears as sentinels of a changing arctic environment

The potential impacts of environmental changes may be most prominent in arctic apex predators such as polar bears that integrate the direct and indirect effects (i.e., changes in habitat and toxicant exposure) from lower trophic levels. Polar bears range throughout the circumpolar Arctic and their worldwide population is estimated to be 20,000 to 25,000 animals (Amstrup et al. 2007). Under projected climate models and changes in sea-ice habitat, polar bears are predicted to decline by 30 – 80 % of their current numbers (Amstrup et al. 2007; O'Neill et al. 2008). Reductions in body condition are also predicted as polar bears undergo longer periods of food deprivation while waiting for ice to freeze (Derocher et al. 2004). Changes in movement and denning in many areas of the polar bear range, including Alaska, are already evident. For example, sightings and denning of polar bears on land (in communities and the oil fields of northern Alaska) have increased during recent years, and correlate to reductions of sea-ice in the

Southern Beaufort and Chukchi Seas (D. Sanzone, BP Alaska, personal communication; K. Rode, USFWS, personal communication). Smaller stature and low cub recruitment of polar bears in the Southern Beaufort Sea (SBS) sub-population has also been related to increases in energetic demands and reductions in sea-ice extent (Regehr et al. 2010; Rode et al. 2010). Changes in the nutritional status and body condition of polar bears are expected to modify circulating concentrations of toxicants and associated defense mechanisms. For example, extended periods of fasting or limited prey availability may lead to behavioral changes in habitat use and / or prey selection in polar bears resulting in changes in exposure to contaminants via ingestion or mobilization of previously stored contaminants from adipose tissue to circulation. After ingestion of contaminants, changes in nutritional status may alter the physiochemical properties of toxicodistribution post-absorption, modify the biotransformation and excretion of toxic compounds, and impact the biological activity of toxicant – receptor interactions (Kelly et al. 2004; Newman 2010; Smith et al. 2007).

The capacity for polar bears to adapt to changes in diet and periods of food deprivation is evident in the natural life history of polar bears, as well as during recorded changes in the distribution of sea-ice (and prey availability). Ringed seal are the primary prey for SBS polar bears, although bearded seal, bowhead whale, beluga whale, and walrus can also make up a large proportion of their overall diet (Bentzen et al. 2007). Polar bears are adapted to seasonal fluctuations in the movement of prey by storing large deposits of adipose (subcutaneous and abdominal) that can be used as energy when food is unavailable (Amstrup 2003; Stirling et al. 2008). Polar bears are also adapted to the arctic climate by physiological and behavioral modifications such as delayed implantation, seasonal breeding, fasting during maternal denning, and extended periods of lactation (Amstrup 2003; Derocher et al. 1993). Female SBS polar bears begin maternal denning

during December, when they give birth to and nurse up to 3 cubs while undergoing a period of fasting. Females can use more than 90% of their fat reserves to support their own maintenance requirements, gestation and lactation (Atkinson and Ramsay 1995). Most studies of fasting polar bears have been performed in the Southern Hudson Bay population. It is generally thought that SBS females act in a similar way, but the time and length of denning, parturition, and nursing while denning is unknown for this population. The duration of maternal denning also likely differs by age, maternal experience, and body condition. Rapid changes in retreating ice platforms for hunting of seals and changes to distribution patterns of prey may extend periods of food deprivation beyond physiological limits of polar bears. Polar bears have a relatively low reproductive rate and produce offspring approximately every 3 years. Studies indicate that polar bears defer reproduction in favor of survival when prey is unavailable and fat reserves are low (Derocher et al. 1992).

General objectives and outline of the dissertation

Previous studies reported that concentrations of contaminants in East Greenland and Svalbard polar bears, and other apex predators, were associated with adverse effects on neuro-endocrine, developmental and immune functions, as well as negative impacts on metabolic and reproductive organs (Ahmad et al. 2008; Brouwer et al. 1998; Letcher et al. 2010; Rolland 2000). However, little is known regarding the current health status of SBS polar bears in response to combined contaminant exposures (i.e., heavy metal and lipophilic contaminants). This dissertation is an effort to bridge this knowledge gap, and discusses the potential environment-toxicant stressors projected for polar bear cohorts.

Chapter 1 of the dissertation builds on previous studies examining the concentrations of lipophilic (i.e., organochlorines including polychlorinated biphenyls, PCBs) contaminants and mercury (Hg) in SBS polar bears as reported by Benzten et al. (2007; 2008a; 2008b) and Cardona-Marek et al. (2009). This chapter focuses on the fact that this and other populations of polar bears are exposed to a combination of contaminants, and that combined exposures of contaminants must be considered in adverse health assessments. Blood concentrations of contaminants were examined instead of those concentrations in visceral (e.g., muscle, liver, kidney) or adipose tissues because the contaminants in blood represent the most recent influence to target sites of toxicity, and most closely match the temporal window of biomarkers measured in blood. This chapter examines the variations of contaminants by sex and age cohorts and the biological and chemical factors driving these variations. A unique addition of this study is the examination of blood lipids using thin layer chromatography coupled with flame ionization detection (TLC/FID), which provides information on the lipid classes (i.e., waxy esters, triglycerides, free fatty acids, cholesterol and phospholipids) in blood, and a more accurate estimate of total lipid concentrations than previous estimates performed using gravimetric methods. This chapter also examines the stable isotope values in packed cells (i.e., red blood cells) and serum to evaluate whether differences in the feeding ecology of polar bear cohorts contributed to variations in circulating toxicants. Relationships between contaminant concentrations and estimates of body condition (i.e., body condition indices) were also examined to assess whether blood concentrations of contaminants resulted from mobilization of previously stored contaminants in subcutaneous adipose, and to assess the combined stressors of toxicants and nutrition in SBS polar bears.

Chapter 2 examined concentrations of Se in whole blood and serum, glutathione peroxidase activity, thyroid hormones and concentrations of albumin to document the current selenium and thyroid status of SBS polar bears. This is the first study to report blood-based biomarkers of selenium status in any free-ranging polar bear population, and the first to report thyroid hormones in the SBS sub-population. Variations in these biomarkers by sex and age cohort are described, as well as the relationships to circulating concentrations of contaminants (Hg and PCBs). These data are presented in the context of key cohorts (e.g., prime reproductive aged males, solitary females in estrous, and females caring for young) and the natural life history of the polar bear. This chapter explores the possible mechanisms of toxicity for contaminants individually, and in combination, with consideration of the physiologic drivers of selenium and thyroid status biomarkers. This effort scrutinizes the use of these biomarkers with respect to the natural variations in the physiological status of female polar bears. This dissertation cautions against the generalization of toxicant impacts among age and sex cohorts.

Chapter 3 examines the maternal transfer of contaminants in female polar bears during mid to late lactation as the fetus, neonate and young are critical cohorts of concern. Although lactation has been described as one of the primary excretion routes for toxicants in female polar bears, this is the first report to document concentrations of PCBs in milk of SBS polar bears, and the first to report Hg concentrations in milk for any free-ranging polar bear sub-population. Toxicant concentrations in polar bear milk are compared to the toxicant concentrations in blood to explore the possible distribution of contaminants from maternal diet to blood to milk. Concentrations of contaminants in milk are compared to stable isotope values, lipid content, maternal body mass, and maternal age to examine the possible biological factors contributing to the variations among female polar bears. Lastly, daily intake levels for Hg and PCBs by milk consumption are

estimated for dependent polar bear cubs (e.g., cubs the year and 1-2 year old cubs) and compared to tolerable daily intake levels and dioxin equivalency thresholds to evaluate the potential health risk of contaminants for nursing young. These health assessments are based on standards developed for humans and non-arctic species, and we caution that this extrapolation is untested. This study provides a toxicological context in which the health risks of toxicants for dependent polar bears can be further evaluated and discussed.

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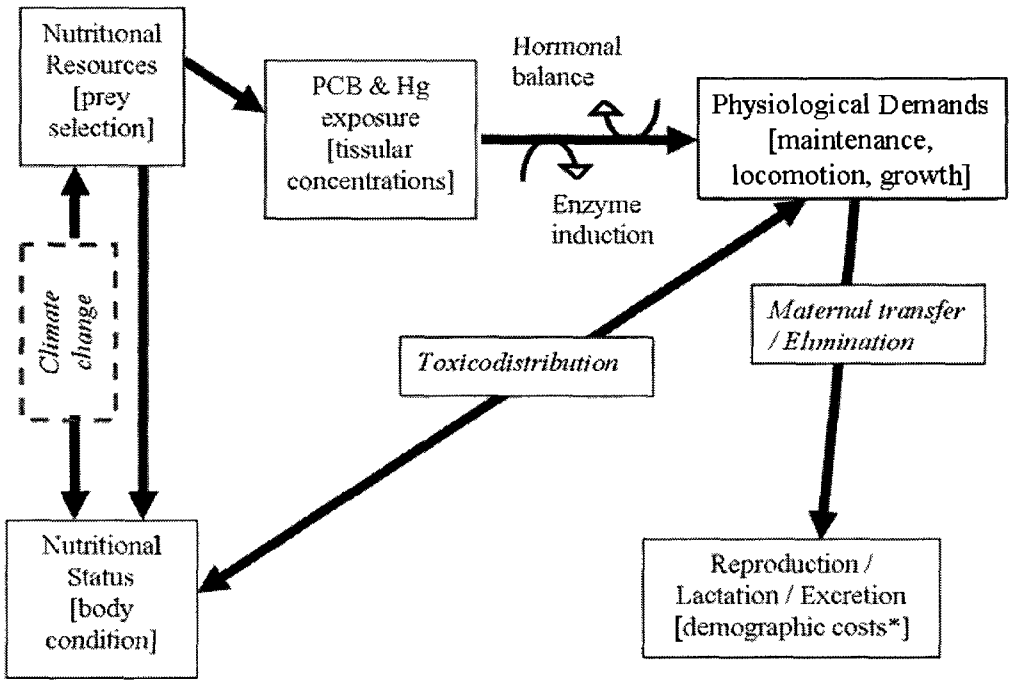
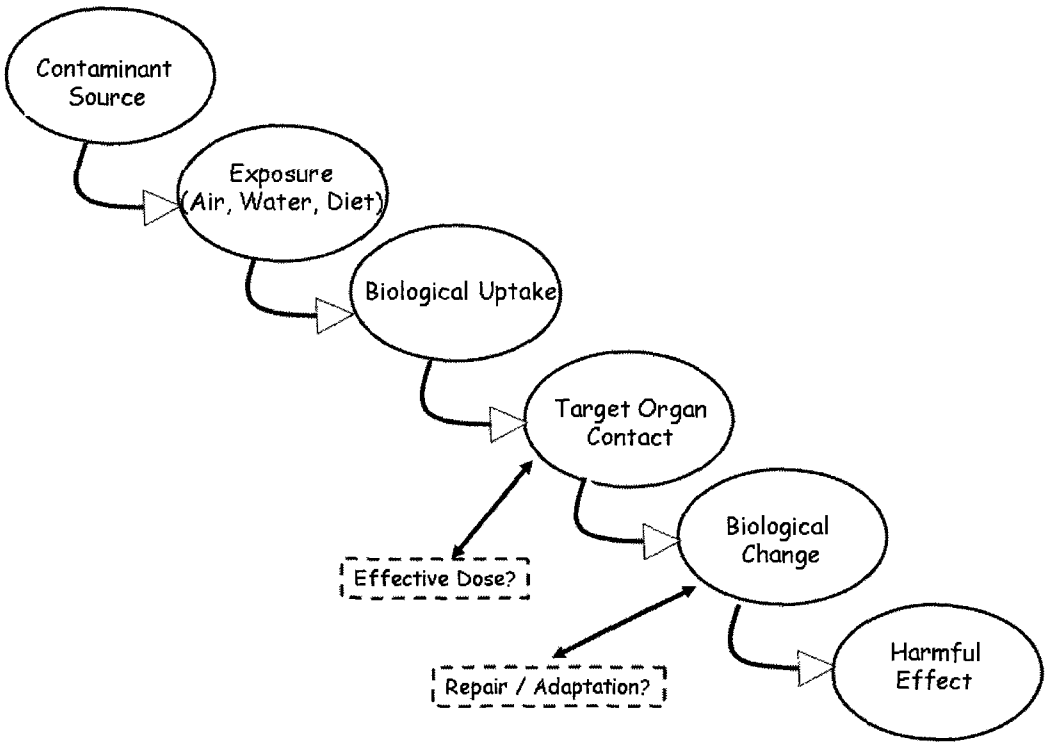


Introduction Figure 1. Conceptual model of the eco-physiological changes (and their interactions) impacting arctic species under the proposed changes in climate. *e.g., reproductive failure, reduced recruitment, etc.



Introduction Figure 2. The cascade of events required to elicit a harmful effect after contaminant exposure.

CHAPTER 1

Concentrations of mercury and polychlorinated biphenyls in blood of Southern Beaufort Sea polar bears (*Ursus maritimus*) during spring: variations with lipids and stable isotope values ($\delta^{15}N$, $\delta^{13}C$)[1]

1.1. ABSTRACT

Polar bears are exposed to heavy metal and lipophilic contaminants that are known to bioaccumulate and biomagnify. Few studies concurrently report both chemical classes in the same individuals and are thus unable to assess drivers of observed tissue concentrations, and the potential adverse biological responses to combined exposures. We examined blood concentrations of mercury (Hg) and the sum of 7 polychlorinated biphenyls (ΣPCB_7) from free-ranging Southern Beaufort Sea polar bears to assess which factors contributed to variations among cohorts (adult males, adult females, young) during spring. Concentrations of Hg ranged from 10.3 – 228.0 ng / g ww, but were similar between males and females independent of age. Concentrations of ΣPCB_7 (range, 2.0 – 132.8 ng / g ww) and were greater among females and young compared to males. Toxicant concentrations were related to packed cell $\delta^{15}N$, an estimate of trophic position, after the inclusion of packed cell $\delta^{13}C$, an estimate of dietary carbon. Concentrations of ΣPCB_7 were also positively correlated with concentrations of neutral lipids (triglycerides and free fatty acids) and inversely correlated to body condition index scores. Elevated concentrations of toxicants and lower body condition indices in females and young compared to males may be a sentinel to a changing arctic environment. Further assessment of the

[1] Knott KK, Boyd D, Ylitalo GM, O'Hara TM. 2011. Concentrations of mercury and polychlorinated biphenyls in blood of Southern Beaufort Sea polar bears (*Ursus maritimus*) during spring: variations with lipids and stable isotope ($\delta^{15}N$, $\delta^{13}C$) values. Canadian Journal of Zoology 89: 999-1012.

potential adverse health impacts of contaminants and nutritional stress in these cohorts is warranted.

1.2 INTRODUCTION

Many contaminants undergo long-range transport from southern latitudes and are deposited in arctic ecosystems via biota, air, and water (Macdonald et al. 2005; Swain et al. 2007). Climate changes are proposed to increase the atmospheric and oceanic transport of contaminants to the abiotic and biotic arctic environment (Outridge et al. 2008; AMAP 2009; Hung et al. 2010), creating new challenges for arctic adapted species. Polar bears (*Ursus maritimus* Phipps, 1774) and other apex predators are exposed to a combination of toxicants and nutrients through trophic transfer (Atwell et al. 1998; Kucklick et al. 2002; Hoekstra et al. 2003). In response to changes in their sea-ice habitat, polar bears are expected to modify their habitat use patterns, use less nutritious food sources, or extend their use of endogenous (stored) lipid and protein (Derocher et al. 2004). These changes are also expected to alter the concentrations of toxicants, and the dynamics of toxicodistribution among polar bear cohorts. Previous studies of the contaminant concentrations in polar bears have examined and reported heavy metal (e.g., mercury, Hg) and lipophilic contaminants (e.g., polychlorinated biphenyls, PCBs) separately (e.g., Bentzen et al. 2008*a*; Cardona-Marek et al. 2009). Researchers are thus unable to examine the similarities or differences of those factors that most influence dietary exposures to these contaminants in a single assessment and report. Separation of studies by chemical classes also limits a researcher's ability to identify and examine the potential adverse impacts of combined exposures.

The concurrent biomagnification of heavy metal and lipophilic toxicants is commonly related to the trophic level at which an animal feeds, and animals that consume higher trophic-

level prey are exposed to greater intakes of some toxicants (Newman 2010). Variations in toxicant concentrations of individuals within a species also vary with the amount and proportion of prey items consumed and the toxicant concentrations of those prey items. In species that undergo periods of fasting, circulating toxicant concentrations may also reflect mobilization of contaminants previously stored in tissues. Bentzen et al. (2007) estimated that ringed seals (*Phoca hispida* Schreber 1775) comprised 47 – 82 % of the overall winter diet of Southern Beaufort Sea polar bears. Polar bears also hunt and scavenge other relatively lower trophic level prey species including bearded seal (*Erignathus barbatus*), beluga whale (*Delphinapterus leucas* Lacepede, 1804), bowhead whale (*Balaena mysticetus* L., 1758*)*, and walrus (*Odobenus rosmaru* L., 1758*;* Bentzen et al. 2007 and references therein). Stable isotope analysis can be used to infer dietary patterns and help identify the transfer of contaminants through ecosystems (Bentzen et al. 2008*a*; Jardine et al. 2006; Horton et al. 2009) that can be age and sex cohort specific. Relative trophic position can be assessed by the enrichment of ^{15}N in predators as compared to prey items, while differences in ^{13}C provide information about sources of dietary carbon (i.e., terrestrial versus marine; pelagic versus sympagic versus benthic) with minimal trophic level enrichment. Previous studies have found that both trophic level and carbon sources were important for identifying exposure routes and the variation in concentrations of heavy metal and lipophilic toxicants to polar bears as reported separately (Bentzen et al. 2008*a*; Cardona- Marek et al. 2009). These and other recent studies have challenged the commonly held dogma that toxicant concentrations are greater among males than females due to the maternal transfer of contaminants to young during gestation and lactation. Although young animals are expected to be the primary cohort of concern for adverse biological effects from exposure to these contaminants, assessment of the toxicant concentrations in this cohort are often underrepresented due to difficulty in capturing and sampling of young animals. Further study of the relationships between stable

isotope values and contaminant concentrations, therefore, can allow researchers to generate hypotheses regarding why the contaminant concentrations of some individuals are greater than others although both occupy the same habitat and consume the same prey (Jardine et al. 2006).

Lipid dynamics also play a significant role in the delivery, toxicodistribution, and biomagnification of many toxicants. Because of their chemical affinity for lipids, the concentration of lipophilic toxicants can often be explained by the concentration of tissular lipids. Many toxicological studies examine lipid concentrations using gravimetric methods that can over estimate true lipid content, thus lowering the actual lipid-adjusted toxicant concentration, and mask important characteristics of the chemical interactions among compounds (e.g., varying solubility and binding between contaminants and lipids). Lipids differ in composition and concentration among dietary sources, between mammalian tissue compartments, and are modified by the biological processes of digestion, lipid mobilization, and storage (Dietschy et al. 1993; Kelly et al. 2004). Polar bears accumulate subcutaneous and abdominal lipid reserves for use as energy stores during periods of limited prey availability. Reductions in body mass from the mobilization of lipid reserves during fasting resulted in an increased concentration of lipophilic contaminants in both adipose and blood of polar bears, but these changes differed by age and sex cohort (Polischuk et al. 2002). Lipid reserves in polar bears have been used as an index of body condition to assess the health of populations, the availability of prey, and predict reproductive success (Cattett et al. 2002, Stirling et al. 2008*a*). Few studies, however, have examined the potential interactions between body condition indices and circulating concentrations of contaminants. An acute increase in the concentrations of toxicants in blood could provoke harmful effects if individuals are already nutritionally compromised or undergoing critical stages of tissue differentiation (e.g., growth, development of cubs). The biological impacts of toxicant exposure are determined by the distribution and the systemic delivery of

contaminants to target cells (Klassen 2001; Haschek et al. 2002; Clarkson et al. 2007; Newman 2010). Thus, an understanding of current contaminant exposure is best explored through examination of toxicant concentrations in blood versus other storage tissue (e.g., visceral organs and lipid deposits) that are more commonly examined. A better understanding of the lipid composition by detection of the lipid classes in blood and inclusion of body condition indices may help to explain variations in circulating toxicant concentrations among polar bear cohorts and determine temporal periods of exposure (e.g., season, stage of life) when animals are most vulnerable.

The objective of this study was to examine the chemical and biological factors that influenced blood concentrations of toxicants in Southern Beaufort Sea (SBS) polar bears during spring. We measured concentrations of Hg and ΣPCB_7 in whole blood and compared these among polar bear cohorts (adult males, adult females, and young). We evaluated the correlations between circulating concentrations of toxicants and stable isotope values ($\delta^{15}N$, $\delta^{13}C$) in packed cells (red blood cells) and serum. We also examined the lipid content of blood and body condition indices among polar bear cohorts to identify which factors (age, sex, diet, lipid content, body condition index, location) were most related to variations in blood Hg and ΣPCB_7. These data allow for an evaluation of toxicant concentrations during spring, as well as an examination of the similarities and differences in the exposure to heavy metal and lipophilic contaminants. To understand the potential future threats to the health of SBS polar bears, the concentrations of toxicants, lipid concentrations, stable isotope values, and body condition indices in recent years (2007) were compared to previous reports of these measures in SBS polar bears. As this study only examines the differences between two years, these data are not expected to identify temporal changes, but report concentrations as a baseline for future monitoring. Further assessment of

contaminant exposure and associated biomarkers of physiological responses in cohorts of concern (e.g., reproductive females and young) will be reported separately.

1.3 MATERIALS AND METHODS

1.3.1 Sample collection

Free-ranging polar bears were captured in the Southern Beaufort Sea during spring (March – May) 2007 by the U.S. Geological Survey Ursid Research program (Anchorage, Alaska) as part of a longterm study of polar bear ecology and population dynamics. Capture efforts began each year in Barrow and continued eastward toward Prudhoe Bay, and Kaktovik, Alaska, USA. A vestigial premolar was removed from anesthetized sub-adult and adult bears to estimate age by counting the cementum annuli (Matson Laboratory, Milltown, MT). Ages of cubs of the year through 3-year old animals were estimated by size and inclusion in a family group. Blood samples were collected from either the femoral vein or artery into non-additive and K3EDTA VacutainersTM (BD Vacutainers, Preanalytical Solutions). Blood samples in non-additive tubes were centrifuged within six hours of collection to separate serum from the packed cells (clotting proteins and blood cells). Standard length (length) was recorded as the straight line length above the bear measuring the distance from the tip of the nose to the tip of the bony part of the tail while in sternal recumbency. Body mass (using a tripod, hoist, scale and net) and capture location (latitude, longitude) were recorded for each bear. Body condition index (BCI) was determined from the natural log (ln) of mass and standard body length for each bear [BCI = (ln mass – 3.07*ln length + 10.76) / (0.17 + 0.009 * ln length); Cattet et al. 2002]. Capture and sampling procedures have been described previously (Bentzen et al. 2008*a*; Cardona-Marek et al. 2009). Animal handling procedures were approved by animal care and use committees at the

USGS and the University of Alaska Fairbanks (UAF Institutional Animal Care and Use Committee Protocol Number 04-58).

1.3.2 Mercury analysis

Whole blood from 31 males and 27 females was analyzed for total Hg concentration at the Wildlife Toxicology Laboratory (WTL), University of Alaska Fairbanks. Concentrations of Hg were determined on a DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT) using an eleven point calibration curve ranging from 1 to 400 ng ($r^2 = 0.998$; method detection limit approximately 50 pg). Standards were analyzed before unknown samples and after each set of 20 individual samples. Standard reference materials included freeze-dried human hair (IAEA-085, 0.573 mg/kg; IAEA-086, 23.2 mg/kg, International Atomic Energy Agency, Vienna, Austria), freeze-dried fish muscle homogenate (Lake Superior Standard Reference Material 1946, National Institute of Standards and Technology), and standard spiking solution (0.10 mg/kg, Perkin-Elmer, Waltham, Massachusetts). Percent recoveries of standard reference materials and spikes were within 88 - 110 %. Concentrations of Hg detected by DMA- 80 was comparable to those assessed by cold vapor atomic fluorescence spectroscopy (percent recovery for hair, 87 – 102 % and blood, 76 – 97%; DMA blood Hg = (0.781*CVAFS blood Hg) + 4.44, $r^2 = 0.993$) as previously performed at the WTL and described in Cardona-Marek et al. (2009). Total mercury (THg) concentrations were considered analogous to methyl-mercury concentrations since approximately 95% of the total mercury concentrations in blood of mammals have been determined to be in the methylated form (Klassen 2001). Data are presented as total Hg in whole blood on a wet weight basis (Hg ng / g ww).

1.3.3 Polychlorinated biphenyl analysis

Concentrations of polychlorinated biphenyls (PCBs) were analyzed in whole blood from 24 males and 22 females. Procedures were followed as described in Sloan et al. (2005). Briefly,

0.91 ± 0.12 g of whole blood was mixed with sodium sulfate and magnesium sulfate (1.5:1, *v/v*) as drying agents. PCBs and lipids were extracted with dichloromethane on an Accelerated Solvent Extractor (ASE 200, Dionex Corp., Sunnyvale, CA) at the WTL, University of Alaska Fairbanks. Extracts were delivered to the National Oceanic and Atmospheric Administration Fisheries laboratory in Seattle, WA. Each sample extract was filtered by gravity flow columns containing silica gel and alumina to remove interfering polar compounds and then further clean-up was conducted using size exclusion high performance liquid chromatography (SEC-HPLC). Individual PCB congeners (ΣPCB_{40}; 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170/190, 171, 177, 180, 183, 187, 191, 194, 195, 199, 205, 206, 208, 209) were detected using a low-resolution quadrupole gas chromatograph/mass spectrometer (GC/MS) system equipped with a 60-meter DB-5 capillary GC column (0.25 mm i.d. and 0.25 μm film thickness) and a 10-meter guard column (0.53 mm i.d.).

Each sample batch (n = 14) included a series of external standards and concentrations were assessed based on a five-point calibration curve. Percent recoveries (mean ± SD) were 98.7 ± 1.7%, 102.1 ± 1.9%, and 100.0 ± 15.9% for internal spike (PCB103) in blood/SRM/method blank, certified congeners from SRM, and congeners of duplicates, respectively. PCB 101 was below detection in all animals. PCB concentrations were reported as the sum of the seven PCB congeners (99, 105, 138, 153, 170, 180, and 194; ΣPCB_7) detected in ≥ 41% of animals sampled (cut—off based on an apparent break in the proportion of animals having concentrations above detection [remaining congeners were detectable in < 7% of animals sampled]. The difference between concentrations reported as ΣPCB_{40} and ΣPCB_7 was ± 2 ng / g ww. All PCB concentrations are reported as ng / g wet weight in whole blood.

1.3.4 Lipid analysis

Lipid composition of whole blood was determined by thin layer chromatography coupled with flame ionization detection (TLC/FID) using the Iatroscan Mark 6 (Iatron Laboratories, Tokyo, Japan). This analysis estimated the lipid concentration of five lipid classes in blood (sterolesters/wax esters, triglycerides, free fatty acids, free cholesterol and phospholipids; Ylitalo et al. 2005). The limits of detection for each of the five lipids were the following: lauryl stearate (wax ester) 0.20 mg / ml, triolein (triglyceride) 0.20 mg / ml, oleic acid (fatty acid) 0.30 mg / ml, cholesterol 0.25 mg / ml and L-alpha-phosphatidylcholine (phospholipid) 0.25 mg / ml. All blood samples were above detection for wax esters and cholesterol. For those samples below detection for triglycerides, free fatty acids, and phospholipids, the value of 0.1 mg / mL (½ the detection limit of the lowest calibration; generally equivalent to < 5 mg lipid class / dL of whole blood) was used. The mean percent recovery of duplicate samples was 107.4 ± 20.7% (mean ± SD) for lipid classes. The concentration (mg / dL) of each lipid class and total lipid in whole blood were determined from sample mass (g) of blood used during extraction. Data were reported as the percent total lipid in blood ([sum all lipid class / total sample wet weight] *100), as well as the percent of each lipid class making up the total lipid content of blood (e.g., [g free cholesterol / g total lipid] * 100)

1.3.5 Stable isotope analysis

Stable isotope ratios (15N / 14N, 13C / 12C) were examined in packed cells and serum (males = 29, females = 26). Stable isotope analysis was performed at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks as previously described (Bentzen et al. 2007; Cardona-Marek et al. 2009). Briefly, serum and packed cells were freeze dried, homogenized, and weighed (0.3 - 0.5 mg) into tin capsules. Stable isotope values ($\delta^{15}N$, $\delta^{13}C$) were determined from the mean of duplicate samples by EA-IRMS using a Costech Elemental Analyzer (ESC

4010), and Finnigan MAT Conflo III interface with a Delta+XP Mass Spectrometer. Stable isotope values were expressed in δ notation as parts per thousand (‰) according to the following:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000\ (‰)$$

where R is the ratio of heavy to light isotope ($^{15}N / ^{14}N$ or $^{13}C / ^{12}C$). Atmospheric N_2 (air) and Pee Dee Belemnite (PDB) were used as standards. Peptone ($\delta^{15}N = 7.0$, $\delta^{13}C = -15.8$; meat based protein; Sigma Chemical Company) was used as a working laboratory standard to ensure accuracy and precision for stable isotope values of unknown samples. Peptone was analyzed before unknown samples and after each set of 10 samples (percent recovery of peptone ranged from 99 – 101% for both $\delta^{15}N$ and $\delta^{13}C$). Stable isotope values are reported as the mean of duplicate samples (all duplicates were less than 0.2 per mil).

1.3.6 Statistical analyses

Statistical analyses were performed using Systat 11 and MYSTAT Student's version (Systat Software Inc.). We assigned cohorts based on sex and age that were expected to contribute to variations in feeding ecology and thus influence toxicant exposure. We identified three groups for analysis: adult males (≥ 5 years), adult females (≥ 5 years), and young (1 - 4 years of age). Although dependent young (1 – 2 years) are expected to consume greater amounts of milk, dependent young were merged with data from sub-adults (3 - 4 years) due to small sample size. Concentrations of toxicants (ng / g ww) and lipids (total and by lipid class, mg / dL) were log transformed to meet normality. Pearson's pairwise correlations with Bonferroni probabilities were used to assess relationships among individual continuous variables. Mean differences among cohorts were examined using analysis of covariance (ANCOVA) with Tukey's adjustments for multiple comparisons. Covariates included year or age depending on the specific comparison. Mean concentrations of lipid classes were compared by cohort using

multivariate analysis of variance (MANOVA). Factors contributing most to variations in circulating Hg and ΣPCB_7 concentrations were selected using a multiple linear regression model with backward stepwise selection (α to remove set at 0.05). The original full model included sex, age, each lipid class, longitude, body condition index, and stable isotope values in serum and packed cells. Only data from 2007 could be included as 2007 was the only year which all relevant variables were measured. Significant differences were determined to be at $\alpha < 0.05$. The relationship between variables in linear regressions were based on the r^2 values, where 0.36 to 0.55 was weakly to moderately related, 0.56 to 0.75 was moderately to strongly related, and $\geq$ 0.75 strongly related. Variables in Pearson's correlations were considered to be moderately related when correlation coefficients (r) were between 0.4 and 0.69, and strongly related if $r \geq$ 0.7.

1.4 RESULTS

1.4.1 Morphometrics

Adult polar bears ranged from 5 to 25 years of age (mean ± SD; males, 11.5 ± 4.6; females, 10.8 ± 5.3 years). Adult males were 2.1 times heavier (males, 221.4 to 586.0 kg; females, 118.2 to 231.8 kg) and 1.2 times longer than adult females (males, 220.9 ± 11.2 cm; females, 195.5 ± 7.6 cm). Body condition indices for males were greater than females and young (**Table 1.1**; 2007; $f = 20.378$, $p < 0.001$; females $\leq$ young < males; Tukey's adjustments, $p <$ 0.006) in all years examined. Overall, body condition indices of polar bear cohorts during 2007 were similar to 2005, but lower than 2003 (**Table 1.1**; year; $f = 9.95$, $p < 0.001$).

1.4.2 Toxicant and lipid concentrations in blood

Concentrations of Hg in whole blood ranged from 10.25 to 228.05 ng / g ww and were similar between males and females regardless of age (ANCOVA; sex, $f = 1.552$, $p = 0.219$; age

as a covariate, $f = 1.082$, $p = 0.303$). Concentrations of blood ΣPCB_7 (sum of PCB congeners 99, 105, 138, 153, 170, 180, 194) ranged from 2.03 to 132.8 ng / g ww (568 to 23,200 ng /g lw). Wet weight concentrations of ΣPCB_7 were significantly higher among females than males and decreased with age for both sexes (ANCOVA; sex, $f = 21.906$, $p < 0.001$; age as covariate, $f = 8.608$, $p < 0.005$). Concentrations of Hg were similar among polar bear cohorts ($f = 1.275$, $p = 0.288$), whereas concentrations of ΣPCB_7 were lower in males compared to female and young (2007: $f = 20.456$, $p < 0.001$; **Table 1.1**). Toxicant concentrations among polar bear cohorts were similar to previous years examined (**Table 1.1**).

Percent lipid of whole blood estimated by TLC/FID ranged from 0.16 to 0.60 % (149.1 to 594.8 mg / dL ww; **Table 1.1,** 2007). Total lipid concentrations in blood were greater for females than males, even after accounting for variations with age (males, 295.9 ± 104.1 mg / dL; females, 386.7 ± 113.6 mg / dL; ANCOVA; sex, $f = 11.989$, $p < 0.001$; age as covariate, $f = 7.033$, $p = 0.011$). Among cohorts, mean concentrations of lipid in blood (mg / dL ww) were similar between females and young, and both were greater than males (young ≥ females > males; males, 319.7 ± 92.3; females, 456.9 ± 131.2; young, 478.9 ± 43.1; ANOVA, $f = 13.645$, $p < 0.001$). Lipid concentrations did not correlate with circulating concentrations of Hg ($p > 0.25$).Concentrations of total lipids in blood, however, were positively correlated with ΣPCB7 ($r = 0.545$, $p < 0.001$) and inversely correlated with body condition index ($r = - 0.570$, $p < 0.001$). Lipid-adjusted concentrations of ΣPCB_7 using the total lipid concentrations estimated by TLC/FID, therefore, also indicated greater ΣPCB_7 concentrations (ng / g lw) in females and young than males during 2007 (**Table 1.1**).

Lipids in whole blood were primarily waxy esters (54.7 ± 7.7 %) and cholesterol (33.5 ± 6.0 %). Other lipid classes were found in lower amounts, and were more variable among individuals (triglycerides, 2.8 ± 5.7 %; free fatty acids, 5.5 ± 6.3%; phospholipids, 4.9 ± 2.3%).

Neutral lipids (FFA and TRIGs) were above detection limits in 41 % of adult males (10 of 27), 63 % of adult females (15 of 24), and 100% of young (7 of 7, all with FFA and 1 with TRIGs) sampled during 2007 (see **Appendix 1.1**). Concentrations of all lipid classes but phospholipids varied among cohorts (MANOVA, Wilks' Lambda, f 10, 102 = 3.260, p = 0.001; **Figure 1.1**). Among the neutral lipids, females had the greatest concentrations of triglycerides, whereas free fatty acids were highest among young. Because the methods to estimate lipid concentrations differed between years, variations in lipid content or lipid corrected ΣPCB_7 concentrations between years could not be examined.

1.4.3 Stable isotope values in blood

The mean of $\delta^{15}N$ and $\delta^{13}C$ values by cohort were similar between years examined (**Table 1.1**). Stable isotope values in packed cells ranged from 18.0 to 21.0 ‰ for $\delta^{15}N$, and from -19.7 to -16.8 ‰ for $\delta^{13}C$ (**Table 1.1**). Stable isotope values in serum (range; $\delta^{15}N$, 18.7 to 22.5 ‰; $\delta^{13}C$, -21.9 to -17.8 ‰) correlated with packed cell values ($\delta^{15}N$, r = 0.842, $p < 0.001$; $\delta^{13}C$, r = 0.764, $p < 0.001$). The mean packed cell $\delta^{15}N$ value for males was 0.77 per mil lower than females and young (**Table 1.1**). Serum $\delta^{15}N$ was similar among cohorts. Blood $\delta^{15}N$ values were not correlated to the concentration of blood lipids or body condition scores ($r < 0.21$, $p > 0.12$). Packed cell $\delta^{13}C$ was also similar among cohorts, whereas serum $\delta^{13}C$ differed as young > females > males (**Table 1.1**; Tukey adjustments, $p < 0.05$). Packed cell $\delta^{13}C$ values were negatively correlated to longitude of capture, but were independent of blood lipids (**Figure 1.2 A and B**). Conversely, serum $\delta^{13}C$ values were negatively correlated with both longitude and the concentration of total lipids (**Figure 1.2 C and D**).

Pearson's correlations indicated no individual relation between toxicant concentrations in blood and packed cell $\delta^{15}N$ (Hg, r = 0.129, $p > 0.900$; PCB ww, r = 0.272, p = 0.255; PCB lw, r

= 0.303, p = 0.153; **Figure 1.3 A and B**). Concentrations of blood Hg and lipid adjusted concentrations of ΣPCB_7 were negatively correlated with packed cell $\delta^{13}C$ (Hg, r = -0.495, p = 0.001, **Figure 1.3 C**; PCB lw, r = -0.401, p = 0.025). Wet weight concentrations of ΣPCB_7, however, were unrelated to packed cell $\delta^{13}C$ (r = -0.303, p = 0.140, **Figure 1.3 D**).

1.4.4 Factors explaining toxicant concentrations in polar bears (spring 2007)

Blood mercury concentration was best explained by packed cell $\delta^{13}C$ and $\delta^{15}N$, and longitude of capture (**Table 1.2A**). Blood ΣPCB7 concentrations varied by packed cell $\delta^{13}C$ and $\delta^{15}N$, were positively correlated with the concentration of neutral lipids (triglycerides and free fatty acids), and inversely correlated with body condition index (**Table 1.2B; Figure 1.4 A - C**).

1.5 DISCUSSION

Blood mercury concentrations of polar bears during spring varied over 20 fold, but did not differ by sex, age, or year. Mercury concentrations in blood are comprised of primarily organic Hg forms (e.g., methyl and ethyl mercury compounds) that have a relatively short half-life in circulation before excretion (30 - 40 days), whereas the half life for PCBs can be up to 5 years depending on the congener and tissue type examined (Klassen 2001; Dietz et al. 2004; Kelly et al. 2004). Circulating concentrations of Hg, therefore, likely reflect the toxicant concentrations of diet items recently consumed. Dietary Hg can cross the blood- placenta barrier during gestation, and has been detected in the milk of lactating females (Polischuk et al. 2002; Clarkson et al. 2007). Similar blood Hg concentrations in young and adult polar bears suggest that the maternal transfer of heavy metals to young is minimal, or that the number of animals in the young cohort of the present study was too small to detect a statistical difference. The target of toxicity for methylmercury is the central nervous system (including fetal), whereas inorganic Hg

forms (mercurous, $Hg–Hg^{+2}$, and mercuric, Hg^{+2}, cations) target the kidney, liver and gastrointestinal tract (Haschek et al. 2002; Clarkson et al. 2007). Methylmercury is considered toxic due to its ability to cross the blood-brain barrier, which can result in oxidative damage, inflammatory responses (involving macrophages and cytokines), and the interference of selenium dependent protein activities (Haschek et al. 2002; Clarkson et al. 2007; Berry and Ralston 2008; Ralston 2008). In polar bears, concentrations of inorganic mercury have been associated with an increase in renal lesions, and methylmercury concentrations related to changes in neurotransmitters in the brain (Sonne et al. 2007; Basu et al. 2009). The concentration of Hg in blood of SBS polar bears in the present study (range, 10.3 to 228.0 ng/g ww) were above the highly conservative toxicity thresholds for humans (5.8 ng / g in human cord blood, USEPA). Blood concentrations of Hg for other polar bears sub-populations have not been reported. Polar bears and other marine mammals are suspected to tolerate higher concentrations of Hg than terrestrial species through the demethylation of methylmercury to less toxic inorganic forms and sequestration as mercuric selenide in some organs (discussed in Woshner et al. 2002; AMAP 2009). The impacts of blood Hg concentrations in polar bears remain largely unknown.

Concentrations of ΣPCB7 in adult SBS polar bears in the present study (range, 2.0 to 132.8 ng / g ww) were similar to circulating concentrations reported for adult polar bears in Svalbard (48.8 ± 28.8 ng / g ww plasma, Polischuk et al. 2002; mean = 32.3 ng / g ww plasma, Olsen et al. 2003). This finding is contrary to previous reports that described concentrations of contaminants in SBS polar bears to be lower than those in other sub-populations of polar bears (Kucklich et al. 2002; Letcher et al. 2010) based on tissues other than blood. Elevated ΣPCB7 concentration in blood of young polar bears was expected as the result of the exposure to contaminants transferred from mothers during gestation and lactation. Lactational transport of lipophilic contaminants from females to young is suspected to be high due to the high lipid

concentrations of polar bear milk (Polischuk et al. 2002), although this relationship has not been fully examined. Comparably higher toxicant concentrations in young relative to adults have previously been reported in polar bears, as well as other arctic and sub-arctic marine mammals (Hansen et al. 1990; Polischuk et al. 1995; Espeland et al. 1997; Beckmen et al. 2003). Concentrations of PCBs in blood versus those in lipid stores are more immediately bioavailable to target organs and could result in potentially harmful biological responses. Reproductive impairments, endocrine disruption, and immune depression in Svalbard polar bears were associated with circulating concentrations of PCBs within the range shown by SBS bears (1.8 to 140 ng / g ww whole blood, present study; 21 to 228 ng / g ww plasma, Svalbard polar bears, Bernhoft et al. 2000). Concentrations of ΣPCB7 in young SBS polar bears were similar to the concentrations of PCBs associated with poor immune responses in perinatal free-ranging northern fur seals (*Callorhinus ursinus* L., 1758; mean ± SE, 22.84 ± 1.58 ng / g ww whole blood; Beckmen et al. 2003). Whole blood, plasma, and visceral tissues, however, vary in water, protein and lipid components, which can affect the partitioning and binding affinity of toxicants. Toxicant – matrix interactions are also modified by reproduction, dehydration, and immune status (Lyderson et al. 2002). These confounding variables should be examined concurrently with tissular concentrations of contaminants to provide a better understanding of these interactions in ursids.

Greater concentrations of lipophilic contaminants in adult females compared to males is a new trend occurring in polar bears. Similar trends among cohorts (young ≥ adult females ≥ adult males) were reported in SBS polar bears when assessing PCB concentrations in subcutaneous lipid biopsies (Bentzen et al. 2008*a*). The elevation of circulating ΣPCB7 concentrations in females was associated with their greater concentrations of blood lipids. Although associations between PCBs and lipids have been previously reported in polar bears and

other species (Bentzen et al., 2008*b*; Letcher et al. 2010), to our knowledge, this is the first study to describe that lipid composition (primarily the neutral lipids triglyceride and free fatty acids), as well as total lipid content, influences PCB concentrations. The present study examined lipid concentration and composition using TLC/FID, resulting in lower estimates than previous gravimetric measures, and a > 2 fold increase in lipid-adjusted PCB concentrations during 2007 compared 2003. The large difference in lipid-adjusted concentrations of PCB between years also highlights the importance of assay selection and accurate estimations of lipid content when comparing toxicant concentrations among individuals, populations, or years.

Female and young polar bears had greater concentrations of blood lipids and greater proportions of triglycerides and free fatty acids than males. The high proportions of sterol esters/wax esters in blood of polar bears likely reflect the large deposits of these lipids in the phytoplankton and zooplankton that are at the base of the arctic marine food web (Lee et al. 1971). Greater cholesterol concentrations in the blood of females and young compared to males is common among mammals, and is suspected to support their greater metabolic requirements for gestation, lactation, sexual maturation and growth, as well as the synthesis of steroid hormones that regulate these processes (Dietschy et al. 1993). Among the neutral lipids, females had the greatest concentrations of triglycerides, whereas free fatty acids were highest among young. Triglycerides and free fatty acids in blood could indicate early metabolic processing of dietary lipids before deposition to adipose, the mobilization of lipids from adipose to circulation, or the promotion of triglycerides in support of lactation. The source of lipids in blood of specific individuals is difficult to determine with the data available due to complex dynamics of lipid mobilization and energy conservation in ursids (Nelson et al. 1983; Grahl-Nielsen et al. 2003). Further information is required, such as the size of lipoproteins in circulation or consequent hormonal controls of glucagon and epinephrine that would signify the mobilization of lipid from

adipose. As fatty acid synthesis can also occur during periods of increased physical activity, the effects of polar bear breeding behaviors and recent energy expenditures on lipid distrbution should also be considered. Further monitoring of lipid dynamics in polar bears is warranted to better understand the seasonal fluctuations in lipid classes among cohorts, the influence of recent changes in physiologic states (e.g., feeding, fasting and lactation) on lipid mobilization, and the consequent impact to circulating concentrations of lipophilic contaminants. These interactions are of particular interest in species such as polar bears that have been described to have increased energetic costs during periods of reduced sea-ice availability (Olsen et al. 2003; Durner et al. 2009).

Our data suggested negligible differences in the overall feeding ecology of polar bears by cohort or years examined. Stable isotope values in packed cells in the present study were similar to those reported by Bentzen et al. (2007), indicating that the diet of SBS polar bears likely continues to include a high proportion of ringed seals. The proportion of prey items and tissues in the recent diet, however, likely differs among individuals. Feeding ecology of polar bears has previously been estimated by stable isotope analyses using the diet-tissue discrimination from captive feeding studies of black bears (*Ursus americanus* Pallus, 1780; Hildebrand et al. 1996; Bentzen et al. 2007; Cardona-Marek et al. 2009). Using these same estimates, the stable isotope values in packed cells of polar bears in the present study would reflect the diet 2 – 3 months previous to spring sampling (winter; late October to January), and stable isotope values in serum would reflect a more recent dietary influence of 2 – 4 weeks. Stable isotope values in packed cells and serum were correlated, suggesting relatively steady dietary sources for SBS polar bears during the previous winter and spring. Movements of SBS polar bears are reported to vary by season in response to changes in sea-ice conditions and accessibility to prey (Durner et al. 2009). Future monitoring of dietary habits of polar bears should include an evaluation of stable isotope

values across several years and regions, and include the assessment of tissues representing different dietary periods. Differences in the stable isotope values could indicate dietary segregations among cohorts, or changes in physiology that alter diet-tissue discrimination of stable isotope ratios between seasons. These differences in feeding, movement and polar bear physiology would also influence the circulating concentrations of toxicants and potential adverse effects.

Increases in toxicant concentrations were most related to decreases in $\delta^{13}C$ values. More negative $\delta^{13}C$ values have been related to an increased concentration of lipid associated with the depletion of ^{13}C during lipid synthesis (Post et al. 2007; Wolf et al. 2009). Our data support this finding as polar bears with greater concentrations of blood lipids also had more negative serum $\delta^{13}C$ values. The difference in blood lipid concentrations among polar bear cohorts also partly explain why serum $\delta^{13}C$ values were more negative in females and young compared to males. Packed cell $\delta^{13}C$ values, however, were more reflective of the longitude at which animals were sampled. The types of species included and length of food webs are known to alter the dynamics of Hg bioaccumulation and biomagnification (Outridge et al. 2008; Horton et al. 2009), and these factors likely contributed to the inverse correlation of blood Hg concentrations with packed cell $\delta^{13}C$ values and longitude. A decrease of $\delta^{13}C$ values from western to eastern longitudes of the Southern Beaufort Sea has been described for several marine species (phytoplankton, ringed seal, beluga, bowhead) and has been related to the influx of terrestrial carbon from the Mackenzie River (discussed in Bentzen et al. 2007 and Cardona-Marek et al. 2009). More negative packed cell $\delta^{13}C$ values may also represent animals feeding in more pelagic (offshore ocean associated) versus sympagic (sea-ice associated) based food webs (pelagic, -24.0 ‰; sympagic,-20.0 ‰; Soreide et al. 2006; Hobson and Welsh 1992). A more accurate interpretation of the ecological

significance of the correlations between toxicants and $\delta^{13}C$ values are complicated by the similarities of $\delta^{13}C$ values in sympagic and benthic communities, and the multiple factors that alter ^{13}C fractionation in the marine environment.

After accounting for the variations with $\delta^{13}C$ values, toxicant concentrations in the blood of polar bears increased with trophic position as measured by packed cell $\delta^{15}N$. The coefficients for packed cell $\delta^{15}N$ were similar for wet weight concentrations of Hg and lipid-adjusted concentrations of ΣPCB_7, indicating a similar influence of trophic transfer and biomagnification for both compounds. Ringed seals and other pelagic feeding species such as spotted seal and beluga whale, have higher toxicant concentrations in tissues reflecting their greater fish-based diet (Kucklick et al. 2002; Dehn et al. 2005). Benthic feeding species such as bearded seal and walrus consume relatively lower trophic prey (e.g., clams, crabs and shrimp) and consequently have lower tissular residues of these toxicants (Dehn et al. 2007). Many male polar bears during the spring of 2007 had lower toxicant concentrations in blood than adult females. Benthic prey species are often larger than pelagic prey and may only be accessible to large polar bears without dependent young. The proportions of non-methylene-interrupted fatty acids in fat (subcutaneous lipid) biopsies from polar bears also suggested a greater benthic diet in larger animals (Thiemann et al. 2007). Conversely, female polar bears have been reported to hunt younger age classes of seals (i.e., juvenile ringed seals, seal pups in subnivian birth lairs) compared to males (Furgal et al. 1996). The $\delta^{15}N$ values and concentrations of many toxicants in very young ice seals are often higher than those in older animals (Espeland et al. 1997). Similarities in the stable isotope composition of prey species and tissues limit a more detailed assessment of the proportion of prey species consumed by polar bear cohorts.

Toxicant concentrations, lipid concentrations and stable isotope values in young polar bears (ages 1 – 4 years of age) were similar to adult females. Polischuk et al. (1995) also found similar toxicant concentrations and stable isotope values in polar bear mother and cub pairs. Polar bear cubs consume milk until they are approximately 1.5 years of age and also consume prey tissues provided by the mother. The proportion of milk versus prey items would differ among young and be reflected in their concentrations of blood toxicants and composition of stable isotopes. Young animals have been theorized to have a lower diet-tissue discrimination of 15N in comparison to adults because of their lower excretory loss of nitrogen relative to the high nitrogen intake necessary for growth (Martinez del Rio and Wolf 2005). Robbins et al. (2005) also reported that $\delta^{15}N$ values in young animals consuming milk were lower than adult carnivores consuming muscle as a result of the greater protein quality of the milk diets, and greater protein requirements of young. Growth rates of young polar bears are high and survival of cubs depends on the accruement of adequate reserves of lipid and protein during the first 3 years of life (Derocher and Stirling 1996). The fractionation dynamics of stable isotope ratios in young animals due to rapid growth and nitrogen requirements, therefore, limit further assessment of dietary inputs and subsequent contaminant exposure pathways via stable isotope analysis.

Variation in the concentration of toxicants among polar bear cohorts was likely the result of a combination of biological and ecological factors. As described, the greater concentration of circulating ΣPCB_7 in females versus males likely relates to differences in the proportion of prey items recently consumed, greater total blood lipids, and the greater concentration of neutral lipids in females associated with physiological processes required to fuel metabolic requirements (e.g., feeding or mobilization of stored lipids) and / or support lactation. Elevated lipid and toxicant concentrations in blood were similarly reported after periods of fasting and lactation in Hudson Bay polar bears (Polischuk et al. 1995; Polischuck et al. 2002),

and reductions in body condition were related to an increase of toxicants in arctic fox (Fuglei et al. 2007). Differences in enzyme induction and / or inhibition have also been described to alter the distribution, detoxification and excretion of contaminants in polar bears (Letcher et al. 2010), but these processes were not examined in the present study. Polar bears are expected to have a similar fasting physiology as other ursids and make use of their fat reserves for energy to minimize their use of protein (Nelson et al. 1983; Hellgren 1998). Stirling et al. (2008*b*) described unusual predation attempts in SBS polar bears that were associated with changing spring sea-ice condition in the same years examined (2003, 2005, 2007, east of the Alaska: Canada border), and indicated that bears were likely nutritionally stressed during these years due to thick ice, open water, and lack of apparent ice seals. The lower body condition index scores of females compared to males suggests that the period of limited sea-ice that occurred during 2007 had a greater impact on females than males. Further study of the movement of polar bears cohorts and changes in body mass are needed to assess how the proposed yearly and seasonal changes in arctic sea-ice will affect contaminant exposure to polar bears.

1.6 CONCLUSIONS

Females and young are the most critical components of the population from a population dynamics perspective, and the present data indicates that many also have elevated circulating concentrations of Hg and PCBs as compared to adult males. Greater concentrations of contaminants in females than males is a new trend occurring among polar bear populations. Furthermore, lipophilic concentrations in Southern Beaufort Sea polar bears were similar to other sub-populations contrary to previous reports. The coefficients for packed cell $\delta^{15}N$ values were similar for wet weight concentrations of Hg and lipid-adjusted concentrations of ΣPCB_7 indicating a similar influence of trophic transfer and biomagnification of both compounds to

polar bears. Blood $\delta^{13}C$ values were most related to lipid content of blood and polar bear location at sampling. Our data found small but significant differences in overall feeding ecology between polar bear cohorts as determined by stable isotope analysis of blood. The proportions of prey species and tissue items recently consumed, therefore, may have contributed to the differences in toxicant concentrations among cohorts. Concentrations of ΣPCB_7 were also positively associated with the neutral lipids triglycerides and free fatty acids, which were greater among females and young compared to males. Concentrations of ΣPCB_7 were also inversely related to body condition indices. Further studies are needed to understand how changes in physiological demands such as increased energy expenditures, fasting, and lactation influence blood lipids in polar bears and thereby impact the distribution of lipophilic contaminants. Special attention should be focused on females and young that may be the first to exhibit adverse biological responses to contaminants and nutritional stress as a result of the changing arctic environment.

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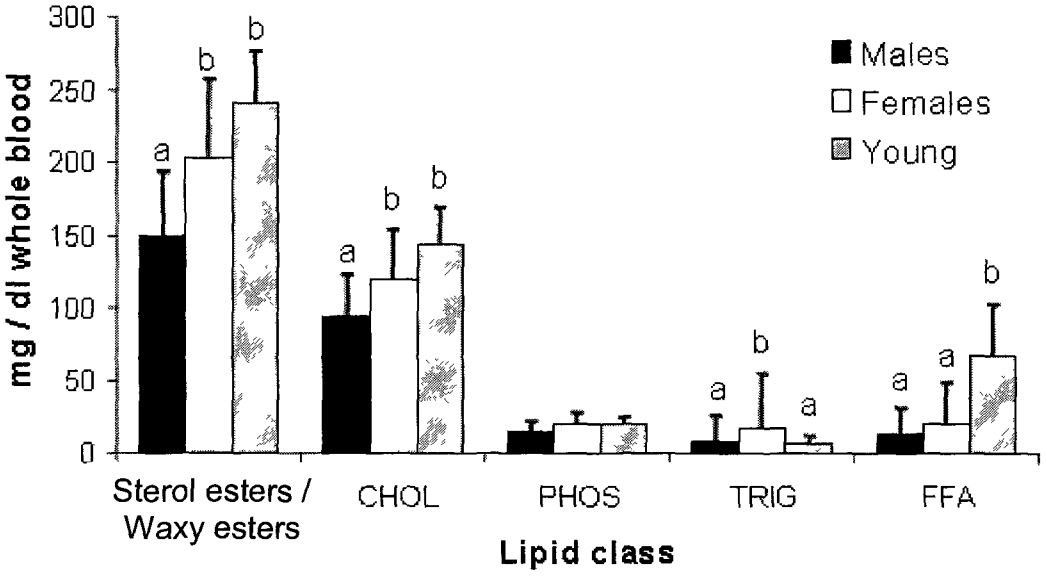

Figure 1.1 Lipid classes (sterol esters / waxy esters; CHOL, cholesterol; PHOS, phospholipids; TRIG, triglycerides; FFA, free fatty acids) detected in whole blood of polar bears sampled during spring 2007 by cohort. Different letters within each lipid class indicate significant differences among cohorts. Lipid concentrations compared by multiple analysis of variance (MANOVA) using log transformed data.

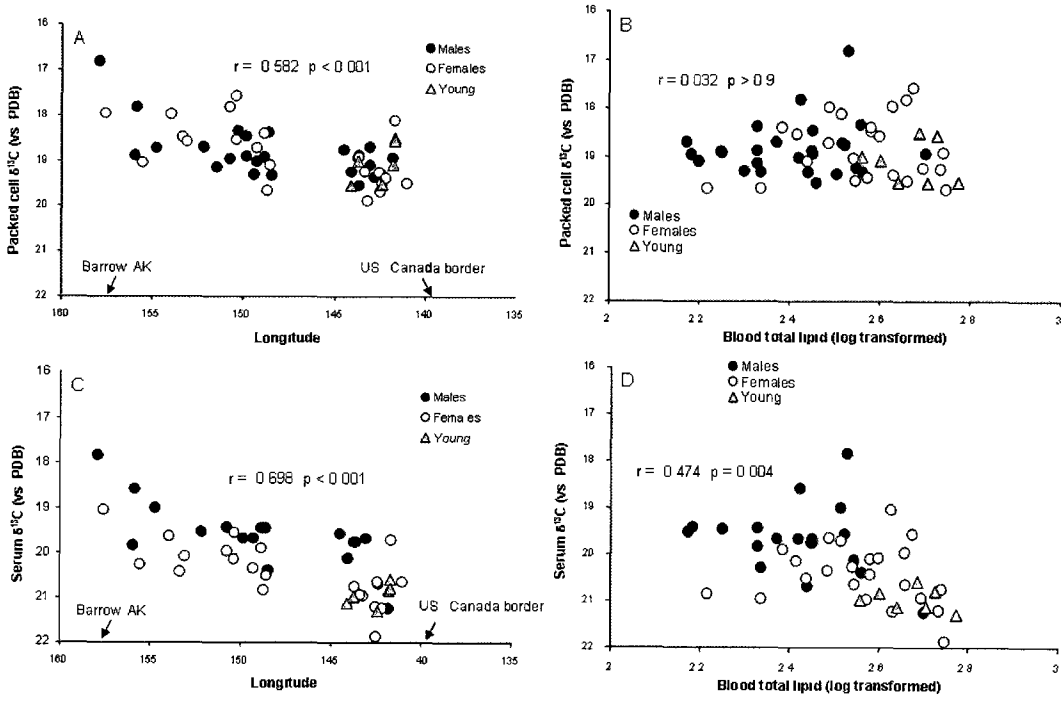

Figure 1.2 Correlation of the $\delta^{13}C$ values in packed cells (A and B) and serum (C and D) to longitude and total blood lipids by polar bear cohort. Arrows in A and C represent the longitude of Barrow, Alaska (- 157.8 W) and the United States (Alaska) : Canada border (-141.0 W).

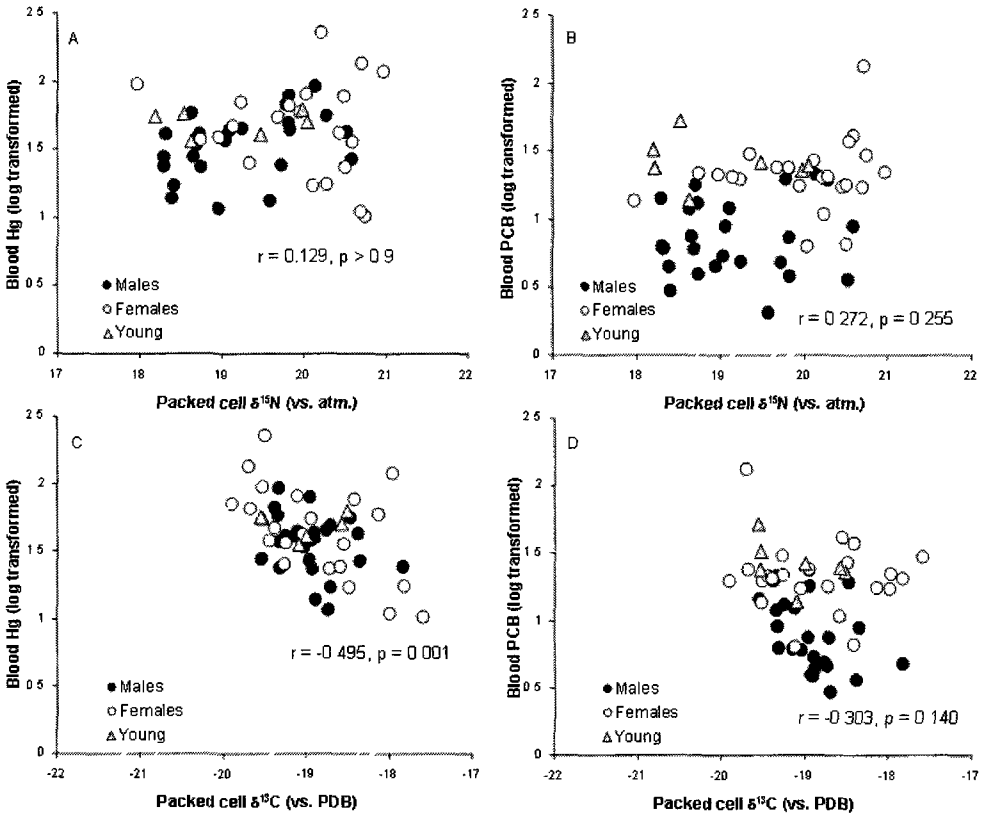

Figure 1.3 Circulating concentrations of Hg and PCBs in relation to stable isotope values in packed cells of polar bears by cohort.

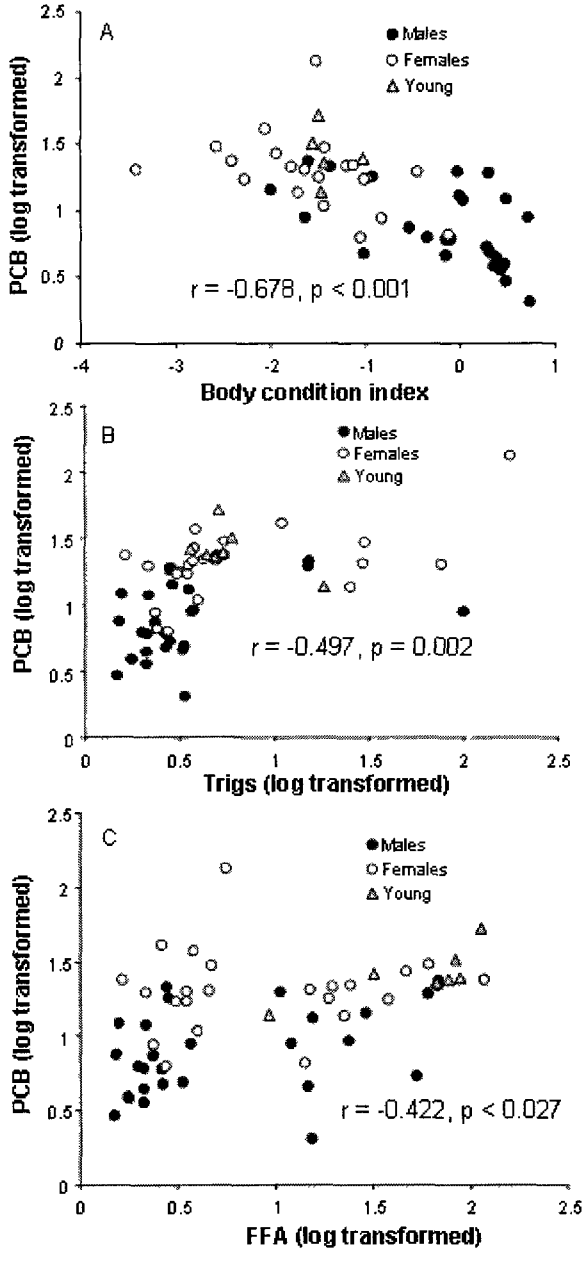

Figure 1.4 Concentrations of PCBs in adult males, adult females, and young (ages, 1 - 4 years) polar bears in relation to (A) body condition index; (B) blood concentrations of triglycerides (Trigs); and (C) blood concentrations of free fatty acids (FFA).

Table 1.1 (A - B) Variables measured in polar bears by cohort during spring 2007 compared to previous studies. Data are shown as mean ± SD, range, and the median. Procedures for 2003 and 2005 described in Bentzen et al. (2008) and Cardona-Marek et al. (2009), respectively. Different letters indicate significant differences of the mean values in each row by analysis of covariance (ANCOVA, year was included as a covariate, Tukey's adjustment for pairwise comparisons, toxicant concentrations were compared using log transformed data). Lipid adjusted PCB concentrations and percent blood lipids could not be compared between years because of differences in analytical technique. All body condition index scores were lower in 2007 versus 2003 ($p < 0.05$). Sample sizes, 2003: male, n = 13 - 15; female, n = 10 – 13; young, n = 6 – 9; 2005: male, n = 12 - 25; female, n = 15 – 24; young, n = 7 – 11; 2007: male, n = 18 - 26; female, n = 22 – 24; young, n = 5 – 10; na = data not available.

A. 2003			
Variable	**Males**	**Females**	**Young**
Hg, ng / g ww	na	na	na
$\sum PCB_7$, ng / g ww	14.90 ± 11.92[a] 3.04 to 51.22 12.03	23.64 ± 16.36[b] 11.07 to 72.89 20.25	31.63 ± 11.99[b] 9.34 to 49.48 35.20
$\sum PCB_7$, ng / g lw	1335.08 ± 1240.88 212.58 to 5263.68 1025.94	1749.34 ± 959.97 760.00 to 4087.95 1356.71	2045.63 ± 854. 48 676. 95 to 3338.83 1952.97
Lipid, %	1.19 ± 0.17 0.85 to 1.43 1.22	1.34 ± 0.29 1.00 to 1.81 1.34	1.59 ± 0.29 1.19 to 2.19 1.54
Serum $\delta^{15}N$, ‰	na	na	na
Serum $\delta^{13}C$, ‰	na	na	na
Packed cell $\delta^{15}N$, ‰	19.34 ± 0.80[a] 18.31 to 20.65 19.65	19.90 ± 0.33[b] 19.30 to 20.42 19.89	19.77 ± 0.52[ab] 18.57 to 20.32 19.97
Packed cell $\delta^{13}C$, ‰	-18.77 ± 0.41 -19.49 to -18.00 -18.84	-18.90 ± 0.33 -19.52 to -18.5 -18.86	-19.06 ± 0.25 -19.50 to -18.71 -19.03
Body condition index	-0.05 ± 0.57[a] -1.12 to 0.89 -0.48	-0.57 ± 0.40[b] -1.32 to -0.02 -0.44	-0.30 ± 0.47[b] -0.98 to 0.41 -0.34

Table 1.1 continued.

B. 2005			
Variable	**Males**	**Females**	**Young**
Hg, ng / g ww	66 74 ± 28 23 25 20 to 130 00 64 90	63 15 ± 55 90 7 00 to 213 00 50 30	74 62 ± 189 50 21 05 to 189 50 49 33
$\sum PCB_7$, ng / g ww	na	na	na
$\sum PCB_7$, ng / g lw	na	na	na
Lipid, %	na	na	an
Serum $\delta^{15}N$, ‰	20 93 ± 0 66 19 49 to 21 81 21 11	20 89 ± 0 74 19 74 to 22 52 20 97	21 06 ± 0 46 20 30 to 21 67 21 14
Serum $\delta^{13}C$, ‰	-19 57 ± 0 46[a] -20 28 to -18 33 -19 57	-20 34 ± 0 37[b] -21 04 to -19 43 -20 39	-20 72 ± 0 49[c] -21 54 to -19 88 -20 54
Packed cell $\delta^{15}N$, ‰	19 10 ± 0 93[a] 16 99 to 20 08 19 44	19 30 ± 0 88[b] 16 90 to 20 59 19 60	19 27 ± 0 49[ab] 18 73 to 20 13 19 11
Packed cell $\delta^{13}C$, ‰	-18 61 ± 0 53 -19 27 to -17 58 -18 73	-18 82 ± 0 52 -19 43 to -17 57 -19 04	-19 23 ± 0 36 -19 81 to -18 71 -19 19
Body condition index	-0 43 ± 0 89[a] -1 69 to 1 44 -0 18	-1 44 ± 0 71[b] -2 95 to -0 26 -1 33	-1 41 ± 0 95[b] -3 10 to 0 04 -1 18

C. 2007			
Variable	**Males**	**Females**	**Young**
Hg, ng / g ww	39 05 ± 19 39 22 70 to 92 31 38 48	56 87 ± 49 25 10 25 to 228 05 40 08	50 82 ± 9 39 35 99 to 62 58 52 87
$\sum PCB_7$, ng / g ww	8 16 ± 5 72[a] 1 80 to 21 00 6 00	25 92 ± 27 60[b] 6 20 to 140 00 25 92	27 80 ± 15 40[b] 14 0 to 54 00 25 00
$\sum PCB_7$, ng / g lw	2917 71 ± 1881 95[a] 568 19 to 6774 194 2199 71	6026 59 ± 4798 57[b] 1945 68 to 23215 09 4719 98	6309 43 ± 3405 33[b] 3255 81 to 11067 79 5457 06
Lipid, %	0 29 ± 0 07[a] 0 16 to 0 43 0 29	0 42 ± 0 11[b] 0 23 to 0 60 0 45	0 44 ± 0 07[b] 0 35 to 0 54 0 43
Serum $\delta^{15}N$, ‰	20 21 ± 0 62 18 73 to 21 33 -19 68	21 07 ± 0 80 19 50 to 22 11 21 07	20 45 ± 0 46 19 67 to 20 97 20 54
Serum $\delta^{13}C$, ‰	-19 60 ± 0 66[a] -20 70 to -17 84 -19 68	-20 43 ± 0 64[b] -21 88 to -19 05 -20 43	-21 02 ± 0 24[c] -21 60 to -10 59 -21 06
Packed cell $\delta^{15}N$, ‰	19 21 ± 0 72[a] 18 31 to 20 59 19 06	19 98 ± 0 76[b] 17 98 to 20 98 20 22	19 02 ± 0 80[ab] 18 21 to 20 05 18 64
Packed cell $\delta^{13}C$, ‰	-18 85 ± 0 56 -19 55 to -16 82 -18 85	-18 84 ± 0 67 -19 89 to -17 59 -18 94	-19 12 ± 0 05 -19 56 to -18 52 -19 10
Body condition index	-0 23 ± 0 76[a] -2 00 to 0 73 -0 07	-1 47 ± 0 63[b] -2 57 to 2 27 -1 46	-1 27 ± 0 42[b] -1 78 to -0 56 -1 47

Table 1.2 Factors describing variations in concentrations of Hg (A) and $\sum PCB_7$ (B) in whole blood (ng / g ww) of polar bears sampled during 2007 (n = 50). Variables were selected using a multiple linear regression with backward stepwise selection. The original full model included: Sex (categorical), Age, log Waxy esters, log CHOL, log PHOS, log TRIG, log FFA, Longitude, Body condition index (BCI), Serum $\delta^{13}C$, Serum $\delta^{15}N$, Packed cell $\delta^{13}C$, and Packed cell $\delta^{15}N$.

A. Toxicant	Variable	Coefficient	p	Overall model: f, p, r^2
Hg	Longitude	0.028	<0.001	28.784, < 0.001, 0.652
	Packed cell $\delta^{13}C$	-0.288	<0.001	
	Packed cell $\delta^{15}N$	0.152	<0.001	

B. Toxicant	Variable	Coefficient	p	Overall model: f, p, r^2
$\sum PCB_7$	TRIGs	0.171	<0.001	28.877, < 0.001, 0.746
	FFA	0.161	0.014	
	BCI	-0.140	0.002	
	Packed cell $\delta^{13}C$	-0.280	< 0.001	
	Packed cell $\delta^{15}N$	0.162	<0.001	

CHAPTER 2

Blood-based biomarkers of selenium and thyroid status indicate possible adverse biological effects of mercury and polychlorinated biphenyls in Southern Beaufort Sea polar bears[2]

2.1 ABSTRACT

We examined biomarkers of selenium status (whole blood Se, serum Se, and glutathione peroxidase activity) and thyroid status (concentrations and ratios of thyroxine, T4, and tri-iodothyronine, T3, and albumin) in polar bears to assess variations among cohorts, and relationships to circulating concentrations of contaminants. Concentrations of total mercury (Hg) in whole blood were similar among cohorts (prime aged males and females, older animals ages ≥ 16 years, and young animals ages 1 – 5 years; 48.44 ± 35. 81; $p = 0.253$). Concentrations of sum of seven polychlorinated biphenyls ($\sum PCB_7$) in whole blood were greater in females (with and without cubs, 26.44 ± 25.82 ng / g ww) and young (26.81 ± 10.67 ng / g ww) compared to males (8.88 ± 5.76 ng / g ww, $p < 0.001$), and significantly related to reduced body condition scores ($p < 0.001$). Concentrations of Se and albumin were significantly greater in males than females (whole blood Se, males, 42.34 pmol / g ww; females, 36.25 ± 6.27 pmol / g ww, $p = 0.019$; albumin, males, 4.34 ± 0.34 g / dl, females, 4.10 ± 0.29 g / dL, $p = 0.018$). Glutathione peroxidase activity ranged from 109.1 – 207.8 mU / mg hemoglobin, but did not differ significantly by sex or age ($p > 0.08$). Thyroid hormones were greater in females (solitary females and females with cubs) compared to males ($p < 0.001$). Biomarkers of Se status and concentrations of T3 were significantly positively related to Hg in all prime aged polar bears ($p < 0.03$). Albumin concentrations were significantly positively related to total TT4, and significantly

[2] Knott KK, Schenk P, Beyerlein S, Boyd D, Ylitalo GM, O'Hara TM. 2011. Blood-based biomarkers of selenium and thyroid status indicate possible adverse biological effects of mercury and polychlorinated biphenyls in Southern Beaufort Sea polar bears. Environmental Research doi:10.1016/j.envres.2011.08009

negatively related to concentrations of $\sum PCB_7$ ($p < 0.003$). Total thyroxine (TT4) was significantly negatively associated with blood concentrations of $\sum PCB_7$ in solitary females ($p = 0.045$). These data suggest that female polar bears were more susceptible to changes in blood-based biomarkers of selenium and thyroid status than males. Further classifications of the physiologic states of polar bears and repeated measures of individuals over time are needed to accurately assess the biological impact of combined toxicant exposures.

2.2 INTRODUCTION

Polar bears (*Ursus maritimus*) are apex predators of the arctic marine ecosystem and exposed to a combination of inorganic and organic toxicants that bioaccumulate and biomagnify (Atwell et al. 1998; Bentzen et al. 2008; Cardona-Marek et al. 2009; Kucklick et al. 2002). Climate changes may negatively impact the health of polar bears and other arctic marine mammals by altering the transmission of disease agents and exposure to contaminants (Burek et al. 2008; Jenssen 2006; Letcher et al. 2010). Changes in sea-ice are also predicted to reduce feeding opportunities for polar bears and lead to declines in body condition and mass (Derocher et al. 2004). The result of these multiple stressors on polar bear health will likely impact their survival. Correlative analyses have suggested that elevated concentrations of contaminants negatively impact health of polar bears by altering concentrations of hormones, vitamins, and immune status (Bernhoft et al. 2000; Braathen et al. 2004; Skaare et al. 2001). These studies focused on the changes in health biomarkers associated with concentrations of lipophilic contaminants including polychlorinated biphenyls (PCBs), but the possible effects of heavy metals such as mercury (Hg) were not directly examined. The life history characteristics of ursids are known to influence both the concentrations of toxicants and many of the biomarkers assessed (Hellgren 1998; Polischuk et al. 2002; Tomasi et al. 1998). The complicated variations in

physiology and biochemistry among cohorts, therefore, require critical assessment before, or in parallel, with the assessment of the role of contaminants on polar bear health.

Biomarkers in toxicology are used to assess the potential responses of biological systems to contaminant exposure. Biomarkers have been categorized as biomarkers of exposure, biomarkers of effect, or biomarkers of susceptibility in comparisons between exposed (or gradients of exposures) and unexposed individuals, cohorts, or populations (Newman 2010). The lack of unexposed or control (reference) animals in studies of free-ranging species makes these comparisons difficult, and researchers have relied on the use of correlative studies, a weight-of-evidence based approach, clinical diagnostics, or comparisons to threshold effect levels of health risks established for unrelated species (Letcher et al. 2010; Sonne 2010). The use of biomarkers as indicators of adverse biological impact, however, is complicated by the physiological mechanisms organisms use to maintain homeostasis and optimize metabolic and reproductive activities. For example, polar bears are adapted to the arctic climate by physiological and behavioral modifications such as delayed implantation, seasonal breeding, fasting during maternal denning, and extended periods of lactation (Amstrup 2003) that are known to be regulated by changes in the concentration of hormones, associated binding proteins, and co-factors (Hellgren 1998; Norris 2006; Tomasi et al. 1998). The natural variations of biomarkers due to non-toxicological mechanisms are largely unknown for most free-ranging species. Thus, dealing with confounding variables can be difficult and elusive. Furthermore, cross-sectional studies of free-ranging species cannot control for factors such as the time and dose of contaminants that ultimately led to the changes in the biomarkers assessed and may direct researchers to erroneous conclusions of adverse biological impacts.

Selenium (Se) supports many biological functions and also acts in the protection against toxicosis. Selenium is essential for the formation of many selenocysteine-containing proteins that

regulate gonadal maturation, immune function, and the formation of thyroid hormones (Kohrle et al. 2005; Van Lente and Daher 1992). Seleno-proteins are also involved in biological activities in the brain, thyroid, and liver that limit oxidative damage of free radicals induced by aging, pathogens and contaminants (Berry and Ralston 2008; Khan and Wang 2010; Mayne 2003; Ralston and Raymond 2006; Scandalios 2005). Seleno-proteins reduce oxidative stress through non-enzymatic antioxidant activities that react with oxyradicals directly, and by enzymatic antioxidant activities that catalyze reactions and reduce the number of oxyradicals present. Non-enzymatic antioxidant activities, such as the sequestration of Hg by seleno-compounds, are thought to be the primary mechanism by which marine mammals tolerate high concentrations of dietary Hg (AMAP 2004a). An equimolar ratio (1: 1) between Se and Hg has been suggested in marine organisms to maintain detoxification via sequestration of Hg (Dietz et al. 2000; Woshner et al. 2002; Yoneda and Suzuki 1997a). Selenium binds to mercury in kidney and liver as mercuric selenide (HgSe), an inert end product of the detoxification of methylmercury by demethylation (Khan and Wang 2010; Wagemann et al. 2000; Woshner et al. 2002), whereas many mercury compounds in circulation are bound to selenoprotein P (Fairweather-Tait et al. 2010). Glutathione peroxidase (GPx) is a Se dependent protein that acts as an enzymatic antioxidant by reduction of potentially damaging hydrogen peroxides (Newman 2010). Glutathione peroxidase activity has been reported to be altered by the bioaccumulation of Hg, although the exact mechanism is under debate (Brigelius-Flohe 1999; Carmagnol et al. 1983; Chen et al. 2006).

Thyroid hormones have been used as biomarkers of contaminant exposure as well as the assessment of overall health of marine and terrestrial animals (Rolland 2000; Rosa et al. 2007; Woshner et al. 2008). The biological actions of thyroid hormones include the regulation of metabolism, growth, cellular differentiation, and reproduction, as well as permissive actions that

enable cells to exert an optimal response to other endogenous and exogenous stimuli (Cunningham and Klein 2007; Norris 2006). Many factors alter thyroid hormone production including age, sex, reproduction, temperature, diurnal and seasonal cyclicity, and nutrition. Concentrations of PCBs, polybrominated diphenyl ethers (PBDEs), and their metabolites have been associated with alterations in the thyroid hormone levels in polar bears and other species, but these interactions have not been consistent across studies (Braathen et al. 2004; Letcher et al. 2010; Skaare et al. 2001; Sonne 2010). Heavy metals such as Hg have also been implicated in the disruption of the hypothalamus-pituitary- thyroid (HPT) system (Rolland 2000), but have not been studied in polar bears. Thyroid status is determined by measuring the concentrations of bound and free fractions of thyroxine (T4) and tri-iodothyronine (T3) that are maintained at optimal levels through negative (and positive) feedback mechanisms to the pituitary (**Figure 2.1**). Disruption of thyroid function can occur through deficiencies in iodine or selenium, changes to the binding proteins in circulation, decreases in the transformation of T4 to T3, or disruption of feedback systems (Bruggemann and Darras 2009; McNabb 1995). Baseline data are needed on the selenium and thyroid hormone concentrations of Southern Beaufort Sea (SBS) polar bears to identify variations among cohorts, as well as the possible multiple and interactive effects of toxicants.

In the present study, we question whether blood-based biomarkers of selenium and thyroid status were adequate measures of adverse biological effects of toxicants in polar bears. The biomarkers examined included whole blood and serum concentrations of selenium, glutathione peroxidase activity, thyroid hormone concentrations and ratios, and albumin concentrations. Our first objective was to report the levels of toxicants (Hg and PCBs) and biomarkers in SBS polar bears, describe variations among sex and age cohorts, and compare these to the known values reported in other polar bear sub-populations. The second objective was

to examine whether selenium and thyroid status of polar bears was associated with circulating concentrations of Hg and PCB as predicted under the proposed mechanisms of toxicity for these chemicals individually and in combination, with consideration of the physiologic drivers of these biomarkers. These data are presented in the context of key cohorts (e.g., prime reproductive aged males, solitary females in estrous, and females caring for young) and the natural life history of the polar bear.

2. 3 MATERIALS AND METHODS

2.3.1 Sample collection

Animal handling procedures were approved by animal care and use committees at the U.S. Geological Survey Research Program and the University of Alaska Fairbanks (UAF Institutional Animal Care and Use Committee Protocol 04-58). A total of 58 free-ranging SBS polar bears (31 males, 27 females) were captured by immobilization with Telazol (Warner–Lambert)-filled projectile darts fired from a helicopter during spring (March – May) 2007 in collaboration with the U.S. Geological Survey Ursid Research program as described previously (Bentzen et al. 2008; Cardona-Marek et al. 2009; Kirk et al. 2010). Ten females were accompanied by cubs (cubs of the year to 2 year olds). A vestigial premolar was removed from subadult and adult bears to estimate age by counting the cementum annuli (Matson Laboratory, Milltown, MT). Age of cubs of the year (COYs) through 3 year old animals was estimated by body size and inclusion in family group. Standard length (length) was recorded as the straight line length above the bear measuring the distance from the tip of the nose to the tip of the bony part of the tail while in sternal recumbency. Body mass (using a tripod, hoist, scale and net) was recorded for each bear. Body condition index (BCI) was determined from the natural log (ln) of mass and standard body length for each bear [BCI = (lnMass – 3.07*lnLength + 10.76) / (0.17 +

0.009 * lnLength); Cattet et al. 2002]. Blood samples were collected from either the femoral vein or artery into non-additive and K_3 EDTA Vacutainers™ (BD Vacutainers, Preanalytical Solutions). Blood samples were prevented from freezing and non-additive tubes centrifuged within 6 hours of collection to separate serum from the packed cells (clotting proteins and blood cells). After gentle remixing, subsamples of blood collected into K_3 EDTA tubes were transferred to cryogenic vials (Nalge Nunc International, Rochester, NY) or Teflon (fluoropolymer) vials (Savillex, Minnetonka, MN) for Hg and PCB analysis, respectively. Hematocrit (% packed cell volume) was estimated after transfer of whole blood into heparinized microhematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA) and centrifugation for 3 minutes at 10,400 RPM (TRIAC Centrifuge 0200/0206, Becton, Dickinson, and Company). One cryogenic vial (~2 mL) remained unfrozen and transferred chilled (~4°C) to the Wildlife Toxicology Laboratory (WTL, University of Alaska Fairbanks) for analysis of glutathione peroxidase (GPx) activity. Samples for GPx activity were shipped by commercial airline two times per week (frequency dependent on capture and flight schedules).

2.3.2 Mercury (Hg) analysis

Whole blood was analyzed for the concentration of total Hg at the WTL. Concentrations of Hg were determined on a DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT) using an eleven point calibration curve ranging from 1 to 400 ng ($r^2 = 0.998$; method detection limit approximately 50 pg) and following modified Environmental Protection Agency methods. Standards were analyzed before unknown samples and after each set of 20 individual samples. Standard reference materials included freeze-dried human hair (IAEA-085, 0.573 mg / kg; IAEA-086, 23.2 mg / kg, International Atomic Energy Agency, Vienna, Austria), freeze-dried fish muscle homogenate (Lake Superior Standard Reference Material 1946, National Institute of Standards and Technology), and standard spiking solution (0.10 mg / kg, Perkin-Elmer,

Waltham, Massachusetts). Percent recoveries of standard reference materials and spikes were within 88 - 110 %. The coefficient of variation for duplicate samples was less than 14%. Concentrations of Hg in whole blood are reported on a wet weight basis (Hg ng / g ww). Mercury concentrations in whole blood were also converted to molar concentrations (nmol / g; molecular weight of Hg = 200.59 g / mol) for comparison to molar Se concentrations.

2.3.3 Polychlorinated biphenyl (PCB) analysis

Procedures for PCB determination in whole blood followed modified procedures of Sloan et al. (2005). Briefly, 0.91 ± 0.12 g of whole blood was mixed with sodium sulfate and magnesium sulfate (1.5: 1, v / v) as drying agents, and extracted with dichloromethane (DCM) on an Accelerated Solvent Extractor (ASE 200, Dionex Corp., Sunnyvale, CA) at the WTL. Extracts were delivered to the NOAA / NMFS Montlake Laboratory in Seattle, WA. Each sample extract was filtered on gravity flow columns containing silica gel and alumina to remove interfering polar compounds and then further clean-up was conducted using size exclusion high performance liquid chromatography (SEC-HPLC). Individual PCB congeners were detected at NOAA / NMFS using a low-resolution quadrupole gas chromatograph/mass spectrometer (GC/MS) system equipped with a 60-meter DB-5 capillary GC column (0.25 mm i.d. and 0.25 μm film thickness) and a 10-meter guard column (0.53 mm i.d.). Each sample batch (n = 14) included a series of standards and were based on a five-point calibration curve. Percent recoveries were 98.7 ± 1.7%, 102.1 ± 1.9%, and 100.0 ± 15.9% for internal spike (PCB103) in blood / SRM / method blank, certified congeners from SRM, and congeners of duplicates, respectively. Method detection limits ranged from 0.07 - 0.79 ng / g (lowest of calibration range) and all method blanks were below detection. Samples with PCB concentrations below method detection limits were set at ½ the minimum detection limit (0.035 ng / g). Only 7 of the 40 PCB congeners examined, were detected in blood of polar bears (99, 105, 138, 153, 170, 180, and 194; $\sum PCB_7$). These

congeners were selected based on those found in ≥ 41% of animals sampled (threshold based on an apparent break in the proportion of animals having concentrations above detection as remaining congeners were present in < 7% of animals sampled). Lipid in whole blood was determined through detection and summation of five lipid classes (e.g., wax esters, triglycerides, free fatty acids, free cholesterol and phospholipids) using thin layer chromatography coupled with flame ionization detection (TLC/FID) on an Iatroscan Mark 6 (Iatron Laboratories, Tokyo, Japan). The concentration of $\sum PCB_7$ was reported as ng / g wet weight (ng / g ww) and ng / g lipid weight (ng / g lw) of whole blood.

2.3.4 Selenium (Se) status

Estimates of Se status were determined through the analysis of Se concentrations in whole blood and serum, and glutathione peroxidase (GPx) activity. Concentrations of Se in both serum and whole blood were included because these values have not been previously reported for free-ranging polar bears, and to assess whether concentrations of Se in serum represented more acute changes in Se status versus whole blood. All assays were performed at the WTL. Samples for Se analysis were heated using a two step digestion in a Perkin Elmer Multiwave 3000 microwave oven. Whole blood and serum (0.350 ± 0.076 g) were placed in a 3: 1 nitric acid: hydrogen peroxide (v / v) mixture and heated to 170 °C for 15 minutes. The initial digest was diluted to 20 mL with ultrapure water (NANOpure Model D4751, Barnstead International, Dubuque, IA). A sub-sample of the diluted digest (2 mL) underwent a secondary digest (heated to 95°C for 60 minutes) with excess hydrochloric acid (HCl) (1:1 v / v) to reduce Se (VI) to Se (IV). Quality control samples (blanks, spikes, duplicates, matrix spikes, and standard reference materials) were included in each digestion batch. Standard reference materials included Lake Superior Fish Tissue 1946 (0.491 ± 0.043 mg Se / kg, National Institute of Standards and Technology (NIST), Gaithersburg, MD) and DOLT-4 (8.3 ± 1.3 mg Se / kg; Dogfish Liver

Certified Reference Material for Trace Metals, National Research Council Canada, Institute for National Measurement Standards, Ottawa, Ontario, Canada). Selenium concentrations were determined using a 7 point calibration curve (0.04 to 4.48 ng / g) through mercury / hydride system - flame ionization atomic spectrometry (MHS-FIAS) on a Perkin Elmer AAnalyst 800 atomic absorption spectrometer (AAS). Sodium borohydride (0.2% NaBH4 in 0.05% NaOH) was used as the reductant in a 10% HCl carrier solution. The mean percent recovery of quality control samples were 113%, 114%, 100%, 126%,, and 119% for spikes, sample spikes, duplicates, Lake Superior Fish SRM, and DOLT4 SRM, respectively. Selenium in serum and whole blood are reported in ng / g ww and as molar concentrations (molecular weight of Se = 78.96 g / mol).

Whole blood GPx activity was measured by a modified procedure described by Carmagnol et al. (1983) using a refrigerated Eppendorf 5810R spectrophotometer (DU Series 520; Beckman Instruments, Inc., Fullerton, CA). Whole blood samples delivered to the WTL were centrifuged at 1500 g to separate erythrocytes from plasma. Erythrocytes (packed cells) were washed twice in cold 0.9% NaCl and lysed in 4 volumes of ultrapure water. The hemolysate was isolated through centrifugation at 10,000 g. Hemoglobin (Hgb) concentrations of the packed cells were determined using Drabkin's Reagent (Sigma-Aldrich Corp., St. Louis, MO). This procedure is based on the oxidation of hemoglobin to methemoglobin (MetHgb) by potassium ferricyanide, which reacts with potassium cyanide to form cyanMetHgb, and has a maximum absorbance at 540 nm. Hemoglobin concentration of the hemolysate (mg Hgb / mL hemolysate) was determined through comparison to a calibration curve [5 – 20 mg Hgb / mL; 100 mg Protein (bovine erythrocytes) Hemoglobin A_o Ferrous; Sigma-Aldrich Corp., St. Louis, MO] and multiplied by the dilution factor. Total hemoglobin content of whole blood was estimated by multiplying the concentration of hemoglobin in the hemolysate by the percent hematocrit (mL

packed cells / 100 mL whole blood) estimated in the field. Total hemoglobin content of whole blood was reported as g Hgb / dL whole blood.

GPx activity was determined using the Glutathione Peroxidase Cellular Activity Assay Kit (Sigma-Aldrich Corp., St. Louis, MO). This kit included a phosphate buffer, NADPH (B NADPH; B-Nicotinamide Adenine Dinucleotide Phosphate, Reduced) assay reagent, and 30 mM tert-butyl hydrogen peroxide solution. A GPx standard was used to ensure instrument and procedural accuracy (100 units diluted to 0.25 units / mL in phosphate buffer; Sigma-Aldrich Corp.). The kit measures GPx activity through an indirect method based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The method employed detection of decreased NADPH absorbance at 340 nm (initial delay of 15 seconds, followed by 6 readings at 10 second intervals), which is indicative of GPx activity, since GPx is the rate-limiting step for the reaction (per product info sheet, Sigma-Aldrich Corp.). Analysis was performed on duplicate samples. GPx activity in this method, therefore, only includes selenium dependent enzymes located within erythrocytes and was calculated as:

GPx umol / min (Units) / mL hemolysate = $(A_{sample} - A_{blank})$ * DF / (6.22 * 0.05 mL)

where, DF = assay dilution factor = 10; 6.22 = e^{mM} for NADPH (extinction rate); 0.05 mL = amount of erythrocyte lysate. GPx Units / mL were divided by the hemoglobin content of the hemolysate (mg Hgb / mL hemolysate) to determine final GPx activity:

GPx activity (mUnits / mg Hgb) = ((GPx Units / mL hemolysate) / (mg Hgb / mL hemolysate)) * 1000.

2.3.5 Thyroid Status

Thyroid hormone concentrations (total thyroxine, TT4; free thyroxine, FT4; total triiodothyronine, TT3; free triiodothyroinine, FT3) in serum were determined at Michigan State

University Pathobiology and Diagnostic Investigation Center following published procedures (Rosa et al. 2007). Validations of thyroid assays for use in ursids were performed through a serial dilution of 2 pooled samples. Serial dilutions of polar bear serum added to the zero standard or protein buffer of the radioimmunoassay were 92%, 97%, 92%, and 99% the projected concentrations of TT4, TT3, FT4, and FT3, respectively. Inter-assay and intra-assay correlations of variations (CVs) ranged from 8 - 24 % with good parallelism (correlation mean > 0.9) between dilution of pooled samples and standard dilutions in protein buffer. Concentrations of hormones (total fractions, nmol / l; free fractions, pmol / l) and molar ratios (TT4: FT4, TT3: FT3, TT4: TT3, FT4: FT3) were reported (molecular weight of T3 = 650.97 g / mol; molecular weight of T4 = 776.87 g / mol).

Albumin concentration was determined using a serum chemistry comprehensive panel on a DRI-CHEM® Veterinary Chemistry Analyzer (Heska Corp.) at the UAF. A human serum reference sample (Heska ® Chemistry Control, Heska, Corp.) with known amounts of albumin was measured to ensure procedure and instrument accuracy.

2.3.6 Statistics

Cohorts were based on sex, age, and natural life history characteristics (dependent young, rapid growth rate, reproductive status, and lactational demand) as used previously for polar bears (Amstrup 2003; Kirk et al. 2010; Rode et al. 2010; Tryland et al. 2002). Cohorts included: young, ages 1 – 5 years; prime reproductive age males, 6 to 15 years; prime reproductive age females, 6 to 15 years; and older animals, ≥ 16 years of age without cubs. Females were further grouped as solitary or with dependent cubs. Two 14-year-old females had cubs of the year, and one 24-year-old female had 2 yearling cubs. Thus, the older animal cohort was considered to be ≥ 16 years of age and without cubs (rather than ≥ 13 years of age used previously in Tryland et al. 2002). The young cohort included both dependent young (ages 1 – 2, n = 5) and sub- adults (ages 3 – 5, n =

4) due to the small number of young animals sampled, and because both were considered to be in a rapid growth phase. Because the young cohort was not sexually mature, the gender of this cohort was not considered a significant variable in analyses.

Statistics were performed using Systat 11 (Systat Software Inc.). As all PCB congeners are considered to be readily bioavailable in blood regardless of the association with lipids, wet weight concentrations of PCBs were used to assess relationships. Variables were log transformed as needed to meet normality criteria and to improve distributional properties. Log transformations did not improve the distribution of whole blood Se : serum Se ratio and albumin concentrations, and were thus examined with no transformation. Analysis of covariance (ANCOVA) was used to assess differences in biomarkers between sexes with age as a covariate. Analysis of variance (ANOVA) was used to assess differences between biomarkers and toxicants by cohort. Pearson's correlations were used to assess correlations between toxicants, biomarkers, and body condition index. Generalized linear models (GLM) were used to assess whether biomarkers of Se status and thyroid status were related to the concentrations of toxicants (Hg, PCBs) while controlling for the effect of sex (i.e., sex was used as a covariate). Body condition index (BCI) was removed from these models due to the lack of significance ($p > 0.3$). Significant differences were determined to be at $p < 0.05$.

Biomarkers of thyroid status were expected to relate to each other because of the feedback mechanisms inherent to the HPT axis. A correlation-based principal components analysis (PCA) with varimax rotation, therefore, was used to investigate simultaneous relationships between toxicants (Hg, $\sum PCB_7$) and measures of thyroid status (total T4, free T4, total T3, free T3, and albumin) by prime aged cohorts (males, solitary females, and females with cubs). Absolute values of component loadings of ≥ 0.6, $0.3 - 0.5$, and ≤ 0.3 were used to describe whether relationships among variables were strongly, moderately, or weakly correlated,

respectively. Signs (+ or -) between variables indicated whether these relationships were positively or negatively correlated. Young and older cohorts were excluded from these analyses due to low sample size. Variables that were strongly correlated by PCA, were further explored using GLM and Pearson's pairwise comparisons.

2.4 RESULTS

2.4.1 Concentrations of circulating toxicants

Concentrations of Hg and $\sum PCB_7$ in whole blood (ng / g ww) were positively correlated in males ($p < 0.001$), but unrelated in females (solitary females and females with cubs combined, $p = 0.834$). Concentrations of Hg in whole blood ranged from 10.25 to 228.05 ng / g ww and were similar between cohorts (SEM ± SD; solitary females, 69.8 ± 65.2 ng / gww; females with cubs, 41.6 ± 29.0 ng /g ww; males, 38.2 ± 18.4 ng / g ww; young, 52.4 ± 14.7 ng /g ww; older animals, 63.2 ± 44.9 ng / g ww; ANOVA, $f = 1.383$, $p = 0.253$; **Figure 2.2A**). Concentrations of blood $\sum PCB_7$ ranged from 2.03 to 132.8 ng / g ww (568 to 23,200 ng / g lw). The concentrations of individual PCB congeners were correlated ($r = 0.47$, $p < 0.005$), and 53% of the $\sum PCB_7$ consisted of PCB 153. Wet weight and lipid weight concentrations of $\sum PCB_7$ were significantly greater in females and young compared to males (solitary females ≥ females with cubs ≥ young > males; SEM ± SD; females with and without cubs, 26.4 ± 25 82 ng / g ww; young, 26.8 ± 10.7 ng / g ww; males, 8.9 ± 5.8 ng / g ww; $\sum PCB_7$ ww, ANOVA, $f = 12.255$, $p < 0.001$, **Figure 2.2A**; $\sum PCB_7$ lw, ANOVA, $f = 5.467$, $p < 0.001$). Elevations in $\sum PCB_7$ were inversely correlated with BCI ($r = -0.675$, $p < 0.001$), and body condition index was lowest among females and young (ANOVA, $f = 13.145$, $p < 0.001$, **Figure 2.2B**). There was no relationship between Hg and BCI ($p > 0.9$).

2.4.2 Biomarkers of selenium and thyroid status

Selenium concentrations in whole blood were significantly greater in males versus females (whole blood Se; males, 536.2 ± 23.4 ng / g ww; females, 459.1 ± 16.2 ng / g ww; ANCOVA, f = 5.956, p = 0.019; age as a covariate, f = 0.482, p = 0.492; **Table 2.1**). Whole blood Se and serum Se concentrations were correlated (r = 0.488, p = 0.001), with whole blood Se generally two times greater than serum Se. The range of GPx activity varied over 2 fold among individual bears (range 109.31 – 206.84 mUnits GPx / mg Hgb), and increased as follows: males and older ≤ young ≤ solitary females ≤ females with cubs (**Table 2.1; Figure 2.3**).

Concentrations of thyroid hormones (TT4, FT4, TT3, FT3) were greater in females than males (**Table 2.2**). Only FT3 concentrations exhibited a significant decrease with age. The ratio of total to free concentrations of thyroxine (TT4: FT4) was similar among sex and age cohorts. The ratio of total T4 to total T3 (TT4: TT3) varied by 4 fold among individuals, but mean values did not differ by sex or age. Males exhibited a greater mean value and range in the ratio of total to free concentrations of tri-iodothyronine (TT3: FT3) and in the ratio of free T4 to free T3 (FT4: FT3). Concentrations of albumin were greater in males than females (**Table 2.2**).

2.4.3 Relationships among biomarkers of selenium status, thyroid status and toxicant concentrations

Concentrations of Se (whole blood and serum) and GPx activity were significantly positively correlated with circulating concentrations of Hg in prime aged polar bears (**Table 2.3; Figure 2.4A - C**). GPx activity was also significantly negatively related to the Se: Hg molar ratio (**Figure 2.4D**). Concentrations of T3 were significantly positively related to the concentrations of Hg even after controlling for the variation by sex (**Table 2.3; Figure 2.5A**). TT3 levels were significantly positively related to the concentrations of Se, and significantly negatively related to the Se: Hg molar ratio (**Figure 2.5B and C**).

The first two components of the correlation-based PCA represented 73 - 82% of the total variation in the variables examined (**Figure 2.6; Table 2.4**). The individual or combined concentrations of toxicants explained less than 39% of this variation (represented by Factor 2, **Figure 2.6 A – C; Table 2.4**). Strongly positive correlations between Hg and $\sum PCB_7$ were found in males (component loading for Hg, 0.897; component loading for PCB, 0.835) and solitary females (component loading for Hg, 0.789; component loading for PCB, 0.692; **Figure 2.6A and B**), whereas the concentrations of toxicants were inversely correlated in females with cubs (component loading for Hg, 0.777; component loading for PCB, -0.949; **Figure 2.6 C; Table 4**). There was a strong negative correlation between toxicant concentrations and albumin in males (component loading relative to toxicants, -0.624). Further exploration of this relationship using general linear models suggested a significantly positive relationship between albumin and thyroxine, and a significantly negative relationship between albumin and PCBs among all prime age animals (**Figure 2.7A and B**). The correlational-based PCA indicated a strong negative correlation between toxicants and thyroxine (total and free fractions) in solitary females (component loadings relative to toxicants for TT4 and FT4, -0.948 and -0.848; **Figure 2.6B; Table 2.4**) that was further supported by examination of TT4 and toxicant correlations by physiological state (**Figure 2.8A and B**).

2.5 DISCUSSION

This study indicated that the concentrations of circulating toxicants differed among the physiologic states of Southern Beaufort Sea (SBS) polar bears. For example, we document that concentrations of Hg and $\sum PCB_7$ were positively correlated in males and solitary females. Concentrations of Hg and $\sum PCB_7$, however, were negatively correlated and more variable among females with cubs. Circulating Hg concentrations can be used as a proxy of recent feeding as Hg

compounds are bioavailable to blood shortly after prey consumption and the half-life of circulating Hg is approximately 30 days (Klassen 2001). The low Hg concentrations of many of the females with cubs, therefore, suggest that these animals had reduced feeding relative to other polar bears or that Hg was transferred to offspring via lactation. Previous studies found that diet as estimated by stable isotope values of $\delta^{15}N$ and $\delta^{13}C$ were strong predictors of Hg and PCB exposure to SBS polar bears (Bentzen et al. 2008; Cardona-Marek et al. 2009). The positive correlation between Hg and $\sum PCB_7$ suggests that males and solitary females were exposed to these contaminants concomitantly during feeding. These data are consistent with the high Hg and PCB concentrations in ringed seals (*Pusa hispida;* Dehn et al. 2005; Kucklick et al. 2002) reported to be the primary prey of polar bears in this region. Concomitant exposure to lipophilic and heavy metal contaminants has not been previously documented in SBS polar bears, but must be considered when assessing potential adverse biological responses.

Wet weight and lipid-adjusted concentrations of circulating PCBs were greater in prime aged females (with and without cubs) compared to males, and positively correlated to reduced body condition scores. Females recently emerging from maternity dens have undergone fasting, and all females with cubs have high energy demands to maintain lactation for offspring (Atkinson and Ramsay 1995). Lower body condition scores among females versus males suggest that females mobilized a greater amount of lipids from body storage sites than males, resulting in the greater release of lipophilic contaminants to circulation. Similar changes in lipophilic toxicants in blood and subcutaneous adipose have been reported in polar bears and arctic fox (*Alopex lagopus*) and were related to lactation, recent increase in energy demand, or fasting (Fuglei et al. 2007; McKinney et al. 2009; Polischuk et al. 2002). The different disposition dynamics of heavy metal and lipophilic contaminants of females compared to males may also

relate to the different feeding behaviors and sea-ice preferences among polar bear cohorts during late winter / early spring as females are more likely to remain closer to shore, especially if they have dependent young (Amstrup et al. 2000). Previous reports hypothesized that lipid soluble contaminants would be lower in females relative to males due to the gestational and lactational transport of contaminants to offspring (AMAP 2004b). Although this finding may be true for concentrations of toxicants stored in subcutaneous lipid and visceral tissues, this hypothesis does not appear to hold true for the circulatory disposition of toxicants in blood as presented in this study. Polar bears in the SBS are expected to undergo shortened feeding periods and greater energy expenditures to access prey under the proposed future changes in arctic sea-ice (Derocher et al. 2004; Molnar et al. 2010). Further understanding of the disposition of contaminants is needed as blood concentrations of toxicants represent the most recent influence to target sites of toxicity, and most closely match the temporal window of biomarkers measured in blood.

Circulating concentrations of Se in polar bears during spring were greater in males than females, and equal or in molar excess to concentrations of blood Hg. Circulating Se concentrations largely reflect dietary exposure to Se-containing foods (Fairweather-Tait et al. 2010), although Se is also stored in tissues of polar bears (Woshner et al. 2001). The variation of circulating Se among individual bears, therefore, is largely a consequence of the location, species, and proportion of prey tissues recently consumed. Dietary sources of Se for SBS polar bears would be largely from the consumption of ringed seals. The molar ratio of Se: Hg in ringed seal muscle ranged from 3.2: 1 to 5.7: 1, and 72 % of the total Hg measured was in its methylated form (Woshner et al. 2001). Visceral tissues such as kidney and liver of ringed seal contained more inorganic forms of Hg and equimolar ratios of Se: Hg (Dietz et al. 2000; Woshner et al. 2001). The majority of Se in plasma of mammals is part of selenoprotein P which contains several selenocysteines and thus has high binding affinity for heavy metals (Fairweather-Tait et

al. 2010; Yoneda et al. 1997; Yoneda and Suzuki 1997). Mercury forms also bind to a number of other seleno-compounds including bis(methlmercuric)selenide, selenomethioneine, free selenocysteines, and mercuric selenide (tiemanite) (Deitz et al. 2000; Woshner et al. 2001; Kahn and Wang 2010), as well as hemoglobin and albumin (Clarkson et al. 2007). The positive relationship between circulating concentrations of Se and Hg support the hypothesis that Hg in the blood of polar bears is bound, in part, to seleno-compounds. Molar ratios of Se: Hg (up to 19: 1 in the present study) above those found in common diet tissues of polar bears suggest that seleno-compounds in blood may also be retained in circulation by Hg. The negative relationships between TT3 concentrations and the Se: Hg molar ratios support a possible disruption in seleno-protein activities. Seleno-compounds are proposed to limit Hg toxicity through sequestration, Se-aided demethylation of MeHg, and inhibition of damaging free radicals; however, seleno-compounds also distribute dietary Hg to sensitive target organs such as the brain, liver, and kidney (Kahn and Wang 2010). Elevated Se: Hg molar ratios in the blood of polar bears likely limit oxidative stress initially induced by Hg, and aid in the distribution and elimination of Hg-compounds.

Glutathione peroxidase activity in polar bears increased with elevated concentrations of Hg. The negative correlation between GPx activity and the Se: Hg molar ratio in blood suggests that GPx may protect against Hg induced oxidative damage when Se: Hg ratios are low. This result is in agreement with other studies of Se status where the proportions of selenium forms measured in blood resulted from the bioavailability of dietary selenium, and the saturation thresholds of the various selenoproteins in circulation (Fairweather-Tait et al. 2010). Circulating concentrations of Hg in Hg-exposed humans was associated with an increase in both selenoprotein P and glutathione peroxidase, but the percentage of Hg associated with selenoprotein P increased with increasing concentrations of blood Hg (Chen et al. 2006).

Changes in the concentrations or proportions of specific selenoproteins in response to toxicant exposure have not been examined in polar bears. Furthermore, the bioavailability of dietary Se and the biological saturation points of seleno-compounds are unknown. The elevated GPx activity in female polar bears with cubs suggest that factors such as recent denning or lactation may also increase oxidative stress. A similar increase of enzymatic antioxidants, including GPx, were also reported in peripartum cattle having low body condition scores and increased lactational demand (Bernabucci et al. 2005). It is assumed that a 1: 1 Se: Hg molar ratio in tissues is biologically inert and has no adverse effect (Khan and Wang 2010). Our data suggest that as Se: Hg ratios approach 1, enzymatic antioxidants such as GPx become more important in the sequestration of Hg and the reduction of free radicals by toxicants and other stressors.

The albumin concentrations of polar bears in the present study are considered to be within the normal range for free-ranging polar bears during spring. The concentrations of albumin reported for SBS polar bears in the present study (range 3.3 – 5.0 g / dl) were similar to the albumin concentrations for the presumably healthy free-ranging polar bears sampled in Svalbard (albumin, range 4.3 – 5.1 g / dl; Tryland et al. 2002). Albumin proteins in circulation have several biological roles including the binding and transport of heavy metal and lipophilic contaminants, fatty acids, amino acids, and thyroid hormones (Ganong 2001; Ucan-Marin et al. 2010). Albumins contain multiple sulfhydryl groups and thus can act as antioxidants, similar to circulating seleno-compounds, and limit toxicity by sequestration of Hg and other toxicants. Albumin has been reported to be a major binding protein for inorganic and organic forms of Hg in humans and mice (Lau and Sarka 1979; Yasutake et al. 1989), and albumin-bound Hg has been proposed to be the primary mechanism for disposition and elimination of Hg via the kidney (Clarkson et al. 2007; Diamond and Zalups 1998; Khan and Wang 2010). The lower albumin

concentrations in female polar bears compared to males, therefore, may contribute to a lower non-enzymatic antioxidant status of this cohort.

The significantly positive relationship between albumin and thyroxine, and the significantly negative relationship between albumin and PCBs, suggest that reductions in albumin may also contribute to a decreased binding capacity for the circulatory transport of thyroid hormones, especially in individuals also exposed to Hg. Thyroid hormone disruption in polar bears and other species has been hypothesized to involve the competitive binding of transthyretin by PCB metabolites in circulation (Braathen et al. 2004; Skaare et al. 2001). Concentrations of T4 and T3 released into the blood stream of mammals are transported bound to carrier proteins thyroxine-binding globulin, transthyretin, and albumin (Yamauchi and Ishihara 2009). These proteins maintain adequate concentration of thyroid hormones in plasma and their distribution to target cells (Gagong 2001). The relative proportions of these carrier proteins differ by species, season, and physiological state (Yamauchi and Ishihara 2009), and are unknown for polar bears. The circulating concentrations of $\sum PCB_7$ of polar bears in the present study ranged from 2.03 to 132.8 ng / g ww. Svalbard polar bears having mean concentrations of 155 ng / g ww of PCBs in blood also had elevated concentration of hydroxy-metabolites of PCBs (e.g., 4-OH-CB107, 4-OH-CB146, and 4-OH-CB187) that saturated available transthyretin (Gutleb et al. 2010). Thyroid hormones that are not bound to transport proteins are more labile, more quickly degraded, and eliminated, which results in decreased circulating concentrations of hormones (**Figure 2.9**). PCB metabolites have also been proposed to increase the biliary elimination of hormones by the liver through the induction of biotransforming CYP-enzymes (Brouwer et al. 1998; McNabb and Fox 2003). Polar bears likely maintain thyroid homeostasis during periods of high toxicant exposure through release of negative feedback loops, and an increased production of T4 by the thyroid. Initial decreases in circulating concentrations of T4 (free and total

fractions), therefore, can be compensated by a HPT level response (i.e., release of negative feedback to the pituitary) and an ultimate increased production of T4 by the thyroid gland. Activation of the HPT axis in response to subtle decreases in thyroid hormones has previously been described in vertebrates as evidenced by increased thyroid gland mass, alterations in thyroid gland histology, and elevated concentrations of thyroid hormones (Hall and Thomas 2007; Van Lente and Daher 1992; Webb and McNabb 2008). Positive correlations between contaminants and circulating concentrations of thyroid hormones have also been previously described in male polar bears (Sonne 2010), although the mechanisms driving these relationships are not currently understood. Our data suggest that as transthyretin becomes saturated by PCBs and their metabolites, elevations of T4 may involve binding with available albumin (**Figure 2.9**). Similar positive relationships between thyroid hormones and albumin have been described in euthyroid sick syndrome in humans (Van Lente and Daher 1992). HPT axis-level responses to different chemical mixtures, and the potential toxicological mechanisms driving these responses, have not been fully explored in polar bears and warrant further examination.

As was expected, female polar bears had greater thyroid hormone concentrations (TT4, TT3, FT4, FT3) than males during spring. Greater thyroid hormone concentrations in females versus males are common among vertebrates, including polar bears, and suspected to relate to the regulation and interaction with estrogens (Braathen et al. 2004; Leatherland and Ronald 1981; Norris 2006; Skaare et al. 2001). Biological responses to toxicants in female polar bears would be expected to be more complex than males as females experience different physiologic and homeostatic states during spring such as recent fasting, lactation, caring for young, breeding activities, and estrous. These physiologic states are regulated by a combination of hormonal signals that include many of the biomarkers used in the assessment of toxic effects in polar bears. For example, thyroid hormones have reported to vary with lactation, pregnancy, molting, and

fasting in vertebrates (Ahmed et al. 2008; Hall and Thomas 2007; Hellgren 1998; Schussler and Orlando 1978). Thus, the examination of potential thyroid disruption by contaminants through analysis of circulating thyroid hormones alone may not accurately reflect an adverse biological effect in female polar bears. The negative correlation between toxicants and T4 in solitary females does not infer that this biological response is adverse, and correlation does not necessary reflect causation. Changes in thyroid status are tightly coupled to dose dependent changes in circulating toxicants and thus an acute biological response to toxicants can be compensated by HPT axis level responses (**Figure 2.1**). Our results suggest that multiple mechanisms including both toxic exposure and shifts in physiologic states (e.g., emergence from maternal denning, lactation, breeding activities) may be involved in the observed variations in thyroid status among polar bears. The use of blood-based biomarkers of thyroid status as indicators of adverse polar bear health are difficult to interpret without further information on thyroid gland morphology (e.g., status) or clinical signs of impairment that have not been identified in this sub-population of polar bears.

Although highly variable, the mean of total T4 concentration of polar bears in the present study (mean, 15 nmol / l) was three times lower than previously reported for presumably healthy captive and free-ranging polar bears (TT4, 50 nmol / l), although TT3 concentrations (present study, mean 1.4 nmol / l) were similar (TT3, 1.5 nmol / l; Churchill, Canada, Leatherland and Ronald 1981). Thyroid hormone concentrations of SBS polar bears were also lower than normal values for humans (TT4, 103 nmol / l; TT3, 2.3 nmol / l; Ganong 2001). Thyroid hormone concentrations of SBS polar bears were similar to those reported in Svalbard polar bears, but both populations also had similar concentrations of lipophilic toxicants (SBS polar bears in the present study, $\sum PCB_7$, 2.03 to 132.8 ng / g ww; Svalbard bears, $\sum PCB_5$, 16.7 to 203 ng / g ww; Braathen et al. 2004). TT3 concentrations for SBS polar bears were similar, but TT4 were lower, than

female Greenland sledge dogs (*Canis familiaris*, TT3, 1.59 ± 0.12 nmol/l; TT4, 27.9 ± 1.65 nmol/l) exposed to an environmentally relevant concentration of contaminants (dietary PCBs, mean 2996 ng/g lw, Hg unreported; Sonne 2010). Congenital hypothyroid states have been reported in other ursids such as black bears (*Ursus amerinanus*) and associated with clinical signs including lethargy, inappetence, alopecia, and skeletal deformities (Duncan et al. 2002; Storms et al. 2004). The disruption of thyroid status has also been associated with the disturbance of neurotransmitters and antioxidant systems in the central nervous system in birds and mammals (Ahmed et al. 2008; McNabb and Fox 2003). Thyroid hormone associated effects in polar bears could not be determined in the present study, but a short-term disruption of thyroid hormone mediated activities in SBS polar bears is a plausible biological response to their present toxicant burden. Our data suggest that female and young polar bears are the cohorts of concern for chronic low-level exposure to chemical mixtures.

2.6 CONCLUSIONS

Circulating concentrations of Hg and $\sum PCB_7$ were correlated in male and solitary female polar bears indicating concomitant exposure to heavy metal and lipophilic contaminants. In addition to dietary sources of contaminant exposure, the greater blood $\sum PCB_7$ concentration of animals with low body condition scores suggests that mobilization of lipophilic contaminants from body storage sites to circulation was greater for females than males. Elevations of Se (whole blood and serum concentrations) with Hg suggest that seleno-compounds sequester and likely retain Hg compounds in circulation. Glutathione peroxidase activity increased with circulating concentrations of Hg suggesting that enzymatic antioxidants are also involved in the sequestration of Hg compounds and the reduction of free radicals. Polar bears likely maintain thyroid homeostasis during periods of toxicant exposure through HPT axis level responses and

release of negative feedback loops, but this is uncertain. The use of blood-based biomarkers of selenium and thyroid status in polar bears for the assessment of adverse biological effects due to contaminants is not conclusive, especially in females that undergo changes in physiologic state throughout the year. Continued investigation of the physiologic states of polar bears, and potential clinical signs of impairment at the organ, individual, and population levels are needed to adequately address the biological impact of combined chemical exposures. A combination of studies using free-ranging and captive (zoo) bears, as well as histological assessments of tissues collected from animals obtained through legal subsistence hunts or found dead, could benefit these research efforts.

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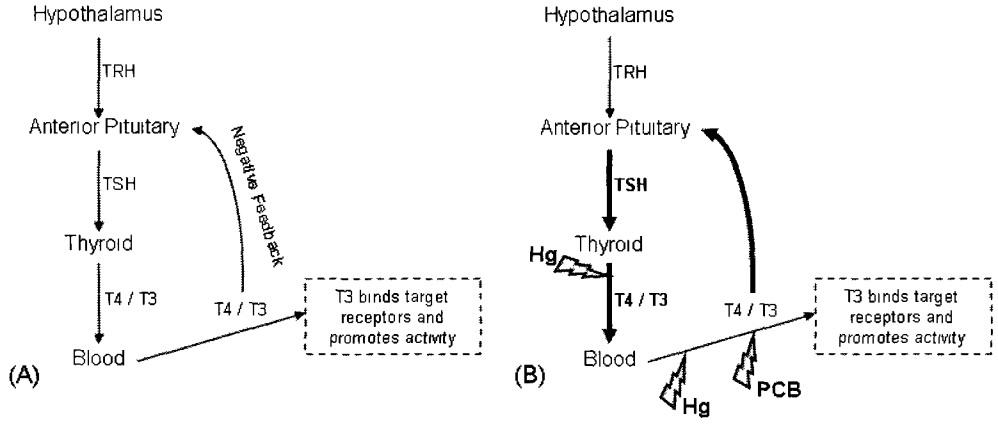

Figure 2.1 (A) Conceptual model of the homeostatic regulation of hypothalamus – pituitary – thyroid axis. When circulating thyroxine (T4) and tri-iodothyronine (T3) concentrations are within optimal limits, further production of T4 and T3 by the thyroid is inhibited. (B) Conceptual model of the response of the hypothalamus – pituitary – thyroid axis when exposed to contaminants. Mercury (Hg) reduces circulating concentrations of T4 and T3 through disruption of thyroid peroxidase activity during hormone synthesis in the thyroid and through competitive binding of transport protein albumin in the blood. Polychlorinated biphenyls (PCBs) reduce circulating concentrations of T4 and T3 through competitive binding of transport protein transthyretin. The hypothalamus – pituitary – thyroid axis responds to the reduction of circulating T4 and T3 concentrations by release of negative feedback mechanisms to the pituitary that signal an increase production of thyroid stimulating hormone (TSH) that consequently increases production of T4 and T3 by the thyroid.

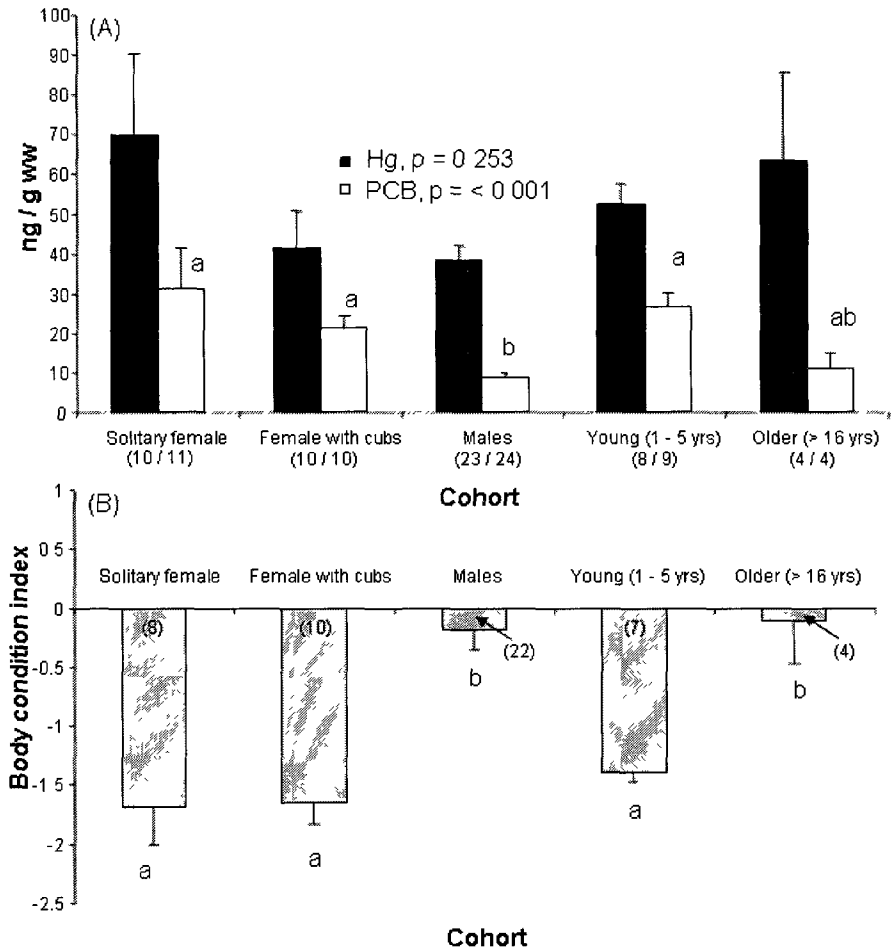

Figure 2.2 (A) Wet weight concentrations of circulating mercury (Hg ng / g ww) and polychlorinated biphenyls ($\sum PCB_7$; ng / g ww) in free ranging polar bears by cohort. (B) Body condition index of polar bears by cohort. Data are shown as mean ± standard error. Mean differences examined by analysis of variance (ANOVA) on log transformed data. The number of animals in each cohort is listed in parentheses.

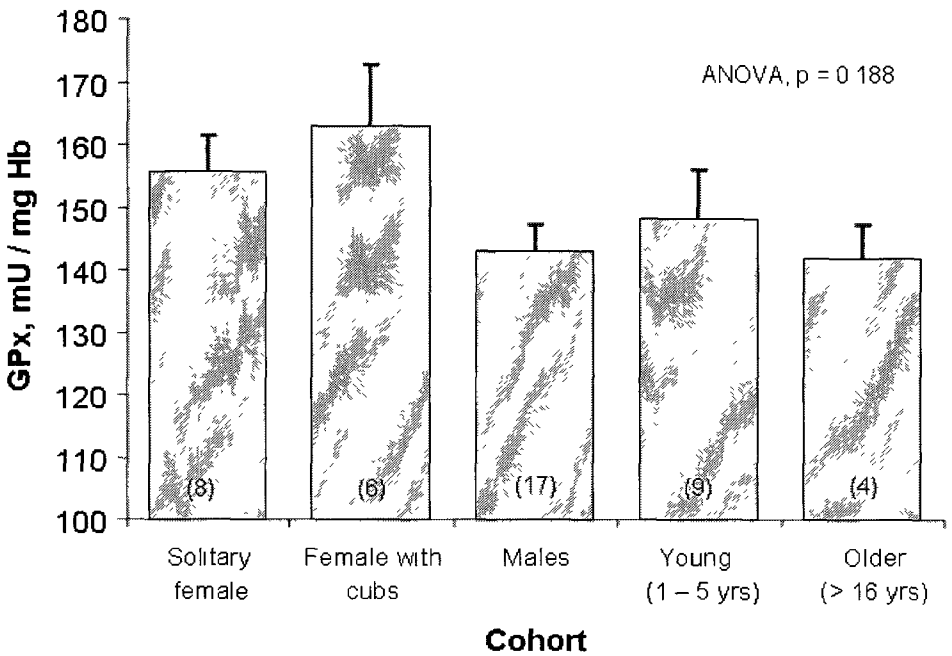

Figure 2.3 Glutathione peroxidase (GPx) activity (mUnits / mg hemoglobin) of polar bears by cohort. Data are shown as mean ± standard error. Mean differences examined by analysis of variance (ANOVA) on log transformed data. Hb = hemoglobin. The number of animals in each cohort is listed in parentheses.

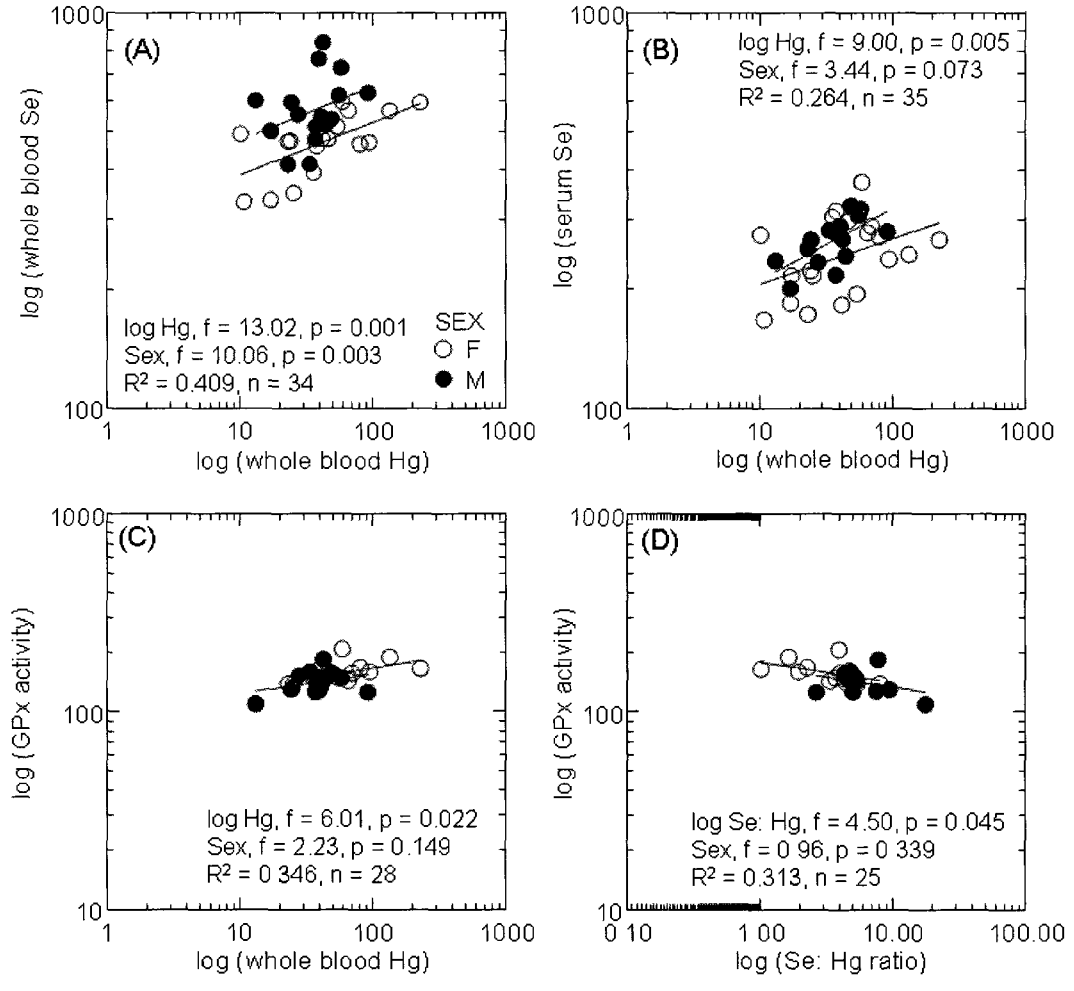

Figure 2.4 Concentrations of selenium (Se; ng / g ww) in whole blood (A) and serum (B) in relation to the concentrations of Hg in blood of prime aged polar bears. Glutathione peroxidase activity (mUnits / mg Hb) in relation to concentrations of Hg (C) and the molar ratio of Se: Hg (D) in blood of prime aged polar bears. General linear models were used to assess relationships between biomarkers and toxicants with sex as a covariate.

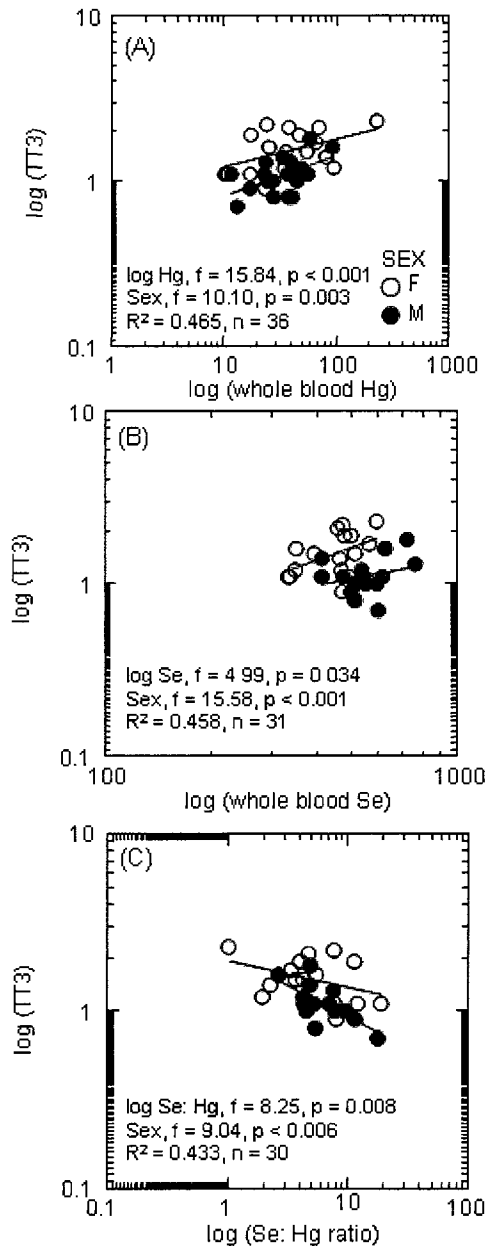

Figure 2.5 Concentrations of total tri-iodothyronine (TT3) and the concentration of Hg (A), whole blood Se (B), and the Se: Hg molar ratio (C) in prime aged polar bears. General linear models were used to assess relationships between biomarkers and toxicants with sex as a covariate.

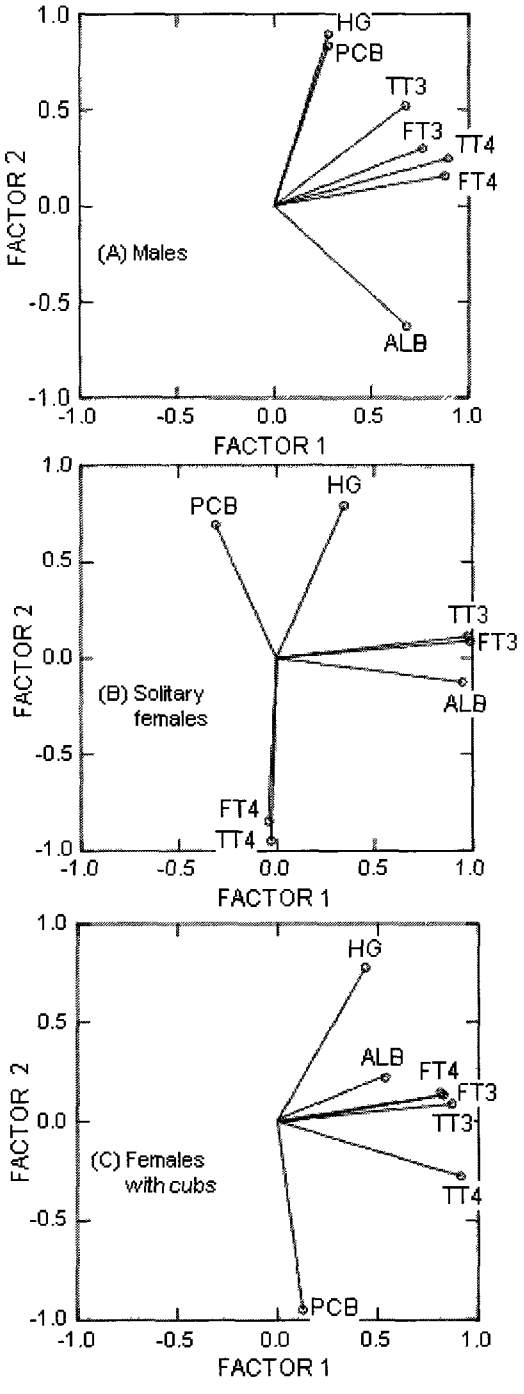

Figure 2.6 Correlation based principal components analysis of the relationships between thyroid status and toxicants in prime aged males (A), prime aged solitary females (B), and prime aged females with cubs (C). Statistics performed on log transformed data, except for albumin concentrations which were included as is.

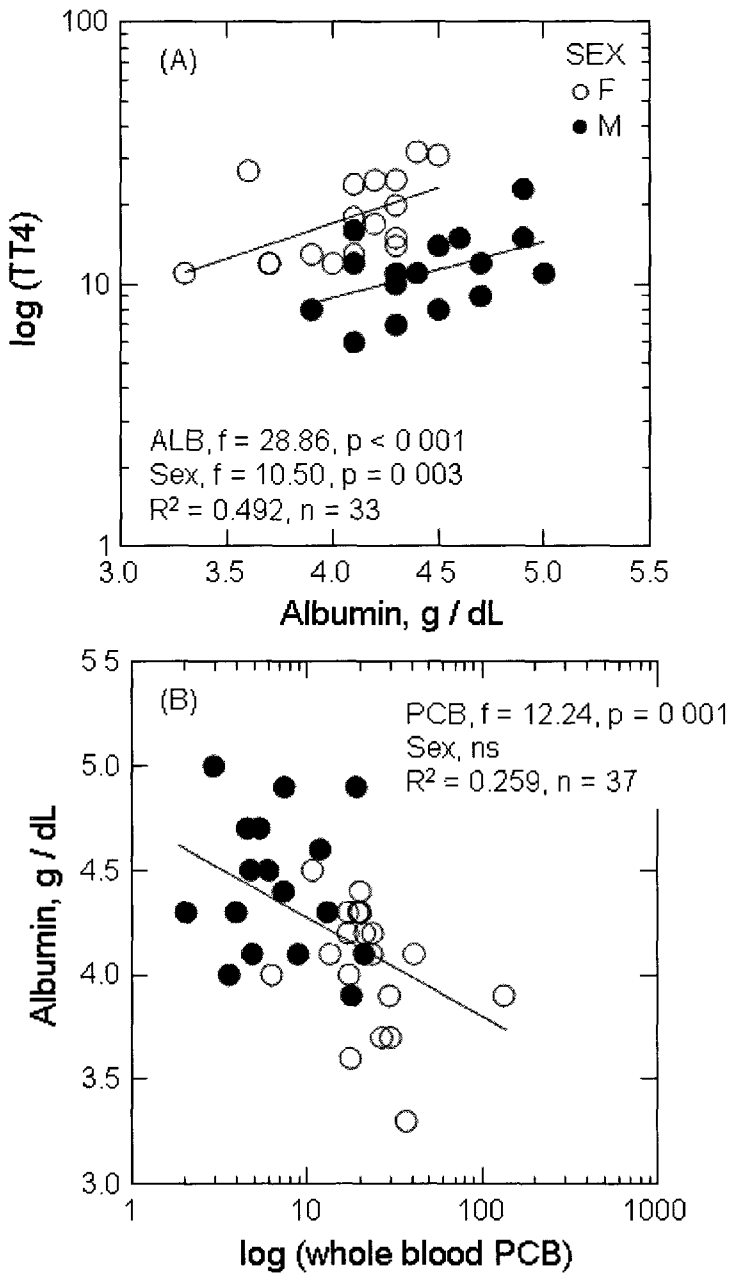

Figure 2.7 The relationship between concentration of total thyroxine (TT4) and albumin (A), and albumin and PCBs (B) in prime aged polar bears. General linear models were used to assess relationships between biomarkers and toxicants with sex as a covariate.

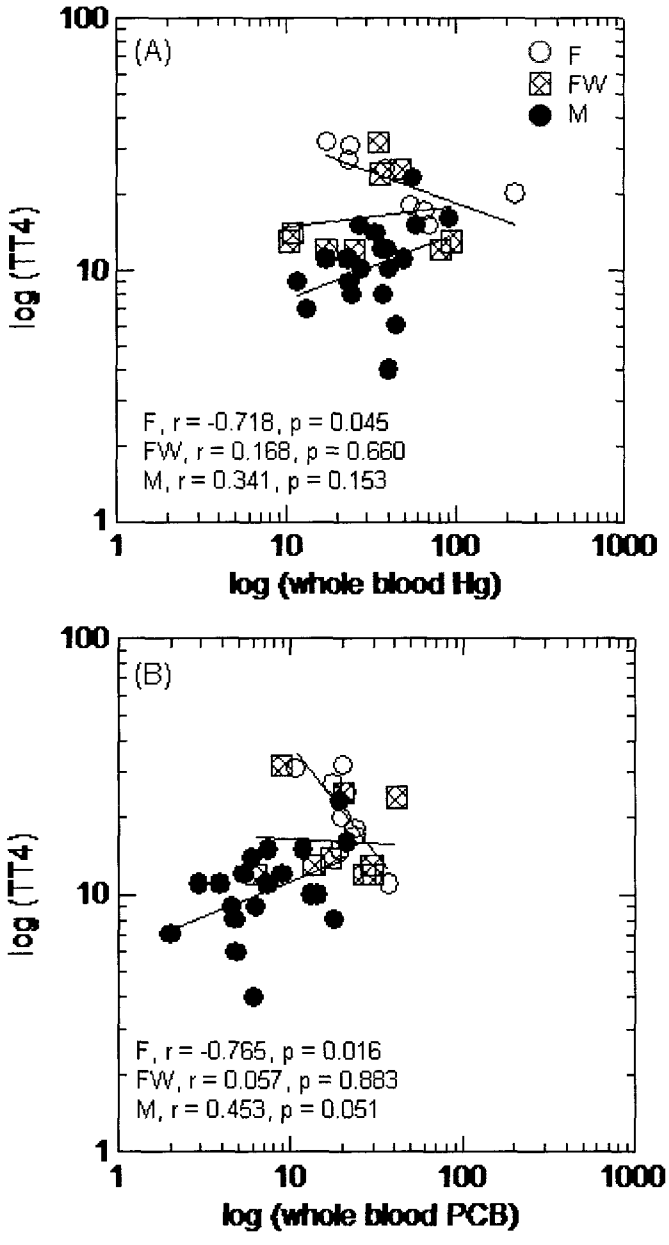

Figure 2.8. Correlations between total thyroxine (TT4) concentrations and the concentration of Hg (A) and the concentration of PCBs (B) in solitary females (F), females with cubs (FW), and adult males (M). Correlations examined using Pearson's pairwise correlations on log transformed data.

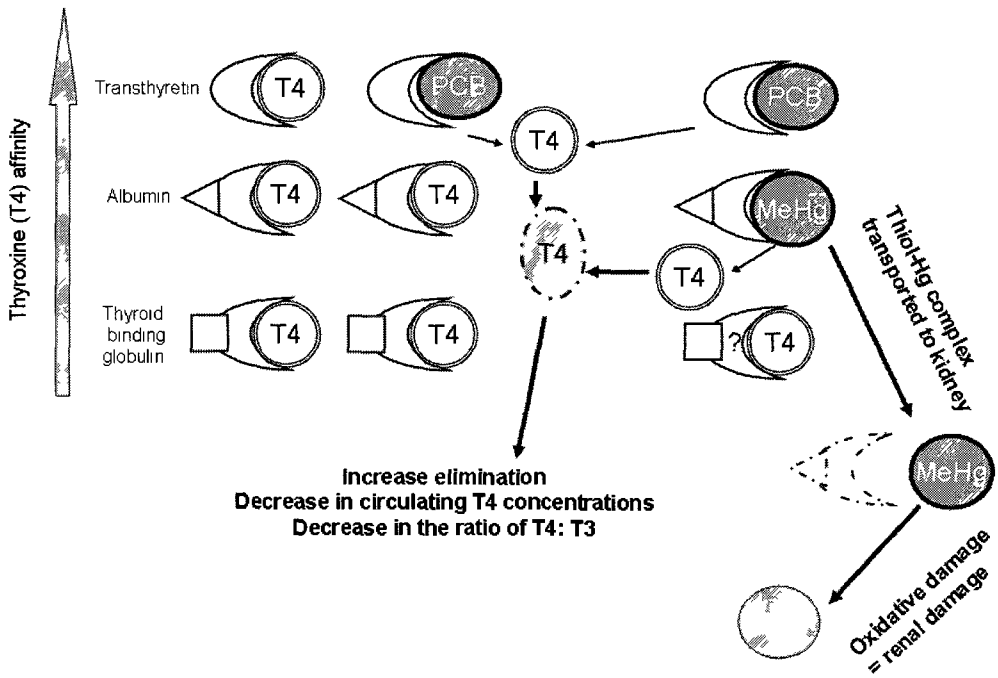

Figure 2.9 Conceptual model of the competitive displacement of thyroid hormone binding proteins (transthyretin, albumin, and thyroid binding globulin) by circulating toxicants.

Table 2.1 Selenium (whole blood and serum) concentrations, glutathione peroxidase (GPx) activity, hemoglobin concentration, and percent hematocrit in the blood of male and female polar bears. Data are shown as mean ± standard deviation, range and median. Ratios were calculated using molar concentrations. Analysis of covariance performed on transformed data.

	Males n = 24 – 29*	**Females** n = 20 – 26*	**Sex** f, p	**Age** f, p
Whole blood Se, pmol/g	42.34 ± 9.78 28.02 – 66.24 40.77	36.25 ± 6.27 26.25 – 46.88 36.25	5.956, 0.019	0.482, 0.492
Serum Se, pmol/g	20.26 ± 3.09 14.88 – 25.65 20.96	19.51 ± 4.04 13.22 – 29.45 19.81	0.662, 0.420	0.015, 0.902
Whole blood Se: Serum Se	2.10 ± 0.42 1.42 – 3.14 2.14	1.94 ± 0.39 1.30 – 2.74 1.94	2.298, 0.137	0.344, 0.561
Whole blood Se: Whole blood Hg	6.38 ± 3.62 2.49 – 17.84 5.07	5.24 ± 4.19 1.02 – 18.94 4.03	2.207, 0.146	1.102, 0.300
Serum Se: Whole blood Hg	3.01 ± 1.46 1.13 – 7.02 2.60	2.74 ± 2.13 0.46 – 10.52 2.13	1.102, 0.300	1.709, 0.199
GPx, mUnits / mg Hgb†	143.71 ± 18.99 109.31 – 194.41 143.09	155.16 ± 18.99 121.75 – 206.84 155.51	3.106, 0.086	0.129, 0.722
Hemoglobin, g / dL whole blood	15.99 ± 3.70 10.21 – 25.96 15.77	13.97 ± 2.85 10.27 – 22.43 13.26	3.232, 0.080	0.057, 0.813
Hematocrit, percent	44.21 ± 5.16 31.50 – 54.70 44.50	42.06 ± 4.20 33.50 – 49.50 41.88	2.260, 0.141	0.056, 0.814

* sample size for GPx, HgB, and hematocrit was 24 for males and 20 for females; †Hgb = hemoglobin

Table 2.2 Thyroid hormone concentrations, thyroid hormone molar ratios, and albumin concentrations of male and female polar bears. Data are shown as mean ± standard deviation, and median. Analysis of covariance performed on log transformed data except for albumin that was used as is. $^{\dagger} * 10^{3}$

	Males n = 28	Females n = 24	Sex f, p	Age f, p
TT4, nmol/l	10.89 ± 4.33 4.0 – 23.0 10.50	19.30 ± 6.82 11.0 – 32.0 18.50	25.06, <0.001	0.99, 0.326
FT4, pmol/l	6.68 ± 2.18 4.0 – 11.0 6.00	10.75 ± 2.64 7.0 – 17.0 10.00	32.99, <0.001	0.046, 0.832
TT3, nmol/l (excludes 1 outlier)	1.12 ± 0.35 0.30 – 2.10 1.10	1.62 ± 0.45 0.90 - 2.50 1.55	21.08, <0.001	1.98, 0.166
FT3, pmol/l	0.37 ± 0.28 0.10 – 1.30 0.30	0.72 ± 0.35 0.3 – 1.8 0.70	24.40, <0.001	6.55, 0.014
TT4:FT4†	1.61 ± 0.29 1.0 – 2.2 1.60	1.77 ± 0.33 1.2 – 2.5 1.71	1.48, 0.229	3.24, 0.079
TT3:FT3†	3.85 ± 1.65 1.40 – 8.00 3.33	2.46 ± 0.55 1.39 – 3.67 2.40	15.73, <0.001	4.39, 0.042
TT4:TT3	10.00 ± 3.42 5.00 – 20.91 9.62	12.53 ± 5.49 7.12 – 30.0 11.62	3.16, 0.082	0.82, 0.370
FT4:FT3	24.21 ± 13.21 7.69 – 60.00 20.00	17.78 ± 8.40 6.67 – 43.33 15.36	6.15, 0.017	10.89, 0.002
Albumin, g/dl	4.34 ± 0.34 3.90 – 5.00 4.30	4.10 ± 0.29 3.30 – 4.50 4.10	6.010, 0.018	0.069, 0.794

Table 2.3 Analysis of variance tables from general linear regression models (GLM) of the relationships between biomarkers and whole blood concentrations of toxicants in prime aged (6 – 16 years) polar bears Analyses were performed on log transformed data for all biomarkers and toxicants, except for albumin which was used with no transformation. Se = selenium; GPx = glutathione peroxidase activity; TT4 = total thyroxine, TT4:TT3 = molar ratio of total thyroxine to total tri-iodothyronine; FT4: FT3 = molar ratio of free thyroxine to free tri-iodothyronine. Values highlighted in bold indicate a significant relationship at $p < 0.05$.

Variable	f-value	P value	R^2
Whole blood Se (n = 34)			
Sex	4.702	0.038	0.419
Hg	**10.329**	**0.003**	
$\sum PCB_7$	0.539	0.472	
Serum Se (n = 35)			
Sex	2.221	0.146	0.264
Hg	**7.518**	**0.010**	
$\sum PCB_7$	0.039	0.845	
GPx (n = 28)			
Sex	1.449	0.240	0.340
Hg	**7.518**	**0.031**	
$\sum PCB_7$	0.039	0.845	
TT4 (n = 36)			
Sex	**7.349**	**0.011**	0.429
Hg	0.155	0.697	
$\sum PCB_7$	1.503	0.229	
TT3 (n = 36)			
Sex	**6.974**	**0.013**	0.470
Hg	**8.608**	**0.006**	
$\sum PCB_7$	0.305	0.585	
FT4 (n = 36)			
Sex	**12.434**	**0.001**	0.468
Hg	0.783	0.383	
$\sum PCB_7$	0.248	0.622	
FT3 (n = 36)			
Sex	**7.731**	**0.003**	0.444
Hg	**6.350**	**0.017**	
$\sum PCB_7$	0.153	0.699	
TT4: TT3 (n = 36)			
Sex	1.352	0.253	0.196
Hg	2.493	0.124	
$\sum PCB_7$	0.933	0.341	
FT4: FT3 (n = 36)			
Sex	0.783	0.383	0.199
Hg	**5.254**	**0.029**	
$\sum PCB_7$	0.015	0.903	
Albumin (n = 36)			
Sex	2.756	0.107	0.290
Hg	0.015	0.905	
$\sum PCB_7$	1.390	0.247	

Table 2.4 Relationships between toxicants and biomarkers of thyroid status in polar bears by prime aged (6 – 15 years) cohorts. Component loadings were assessed using a correlation based principal components analysis. Values highlighted in bold are were strongly correlated (≥ 0.6). Signs (+ or -) in each column reflect whether the variable was positively or negatively correlated with the other variables in that column (i.e., relative to other variables making up that component loading).

	Prime Aged Males		**Prime Aged Solitary Females**		**Prime Aged Females with Cubs**	
	Loadings for Factor 1	**Loadings for Factor 2**	**Loadings for Factor 1**	**Loadings for Factor 2**	**Loadings for Factor 1**	**Loadings for Factort 2**
PCB	0.278	**0.835**	-0.312	**0.692**	0.127	**-0.949**
Hg	0.280	**0.897**	0.346	**0.789**	0.439	**0.777**
TT4	**0.894**	0.247	-0.029	**-0.948**	**0.912**	-0.278
TT3	**0.676**	0.523	**0.976**	0.112	**0.868**	0.088
FT4	**0.876**	0.156	-0.042	**-0.848**	**0.824**	0.134
FT3	**0.763**	0.301	**0.986**	0.088	**0.809**	0.144
Albumin	**0.679**	**-0.624**	**0.947**	-0.125	0.537	0.222
% total variation	55 %	33 %	43 %	39 %	49 %	24 %

CHAPTER 3

Lactational transfer of mercury and polychlorinated biphenyls in polar bears[3]

3.1 ABSTRACT

Concentrations of mercury (Hg) and polychlorinated biphenyls (PCBs) were examined in blood and milk from free-ranging polar bears (*Ursus maritimus*) to assess maternal transfer of contaminants during lactation and the potential health risk to nursing young. Concentrations of toxicants in the blood of dependent and juvenile animals (ages 1 – 5 years) ranged from 35.9 – 52.2 ng / g ww for Hg and 13.9 – 52.2 ng / g ww (3255.81 to 11067.79 ng / g lw) for ΣPCB_7s, similar to those of adult females, but greater than adult males. Toxicant concentrations in milk ranged from 5.7 to 71.8 ng Hg / g ww and 160 to 690 ng ΣPCB_{11} / g ww (547 to 5190 ng / g lw). The greatest concentrations of PCB congeners in milk in descending order consisted of PCB153, PCB180, PCB99, PCB138 and PCB170 (all > 10 ng / g wet weight). Lower blood concentrations of toxicants in females with cubs compared to solitary females suggested that maternal transfer of contaminants to young limited the bioaccumulation of dietary contaminants in lactating female polar bears during early spring. The daily intake levels for Hg via milk consumption estimated for dependent young were below the tolerable daily intake level (TDIL) of Hg established for adult humans. Although the daily intake levels of PCBs through milk consumption for cubs of the year exceeded the TDIL thresholds, calculated dioxin equivalents for PCBs in milk were below adverse physiological thresholds for aquatic mammals. We were unable to determine which factors most contributed to elevated toxicant concentrations in polar bear milk. Continued studies should include a larger sample size, more information on the reproductive history of

[3] Knott KK, Boyd D, Ylitalo G, O'Hara TM. Lactational transfer of mercury and polychlorinated biphenyls in polar bears. Formatted for Chemosphere (submitted Aug 4, 2011).

female polar bears, and further details regarding the composition of blood and milk (e.g., protein compartments that may bind Hg and / or PCBs) to address the potential health impacts of contaminant exposure to dependent cubs.

3.2 INTRODUCTION

Elevated mercury (Hg) and polychlorinated biphenyl (PCB) concentration in humans and wildlife have been associated with impairments of hepatic and renal systems, endocrine function, and immunity, as well as reproductive and neurodevelopmental deficits (ATSDR 2004; Grandjean et al. 1995; Letcher et al. 2010; Mead 2008; Needham et al. 2011; Sonawane 1995; Sonne 2010). These toxicants are passed from mammalian females to offspring across the placenta and during lactation (Kelly et al. 2004; Clarkson et al. 2007; Wang et al. 2011). Concentrations of Hg and PCBs in milk, therefore, reflect the dietary and body stores of toxicants of the mother, and the lactational exposure to her offspring. Although fetal, neonatal, and juvenile animals have been identified as the cohorts of concern for adverse health effects, these life stages are seldom examined in toxicological studies. Scientists have recognized the need to use data collected from animals to better understand contaminant pathways, transport mechanisms and potential adverse health impacts in humans, and vice versa (Lindstrom et al. 1995). Polar bears (*Ursus maritimus*) have been used as indicators of ecosystem health because concentrations of contaminants persisting in lower trophic levels bioaccumulate and biomagnify in these apex predators (AMAP 2009). Because of their similar dietary routes of contaminant exposure and chemical transport mechanisms, polar bears may also be a useful model for understanding the possible impact of contaminants to the health of northern human residents that consume a high proportion of marine mammals and fish as a part of their diet (11 to 30% dietary energy, AMAP 2009; Grandjean et al. 1995).

Pregnant female polar bears in the Southern Beaufort and Chukchi Seas undergo a fasting period for up to 6 months during maternal denning, and body lipid stores are used to support themselves and the production of milk for 1 to 3 offspring (Atkinson and Ramsay 1995; Amstrup 2003). Polar bear cubs consume milk until they are approximately 1.5 years of age and are dependent on prey provided by the mother for up to 3.5 years (Derocher et al. 1993; Derocher and Stirling 1996). Recent studies of contaminants in Southern Beaufort Sea polar bears reported that many females and young had greater concentrations of Hg and PCBs than adult males (PCB in lipid and blood, Bentzen et al. 2008; Hg in hair and blood, Cardona-Marek et al. 2009; PCB and Hg in blood, Knott et al. 2011). Greater concentrations of toxicants in females versus males is a new trend occurring in polar bear populations, and challenges the theory that reproductive females reduce their toxicant burdens during gestation and lactation. Greater toxicant concentrations among females and young polar bears compared to males also prompt questions regarding whether sex or age-dependent mechanisms of toxicant exposure or excretion are contributing to decreases in reproductive success or juvenile condition. Life history parameters such as age, sex, reproductive status, and birth order have been found to influence toxicant concentrations in pinnipeds and cetaceans (Ylitalo et al. 2001; Lyderson et al. 2002), but have not been examined in polar bears. The study of the lactational transport of contaminants in polar bears has implications for the conservation of this species, and also aids in the understanding of the potential health risks associated with contaminant exposure during the mammalian postnatal period.

Milk provides energy, nutrients, and bioactive substances (i.e., growth factors, hormones) needed for growth and postnatal development, and contains immunoprotective components that support neonatal immunity (Jenness et al. 1972; Derocher et al. 1993; Arnould and Ramsay 1994; Oftedal 2000; Mead 2008). Previous studies have found that contaminant

concentrations biomagnify with trophic level as measured by an enrichment of stable isotopes of nitrogen ($^{15}N/^{14}N$; $\Delta^{15}N$; Atwell et al. 1998; Kucklick et al. 2002; Bentzen et al. 2008; Cardona-Marek et al. 2009). Because young rely on milk for nutrients, they are often considered a trophic level above their mother and thus potentially receive higher concentrations of toxicants while nursing compared to toxicant exposure directly from the consumption of prey. The relatively high concentrations of PCBs found in polar bear milk have also been related to the high percentage of lipids (range, 4.9 to 46.3 %) (Derocher et al. 1993; Polischuk et al. 1995; Polischuck et al. 2002), as high lipid concentrations in milk are needed for rapid growth, metabolic activity, and thermoregulation of young bears (Oftedal 2000). Although heavy metal toxicants such as Hg are known to transfer from mothers to their offspring during gestation and lactation in humans and experimental studies of rodents (Grandjean et al. 1995; Sundberg et al. 1999), the transfer of heavy metals to cubs has not been examined in polar bears.

We examined the concentrations of Hg and PCBs in milk and blood from free-ranging polar bears to (1) examine the concentration and disposition of toxicants to milk in polar bears in comparison to previous studies of polar bears and other mammals; (2) examine the possible chemical and biological factors contributing to variations in toxicant concentrations in polar bear milk; and (3) assess the health risk of contaminant exposure for adult female and nursing polar bear cubs based on the common guidelines for these contaminants in humans. These data provide information regarding the toxicological significance of contaminant exposure to young polar bears, and addresses aspects of lactational transport of chemicals that are common among mammalian females.

3.3 MATERIALS AND METHODS

3.3.1 Sample collection

Blood and milk samples were collected from polar bears of the Southern Beaufort Sea and Chukchi Sea sub-populations during springs (March-May) of 2007 and 2008 by the U.S. Geological Survey Ursid Research program and the U.S. Fish and Wildlife Service as part of long-term studies of polar bear ecology and population dynamics. Ages of cubs of the year through 3-year old animals were estimated by size and inclusion in a family group. Blood samples were collected from 27 adult males, 23 females (13 solitary females, 10 females with dependent young) and 9 young (2 yearlings, 3 two-year olds, 3 three and four-year old juveniles, and 1 of unknown age) polar bears. Blood was collected from either the femoral vein or artery into K_3EDTA Vacutainers™ (BD Vacutainers, Preanalytical Solutions). Milk samples were collected from 18 adult lactating female polar bears (4 from Chuckchi Sea; 14 from Southern Beaufort Sea) ranging from 7 to 24 years of age (mean, 12 years; body mass ranged from 162 to 488 kg). Milk was collected via intramuscular administration of oxytocin (Bimeda, Inc., Le Suer, MN, 15 - 20 international units per 100 kg body mass) and manual expression directly into sampling containers as previously described for polar bears (Polischuk et al. 2002). Body mass was taken using a tripod, hoist, scale and net. Capture and sampling procedures have been described previously (Bentzen et al. 2008; Cardona-Marek et al. 2009; Regehr et al. 2010; Rode et al. 2010). Animal handling procedures were approved by animal care and use committees at the USGS and the University of Alaska Fairbanks (UAF Institutional Animal Care and Use Committee Protocol Number 04-58 and 08-02).

3.3.2 Mercury analysis

Whole blood and milk samples were analyzed for total Hg concentration at the Wildlife Toxicology Laboratory (WTL), University of Alaska Fairbanks as previously described (Knott et

al. 2011). Concentrations of Hg were determined from the mean of duplicate samples on a DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT) using an eleven point calibration curve ranging from 1 to 400 ng ($r^2 = 0.998$; method detection limit approximately 50 pg). Duplicate samples had a coefficient of variance less than 10%. Standards were analyzed before milk and blood samples and after each set of 20 unknown samples. Standard reference materials included freeze-dried human hair (IAEA-085, 0.573 mg / kg; IAEA-086, 23.2 mg / kg, International Atomic Energy Agency, Vienna, Austria), freeze-dried fish muscle homogenate (0.433 mg / kg, Lake Superior Standard Reference Material 1946, National Institute of Standards and Technology), freeze-dried fish protein (0.414 mg / kg, DORM-3, certified material for trace minerals, National Research Council Canada), and standard spiking solution (0.10 mg / kg, Perkin-Elmer, Waltham, Massachusetts). Percent recoveries of standard reference materials and spikes were within 96 -111 %. Data are presented as total Hg (THg) on a wet weight basis (THg ng / g ww).

3.3.3 Polychlorinated biphenyl analysis

Concentrations of polychlorinated biphenyls (PCBs) were analyzed in whole blood and milk following procedures previously described (Sloan et al. 2005; Knott et al. 2011). Briefly, ~1 g of the sample was mixed with sodium sulfate and magnesium sulfate (1.5:1, v/v) as drying agents. PCBs and lipids were extracted with dichloromethane on an Accelerated Solvent Extractor (ASE 200, Dionex Corp., Sunnyvale, CA) at the WTL, University of Alaska Fairbanks. Extracts were delivered to the National Oceanic and Atmospheric Administration Fisheries laboratory (Seattle, WA). Each sample extract was filtered by gravity flow columns containing silica gel and alumina to remove interfering polar compounds. Further clean-up was conducted using size exclusion high performance liquid chromatography (SEC-HPLC). Individual and co-eluted PCB congeners (ΣPCB_{40}; 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90,

105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170/190, 171, 177, 180, 183, 187, 191, 194, 195, 199, 205, 206, 208, 209) were detected using a low-resolution quadrupole gas chromatograph/mass spectrometer (GC/MS) system equipped with a 60-meter DB-5 capillary GC column (0.25 mm i.d. and 0.25 μm film thickness) and a 10-meter guard column (0.53 mm i.d.).

The concentration of PCB congeners were based on a five-point calibration curve. PCB concentrations in milk were reported as the sum of the eleven PCB congeners (74, 99, 105, 118, 138, 153, 156, 170, 180, 183, and 194; ΣPCB_{11}) that were detected in $\geq$ 78% of milk samples. Only 7 of the 40 PCB congeners examined were detected in blood of polar bears (99, 105, 138, 153, 170, 180, and 194; ΣPCB_7). These congeners were selected based on those found in $\geq$ 41% of animals sampled (threshold based on an apparent break in the proportion of animals having concentrations above detection as remaining congeners were present in < 7% of animals sampled). All method blanks were below detection. Percent recoveries (mean ± SD) were 98.7 ± 1.7%, 102.1 ± 1.9%, and 100.0 ± 15.9% for internal spike (PCB103) in sample/SRM/method blank, certified congeners from SRM, and congeners of duplicates, respectively. Method detection limits ranged from 0.07 - 0.79 ng / g ww (lowest of calibration range) for specific congeners. Concentrations of PCBs were reported on a wet weight (ng / g ww) and lipid weight (ng / g lw) basis.

3.3.4 Lipid analysis

Lipid composition of whole blood and milk was determined by thin layer chromatography coupled with flame ionization detection (TLC/FID) using the Iatroscan Mark 6 (Iatron Laboratories, Tokyo, Japan). This analysis estimated the lipid concentration of five lipid classes in blood (sterol esters/wax esters, triglycerides, free fatty acids, free cholesterol and phospholipids; Ylitalo et al. 2005). The limits of detection for each of the five lipids were: lauryl

stearate (wax ester) 0.20 mg/ml, triolein (triglyceride) 0.20 mg/ml, oleic acid (fatty acid) 0.30 mg/ml, cholesterol 0.25 mg/ml and L-alpha-phosphatidylcholine (phospholipid) 0.25 mg/ml. The mean percent recovery of duplicate samples was 107.4 ± 20.7% (mean ± SD) for lipid classes. Data were examined as the percent total lipid in the sample ([sum all lipid class / total sample wet weight] * 100), as well as the percent of each lipid class making up the total lipid content of the sample (e.g., [g free cholesterol / g total lipid] * 100).

3.3.5 Stable isotope analysis

Analyses were performed at the Alaska Stable Isotopes Facility at the University of Alaska Fairbanks as previously described (Bentzen et al. 2008; Cardona-Marek et al. 2009; Knott et al. 2011). Briefly, samples were freeze dried, homogenized, and weighed (0.3 - 0.5 mg) into tin capsules. The mass of milk samples before and after freeze drying was used to estimate moisture content. Stable isotope values ($\delta^{15}N$, $\delta^{13}C$) were determined from the mean of duplicate samples by EA-IRMS using a Costech Elemental Analyzer (ESC 4010), and Finnigan MAT Conflo III interface with a Delta+XP Mass Spectrometer. Stable isotope values were expressed in δ notation as parts per thousand according to the following calculation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is the ratio of heavy to light isotope (^{15}N / ^{14}N or ^{13}C / ^{12}C). Atmospheric N_2 (air) and Pee Dee Belemnite (PDB) were used as standards. Peptone ($\delta^{15}N$ = 7.0, $\delta^{13}C$ = -15.8; meat based protein; Sigma Chemical Company) was used as a working laboratory standard to ensure appropriate quality control and assurance. Peptone was analyzed before unknown samples and after each set of 10 samples (percent recovery of peptone ranged from 99 – 101% for both $\delta^{15}N$ and $\delta^{13}C$).

3.3.6 Statistical analyses and calculations

Statistical analyses were performed using Systat 11 (Systat Software Inc.). Significant differences were set at $p < 0.05$. Concentrations of toxicants (ng / g ww) and percent total lipid were log transformed to meet normality assumptions. Transformations were not performed on stable isotope values as no transformation improved distributional properties. Pearson's pairwise correlations with Bonferroni probabilities were used to assess relationships among individual continuous variables. Relationship between the concentrations of toxicants and select biological variables (lipid content, maternal body mass, maternal age, etc) were assessed using general linear models (GLM). Mean differences among cohorts, matrices, and congeners were examined using analysis of variance (ANOVA) with Tukey's adjustments for multiple comparisons. Multiple analysis of variance (MANOVA) was used to assess the differences in PCB congener concentrations among cohorts (young, solitary females, females with cubs, adult males). Ratios (wet weight) between the mean concentration of toxicants in milk and blood from lactating females (milk: blood ratios) were used to assess whether the dispositional properties of contaminants between these matrices in polar bears were similar to those described for humans (e.g., Grandjean et al. 1995; Kelly et al. 2004).

Daily intake levels of toxicants through milk consumption were estimated using the toxicant concentrations in milk from females with COYs and 1 - 2 year olds, mean body mass of dependent young (COYs, 30 kg; yealings, 80 kg), and estimates of milk consumption for nursing polar bears previously reported (COYs, 469 g / day; yearlings, 131 g / day; Arnould 1990) using the following calculation:

μg of Hg or PCB / kg body mass / day = ((toxicant concentration in milk, μg /g) * (milk consumption, g / day)) / body mass (kg).

Of the congeners with dioxin-like toxicities, only PCB 105, PCB 118, and PCB 156 were detected in polar bear milk. The 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalency factors for these compounds are 0.00003 (Van den Berg et al. 2006). Estimates of 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in polar bear milk were determined by the following calculation:

dioxin equivalent ng / g ww = (0.00003*[PCB 105]) + (0.00003*[PCB 118]) + (0.00003*[PCB 156]),

where [PCBx] is the concentration of that congener in milk. The range of TDIL and dioxin equivalent values for the PCBs congeners measured were compared to toxicity thresholds from the literature (Grandjean et al. 1988; Arnould 1990; World Health Organization 1990; World Health Organization 2003; Kannan et al. 2000; Van den Berg et al. 2006) to estimate the health risk of toxicant exposure to young polar bears during milk consumption. These estimates only reflect the PCB-based dioxin equivalency in polar bear milk, and not the total dioxin equivalencies as other dioxin-like compounds (e.g., dioxin, polychlorinated dibenzofurans, polychlorinated dibenzo-p-dioxins) were not examined. Concentrations of toxicants in polar bear blood and milk could only be compared to threshold guidelines in humans (AMAP 2009), as no guidelines are available for ursids.

3.4 RESULTS

3.4.1 Composition of polar bear milk and maternal information

Toxicant concentrations in milk ranged from 5.7 to 71.8 ng / g for Hg (**Table 3.1, Figure 3.1**). Concentrations of ΣPCB_{11} ranged from 160 to 690 ng / g ww (547 to 5190 ng / g lw) (**Table 3.1, Figure 3.1**) of which 48 – 57% consisted of PCB 153. The percentages of the remaining PCB congeners found in milk as listed in descending order were as follows: PCB180 ≥ PCB99 ≥ PCB138 ≥ PCB170 ≥ PCB118 ≥ PCB194 (**Figure 3.2A**). Concentrations of PCB congeners 74,

105, 156, and 183 were < 9 ng / g ww. Milk contained < 1 to 30% total lipid (**Table 3.1**). The percent of total lipids in milk were not significantly related to maternal body mass (Pearson's correlation; r = 0.592, p = 0.36), cub age, or cub number (ANOVA, p > 0.134). Lipids in milk were predominantly triglycerides (> 86% of total lipid, **Table 3.1**), although 2 milk samples also contained sterol esters/waxy esters (both < 15% of total lipid). The range of stables isotope values varied by 4.5 per mil for $\delta^{15}N$ and $\delta^{13}C$ (**Table 3.1**). Stable isotope values were unrelated to the age, or number of cubs (ANOVA, p > 0.10), and did not correlate with maternal body mass or maternal age (p > 0.3). As expected, milk $\delta^{13}C$ values were correlated with lipid content of milk (r = 0.621, p = 0.031). Lipid content was not correlated with milk $\delta^{15}N$ values (r = -0.362, p = 0.247). Moisture content of milk ranged from < 5% to 25%; however, due to the high lipid content and tackiness of the milk samples after freeze-drying, these estimates are considered to be only a rough estimate.

3.4.2 Relationships among milk toxicants, demographics, stable isotope values, and lipids

The concentrations of toxicants in milk did not vary with the age or number of cubs (ANOVA; age of cubs, Hg, f = 1.2, p = 0.349; PCBs, f = 2.7, p = 0.114; number of cubs, Hg, f < 0.1, p = 0.932, PCB, f = 1.1, p = 0.327). Concentrations of Hg and PCBs were also unrelated to the stable isotope values in milk (p > 0.6). Concentrations of Hg and PCBs in milk were unrelated to maternal age or body mass (Hg, f < 0.8, p > 0.42; PCB, f < 4.3, p > 0.1). Concentrations of PCBs in milk were also not related to the total lipids in milk (p = 0.580, **Figure 3.2**).

3.4 3 Comparisons between milk and blood toxicants

Toxicants concentrations in blood of young polar bears (ages 1 – 4 years) ranged from 35.9 – 52.2 ng / g ww for Hg and 13.9 – 52.2 ng / g ww (3255.81 to 11067.79 ng / g lw) for

PCBs. The wet-weight (and lipid-adjusted) concentrations of Hg and PCBs in the blood of young polar bears were similar to adult females (with and without cubs), but greater than adult males (**Figure 3.1, Figure 3.2**). Concentrations of PCB congeners in solitary females were generally greater than females with cubs, although this difference was not statistically significant (**Figure 3.2B**).

Total Hg concentrations (ng / g ww) in polar bear blood and milk were similar (**Figure 3.1A**). The profiles of PCB congeners in milk and blood were also similar between matrices (**Figure 3.2**). Although wet-weight concentration of PCBs in blood were greater than the PCB concentrations in milk (**Figure 3.1B; Figure 3.2**), lipid-adjusted concentrations of PCBs, were over 3 times greater in blood versus milk (mean ± SD; blood from young, 6309.4 ± 3405.3 ng / g lw; blood from adult females, 6026.6 ± 4798.6 ng / g lw; milk, 2629.2 ± 1486.8 ng / g lw; **Figure 3.1C**). Both milk and blood samples were only available from 3 of the 4 females for Hg and 2 of 4 females for PCBs. The milk: blood ratios for these animals (Hg ww: 0.42:1, 0.72:1, and 0.76:1; PCBs ww: 11.8:1 and 39.6:1) were similar to the milk: blood (ww) ratios calculated using mean toxicant concentration of all milk samples and the mean toxicant concentrations of all blood samples from females with cubs (Hg, 0.6:1; PCBs, 17.1: 1; **Table 3.1**).

3.4.4 A risk assessment of toxicant exposure to young and adult polar bears

On average, cubs of the year would consume 0.42 ug / kg bm / d of Hg and 41.1 ug / kg bm / d of PCBs, and 1 and 2 year olds would consume 0.044ug / kg bm / d of Hg and 4.3 ug / kg bm / d of PCBs. The intake levels of Hg for all nursing polar bears were below the TDIL established for adult humans (**Figure 3.3A**). The range of intake levels of PCBs for 1 and 2 year old polar bears were also below TDIL for PCBs in humans; however, the estimated intake levels of PCBs for cubs of the year exceeded these prescribed toxicity thresholds (**Figure 3.3B**). Polar bear milk had PCB-related 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalent values ranging from

2.07×10^{-4} through 7.77×10^{-4} ng / g wet weight. These concentrations were below the dietary thresholds for adverse physiological effects in aquatic animals (**Figure 3.4**). Concentrations of circulating toxicants in all polar bears exceeded the guideline values of concern for humans (US evaluation level, 5.8 ng Hg / g; Canadian increasing risk threshold, 20 ng Hg / g; Canadian level of concern for pregnant women, 5 ng PCB / g; Canada action level, 100 ng PCB / g; AMAP 2009).

3.5 DISCUSSION

3.5.1 Composition of polar bear milk

Females emerging from dens with cubs of the year (COYs) during spring are considered to be in mid-lactation (Oftedal 2000; Derocher et al. 1993). Samples in the present study, therefore, were limited to collection during mid-lactation for females with COYs, and late lactation for females with cubs 1-year and older due to the logistical and animal welfare concerns of sampling denning females. Concentrations of PCBs and lipid content of milk from Southern Beaufort Sea and Chuckchi Sea polar bears were similar to those reported in other sub-populations of polar bears (Derocher et al. 1993; Polischuck et al. 1995; Polischuck et al. 2002). Lipid concentrations of polar bear milk were similar to the lipid concentrations in milk for pinnipeds and cetaceans (Jenness et al. 1972; Mellish et al. 1999; Lang et al. 2005), but were 5 to 30 times greater than the percentage of lipid in milk from humans (< 1%), consistent with the high energy demands for growth and thermoregulation of polar bear cubs (Atkinson and Ramsay 1995; Derocher et al. 1993; Oftedal 2000). As expected, $\delta^{13}C$ values of the relatively lipid-rich polar bear milk were depleted in ^{13}C similar to previous reports, and consistent with the preference of the lighter carbon isotope (e.g., ^{12}C) in fat (Polischuk et al. 2001; Wolf et al. 2009).

The range of $\delta^{15}N$ values in milk were similar to those reported in the blood of female polar bears (19.3 to 20.4 per mil; Bentzen et al. 2008; Knott et al. 2011). We found no factors that were significantly related to toxicant concentrations in milk; however, our sample sizes were limited. Milk composition has been reported to change with maternal nutrition, activity, duration of fasting, time since last suckling, and stage of lactation (Bauman and Currie 1980; Oftedal 1984; Derocher et al. 1993; Oftedal 2000), but these factors could not be examined in the present study.

3.5.2 Intraspecific and interspecific comparisons of contaminant transfer during lactation

The concentrations and milk : blood ratios of Hg in polar bears (5.7 to 71.8 ng Hg / g ww, 1.5: 1 ww) were greater than those reported for humans consuming marine based diets (1 to 4 ng Hg /g ww, 0.12 : 1 ww, Grandjean et al. 1995) and likely related to the greater intakes of Hg in the polar bear diet (i.e., absolute concentration of Hg in diet items and number of meals / unit of time). The total Hg concentration in polar bear milk likely included both methylated and inorganic forms since both have been detected in milk from humans and mice (Sundberg et al. 1999). Mercury compounds in milk are more associated with proteins (casein and whey) versus lipid, although the proportions differ between inorganic (IHg) and methylated (MeHg) forms (45% and 39 % for MeHg, and 72% and 15% for IHg associated with proteins and lipids respectively; Sundberg et al. 1999). Serum albumin is a primary whey protein in humans and mice (Sundberg et al. 1999), and these proteins have also been identified in the milk and blood from polar bears and other ursids (Jenness et al. 1972; LeBlanc et al. 2001). In the present study, we report that blood Hg concentrations of females with cubs were lower than solitary females and males. Mercury bound to albumin in blood and subsequent transfer to offspring in milk may explain the relatively lower Hg concentrations among lactating versus non-lactating females. This mechanism of lactational transport of Hg has been suggested for humans and mice as the

majority of Hg compounds are bound to serum albumins and passively transferred to milk via blood (Sundberg et al. 1999; Clarkson et al. 2007). The greater lactational transfer of Hg compounds during the production of milk colostrum in humans has also been hypothesized to relate to the higher serum albumin in milk during early lactation (Sundberg et al. 1999). Ursids also have elevated albumin concentrations in plasma and milk during maternal denning and early lactation (Le Blanc et al. 2001). Mercury transferred to neonatal polar bears during early lactation in maternal dens (unavailable for this study), therefore, was likely higher than the concentrations of Hg reported here for mid to late lactation. *In utero* exposure could also present a significant source of Hg to developing offspring, but could not be assessed in the present report.

Lipid sources for milk production in mammals occur on a continuum from animals that primarily use endogenous lipid sources for milk to those that integrate the majority of milk lipids directly from food to milk (Sadleir 1984). High energetic costs and small stature of female polar bears, and the low recruitment of cubs, have been associated with recent changes in the sea-ice of the Southern Beaufort Sea (Regehr et al. 2010; Rode et al. 2010; Durner et al. 2011). Female polar bears use endogenous lipid reserves during milk production, but as fat stores become depleted, the lipids in milk must come from the diet or milk production ceases (Derocher et al 1993; Derocher and Stirling 1996). The congener profiles in polar bear blood and milk reported in the present study were similar to the PCBs congener profiles from dietary sources such as ringed seal (*Phoca hispida*, Kucklick et al. 2002) and consistent with the transport of contaminants dietary sources to blood to milk. Lactational transfer of contaminants during mid to late lactation for polar bears, therefore, may be proportional to maternal dietary intake levels as has been reported for humans and cattle (Matthews and Detrick 1984; Grandjean et al. 1995). The distribution of chemicals into milk is based on the structural properties of PCBs and affinity for lipids (Matthews and Detrick 1984), as well as adsorption to non-lipid components such as

serum albumin (Kelly et al. 2004; Matthews and Dedrick 1984). The lower lipid-adjusted concentrations of PCBs in polar bear milk versus blood (and higher PCB concentrations in milk versus blood on a wet weight basis), therefore, suggest that the maternal transfer of PCBs during mid to late lactation may be more associated with plasma proteins than lipids. As plasma proteins were not examined in the present study, further studies are needed to examine the extent of this possible mechanism of toxicant transfer during lactation. This is the first report of PCB congeners in polar bear milk during mid to late lactation after females and cubs have emerged from maternity dens and are no longer fasting. Previous studies described the chemical transfer of persistent organic pollutants were largely from endogenous lipid stores in pinnipeds and lactating female polar bears fasting on land in Hudson Bay (Polischuk et al. 2002; Wolkers et al. 2004). It is unknown whether PCB congener profiles in milk would differ from those reported here if these chemicals were from endogenous sources alone. Comparisons between fasted and feeding lactating polar bears would be needed to further assess the sources of milk contaminants.

The concentrations of PCBs and the PCB congener profile in milk of polar bears were similar to those in milk from humans consuming a high proportion of marine (pilot whale and fish) based diets (500 – 2000 ng / g lw predominately of congeners PCB 153, PCB 180 and PCB 138, Grandjean et al. 1995; Needham et al. 2011). These PCB congeners have high biomagnification potentials due to higher K_{ow} values (i.e., greater lipid solubility) and lower biotransformation rates compared to other congeners (Matthews and Detrick 1984; Polischuk et al. 2002; Kucklick et al. 2002). Similar PCB congeners in milk and blood of polar bears suggest that there is little selective transfer from diet to maternal blood to milk during mid to late lactation, contrary to the lactational transfer of contaminants that has been described for pinnipeds (Wolkers et al. 2004). The biotransformation of persistent organic pollutants in polar bears is expected to be minimal during fasting due to the lack of fecal production, and

biotransformation and fecal partitioning during feeding is greater for more water soluble congeners (Kelly et al. 2004; Polischuk et al. 2002). Elimination of highly lipophilic toxicants during gestation and lactation has been suggested to be an additional mechanism for significantly decreasing body burdens in mammalian females, but this finding has been challenged (Thomsen et al. 2010; Weijs et al. 2010). The relative decrease of toxicants in female polar bears during early lactation could not be determined in this study. Our data, however, suggest that transfer of contaminants during mid to late lactation does appear to limit the initial bioaccumulation of dietary contaminants when females feed after emergence from maternal denning. These data are consistent with greater variability of the lipophilic and heavy metal toxicant concentrations in blood of adult females compared to adult males reported previously (Hg, adult females range, 10.2 – 228.1 ng / g ww, adult male range, 22.7 – 92.3 ng /g ww; PCBs, adult female range, 6.2 – 140.0 ng / g ww, adult male range, 1.8 – 21.0 ng / g ww; Knott et al. 2011) and in the present study. Relatively lower toxicant concentrations in blood among males during spring is likely the combined result of their relatively greater and more stable body condition, spring breeding activities that deter them from hunting, and greater consumption of lipid from larger prey as previously described for reproductive males (Cherry et al. 2009; Rode et al. 2010; Cherry et al. 2010).

3.5.3 Risk assessments of toxicant exposure in polar bears

The estimated intake levels of Hg through milk consumption in young polar bears were below the provisional tolerable intake level of Hg for adult humans (equivalent to 0.6 ug / kg bm / d, World Health Organization 1990). Estimated intake levels of PCB for cubs of the year exceeded the maximum tolerable daily intake of PCBs for adult humans (1.0 ug / kg bm / d, World Health Organization 2003; 0.2 ug / kg bm / d, Grandjean et al. 1988); however, dioxin equivalency values were below the dietary threshold concentration above which physiological

effects could be determined in aquatic mammals (mean estimate from studies of harbor seals, *Phoca vitulina*, bottlenose dolphin, *Tursiops truncatus*, European otter, *Lutra lutra*, and mink, *Mustella viso:* 14×10^{-4} to 19×10^{-4} ng / g wet weight; Kannan et al. 2000). We emphasize that our estimates only represent the PCBs having dioxin-like toxicities as we did not measure all relevant dioxins and thus total dioxin equivalency values would be expected to be higher. The mechanism of action for dioxin-like compounds is through binding to the aryl hydrocarbon (Ah) receptor, which has been associated with the disruption of normal endocrine function, growth retardation, learning deficits, impaired immune responses, and neoplasia in human and animal studies (Lindstrom et al. 1995). Our data suggest that although the overall concentration of PCBs in polar bear milk were high, those congeners that are expected to elicit the greatest toxic effects were low and pose minimal risk to the health of young polar bears by dioxin-like mechanisms. The effects of toxicants, however, are expected to differ between neonates and adults due to differences in the maturation of immune, endocrine, and organ function (Oskarsson et al. 1998). Our estimates of toxicant concentrations in milk were also from samples collected during mid to late lactation, and the toxicant concentrations during early lactation are expected to be higher than those reported here. Polar bears have a small birth mass (0.3% of maternal mass) and undergo a longer lactational period than other terrestrial mammals (Oftedal 2000; Amstrup 2003). Thus, the duration of lactational exposure of toxicants through milk consumption is longer for young polar bears than many other species and is deserving of a more complete evaluation and appropriate modeling.

Lactation places a great energetic demand on female polar bears and also influences their lifetime reproductive fitness (Derocher et al. 1993; Atkinson and Ramsay 1995; Oftedal 2000). As expected, toxicant concentrations in the blood of all polar bears exceeded guideline thresholds for humans. Our study did not examine other toxic compounds known to bioaccumulate in polar

bears and humans such as arsenic, polybrominated and polyfluorinated contaminants, and dioxins (Grandjean et al. 1995; Polischuk et al. 1995; Polischuk et al. 2002; Needham et al. 2011). The effects of combined concentrations of toxicants (both lipophilic and heavy metal compounds) should also be considered in biological effects assessments. Previous studies have indicated that the low body mass of female and young polar bears associated with limited food availability was the main cause of mortality in young polar bears (Derocher and Stirling 1996; Rode et al. 2010). The benefits of milk consumption (e.g., nutritional, immunologic, selenium, etc) for young polar bears are expected to outweigh the potential costs of toxicant exposure. The absence of any alternative diet sources for dependent young presents a unique 'arctic dilemma' for polar bears and a difficult management scenario. Accessibility to prey and the ability of female polar bears to accumulate adequate reserves prior to gestation and lactation are expected to be the greatest limiting factors to reproductive success and neonatal survival. These prey items, however, are also the source of contaminants for her, the fetus and the neonate. Thus, contamination of the maternal diet is the point of potential intervention for minimizing the maternal transfer of contaminants to offspring. Toxicant exposure during critical stages of neonatal development may exacerbate or compound health effects due to other causes such as nutritional stress (e.g., deficiency in essential nutrients or calories) or pathogen exposure.

3.6 CONCLUSION

Our data suggests that transfer of contaminants during mid to late lactation limits the bioaccumulation of dietary toxicants in female polar bears with dependent nursing young. We were unable to determine which of the measured biological or chemical factors most influenced toxicant concentrations during mid to late lactational milk production indicating that the transfer of chemicals into milk may be a more complex process than assumed (e.g., PCBs do not simply

follow the lipids). Further studies should include a larger sample size, more information on the reproductive history of individual bears (i.e., parity, number of successful births, duration of denning, body mass loss and energy expenditure, etc.), and further details regarding the composition of blood and milk (e.g., protein compartments that may bind Hg and / or PCBs). Exposure to contaminants for young polar bears during mid to late lactation were generally below commonly used thresholds for toxicity established for adult humans and aquatic mammals. Exposure to total sum PCBs through milk consumption in cubs of the year, however, exceeded toxicity guidelines. Neonatal young may be more susceptible to toxicants and these toxicity thresholds do not address the biological impact of chemical mixtures. Polar bears are expected to undergo shortened feeding periods and greater energy expenditures in response to changes in their arctic sea-ice habitat. The adverse health impacts associated with nutritional stress may be exacerbated by mobilized and dietary contaminants, especially in sensitive cohorts such as reproductive females and their young.

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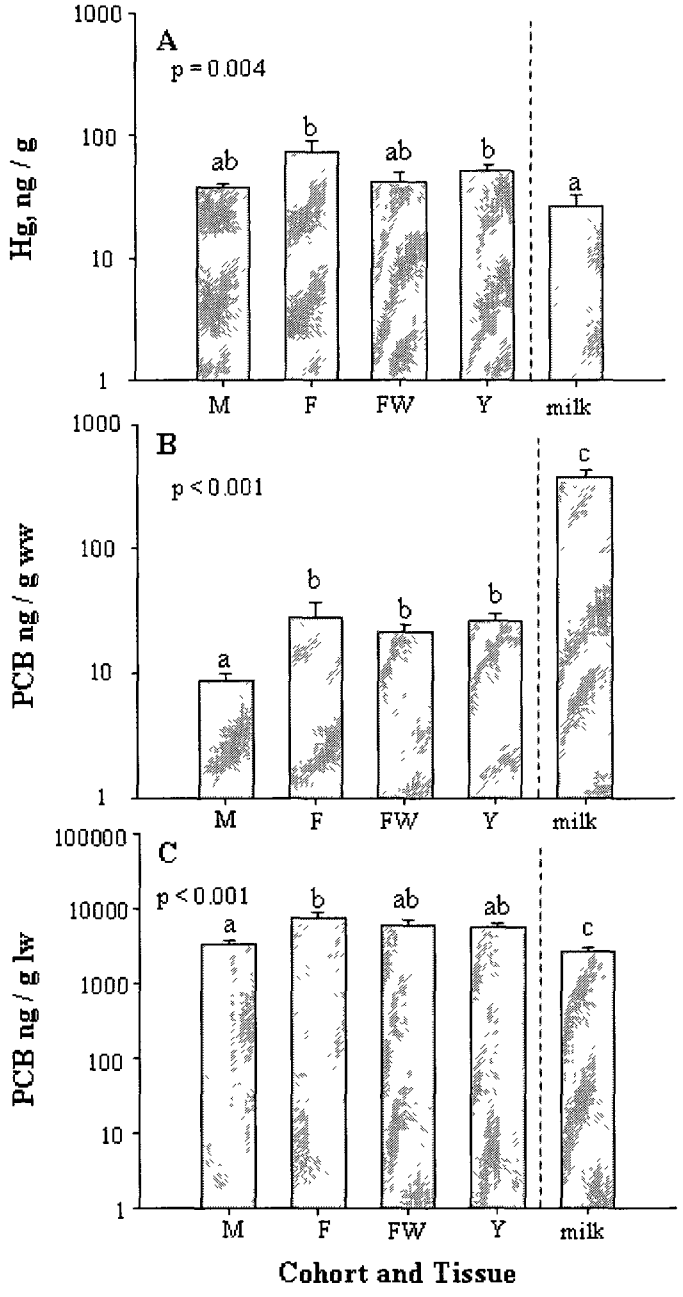

Figure 3.1 Concentrations of toxicants in blood (M = adult males, SF = adult solitary females, FW = adult females with cubs, Y = ages 1 -4 years) and milk of polar bears. (A) Mercury (Hg) concentrations (ng / g wet weight), (B) Polychlorinated biphenyl (PCB) concentrations on a wet-weight-basis (ng /g ww), and (C) PCB concentrations on a lipid-adjusted basis (ng / g lw). Data are shown on a log scale. Different letters indicate significant differences between the mean concentrations by cohort or tissue matrix.

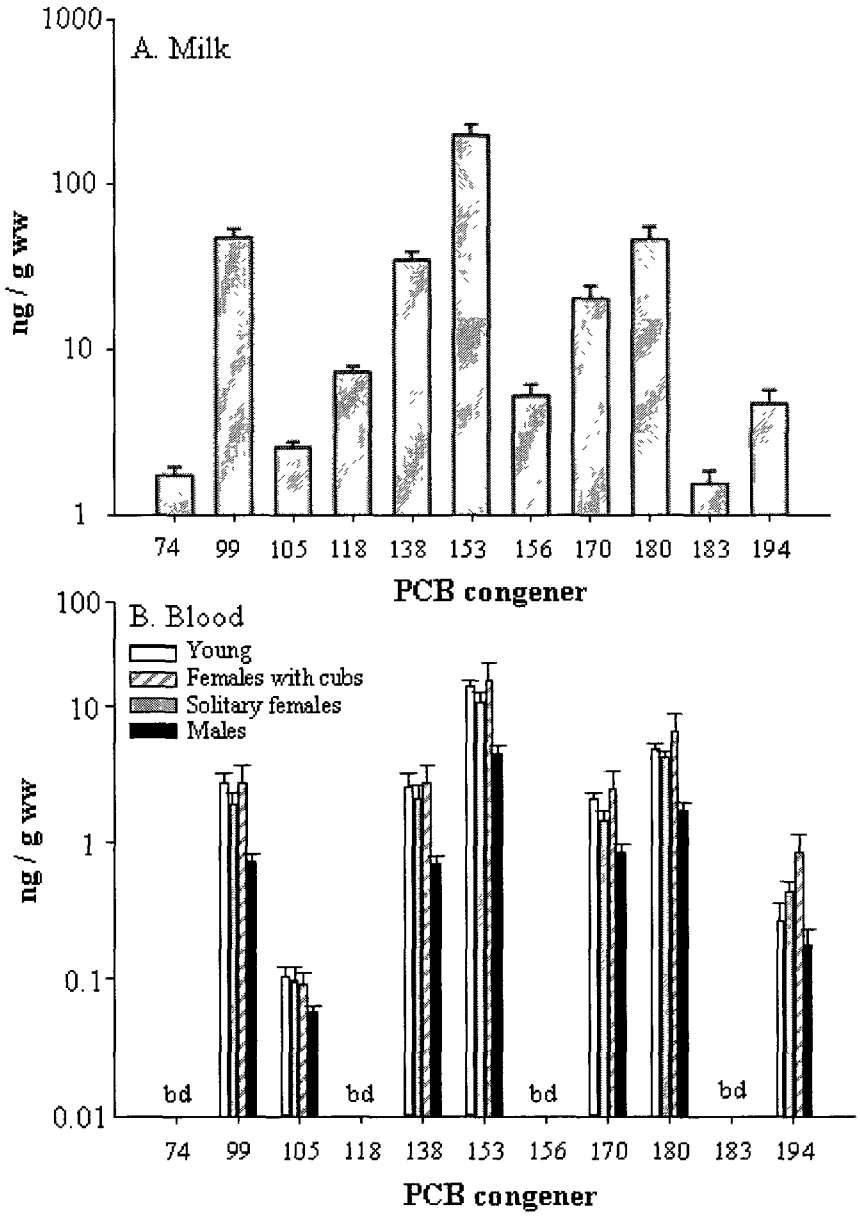

Figure 3.2 The concentrations (ng / g wet weight) of individual polychlorinated biphenyl congeners detected in polar bear milk (A) and polar bear blood by cohort (B). Different letters indicate significant differences between mean concentrations of congeners. Multiple analysis of variance (MANOVA) identified that all concentrations of congeners in blood were lower in males compared to other cohorts ($f = 2.8$, $p < 0.044$), except for PCB 105 ($p = 0.053$). The PCB congeners 74, 118, 156, and 183 were below detectable levels (bd) in blood. Data are shown on a log scale.

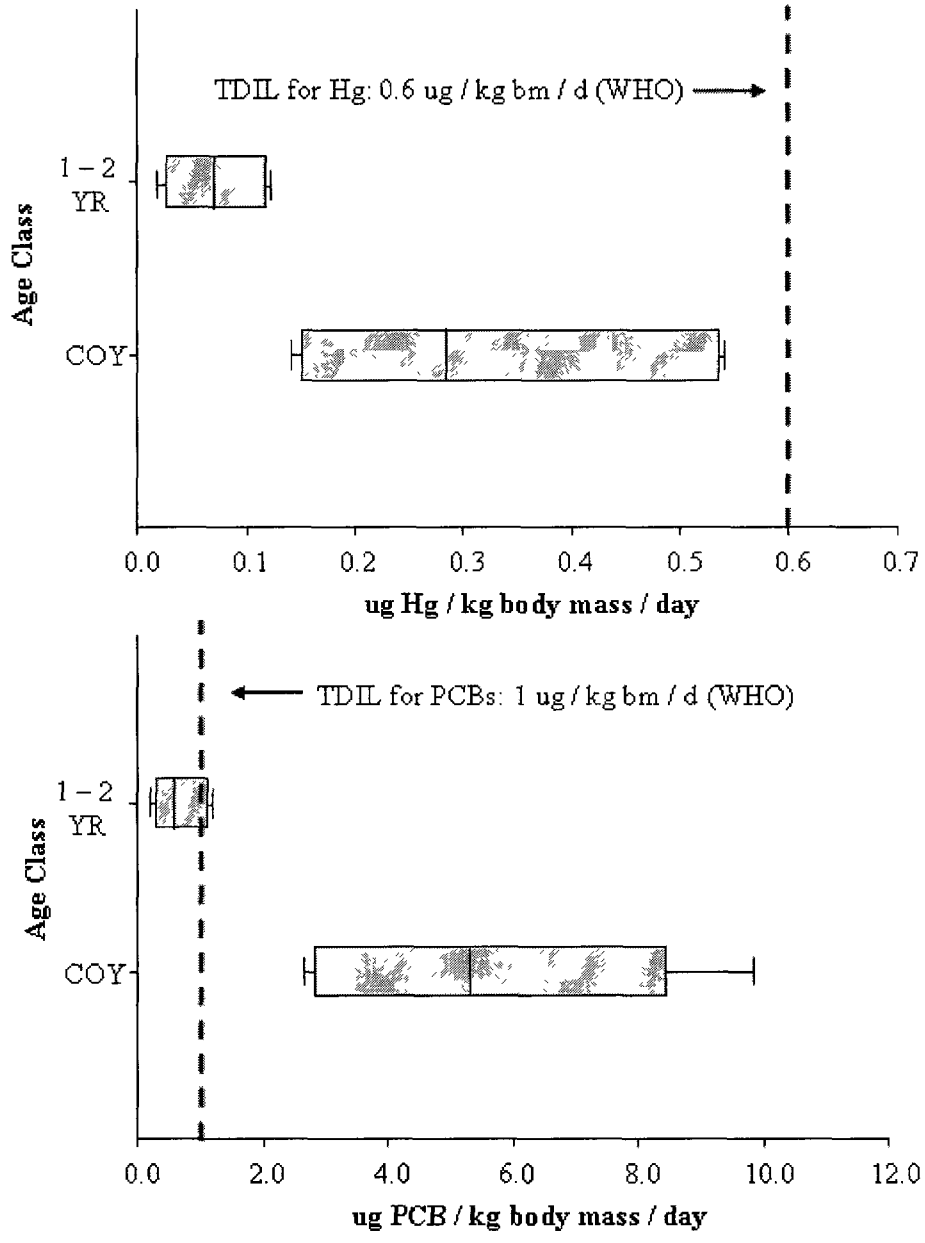

Figure 3.3 Intake levels of toxicants (ug of toxicant / kg body mass / day) through milk consumption for cubs of the year (COYs; Hg, n = 8; PCB, n = 9) and yearlings (YRL; Hg, n = 3; PCB, n = 5) compared to the tolerable daily intake levels (TDIL) established for adult humans for mercury (Hg, A) and polychlorinated biphenyls (PCBs, B). Boxes represent the 10^{th} and 90^{th} percentiles, the line indicates the median value (50^{th} percentile), and the error bars indicate the 5^{th} and 95^{th} percentiles. WHO, World Health Organization.

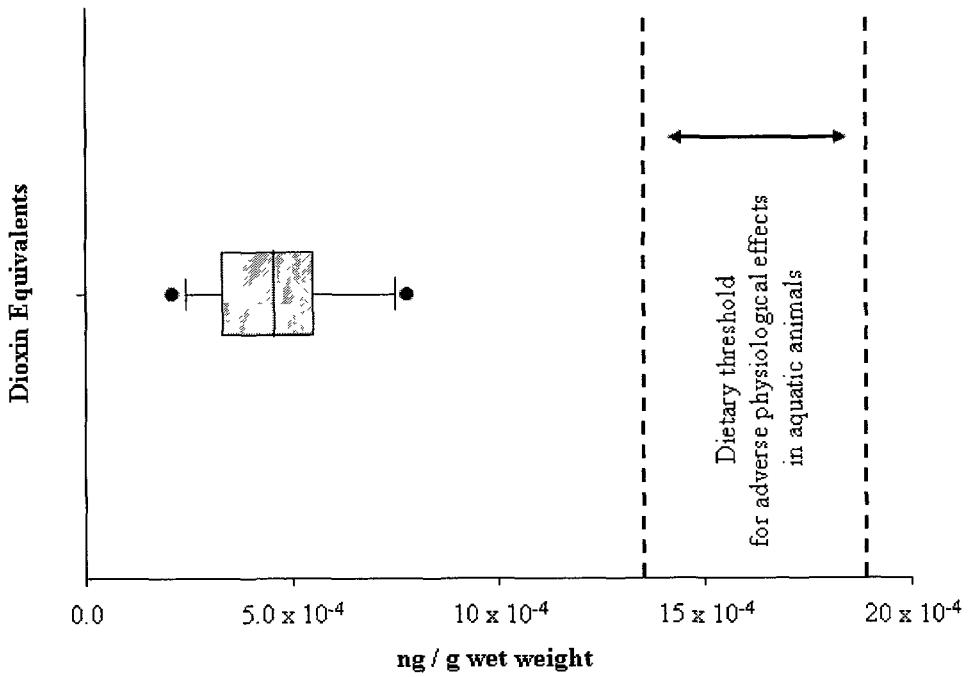

Figure 3.4 Concentrations of dioxin equivalency values (ng / g wet weight weight) for PCBs in polar bear milk compared to the dietary thresholds of adverse physiological responses established for aquatic mammals (Kannan et al. 2000). Boxes represent the 10^{th} and 90^{th} percentiles, the line indicates the median value (50^{th} percentile), and the error bars indicate the 5^{th} and 95^{th} percentile.

Table 3.1 The composition and toxicant concentrations in milk from Southern Beaufort Sea (SBS) and Chukchi Sea (CS) polar bears in comparison to previous data reported for Hudson Bay polar bears. Data are presented as the mean, with the range (if available) in parentheses. Data collected from, [1]Derocher et al. 1993, [2]Arnould and Ramsay 1994, [3]Polischuk et al. 1995, [4]Polischuk et al. 2001, [5]Polischuk et al. 2002. COYs = cubs of the year, YRLGs = yearling cubs.

	Polar bear (SBS/CS; present study)	**Polar Bear (Hudson Bay)[1-5]**
Hg, ng / g ww	26.9 (5.7 – 71.84)	--
PCB, ng / g ww	374.3 (160.0 – 690.0)	--
PCB, ng /g lw	2629.2 (547.3 – 5190.6)	2768 (COYs) 4758 (YRLGs)
Hg, milk : blood ratio (ww basis)	1.5:1	--
PCB, blood: milk ratio (ww basis)	17.1: 1	30:1
Total lipid, %	17.3 (< 1 – 30)	22 17 (COYs; YRLGs)
Triglycerides, g / 100 g lipid	98.7 (85.9 – 100)	--
$\delta^{15}N$, ‰	19.3 (16.0 – 20.5)	19.5 (milk protein)
$\delta^{13}C$, ‰	-24.3 (-26.6 to -22.0)	-25.4 (milk lipids)

CONCLUSION

This dissertation documents the concurrent concentrations of Hg and PCBs in polar bears which are apex predators of the northern Alaska ecosystem. This effort includes the food chain transfer and bioaccumulation/biomagnification of these contaminants, and the use of physiologically-based biomarkers to assess potential adverse biological effects. In addition to the inherent value to polar bear conservation and management, these studies discuss the advantage and limitation of health risk assessments of contaminants in free-ranging species. Limited access and sampling of key life stages (e.g. dependent cubs, immature animals, reproductive females with and without cubs) has resulted in an incomplete assessment of toxicant exposure and associated health risks of cohorts that are critical from both a population dynamics perspective and are expected to be the most impacted by contaminants. The present dissertation has made an effort to bridge this gap, but further study and increased sample sizes are needed for a more complete assessment. This dissertation discusses the possible multiple and interacting impacts of contaminants to polar bears and supports the use of this species as sentinels of environmental change.

Geographically, the present data aligns with previous studies reporting that Hg concentrations are lowest for many species in Alaska, including polar bears, and that concentrations increase eastward toward the Canadian, Greenland, and Norwegian Arctic (AMAP 2011; Born et al. 1991; Cardona-Marek et al. 2009). The causes of the geographical variations in Hg concentration is thought to result from differences in food web dynamics influencing Hg bioaccumulation and biomagnification, and differences in the Hg sources and abiotic transfer within these arctic regions (AMAP 2011; Cardona-Marek et al. 2009). Determining the geographical distribution of lipophilic contaminants such as PCBs is more difficult due to the trophic interactions,

physiological processes, and toxicodynamics that alter exposure and distribution through tissues. For example, the present studies report significant positive relationships between concentrations of PCBs, blood composition of lipids, and reduced body condition indices in Southern Beaufort Sea (SBS) polar bears. Although PCBs are not expected to directly result in reduced body condition (or vice versa), these data are consistent with previous studies reporting correlations between elevated concentrations of contaminants and poor nutritional status (Das et al. 2003; Fuglei et al. 2007; Krahn et al. 2001; McKinney et al. 2009) that have not been previously reported for species in the Alaskan Arctic.

Diet is the primary route for toxicant exposure for polar bears. The stable isotope values in blood support that ringed seal continue to be the primary dietary resource for SBS polar bears as previously reported by Bentzen et al. (2007). Variations in contaminant concentrations among age and sex cohorts, however, may result from varying proportions of ringed seal in the overall diet of individual bears. For example, the stable isotope values and concentrations of blood toxicants in female polar bears were consistent with a diet containing greater proportions of pelagic-feeding ringed seals (or prey of similar chemical composition) versus male polar bears that consumed greater proportions of benthic-feeding walrus and bowhead whale (or prey of similar chemical composition). The present findings are in agreement with macromolecule (protein and lipid) routing-based isotope mixing models performed by Cherry et al. (2010) reporting that male polar bears consumed more larger (i.e., lipid from bearded seal, beluga whale, and bowhead whale) than smaller prey (i.e., protein from ringed seals), and that the diets of female polar bears overlapped with males but tended to include less large prey. Changing climate conditions are expected to modify the composition and distribution of available prey species in

the Arctic. Divergent trophic transfer of contaminants between male and female polar bears is anticipated to widen with the continued loss of arctic sea-ice and changes in habitat use patterns.

Within the SBS sub-population of polar bears, the circulating concentrations of Hg and PCBs were highest in females (we did not have access to neonates). This finding challenges previous expectations that contaminants would be lower in reproductive females compared to males due to the gestational and lactational offload of contaminants to offspring. Recent study of contaminant concentrations of polar bears in Canada and East Greenland sub-populations also reported greater concentrations of contaminants among adult reproductive females compared to adult males (McKinney et al. 2009; McKinney et al. 2011a). Previous studies reported that blood concentrations of many toxicants correlated with those in the adipose tissues of polar bears (Bentzen et al. 2008). Relationships between blood and other tissue compartments, however, may not be consistent across age and sex cohorts limiting the interpretation of possible adverse health impacts. Furthermore, it is uncertain the extent of which toxicant concentrations fluctuate within and between tissue compartments during varying temporal scales. It would appear that the interpretations of contaminant exposure and markers of body burden are indeed very tissue specific and may be changing over time (decades).

Polar bear milk contained detectable concentrations of both Hg and PCBs supporting the theory that lactational transfer limits the bioaccumulation of contaminants in reproductive female polar bears and that lactation represents an additional route of elimination not available to other cohorts. Greater circulating concentrations of PCBs in reproductive females compared to males, despite this lactational transfer of contaminants, could suggest that other factors are driving or disrupting the expected bioaccumulation pattern for toxicants (**Figure 1**), and / or that lactational

elimination of contaminants is limited in the overall scheme. Female SBS polar bears during spring of 2007 had lower body condition scores than spring 2003, but males during these years had similar body condition scores. Previous studies also reported thinner animals, smaller body stature, and reduced cub recruitment of polar bears in the SBS sub-population correlating to recent sea-ice declines (Rode et al. 2010). Reproduction is energetically expensive for female polar bears (Derocher et al. 1992; Derocher and Stirling 1993). It would follow, therefore, that during years that female polar bears cannot accrue adequate reserves to support gestation and lactation that females would abandon maternal denning and limit lactational demands. Although this has been reported for Hudson Bay polar bears (Derocher et al. 1992), abandonment of maternity dens or reductions in the duration of lactation has not been reported for SBS polar bears. Recent reports, however, suggest a greater loss of dependent cubs by presumed drowning and describe possible abandonment of cubs in poor body condition (Durner et al. 2011). Females that forego reproduction or abandon cubs could be placed on a different trajectory leading to an elevation of toxicants among prime aged females (**Figure 1**). The data presented in this dissertation support this hypothesis, but must be corroborated by a study design combining contaminant concentrations with the reproductive history of specific female polar bears.

The present study reports relatively high concentrations of contaminants in young (ages 1 - 5 years) polar bears, consistent with previous reports in polar bears and other arctic and sub-arctic species (Hansen et al. 1990; Polischuk et al. 1995; Espeland et al. 1997; Beckmen et al. 2003; Bentzen et al. 2008). Young polar bears rely on maternal nutrition for up to three years to support their rapid growth demands (Derocher et al. 2003). During this period, females provide lipid-rich milk and also share prey items with dependent young during this critical growth and development phase (Amstrup 2003). Similar concentrations of contaminants and stable isotope values in blood

of female polar bears, in blood of dependent young, and in polar bear milk support equivalent dietary inputs (i.e., incorporation of prey nutrients into milk and prey sharing) among females and their young. Fetal, neonatal, and juvenile animals are expected to be the primary cohort of concern for adverse biological effects due to the development and maturation of immune, endocrine and organ functions (Klassen 2001; Oskarsson et al. 1998). Unfortunately, the young cohort is often underrepresented in studies of risk assessment due to contaminants because of the difficult logistical constraints of safely capturing and sampling of young animals. This study introduced the sampling technique for acquiring milk samples, with matched blood samples, from females with cubs to address these important cohorts once outside the den. We report that estimated daily intake levels through milk consumption for cubs of the year exceeded recommended toxicity thresholds for PCBs (established for human exposures) largely due to their greater reliance on milk for nutrition (COYs, 469 g / day; yearlings, 131 g / day; Arnould 1990). Milk could only be collected from female polar bears during mid to late lactation, and thus these toxicity estimates likely underestimate the true contaminant exposure for nursing young. The present report also did not address gestational transport of contaminants to the developing fetus. Contaminant exposure during critical stages of neonatal development requires further study to determine if toxicants exacerbate or compound health effects due to other causes such as limitations in nutrition or pathogen exposure.

This dissertation focused on blood concentrations of contaminants in polar bears to examine the same temporal window of physiologically-based biomarkers examined in blood. These data and chapters, however, also highlight how toxicodynamics differ between tissue matrices (**Figure 2**) and influence the interpretation of adverse biological impacts. The present findings in polar bear suggest that toxicodistribution of lipophilic contaminants involves interactions between the

specific congener's affinity for lipids (e.g., Kow values) in the blood and adipose compartments. For example, the present study identified the importance of neutral lipids, triglycerides and free fatty acids, in the disposition dynamics of PCBs into blood of polar bears. Previous studies using gravimetric methods have likely masked these interactions that have nutritional and physiological importance in addressing toxicodynamics and adverse effects (including biomarkers).

The assessment of biomarkers for adverse effects of contaminants is challenging in wildlife studies when time and dose of exposure are unknown and cannot be controlled. In the present report, we examined variations in selenium status and thyroid status in polar bears. Cause and effect relationships could not be definitively determined with the study designs described. Hg concentrations were associated with elevations in selenium status in polar bears suggestive of oxidative stress, although these positive correlations may also be a physiological mechanism of sequestering Hg compounds for excretion. Positive and negative associations between contaminants and biomarkers of thyroid status indicated possible endocrine disruption in SBS polar bears consistent with displacement of thyroid transport proteins and deficiencies of selenium. Thyroid hormone changes due to contaminants, however, could not be separated from the ***equally possible impacts of nutritional stress and recent changes in physiological status,*** especially for female polar bears. These data will be useful in predicting and monitoring the current and ongoing contaminant-environmental stressors for polar bears in their changing arctic environment.

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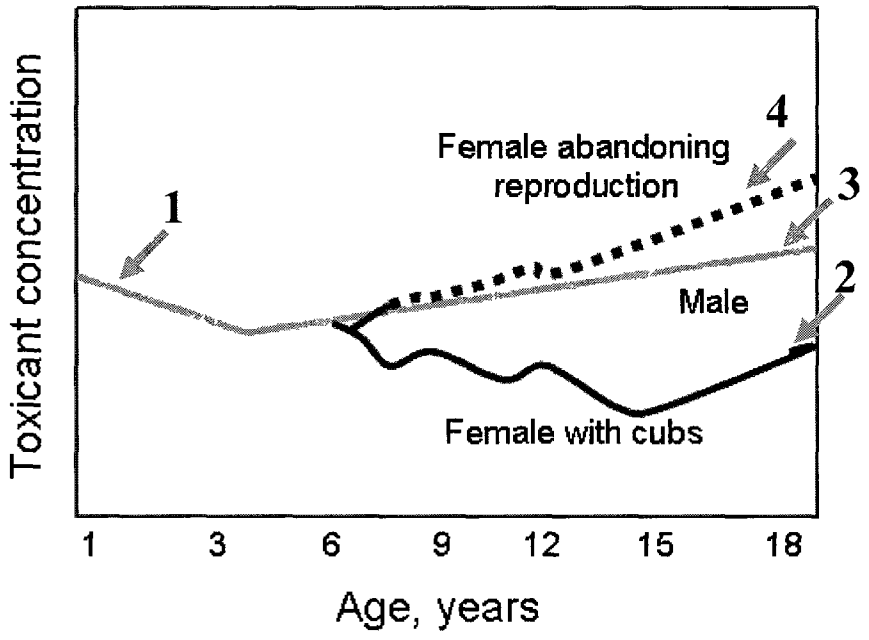
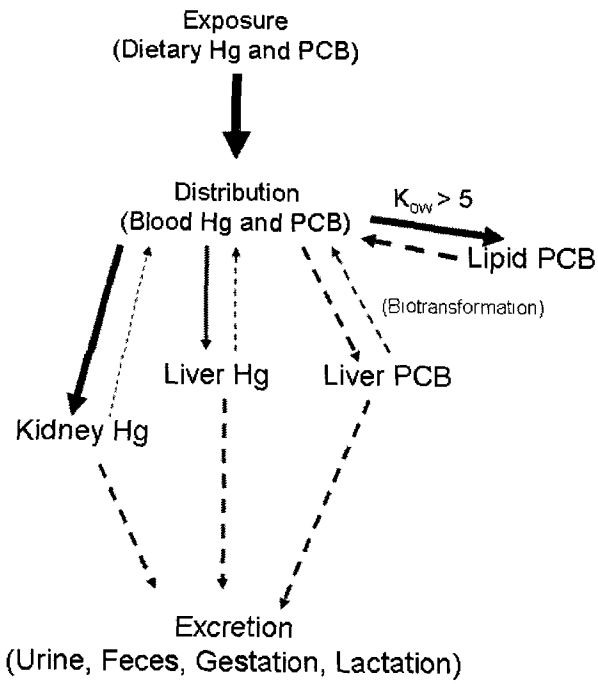


Conclusion Figure 1. Conceptual model of bioaccumulation of contaminants in polar bears. Traditional dogma for the bioaccumulation of contaminants suggests that reproductive female polar bears offload their contaminant burden to their offspring, thus leading to the highest concentrations in young animals (arrow 1), and the lowest concentrations in reproductive females (i.e., females with cubs; arrow 2). Males that cannot offload their contaminant burden via reproductive excretion routes bioaccumulate contaminants as they age (arrow 3). If females cannot accrue adequate body reserves to support gestation and lactation, female polar bears may be placed on a different trajectory resulting in elevated concentrations of contaminants with age (arrow 4).



Conclusion Figure 2. Conceptual model of the distribution of mercury (Hg) and polychlorinated biphenyls (PCBs) in polar bears after dietary exposure. Arrow thickness represents the relative concentration of contaminants deposited in tissue compartments.