Factors Affecting Persistent Organic Pollutant (POP) Accumulation in British Columbia Grizzly Bears (*Ursus arctos horribilis*)

by

Jennie Rebecca Christensen
B.Sc., University of Alberta, 1996
M.Sc., University of British Columbia, 2002

A Dissertation Submitted in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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University of Victoria

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ABSTRACT

This thesis characterizes major factors influencing the accumulation of polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and polybrominated diphenyl ethers (PBDEs), in grizzly bears.

Dietary differences among grizzly bears have significant implications for contaminant concentrations and patterns. While salmon-eating bears were dominated by lipophilic PCBs, OC pesticides, and lower-brominated PBDEs, non-salmon-eating bears were dominated by the more volatile PCBs and OC pesticides and higher-brominated PBDEs (e.g. BDE-209). Overall, the ocean-salmon-bear pathway appeared to preferentially select for those contaminants with an intermediate log K_{ow} ~6.5, with salmon delivering up to 70% of OC pesticides, 85% of PBDEs and 90% of PCBs to grizzly bears.

Fat utilization by grizzly bears during hibernation results in significant contaminant concentration increases in residual fat ("concentration effect"). Overall, ΣPCBs increased by 2.21 times from pre- to post-hibernation, and ΣPBDEs by 1.58

times. Interestingly, the patterns of the two distinct pre-hibernation grizzly bear feeding ecologies (salmon- and non-salmon-eating) converged during hibernation, suggesting that shared metabolic capacities drive POP patterns during hibernation.

Relative to salmon, grizzly bears have extremely low biomagnification factors (BMFs) for PCBs (0.147), compared to other marine mammals. Low BMF values were a result of >90% depuration (loss) of PCBs through contaminant metabolism and excretion. The results suggest that grizzly bears only metabolize PCB congeners with *meta*- and *para*- vicinal hydrogen (H) atoms, suggesting that they have active cytochrome (CYP) P450 2B/3A-like metabolic enzymes. However, congeners structurally resistant to metabolic biotransformation, and those with *ortho*- and *meta*- vicinal H atoms, were not readily metabolized, but rather were lost through excretion. This was evidenced by a significant relationship between total retention (R_{total}) of those congeners and log K_{ow}, as well as a lack of change in that relationship during hibernation.

Vegetation and the terrestrial food web were dominated by PBDEs and volatile OC pesticides and PCBs, while salmon and the marine food web were dominated by lipophilic PCBs and OC pesticides, mirroring patterns in grizzly bears within their respective food web. Following consumption of these various foods by the grizzly bears, fecal material closely resembled food in contaminant pattern, suggesting that many of the contaminants may go unabsorbed.

While previous work identified major factors (e.g. age, sex, diet) influencing POP behaviour in wildlife and food webs, this research highlights the need to refine our ideas about those factors in order to better assess chemical health risk in wildlife by considering: 1) individual differences in feeding behaviour; 2) integrated dietary histories

(temporal changes); 3) unique biological traits affecting POP fate; 4) modes of POP loss other than metabolism; 5) selection of the most recalcitrant congener for more robust analysis of POP behaviour; 6) use of non-invasive techniques to study diet and POP exposure; and, 7) tissue residue guidelines underestimate health risks. Our results also suggest that PBDEs show POP-type characteristics as defined under the Stockholm Convention, and thus should be regulated.

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I want to give special acknowledgement to the Heltsiuk First Nations of Bella Bella, particularly Larry Jorgenson, Martin Campbell and Doug Brown. They generously donated the Koeye River Lodge, their time, their boats, their cooking skills and their friendship to me and to the project. I am so grateful for my extraordinary experiences in Koeye River, fishing, canoeing, bear watching, hiking and laughing with friends. You and Koeye have forever changed me.

Lastly, but most importantly, I want to thank the grizzly bears, especially my little Zoolander, who allowed us to sit with him for hours at a time, and experience his greatness. I only hope his spirit will be in my Lily, who also came from Koeye.

Figure 1. Salmon-eating grizzly bear observed in Koeye River, Coastal British Columbia. Photo by Jennie Christensen.

I dedicate this work to *my* little cub

Lily Anika Chourmouzis

INTRODUCTION

Throughout recent history, grizzly bear (*Ursus arctos horribilis*) populations in North America have been negatively associated with human settlement and the exploitation of environmental resources. Human encroachment into remote wilderness has resulted in the loss of prime grizzly habitat and decreased food availability and quality. Habitat fragmentation is considered the greatest threat to current day grizzly bear populations (1). However, grizzly bears are also facing direct mortality through trophy hunting, poaching, vehicular collisions on logging roads and highways, and other human-bear interactions (2).

In 1991, the prairie population (Alberta, Saskatchewan and Manitoba) in Canada was designated as "*extirpated*" and the northwestern population (British Columbia, Yukon, Northwest Territories, Nunuvat and Alberta) was designated as "*special concern*" (reconfirmed in May 2002) by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). British Columbia (BC) has designated the grizzly bear as "blue-listed". Even long term genetic viability in grizzly bear reserves of the Khutzeymateen Valley (443 km^2) and the Kitlope Valley (3887 km^2) in BC may be compromised through poaching and hunting activities (3). With the exception of Alaska, only dwindling populations remain in a few areas in the USA, such as Yellowstone National Park (Wyoming, Montana and Idaho) (2).

At present, there are conflicting estimates as to the number of grizzly bears in BC. Prior to European settlement, BC was home to 25,000 grizzly bears, a number that is now thought to be closer to the number of grizzly bears in all of present-day Canada (4). The Ministry of Environment (MoE) now estimates that there are 10,000 – 13,000 grizzly

bears in BC (5), while Banci (4) estimates 3,200 grizzly bears on the north coast of BC and only 90 in the more populated areas.

Certain characteristics in the life history of the grizzly bear make it vulnerable to human activities and natural events. Grizzly bears are large, solitary mammals that require vast expanses of undisturbed habitat. It is estimated that females require between 200 and 600 km^2, while males require 900 to 1800 km^2 (6). Grizzlies are also relatively long-lived, reaching ages up to 25 years old. They do not reach sexual maturity until the age of four to eight years for females and five to ten years for males. They also have a very low reproductive rate, breeding in three to four year intervals and producing between one and three offspring at one time, with a lifetime total of approximately eight cubs (5).

Generally, grizzly bear population density is associated with availability of meat-type foods in their diet. The largest source of dietary meat is spawning salmon and in areas where this food is available for the majority of the non-denning season, grizzly bear population densities are the greatest (7,8). Salmon consumption has been linked to larger individual bear size, earlier age at first reproduction, and increased litter sizes, demonstrating the nutritional value and importance of the marine food web to some grizzly bear populations (7). In the interior, grizzly bears are mainly herbivorous and fructivorous, relying on plants, berries, roots, insects and nuts, with some supplementation of terrestrial mammals and freshwater fish, where available (7-9).

Food availability and quality is thus integral to the ability of grizzly bears to store energy for hibernation, reproduce and survive. Hence, the deterioration of food quantity and quality has resulted in negative consequences for grizzly bears at the population

level. For example, in the protected area of Yellowstone National Park, the reintroduced bears are vulnerable due to the potential loss of pine nuts through the looming threat of the pine beetle (10). Coastal salmon-eating grizzly bears are also feeling the effects of depleted salmon stocks due to overfishing, climate change and salmon habitat destruction/loss (11,12). As well, pesticide spraying through current forestry practices hinders the growth of many plant species that grizzly bears rely upon in the spring when they emerge from hibernation (13). Over the horizon, a previously unrecognized risk may also potentially hinder the future of the grizzly bears: dietary exposure to Persistent Organic Pollutants (POPs).

Persistent Organic Pollutants (POPs). Under the Stockholm Convention, POPs are defined as substances that are persistent in the environment, distributed widely geographically, have the propensity to bioaccumulate in fatty tissue and are toxic to wildlife and humans. In this research, the exposure to and behaviour of three major classes of POPs in grizzly bears were assessed, including those that have been largely regulated in industrialized nations (polychlorinated biphenyls [PCBs] and organochlorine [OC] pesticides) and those that are in current use (polybrominated diphenyl ethers [PBDEs]) in North America. However, while only PCBs and some OC pesticides are currently considered POPs under the Stockholm Convention, other OC pesticides (e.g. endosulfan) and PBDEs are under consideration, and thus, in this thesis for simplicity, are also termed POPs.

PCBs are a class of heat-resistant commercial compounds that were used widely in the industrialized world in electricity transformers, heavy industry and a number of consumer applications from the time of the Second World War to the mid-1970s. There

are 209 possible PCB congeners related to their degree of chlorination (Figure 2), although only 135 have been found in environmental samples (14). Between 1930 and 1970, over 600 million kilograms of PCBs were used in North America, 15% of which were released to the environment through legal and illegal use and disposal and accidental releases (15). Their chemical properties make them resistant to degradation; therefore, they persist in the environment for many years and bioaccumulate up both terrestrial and aquatic food webs. They were banned in the US in 1976, as it became clear that they were globally ubiquitous (16), magnified to extremely high concentrations in top predators (17,18), and were highly toxic (19-22). PCBs are considered of greatest global ecotoxicological concern due to their continued use in developing nations, production, discharge, global transport, and biomagnification potential, presenting a risk to vulnerable populations of both humans and wildlife (23).

Organochlorine (OC) pesticides, such as 4,4'-dichlorodiphenyl trichloroethane (DDT), chlordane (ΣCHL), hexachlorocyclohexane (HCH) and hexachlorobenzene (HCB), are persistent and bioaccumulative contaminants that are highly toxic to many organisms, including wildlife and humans (21,24,25). As the "atomic bomb" of pesticides, DDT was first used during World War II, after which it was used to control agricultural pests and insects that carried diseases like malaria and yellow fever. In 1972, the US Environmental Protection Agency (EPA) cancelled all use of DDT on crops, although limited use still continues for disease control. While no longer used in the US, DDT use continues in other parts of the world to control malaria (26). DDT breaks down into two major metabolites, dichlorodiphenyl dichloroethylene (DDE) and dichlorodiphenyl dichloroethane (DDD). DDT and its metabolites are highly lipophilic

and biomagnify in food webs to top predators where toxic effects, such as eggshell thinning, have been observed in birds (21,24).

Chlordane was first registered in 1948 and was used as a pesticide for agricultural crops, residential lawns, gardens, and termite control until a voluntary ban was introduced in 1988 (27). Today, the United States continues to manufacture chlordane, but it can only be used in or sold to foreign countries. Technical chlordane consists of 50 related chemicals, but is primarily composed of *cis*- and *trans*-chlordane, heptachlor, and nonachlor (28,29). *Cis*- and *trans*-isomers of chlordane and the component heptachlor may be metabolized to epoxides, oxychlordane, and heptachlor epoxide. Technical chlordane's parent compounds, as well as its metabolites, have been detected in both human and wildlife tissues (30-32). While the parent chlordanes are generally found in the kidney and liver, the metabolites tend to accumulate in the fat (27).

Hexachlorocyclohexane (HCH) is produced through photochlorination, which yields a mixture of isomers: α-HCH, β-HCH, δ-HCH, and γ-HCH (33). Only γ-HCH (lindane) is still used today as an agricultural insecticide and to control head lice. Like other organochlorines, HCH has relatively high vapor pressure and can, therefore, be easily transported atmospherically to remote locations: as shown through its detection in high elevation snowpacks (34). Although HCH isomers are generally less lipophilic than other organochlorines, they accumulate in wildlife, and is especially observed for γ-HCH (35,36).

Hexachlorobenzene (HCB) was widely used as a pesticide to protect seeds against fungus until 1965. It was also used to make fireworks, ammunition, and synthetic rubber. Currently, there are no commercial uses of HCB in North America (37). However, HCB

breaks down very slowly in the environment and due to its lipophilic nature accumulates through the food web to top predators where it can impair wildlife health (36,38,39).

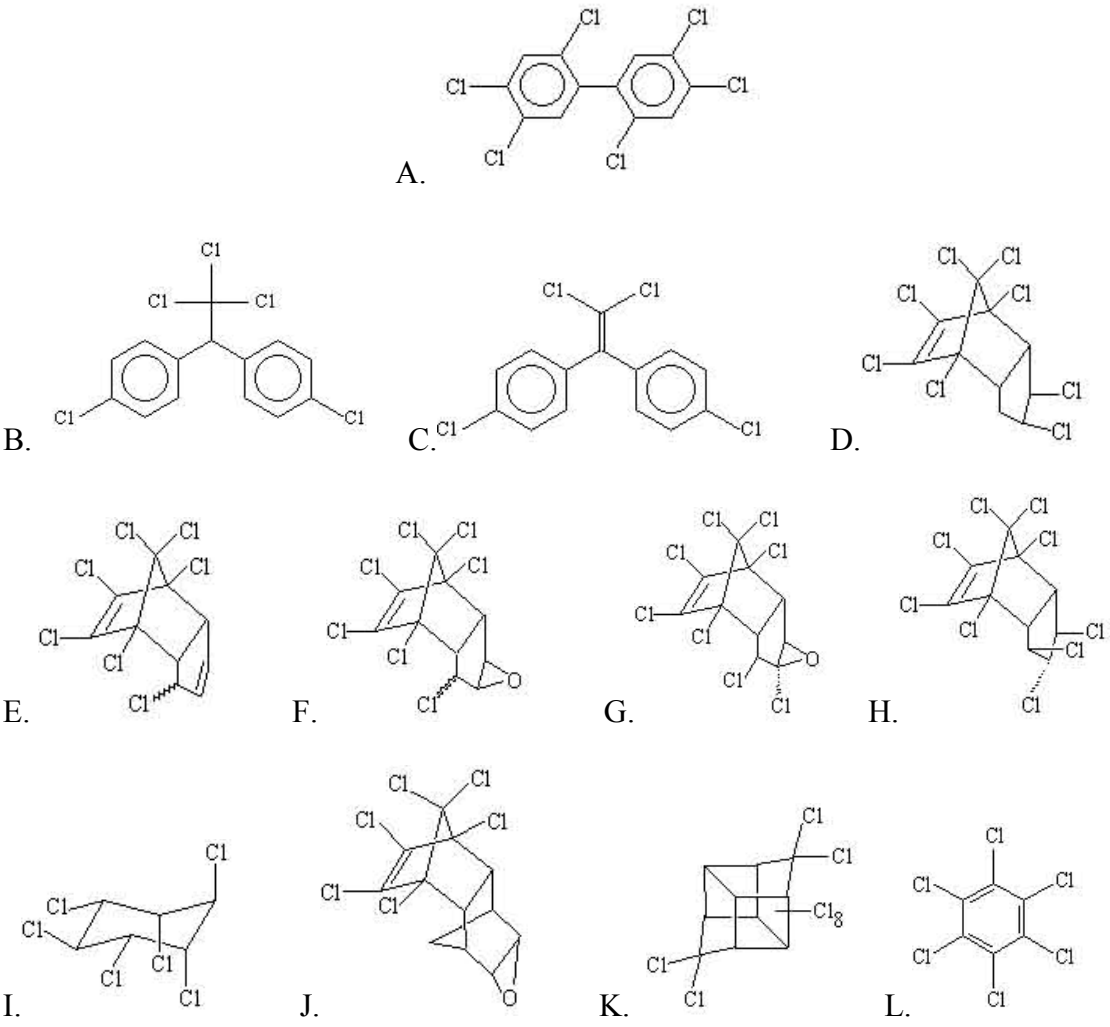

Figure 2. Molecular structures for the major organochlorine contaminants analyzed in this study. A) Polychlorinated biphenyl congener 153 (CB-153), B) 4, 4'-DDT, C) 4, 4'-DDE, D) α-chlordane, E) heptachlor, F) heptachlor epoxide, G) oxychlordane, H) *trans*-nonachlor, I) δ-HCH (lindane), J) dieldrin, K) mirex, and L) HCB. Drawings taken from: http://www.nefsc.noaa.gov/nefsc/publications/tm/tm157/tm157struc.htm

The current-use PBDEs belong to a family of brominated flame retardants used extensively today in textiles, fabrics and consumer electronics. Similar to PCBs, PBDEs have 209 possible congeners reflective of their degree of bromination (Figure 3),

although only about 40 are found in the commercial products. These commercial PBDE products consist of three technical formulations: penta- (PentaBDE), octa- (OctaBDE) and deca-bromodiphenyl ether (DecaBDE), reflecting the dominant degree of chlorination present in the formulation. At present, only the fully brominated DecaBDE is currently used in Europe, as both Penta- and Octa-BDE formulations have been banned since 2004. In most parts of Asia, all formulations are still in use (with DecaBDE dominating the market), while Japan has regulated the use of PentaBDE (40). At present, Penta- and Octa-BDEs are under consideration for regulation in Canada.

PBDEs have been detected in various environmental media, including sediments (41,42), wildlife (43-48) and humans (49-52). Exponential increases of PBDEs in Great Lakes fish (53) and Columbia River whitefish (54) highlight this chemical class as an important and emerging toxicological concern for wildlife in aquatic food webs in North America. However, more recent research suggests that terrestrial wildlife may be at greater risk to exposure due to the predominance of PBDEs in terrestrial food webs (47,48). PBDEs have been associated with both neurological dysfunction and endocrine dysruption (55-60).

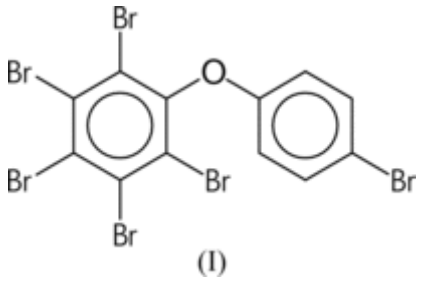

Figure 3. Molecular structure of polybrominated diphenyl ether (PBDE). Drawing taken from: http://journals.iucr.org/e/issues/2002/10/00/cv6147/cv6147scheme1.gif

Global Transport of POPs. Whether the source of these POPs are local or are from developing nations on the other side of the world (e.g. China) their atmospheric and oceanic transfer results in their incorporation into the environments and food webs of every nation, irrespective of national boundaries and regulations. Air concentrations of POPs vary around the world, generally depending on the proximity to the source of the particular contaminant. Of the Asian countries, China has the highest concentrations of OCs, likely as a result of their status as the world's second largest producer and consumer of pesticides, accounting for 14% of the world total (61). To illustrate this point, HCB concentrations in the air of the temperate northern hemisphere, where HCB is no longer in use, were measured at 50 pg/m^3 (62), while in China HCB concentrations were measured at concentrations up to 460 pg/m^3 (63).

Atmospheric POPs are repeatedly volatilized or revolatilized in warmer locations, transported various distances, and then deposited through condensation in cooler environments, such as the oceans and the arctic. This phenomenon is appropriately termed the "grasshopper effect", and has resulted in the air of remote locations containing a plethora of POPs either in a gaseous phase or adhered to particles originating from elsewhere on the earth (64-68).

Vegetation has a large surface area, often covered by a lipid-rich cuticle, and as such, has been suspected of playing an important role in the global cycling and distribution of POPs (69). Therefore, it has been proposed that plants from remote areas are excellent indicators of atmospherically transported POPs. Plants sequester POPs through translocation from soil to roots to xylem, or through deposition from the atmosphere onto the plant surface (adsorption) with possible uptake through stomata or

cuticle into the phloem (70). Specifically, higher log K_{oa} POPs (e.g. higher brominated PBDE congeners) are associated with particulates, therefore, their uptake by the plant occurs via particulate deposition and adsorption onto the plant's surface (71). Conversely, lower log K_{oa} POPs (e.g. lower brominated PBDEs) are associated with gaseous deposition onto plants (71).

When deposition of atmospherically transported POPs occurs in mid- to high-latitude oceans (e.g. Northeast Pacific Ocean), the fate of those contaminants is driven by other biogeochemical processes, such as phytoplankton uptake and subsequent sinking to deep waters (72). Surface sea water in the North Pacific Ocean had Total PCB concentrations (ΣPCBs) of 24 pg/L, ΣDDT of 1.2 pg/L, and ΣHCHs of 250 pg/L. The dominance of ΣHCHs over other OC pesticides and PCBs in ocean water clearly demonstrates the high volatility of ΣHCH and ability to be transported over great distances, as well as the overall lack of ΣHCH loss through sedimentation, food web uptake and/or revolatilization from the surface waters. Conversely, PCBs and other OC pesticides are more lipophilic (higher octanol/water partition coefficient – log K_{ow}) and thus may be more readily taken up and accumulated in aquatic biota relative to HCH, and subsequently, in lower concentrations in water column (73).

Grizzly Bear Exposure to POPs: Why the Concern? As the grizzly bear is a terrestrial mammal, it has been largely overlooked as a potential candidate for significant POP accumulation. This is due to lower POP concentrations thought to occur at the base of terrestrial food webs, in combination with an often shorter and less complex food web precluding POP amplification. However, many coastal populations of grizzly bears rely heavily on Pacific salmon in the fall (7,74), and some interior populations rely on

cutthroat trout (*Oncorhynchus clarki*) for dietary supplementation (75). "Maritime" (defined as a species which feeds and lives at the marine/terrestrial interface) grizzlies are known to consume large quantities of salmon, which have been estimated to make up >60% of their diet during the fall (8). Reliance on foods within an aquatic/marine food web may increase the degree of exposure to POPs, as well as their amplification in this top predator.

The cornerstone of this study, an assessment of the factors affecting the behaviour of POPs in grizzly bears, is the determination and characterization of dietary exposure to POPs through the grizzly bears' reliance on terrestrial and marine food webs. This thesis was built upon the recent work clearly showing that Pacific salmon are returning from the open ocean with POPs, including PCBs, OC pesticides, and PBDEs (76-79). Pacific salmon bioaccumulate POPs through bioconcentration from ocean water (via gills) and biomagnification through their diet (80). Coho (*O. kisutch*), pink (*O. gorbuscha*) and chum (*O. keta*) salmon were found to have ΣPCBs ~ 5 ng/g wet weight (ww), sockeye salmon (*O. nerka*) had ΣPCBs ~10 ng/g ww, and Chinook salmon (*O. tshawytscha*) had ΣPCBs ~15 ng/g ww (81). Hoekstra et al. (82) measured ΣPCBs in pink salmon from the southern Beaufort-Chukchi Sea at 42 ng/g lipid weight (lw), ΣDDT at 29 ng/g lw, ΣCHL at 21 ng/g lw, and ΣHCH at 22 ng/g lw. Hamilton et al. (83) found wild pink salmon from coastal BC had ΣPCBs of ~50 ng/g lw, while wild sockeye salmon had ΣPCBs of 75ng/g lw. In regards to PBDEs, wild BC Chinook salmon had some of the highest concentrations at 2.26 ng/g lw relative to other BC salmon (0.130 ng/g lw), as they tend to feed at higher trophic levels (79).

Pacific salmon represent an important food source for a number of species in the marine environment, such as killer whales (7). BC's male southern resident killer whales that rely heavily on Chinook salmon are some of the most contaminated marine mammals in the world, having concentrations of PBDEs measuring 942 ± 582 ng/g lw (43) and 146,000 ng/g lw ΣPCBs (18), approximately 100 times the concentrations observed in other wildlife (84-86). Thus, terrestrial mammals that rely heavily on BC salmon, such as the maritime grizzly bears, may also be at risk for significant POP accumulation.

The terrestrial food web may also pose a threat to grizzly bears. Mounting evidence indicates that top predators of terrestrial systems may be at greater risk of exposure to PBDE flame retardants than those of aquatic systems, especially to deca-BDE (44,45,47,48,87). BDE-209 concentrations in the liver of red fox (*Vulpes vulpes*), a terrestrial food-based organism, reached up to 760 ng/g lw (47). So, while salmon is available to only some populations of grizzly bears in BC, all grizzly bears rely heavily on terrestrial foods throughout their non-denning season.

Accumulation of POPs can eventually lead to impacts on the health of top predators like the grizzly. Their susceptibility and sensitivity to these POPs is presently unknown, since no previous investigations have been conducted on this species. In order to assess the risks of POPs to grizzly bears, ecological (e.g. hibernation) and physiological (e.g. metabolic capacity) aspects needed to be explored, alongside prerequisite dietary exposures. These facets of the grizzly bear may have profound influence on the behaviour and accumulation of POPs, as well as on the health risks associated with direct exposure to the individual or indirect exposure through POP transfer to offspring.

Grizzly bear hibernation is one such unique attribute of their ecology that could have profound influence on risks posed by POP exposure and accumulation. Grizzly bears rely heavily on stored fat reserves during their approximate five-month hibernation, as fat is of high energy yield per unit weight and is in a non-hydrogenated form (88). Through utilization of these fat reserves during this fasting episode, POPs may be remobilized into the blood stream and ultimately concentrate (increase in concentration) in the residual fat, as demonstrated in fasting polar bears (89,90).

While biotransformation of parent compounds into less lipophilic metabolites potentially aids in their elimination, these metabolites are still lipophilic and can be highly toxic and endocrine-disruptive (91,92). Thus, metabolites may also pose increased health risks to exposed grizzly bears. Metabolic capacity is dictated by the presence or absence of certain cytochrome (CYP) P450 xenobiotic metabolizing enzymes in an organism. There are 22 CYP families known to exist in mammals, three of which are important to the metabolism of anthropogenic substances: CYP1, CYP2 and CYP3. Planar aromatics, such as planar PCBs, generally induce CYP1A enzymes, while globular molecules, such as *ortho*-substituted PCBs, induce CYP2B and CYP3A (93). Phase I metabolites are hydroxylated (-OH group added) and can be further metabolized (Phase II) through conjugation (93). Some of these metabolites, although more water soluble than their parent counterparts, are lipophilic and can, therefore, bioaccumulate (94). Metabolites are also highly toxic, sometimes to a larger degree than the parent compounds (95-97).

Thesis Objectives

Aspects of the grizzly bear, that influence POP behaviour, accumulation and associated health risks, including feeding ecology, hibernation, metabolic capacity, uptake, and excretion, are examined within the following chapters of this thesis:

Chapter One examines how the varying reliance on terrestrial and marine food webs by grizzly bears plays a vital role in POP patterns and accumulation.

Chapter Two examines how hibernation, a unique facet of grizzly bear ecology, plays a role in POP concentrations and patterns. It is also inferred which POPs are more liable to accumulate and those that are more likely to be depurated by the bears.

Chapter Three assesses the ability of grizzly bears to metabolize PCBs and infers which metabolic enzymes may be present and active in the bears during feeding and fasting phases. With the unique grizzly bear model developed for this work, previous methods used to infer accumulation and metabolism (biomagnification factors and metabolic indices) are expanded upon by a) minimizing assumptions used in those calculations and b) differentiating between metabolism and excretion, as modes of depuration.

Chapter Four examines the behaviour of POPs in a remote grizzly bear population by analyzing and comparing POPs in grizzly bear foods to that of both their fecal material and fat tissue. This chapter brings together all previous work on the bears from Chapters One through Three, and characterizes dietary influence (terrestrial vs. marine food webs) on POP exposure, depuration of POPs through excretion and metabolism, and overall POP accumulation in the bears.

And finally, in *Chapter Five*, the information obtained on the behaviour of POPs in grizzly bears from the research conducted within this thesis is used to reassess the chemical risk assessment process for wildlife. Research areas that require further investigation, before the full implications to POP-associated health effects for grizzlies can be realized, are also discussed.

CHAPTER 1: PERSISTENT ORGANIC POLLUTANTS IN BRITISH COLUMBIA GRIZZLY BEARS: THE CONSEQUENCE OF DIVERGENT DIETS

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Introduction

Atmospheric transport readily delivers contaminants from Asia and other sources to North America and the North East Pacific Ocean (98,99). Subsequent deposition of contaminants into marine and terrestrial environments introduces persistent organic pollutants (POPs) into the lipid compartment of food webs (100), where the POPs may readily bioaccumulate, particularly through aquatic food webs, to top predators (18,101,102).

Grizzly bears (*Ursus arctos horribilis*) in British Columbia (BC), Canada, are typically regarded as terrestrial predators, consuming a wide variety of plants, berries, insects, mammals and carrion. Therefore, grizzly bears might be considered unlikely to accumulate significant concentrations of POPs as a result of the lower concentrations that typify the base of terrestrial food webs and the shorter food chains that limit POP amplification (103-105). In this way, grizzlies have been overlooked in contaminant studies. However, some grizzly bears rely heavily on Pacific salmon in the fall (7), and recent reports highlight the role that migratory Pacific salmon play as biological vectors for ocean contaminants to coastal North American watersheds (76,106). Given that North American grizzly bear populations continue to face increased habitat loss, decreased food availability, and mortality associated with human settlements (2), POP exposure may present an additional conservation concern.

The obvious challenges associated with studying grizzly bears (e.g. their elusive nature, difficulty in capture, potentially dangerous disposition) have largely precluded a detailed assessment of their foraging ecology, a critical foundation for any contaminant exposure assessment. Stable isotope analysis of various animal tissues, such as blood and

hair, have been used as a surrogate for the assessment of both short- and long-term diet, respectively, in wildlife (107-110). Carbon (^{13}C:^{12}C; $\delta^{13}C$) and nitrogen (^{15}N:^{14}N; $\delta^{15}N$) are the most widely utilized stable isotopes in ecological applications. While elevated $\delta^{13}C$ values indicate the extent of marine influence in diet, elevated $\delta^{15}N$ provides relative trophic position of the consumer, as there is a general enrichment in $\delta^{15}N$ of 3 to 4‰ with every increase in trophic level (111).

Available stable isotope information for grizzly bears is limited to homogenized whole hair strands to gather integrated dietary information over extended periods (e.g. annual) (109,112,113). While useful to observe gross differences in diet preferences, whole hair sheds little light on seasonal diet variation. Hair is a metabolically inert tissue and therefore records stable isotopes chronologically along the length of the strand (114), where the root represents the most recent diet prior to sample collection. Studies on variation in stable isotopes along the hair length are limited to captive animals with relatively homogeneous diets (114) and free-ranging wolves (for which two sections were used) (115). By conducting stable isotope analysis in multiple hair sections, especially in animals that undergo large seasonal dietary shifts, we would obtain better resolution of temporal and individual dietary variation. Hair segmentation stable isotope analysis becomes an essential foundation for interpreting the relative contributions of two food webs to POP burdens in grizzly bears in this study.

We studied three classes of POPs in BC grizzly bears: polybrominated diphenyl ethers (PBDEs), organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs). While OC pesticides and PCBs are legacy contaminants that are largely regulated in the

industrialized world, PBDEs are presently increasing exponentially in wildlife and humans (49,52,54,116).

Our objectives in this study were to 1) characterize seasonal variation in the diet of BC grizzly bears using carbon and nitrogen stable isotope analysis; 2) estimate the proportion of salmon consumed by grizzly bears using a diet-to-consumer stable isotope fractionation model (109); 3) quantify PBDE, OC pesticide and PCB concentrations in grizzly bears; and 4) characterize the linkage between POP burdens of individual bears and their dietary preferences. The diverging feeding habits (i.e. marine and terrestrial) of two grizzly bear populations provide novel insight into pathways of exposure and accumulation of contaminants of global concern.

Materials and Methods

Sample Collection. In collaboration with the BC Ministry of Water, Land and Air Protection (MWLAP), compulsory inspectors and conservation officers, we obtained (where possible) fat, muscle, skin and hair samples from 12 legally hunted or management ("problem") grizzly bears (Table 1) from various locations in BC during the fall, 2003. Mule deer (*Odocoileus hemionus*; n=4) and moose (*Alces alces*; n=7) hair samples were also obtained (Terrace, BC) as proxies for purely herbivorous mammals. Additionally, Chinook salmon (*Oncorhynchus tshawytscha*) were collected from Johnstone Strait (2000; n=6), Harrison Lake (2000; n=6), Duwamish River (2001; n=6) and Deschutes River (2001; n=6) in Coastal BC (unpublished data, P.S.Ross). Samples were directly placed in hexane-rinsed aluminum foil, and sealed in water-tight Ziploc bags. All samples were shipped frozen and stored at -20°C immediately upon delivery.

Information on grizzly bears was cross-referenced with the BC MWLAP and where possible, included age (determined using tooth cementum analysis), sex, sampling date, weight, general condition and geographic location.

Table 1. Summary of individual grizzly bear samples in British Columbia, Canada and their putative feeding strategy.

Bear ID #[a]	Sex	Age	Tissue analyzed for POPs[b]	Percent (%) lipid	Feeding group
1	f	3	fat	97.6	interior[c,e]
2	f	unknown	fat	71.7	interior
3	m	15	fat	100.7	interior
4	m	1	fat	88.7	interior
5	m	10	fat	71.4	interior
6	m	5	fat	26.8	maritime[d]
7	m	12	muscle	6.2	maritime
8	m	unknown	fat	83.8	maritime
9	f	5	fat	83.2	maritime
10	f	5	fat	97.2	maritime
11	m	unknown	fat	92.7	interior
12	f	8	fat	100.4	maritime

[a] Bear identification numbers (ID#) can be cross-referenced with Figure 2 for location and contaminant pattern information.

[b] Persistent Organic Pollutants (POPs)

[c] Interior = non-salmon-eating

[d] Maritime = salmon-eating

[e] Bear #1 is considered our herbivorous "baseline" grizzly bear

Stable Isotope Analysis. Grizzly hair was plucked from skin samples (bears #1–10) and sub-divided into 1 cm segments commencing at the root to 5 cm, with each of six segments reflecting approximately 20 days of growth (75). For bear #12, enough hair was available to measure only whole hair stable isotopes. Bear #11 had only skin available to conduct stable isotope analysis. Deer and moose hair samples were not segmented. All hair samples were washed with 2:1 chloroform:methanol solution three

times to remove surface oils and debris, then ground with a mortar and pestle to a powder using liquid nitrogen. Each hair sample was then freeze-dried at -50ºC for at least 24 hours. The skin sample for bear #11 was ground to powder using liquid nitrogen and freeze-dried at -50ºC for 48 hours.

Stable isotope measurements of sub-samples (0.5 ± 0.08mg) were carried out at the Biogeochemistry Facility (School of Earth and Ocean Sciences, University of Victoria, BC) using a Fisons NA 1500 Elemental Analyser-Isotope Ratio Mass-Selective (Milano, Italy) interfaced to a FinniganMAT 252 Isotope Ratio Mass Spectrometer (Bremen, Germany). Results are reported using standard isotope ratio notation (parts per thousand, ‰):

$$\delta X = \left[\left(R_{SAMPLE} / R_{STANDARD}\right) - 1\right] \times 1000 \qquad (1)$$

where δX is $\delta^{13}C$ (‰ vs. PDB) or $\delta^{15}N$ (‰ vs. air N_2), and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, respectively (111). Carbon and nitrogen measurements were made relative to runs of acetanilide (an in-house standard with known isotope ratios) and blanks. Replicates were conducted on random samples to 1) observe within sample stable isotope variation; 2) measure any deviation of stable isotope values over time; and 3) measure differences from one sample rack to another. Isotopic values were adjusted to the standards if any deviation occurred.

Contaminant Analyses. Approximately 3 g fat (n=11) or when this tissue was not available, 20 g muscle (n=1) were analyzed for 39 PBDE congeners and 28 organochlorine pesticides [α-hexachlorocyclohexane (α-HCH), β-HCH, δ-HCH, γ-HCH, hexachlorobenzene (HCB), 2,4'-dichlorodiphenyl dichloroethane (DDD), 4,4'-DDD, 2,4'-dichlorodiphenyl ethylene (DDE), 4,4'-DDE, 2,4'-dichlorodiphenyl trichloroethane

(DDT), 4,4'-DDT, heptachlor epoxide, heptachlor, methoxychlor, oxychlordane, γ (*trans*)-chlordane, α (*cis*)-chlordane, *cis*-nonachlor, *trans*-nonachlor, α-endosulfan, β-endosulfan, endosulfan sulphate, dieldrin, endrin, endrin aldehyde, endrin ketone, aldrin, mirex].

A second set of fat samples pooled by feeding categories (determined following stable isotope analysis) was analyzed for 160 congener-specific PCBs (n=2 pools) to use as a reference contaminant containing congeners with a wide range of octanol/water partition coefficient (log K_{ow}) values (i.e. log k_{ow}~4.2–8.5) (117) that spanned those of the OC pesticides (118,119) and PBDE congeners (120). One sample was a homogenate of 6 interior (non-salmon-eating) grizzly bears (#1-5, 11) and the other sample was a homogenate of 4 maritime (salmon-eating) bears (#6, 8-10). Bear #7 and #12 were not included in the maritime homogenate sample for PCB analysis, as #7 was a muscle sample and #12 had insufficient fat for analysis.

Samples were analyzed using High Resolution Gas Chromotography/High Resolution Mass Spectrometry (HRGC/HRMS) by AXYS Analytical Services, Sidney, BC, according to their laboratory procedures and criteria using an Ultima HRMS equipped with a Hewlett Packard 5890 GC and a DB-5 Durabond capillary column (60m X 0.25mm, 0.10μm film). Percent lipid in samples was determined at AXYS Analytical Services using the gravimetric lipid determination by weight of extract method with dichloromethane.

Samples were spiked with ^{13}C-labelled surrogate standards (n=12 PBDEs; n=29 PCBs; n=21 OC pesticides) and then ground with anhydrous sodium sulphate. Samples were transferred to a soxhlet thimble, surrogate standard was added, and samples were

refluxed for 16 hours with dichloromethane (DCM). The extract was eluted through a gel permeation column with 1:1 DCM:hexane. The extract was applied to a partially deactivated Fluorisil column and eluted with hexane followed by 15:85 DCM:hexane. Eluates were then combined and eluted with 1:1 DCM:hexane and each fraction concentrated.

Mono- and di-BDE data were not used for interpretation as surrogate recoveries were less than 10%. Since the isotope dilution method of quantification produces data that are recovery corrected, the slight variances from the method acceptance criteria are deemed not to affect the quantification of these analytes.

Included with each batch of samples was a procedural blank. The lab blank had concentrations slightly above detectable levels (<20pg/g) for 11 PBDE and 38 PCB congeners. BDE-47, 99 and 209 were detected at 92.5, 67.9 and 167pg/g, respectively. There were no PCB congeners detected above 12.8pg/g. Trace amounts (non-detectable ranges; NDR) of eight OC pesticides were found in the lab blank. HCB was detected at a concentration of 0.021ng/g.

Detection limit substitutions were made for PBDE and OC pesticide analytes that were not detected in cases where at least 8 out of 12 individual bears (>67%) had detectable values for that contaminant. Where less than 8 bears had detectable concentrations of an analyte, 0 ng/kg was substituted for non-detect concentrations. Contaminants were not reported if there were low NDRs in combination with non-detectables (below detection limit.) in all bear samples. Detection limits for PBDE congeners were consistently <10 pg/g wet weight and in most cases, <5 pg/g, with exception to BDE-209 which had detection limits ranging from 2.5 to 562 pg/g.

Whenever the determined concentration of native BDE-209 in samples was not significantly different from that in the lab blank (167 pg/g wet weight), the detection limit for BDE-209 in samples was elevated to the concentration of the detected analyte and considered not detected. For PCB congeners, detection limits were consistently <1 pg/g and in most cases, <0.25 pg/g. For OC pesticides, detection limits were consistently <0.05 ng/g and in most cases, <0.01 ng/g. Results are expressed on a lipid weight basis, and expressed as mean ± 1 standard deviation (SD).

While variable reporting of higher brominated PBDE congeners (e.g. BDE-206 to 209) partly reflects analytical difficulties (51), the inclusion of these congeners is considered important (121). We report here ΣPBDEs (all congeners detected including BDE-206 to 209), as most recoveries were considered within acceptable limits set by AXYS, and the reported concentrations were adjusted based on both those recoveries as well as concentrations found in the lab blank.

For PCB homogenate samples, the toxic equivalency quotient (TEQ) was calculated based on toxic equivalency factors (TEFs) of specific PCB congeners (122) in the following formula:

$$TEQ = \Sigma[PCB_i] \times TEF_i \qquad (2)$$

Theoretical Calculations. Grizzly bears are large mammals with extensive home ranges (123) and their omnivorous diet in coastal areas of BC is poorly described. In general, their diets depend on opportunity and habit. We chose the whole hair isotopic value of bear #1 to act as our "baseline" or "anchor" for all BC grizzlies ($\delta^{15}N$=3.5‰, $\delta^{13}C$=-23.0‰), as this bear most closely resembles the relative trophic position of the sampled herbivores, i.e. moose and deer; $\delta^{15}N$=3.8±0.9‰. This baseline provides the

basis for an internally consistent algebraic approach to define food item end-members that encapsulate the data field. The 100% herbivore reference point then enables the estimation of deviations from an herbivorous diet for each of the other grizzly bears samples.

Although both $\delta^{13}C$ and $\delta^{15}N$ were measured in the grizzly bear hair, only $\delta^{15}N$ is required to calculate the estimated diet because: 1) there was a significant linear correlation between $\delta^{13}C$ and $\delta^{15}N$ (see results) implying that terrestrial meat and salmon diets result in similar changes in $\delta^{13}C$ in relation to their trophic position ($\delta^{15}N$); 2) due to a resultant two end-member diet model, results from only one stable isotope are necessary to estimate diet (107); and 3) the use of $\delta^{15}N$ in characterizing trophic levels in food web-based contaminant studies is well established. The two end-members for the model were vegetation and Chinook salmon. Although some bears do not consume salmon, by using Chinook as the meat end-member (which is the highest trophic-level salmon species), we are in fact calculating what might be considered a meat percent (%) "Chinook Equivalent" (CE). As we cannot accurately determine the composition of salmon species, or terrestrial species consumed by a particular bear, we have simply used the Chinook salmon as the index of meat consumed. This approach is supported by the strong correlation between $\delta^{15}N$ and $\delta^{13}C$ for bears (also observed by Hilderbrand et al. (109)), and by strong correlations observed between $\delta^{15}N$ (trophic level) and POPs in aquatic food webs (73,124).

First, stable isotope deviations ($\Delta\delta^{15}N_{SEG}$) were calculated from the herbivore baseline (3.5‰) in each hair segment ($\delta^{15}N_{SEG}$) for bears #2-10 using:

$$\Delta\delta^{15}N_{SEG} = \delta^{15}N_{SEG} - 3.5 \qquad (3)$$

This calculation was not conducted on bears #11 and 12, for which segmented hair samples were unavailable. Cumulative deviation in $\Delta\delta^{15}N_{SEG}$ from the baseline herbivore diet over the four-month period ($\Sigma\Delta\delta^{15}N$) for bears #2-10 was calculated as:

$$\sum \Delta\delta^{15}N_{HAIR} = \Delta\delta^{15}N_{SEG1} + \Delta\delta^{15}N_{SEG2} + ... + \Delta\delta^{15}N_{SEG6} \quad (4)$$

Using stable isotope data from black bear feeding trials with known diets Hilderbrand et al. (109) derived a linear relationship between the stable isotope values in diet with those of bear plasma (which they suggest is appropriate for all bear tissues except adipose tissue). Generalizing the relationship derived by Hilderbrand et al. (109) for plasma to bear hair, we substituted the assumed relationship:

$$\delta^{15}N_{HAIR} = 4.76 + 0.91\left(\delta^{15}N_{DIET}\right) \quad (5)$$

to calculate the estimated 100% Chinook Equivalent end-member (Chinook: δ^{15}N=15.4±0.6‰; P.S. Ross, unpublish. data). Using the 100% CE calculated from that model ($\delta^{15}N_{HAIR}$=18.8‰) and substituting it into equations 3 and 4 (as a value for each hair segment), we estimate 100% CE over four months equated to $\Sigma\Delta\delta^{15}$N of 91.8‰. By definition, 100% vegetation (baseline) end-member over the sampling period equated to $\Sigma\Delta\delta^{15}$N of 0‰. Both vegetation and meat CE end-members were then incorporated into a mass balance to obtain relative proportions of meat (P_{MEAT}) and vegetation (P_{VEG}) for each grizzly bear (#1-10):

$$\sum \Delta\delta^{15}N_{HAIR} = P_{VEG}\left(\sum \Delta\delta^{15}N_{VEG}\right) + P_{MEAT}\left(\sum \Delta\delta^{15}N_{MEAT}\right) \quad (6)$$

which can be simplified to:

$$P_{MEAT} = \frac{\sum \Delta\delta^{15}N_{HAIR}}{91.8} \quad (7)$$

where

$$P_{VEG} = 1 - P_{MEAT} \quad (8)$$

We estimated the vegetation-derived contaminant concentrations for each grizzly bear ($[POP]_{VEG}$) using:

$$[POP]_{VEG} = [POP]_{BASELINE}(P_{VEG}) \quad (9)$$

where $[POP]_{BASELINE}$ is the contaminant concentration in the anchor bear (bear #1). For PCBs, $[POP]_{VEG}$ is calculated by substituting $[POP]_{BASELINE}$ with the contaminant concentration of the interior bear homogenate, where P_{VEG} is the average proportion of vegetation consumed by the four maritime bears used in the homogenate sample.

To obtain the concentration of each contaminant attributed to meat ($[POP]_{MEAT}$), the $[POP]_{VEG}$ value calculated for each bear was incorporated into:

$$[POP]_{MEAT} = [POP]_{TOTAL} - [POP]_{VEG} \quad (10)$$

where $[POP]_{TOTAL}$ is the contaminant concentration measured in the tissue sample of that individual.

The $[POP]_{MEAT}$ values were plotted against the proportion of meat (P_{MEAT}) in the diet of individual bears to produce "bioaccumulation slopes", which were used to assess contaminant-specific bioaccumulative potential in grizzly bears.

To calculate the proportion of contaminants coming from salmon to the maritime grizzly bears ($P_{[POP]}$), we established which grizzlies had, in highest likelihood, consumed salmon (as opposed to terrestrial meat) by comparing both $\delta^{15}N$ and $\delta^{13}C$ stable isotopic values in the hair with realistic diets of the captive bears from Hilderbrand et al. (109), as well as considering opportunity to access salmon based on geographic location. The proportion of contaminants from salmon was calculated in the salmon-eating bears using only the following equation:

$$P_{[POP]} = \frac{[POP]_{MEAT}}{[POP]_{TOTAL}} \quad (11)$$

For PBDEs and OC pesticides, the POP proportion from salmon was averaged for the four maritime bears. There is only one value for PCBs since there were only two homogenate samples to conduct the calculation. Appendix I provides an example of how to use the theoretical calculations to obtain proportion of contaminants transported from salmon to grizzly bears.

Statistical Analysis. Regression analyses were applied to relationships between 1) total contaminant concentrations and $\Sigma\Delta\delta^{15}N$; 2) $[POP]_{MEAT}$ and proportion of meat (P_{MEAT}) in diet for each grizzly (bioaccumulation slopes); and 3) proportion of PBDE, OC pesticide and PCB contaminants (arcsine transformed) attributed to salmon and log K_{ow}. T-tests (two-tailed) assuming unequal variances were conducted to compare contaminant concentrations between feeding groups. The criterion for significant effects was α=0.05. Normality and constant variance were assessed and data were transformed if those tests resulted in α<0.05. Statistical analysis was not conducted on PCB data between interior and maritime bears, as there was only one homogenized sample from each feeding group.

Results and Discussion

Stable isotopes and feeding ecology in grizzly bears. Changes in $\delta^{15}N$ and $\delta^{13}C$ isotope ratios along the hair strands reflect chronological change in the assimilated diets for individual bears over the course of approximately four months (Figure 4). Five bears (#1-5) exhibit low $\delta^{15}N$ and $\delta^{13}C$, with little variation over time, consistent with a diet of vegetation and, possibly, a small supplement of terrestrial meat. Sharp rises in hair $\delta^{15}N$ and $\delta^{13}C$ towards the fall indicate a fundamental dietary shift in five other individual

bears (#6-10), coincident with the return of adult Pacific salmon in coastal watersheds (125). The correlation between $\delta^{15}N$ and $\delta^{13}C$ (r^2=0.88; $p<0.01$) suggests a marine origin for the observed increase in trophic position.

While interior bears range in cumulative changes in $\Delta^{15}N$ ($\Sigma\Delta\delta^{15}N$) from 6.7 to 13.5‰, the maritime bears show both greater and more varied shifts ranging from 12.3 to 55.6‰ in $\Sigma\Delta\delta^{15}N$. We did not have adequate hair samples from two individuals (#11 and #12) to conduct hair segmentation assessment. Whole hair stable isotope ratios for bear #12 are $\delta^{13}C$ -19.4‰ and $\delta^{15}N$ 14‰, consistent with values observed in maritime study bears. Skin stable isotope ratios for bear #11 are $\delta^{13}C$ -22.5‰ and $\delta^{15}N$ 9.4‰, suggesting the diet of this bear is terrestrial, but fairly high trophically.

In summary, we estimate that the average diets during the period captured by hair growth ranged from 0 to 19% meat (as estimated using CE; see methods) for interior bears; and from 13 to 61% meat for maritime bears. The remaining diet of all bears was assumed to consist of vegetation.

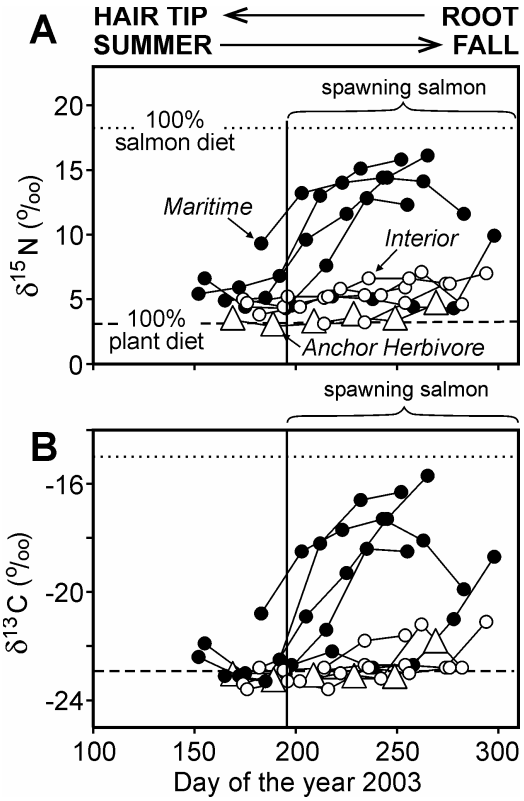

Figure 4. Seasonal changes in diet of individual grizzly bears as revealed by stable isotope ratios in growing hair. Interior bears (○), maritime bears (●) and the herbivorous anchor grizzly bear (△) are plotted, with the latter used to estimate diet proportions of other bears. The lower dashed line denotes a theoretical 100% vegetation diet, while the upper dotted line denotes theoretical 100% Chinook salmon diet. In British Columbia, salmon generally spawn in coastal watersheds after July 15 (Day 196). (A) Increasing $\delta^{15}N$ towards the fall indicates a shift to higher trophic positions by maritime bears. (B) Corresponding $\delta^{13}C$ increases provides additional evidence that this shift relates to marine sources (i.e. salmon).

Contaminant concentrations in grizzly bears. Overall, maritime bears were more contaminated with many POPs than the interior bears. The maritime grizzly bears had higher concentrations of ΣDDT (t-test, p=0.046), ΣCHL (p=0.017), dieldrin (p=0.044) and ΣPCBs (t-test not done, as n=2 pools) than the interior bears. ΣPBDE concentrations did not differ between the two groups (t-test, p=0.313).

Surprisingly, total PBDEs dominate in contaminant concentration rankings of the interior grizzlies: ΣPBDEs>ΣPCBs>HCB>ΣHCH>ΣCHL>ΣDDT, where ΣPBDES:ΣPCB

is 2.34:1. Contaminant profiles in these bears are dominated by both the heavier PBDE congeners (e.g. BDE-209, which constitutes up to 83% of ΣPBDEs for these bears) and the lighter, more volatile pesticides, including HCB and ΣHCH. The relatively low trophic levels occupied by interior bears suggest that air-to-plant partitioning may play an important role in contaminant exposure for this feeding group; their generally low POP concentrations indicate that these levels, for the most part, can be considered as "baseline" for all grizzlies. The dominance of ΣPBDEs in this baseline suggests that vegetation and the terrestrial food web may presently be the important pathway for the heavier congeners of this emerging contaminant of concern (e.g. BDE-209).

For maritime grizzly bears, ΣPBDEs are not as prominent in the overall contaminant rankings, where ΣPCBs>ΣCHL>HCB>ΣDDT>ΣPBDEs>ΣHCH. Rather, these salmon-eating bears are dominated by legacy bioaccumulative contaminants, where the ratio ΣPBDES:ΣPCB is 0.12:1. Contaminant patterns observed in these maritime bears likely reflect the seasonal shift to a higher trophic level through salmon consumption.

Although we observed significant differences in POP concentrations between these two feeding groups of grizzly bears, large variation within each group was also evident. Since diet represents the major contributor to POP contaminant burdens in mammals, the variation likely reflects individual differences in diet. Studies of other mammalian top predators, such as killer whales (18), show strong relationships between age/sex and contaminant concentrations found in individual animals. No statistically significant relationships between age, sex or percent lipid content of the grizzly bears and their contaminant concentrations could be found (results not shown) although our sample

size was small. Therefore, we evaluated our contaminant results on an individual basis using only the individual variation in food choices, as measured by stable isotopes in hair.

For most POPs measured, total concentrations increased with an increasing trophic position ($\Sigma\Delta\delta^{15}N$) of individual bears (Table 2) suggesting that salmon consumption explains the increases in the concentrations of these POPs in the maritime grizzly bears. Increases in total POP concentrations were also observed in interior bears, likely reflecting individual-based increases in the consumption of terrestrial meat.

Contaminant patterns in grizzly bears. Maritime grizzly bears that deviate from a terrestrial to a marine food web not only have increased contaminant concentrations, but also show marked differences in contaminant patterns from the bears that feed exclusively within a terrestrial food web (i.e. interior grizzly bears).

Table 2. Polybrominated diphenyl ether (PBDE), organochlorine (OC) pesticide and polychlorinated biphenyl ether (PCB) concentrations in sampled grizzly bears. Correlation statistics (variation – r^2; significance – p-value) between contaminant concentration and cumulative changes in trophic position ($\Sigma\Delta\delta^{15}N$) over the four-month period captured by segmented hair stable isotope analysis.

Contaminant	Concentration Range (ng/kg lipid weight)[g]	Mean Concentration ± S.D.[g]	r^2 for regression with $\Sigma\Delta\delta^{15}N$[h]	p-value for regression with $\Sigma\Delta\delta^{15}N$[h]
ΣPBDE[a]	1,121–53,470	10,794±16,222	0.03	0.651
ΣDDT[b]	28–20,277	4,461±6,243	0.58	**0.010**
ΣCHL[c]	213–27,606	9,179±8,918	0.73	**0.002**
ΣHCH[d]	304–3,779	1,322±1,364	0.51	**0.020**
DIEL	25–3,354	982±1,175	0.65	**0.005**
HCB	1,023–21,811	5,963±5,980	0.29	0.112
ΣPCB[e]	6,948–43,167	25,058	n/a	n/a
ΣPCB TEQ[f]	0.42–2.01	1.22	n/a	n/a

[a] This ΣPBDE includes the 20 congeners detected out of 39 tested.

[b] ΣDDT includes 4,4'-DDD, 4,4'- DDE and 4,4' - DDT

[c] ΣCHL includes heptachlor epoxide, oxychlordane, α-chlordane, *trans*-nonachlor, γ-chlordane and *cis*-nonachlor

[d] ΣHCH includes α-, β-, δ- and γ-hexachlorocyclohexane (HCH)

[e] ΣPCB includes all 132 congeners detected out of 160 tested; used as reference to other POPs, where only two pools analyzed

[f] Toxic Equivalency Quotient (TEQ)

[g] concentration range and mean concentrations based on all sampled bears (n = 12)

[h] regression statistics based on bears that had $\Sigma\Delta\delta^{15}N$ values calculated (n = 10)

Maritime bears were characterized by a pattern of top PBDE profile of 47>209>99>100>153, while interior bears were dominated by the higher brominated PBDEs: 209>206>47>207>208 (Figure 5). The predominance of the lighter congeners, such as BDE-47, in the maritime bears suggests that this congener may be attributed to marine foods, such as salmon, and/or enhanced atmospheric transport with subsequent accumulation through the terrestrial food web in coastal areas. The heavier PBDE congeners, such as BDE-209, appear to be delivered to the bears through their consumption of terrestrial vegetation, as bears with higher proportions of vegetation reliance (i.e. interior) are dominated by these congeners. The dominance of heavier PBDE congeners in interior bears may also indicate an increasing influence of local (North American) sources in bears inhabiting the interior portions of British Columbia (e.g. Deca-BDE currently at highest production for PBDE formulations (126)).

Interior and maritime grizzly bears had differing OC pesticide patterns in their tissues: interior bears were dominated by HCB>oxychlordane>α–HCH>β–HCH >dieldrin>heptachlor epoxide (Figure 5), consistent with observations in terrestrial herbivores where volatile contaminants (e.g. ΣHCH and HCB) dominate and ΣDDT is generally low (103,127), whereas maritime bears were dominated by oxychlordane>HCB>DDE>*trans*-nonachlor>dieldrin>α–CHL, a pattern that is more reflective of contaminants that bioaccumulate through aquatic food webs and is consistent with patterns observed in salmon (82). Metabolism may affect some OC pesticides, such as *cis*- and *trans*-chlordane: Hites et al. (81) documented these parent compounds in wild B.C. salmon, and yet they are absent in salmon-eating grizzly bears.

Oxychlordane (a major metabolite of chlordane), on the other hand, is found in high concentrations in our maritime bears.

Two exceptions to the contaminant patterns were observed. The maritime bear #7 the interior grizzly #11 had contaminant profiles that did not resemble those predicted isotopically. Switching feeding strategies between years by these individuals may explain these anomalies. In addition, contaminant results from bear #7 may have differed somewhat as muscle was used in place of fat.

Both interior PCB (153>118>180>99>138) and maritime grizzly bear PCB (153>118>180>138>99) patterns were dominated by the same congeners, although the patterns differed slightly. The relative proportions of non- and mono-ortho PCBs were similar between the feeding groups, however, PCB-156/157 contributed the most to total TEQ in maritime bears (ca. 39%), while PCB-126 contributed the most in interior bears (ca. 38%). TEQ values for both maritime and interior bears are low compared to those found in studies of high trophic level aquatic biota (17,18,122,128).

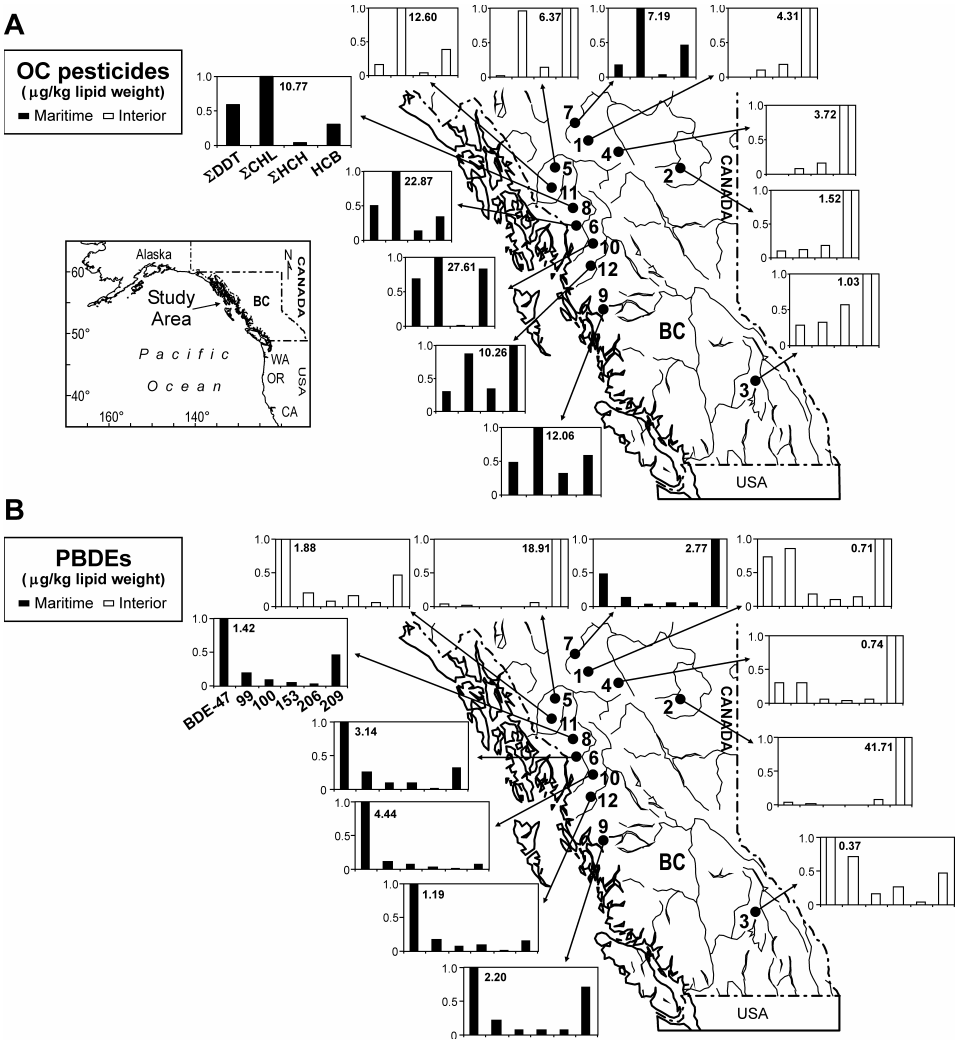

Figure 5. Grizzly bear sampling locations in British Columbia and their persistent organic pollutant (POP) patterns. A) The patterns of the most dominant organochlorine (OC) pesticides (from left bar to right): ΣDDT, ΣCHL, ΣHCH and HCB. B) The patterns of top 6 polybrominated diphenyl ether (PBDE) congeners (from left bar to right): BDE-47, -99, -100, -153, -206 and -209. Bars represent the proportion of the contaminant present at the highest concentration within each bear sample, with actual concentrations indicated numerically for this contaminant (μg/kg). Maritime (salmon-eating) grizzly bears are represented by black bars. Interior grizzlies are represented by white bars.

Bioaccumulation of Individual PBDE congeners and OC pesticides. Most contaminants had significant bioaccumulation slopes (i.e. relative increase in contaminant concentrations with increasing consumption of meat by individual bears; Table 3).

Positive slopes suggest that certain contaminants are transported to the grizzly bears through increased consumption of salmon (maritime grizzlies) or terrestrial meat (interior grizzlies). The slope itself is a reflection of the degree of contaminant bioaccumulation, where oxychlordane and 4,4'-DDE are the most bioaccumulative contaminants, while BDE-47 is the most bioaccumulative PBDE congener. Contaminants with steeper bioaccumulation slopes represent POPs that bioaccumulate more readily through aquatic food webs to grizzly bears, whereas contaminants with less accentuated slopes are more likely to be evenly distributed across food webs (terrestrial=marine) or are readily metabolized by the grizzly bears.

Rankings of these bioaccumulation slopes for OC pesticides and PBDEs are consistent with the observed contaminant pattern in maritime grizzly bears, supporting the conclusion that their contaminant profiles are dominated by those POPs that bioaccumulate in aquatic food webs. Higher brominated PBDE congeners (BDE-206 to -209) had negative (albeit non-significant) bioaccumulation slopes, possibly indicating a preferential exposure to local sources through their consumption of vegetation.

Table 3. The bioaccumulation slopes for individual organochlorine (OC) pesticides and polybrominated diphenyl ethers (PBDE) congeners in grizzly bears listed in order of highest to lowest. Slopes were derived from the relationships between estimated proportion of meat consumed by individual grizzly bears and OC pesticide and PBDE concentrations in their tissues as a result of meat consumption.

Contaminant	Bioaccumulation	95% Confidence	r^2	p-value
oxychlordane	23,057	12,076–34,038	0.68	**0.003**
DDE	21,136	7,233.9–35,038	0.53	**0.018**
hexachlorobenzene (HCB)	20,288	2,179.8–38,396	0.38	0.059
trans-nonachlor	10,600	4,778.8–16,422	0.61	**0.007**
BDE*-47	6,180.7	3,417.9–8,943.5	0.71	**0.002**
Dieldrin	5,168.0	2,930.9–7,405.2	0.72	**0.002**
α-chlordane	3,894.1	2,008.8–5,779.3	0.67	**0.004**
β-hexachlorocyclohexane	3,623.4	1,871.4–5,375.3	0.67	**0.004**
DDT	2,269.2	1,229.7–3,308.7	0.70	**0.003**
heptachlor epoxide	2,158.4	1,314.4–3,002.5	0.76	**0.001**
α-HCH	1,543.8	-868.37–3,956.0	0.16	0.245
cis-nonachlor	1,452.7	547.17–2,358.3	0.55	**0.014**
BDE-99	1,106.1	147.01–2,065.2	0.39	0.054
β-endosulfan	921.18	208.96–1,633.4	0.45	**0.035**
γ-chlordane	563.69	284.10–843.28	0.66	**0.004**
BDE-100	541.09	304.83–777.35	0.72	**0.002**
BDE-153	496.33	383.41–609.25	0.90	**0.000**
Mirex	337.08	199.51–474.64	0.74	**0.001**
α-endosulfan	323.81	-13.918–661.54	0.31	0.097
BDE-28	252.82	86.387–419.25	0.53	**0.018**
BDE-154	126.20	42.708–209.69	0.52	**0.018**
2,4'-DDD	97.857	-30.582–226.30	0.22	0.174
γ-HCH	76.401	-360.42–513.23	0.01	0.741
BDE-66	74.334	18.631–130.04	0.46	**0.031**
BDE-49	67.586	29.948–105.22	0.61	**0.008**
Endrin	65.111	-24.183–154.40	0.20	0.191
Endosulfan sulphate	63.659	-114.37–241.69	0.06	0.503
BDE-77	49.893	15.346–84.440	0.50	**0.022**
BDE-119/120	36.700	27.147–46.253	0.84	**0.000**
δHCH	36.373	14.505–58.241	0.57	**0.016**
BDE-17	30.562	16.489–44.635	0.69	**0.003**
BDE-155	22.848	14.091–31.605	0.77	**0.001**
BDE-183	21.733	-39.544–83.010	0.06	0.507
BDE-85	19.429	-40.710–79.568	0.05	0.544
BDE-140	3.1020	-2.0448–8.2488	0.15	0.271
BDE-138	2.1261	-21.931–26.183	0.00	0.867

*BDE = brominated diphenyl ether (congener number)

Chemical properties govern delivery of contaminants by salmon. Contrasting stable isotope ratio signatures in our two grizzly bear feeding groups provide a unique opportunity to quantify the relative contributions of terrestrial (i.e. vegetation) and marine (i.e. salmon) food webs to POP accumulation. By removing the contaminant proportion derived from vegetation in each maritime bear, we estimate that salmon contribute 70±34% of the OC pesticides, up to 85% of the lower brominated PBDEs and 90% of the PCBs in maritime grizzlies.

The two food webs will preferentially deliver certain individual POPs over others to the grizzly bears, reflecting the role that contaminant physico-chemical properties (e.g. log K_{ow}) play in regulating exchange among environmental compartments and fate in the environment. This is evidenced by an observed "peak" regression between the contaminant concentrations attributed to salmon (modified Gaussian, 4 parameter, r^2=0.52; $p<0.0001$; Figure 6) and log K_{ow} values (117,120). Together, the ocean and the salmon food web provide a small window (log K_{ow} ~5.9-7.5) that strongly favors the delivery of POPs to bears (>85% of total concentration). This range of "enhanced accumulation" for POPs integrates processes involved in atmospheric transport to the North Pacific Ocean, deposition, subsequent uptake into marine food webs and retention in lipids of biota. Similar patterns and peaks have been observed for bioaccumulation of legacy POPs in marine zooplankton (129) and tidal river marsh food webs (130). Consistent with our observations of a zone of enhanced accumulation, biomagnification factors (BMFs) between food and rainbow trout, *Oncorhynchus mykiss*, peaked at ~7.0 log K_{ow} (131) and BMFs in a freshwater food web peaked for PCBs at ~7.0 to 7.5 log K_{ow} (132).

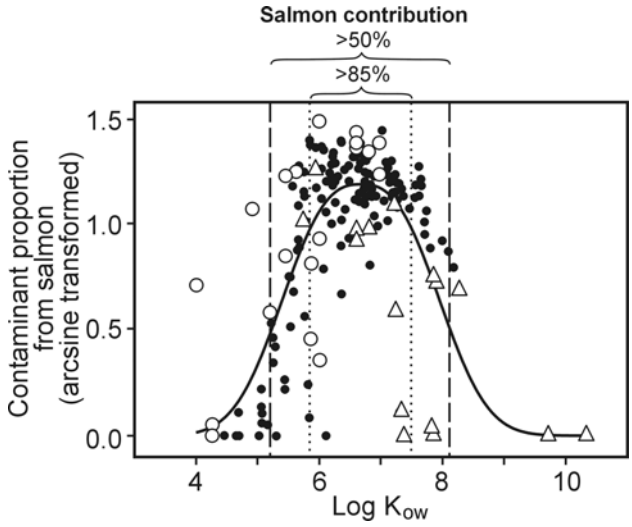

Figure 6. The proportion of contaminants in "maritime" grizzlies attributed to salmon correlates in a modified Gaussian (4 parameters) "peak" to log K_{ow} (solid line: r^2=0.52, p<0.0001). Organochlorine (OC) pesticides (○). Polychlorinated biphenyl ether (PCB) congeners (●). Polybrominated diphenyl ether (PBDE) congeners (△). Area between dotted lines approximates our 'range of enhanced accumulation' (5.9–7.5 log K_{ow}), where the log K_{ow} of the contaminants appears to favor ultimate partitioning into salmon. Salmon contributes >85% of the contaminant concentration within this area. Dashed lines represent the approximate points at which terrestrial and salmon contributions to grizzly bear contaminant concentrations are equal, although metabolism likely plays an unspecified role in elimination of lower log K_{ow} contaminants.

Conversely, more volatile POPs (log K_{ow}<5.3) and heavier PBDEs (log K_{ow}>7.9) are provided to grizzly bears either preferentially through terrestrial foods (where >50% of total concentration is attributable to vegetation consumption), or are low as a result of contaminant-induced metabolism (133). Without a more accurate picture of the composition of grizzly bear diets and their contaminant concentrations, it is not possible to adequately characterize the importance of metabolism in shaping contaminant patterns in the bears. While metabolic elimination may partly explain reduced estimates of salmon contributions for the low log K_{ow} contaminants, the heavier PBDEs are likely provided to the grizzlies predominantly through terrestrial vegetation. The relative abundance of heavy PBDE congeners and virtual absence of light PBDE congeners in interior bears

supports the notion that metabolism is not the key factor in explaining reduced estimates of salmon contributions for the higher log K_{ow} contaminants.

The seasonal pulse of marine-derived nutrients associated with the influx of salmon drives the productivity and diversity of British Columbia coastal rainforests (74,134). While salmon sustain healthy populations of maritime grizzly bears (135), they also deliver potentially endocrine-disrupting POPs to these bears. Our results suggest that although all grizzlies share a common terrestrial food web, pre-hibernation gorging on salmon by some bears leads to an increased risk of contaminant-related health effects. Several studies on polar bears, *Ursus maritimus*, suggest that there may be potential relationships between contaminant concentrations and hormone levels, impaired immune systems, and population-level effects (136-139); however contaminant concentrations in polar bears are much greater than those observed in our grizzlies. The TEQ values for both grizzly bear feeding groups are also well below the no-observed adverse effects level (NOAEL) for reproductive effects in mink (a mammal feeding within both aquatic and terrestrial food webs and highly sensitive to effects of PCBs) of 2,000 ng/g wet weight (140).

Despite contaminant concentrations in our adult grizzly bears being lower than reported in most other species occupying high trophic positions in marine food webs (18,43), the reproductive window may be vulnerable. PBDE concentrations (e.g. BDE-47) in the maritime grizzlies exceed those reported for women's breast milk in Sweden (52); the latter concentrations contributed to the ban of penta- and octa-BDEs in Europe. With low reproductive rates and seasonal cycles of fasting (hibernation) (141), adult

female grizzly bears may supply elevated concentrations of endocrine-disrupting chemicals to their young through transplacental and/or lactational transfer (52,89,142).

British Columbia grizzly bears provide two distinct signals of the fate of legacy and new POPs in the environment. While legacy contaminants have been largely addressed by national regulations and international treaty (e.g. Stockholm Convention), the use of PBDEs continues. Despite the ban of Penta- and Octa-BDE formulations in Europe and their potential ban in Canada and several U.S. States, the unregulated use of Deca-BDE will continue to contaminate the environment through the debromination to lighter PBDE congeners (143), and through the near-source contamination by heavier PBDE congeners. Continued exposure of both interior and maritime grizzly bears in British Columbia to PBDEs may therefore be expected over the coming decades, albeit to PBDE mixtures with contrasting profiles.

CHAPTER 2: HIBERNATION-ASSOCIATED CHANGES IN PERSISTENT ORGANIC POLLUTANT (POP) LEVELS AND PATTERNS IN BRITISH COLUMBIA GRIZZLY BEARS (*URSUS ARCTOS HORRIBILIS*)

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Introduction

Grizzly bears (*Ursus arctos horribilis*) prepare for winter hibernation by gorging on high caloric foods in the late summer and fall. Since dietary intake is the main route of mammalian exposure to persistent organic pollutants (POPs) (18), increased uptake and accumulation of POPs is likely to take place at this time. We previously demonstrated the role that trophic status and reliance on different food webs played in influencing POP concentrations and patterns in grizzly bears (144). While fall grizzly bears had moderate concentrations of contaminants relative to other top aquatic predators (18,89), we speculated that fat loss associated with their approximate five-month hibernation period would result in a concentration of fat-soluble POPs (89).

While all hibernating animals rely heavily on fat reserves for the maintenance of vital body processes, bears are thought to have unique attributes associated with their hibernation. Unlike most other hibernators, bears maintain their body temperature within a few degrees of their active or normal state (145,146). There is a 75% reduction in heart rate (147), with a corresponding 50 to 60% reduction in the basal metabolic rate of the bears (146), a depression thought to be much less than other hibernators. Hibernating bears form a plug in their rectum ("tappen") preventing defecation from occurring during the hibernation period (88), and they also do not urinate during this time (88,148). Of additional interest is the fact that bears are the only carnivores in which pregnancy and lactation coincide with hibernation (149). Reproductively active female bears, therefore, utilize fat reserves for fetal development, and milk production, as well as for their own metabolic needs during hibernation.

POPs may represent an additional conservation concern to the diminishing populations of North American grizzly bears. As grizzly bear hibernation coincides with their reproductive, developmental and lactational phases, the hypothesized increase in POP concentrations associated with the fasting period may increase the risk of endocrine disruption in the hibernating adult bears and/or their offspring (136,150-153).

Given the highly variable feeding habits among individual grizzly bears, even within the same feeding group (i.e. salmon-eating or non-salmon-eating) (144), a simple comparison of POP concentrations in different pre- and post-hibernation individuals would not accurately depict hibernation-associated change. Hence, it is especially important to first consider individual feeding preferences in the interpretation of POP levels and patterns in studies of omnivorous wildlife. Our main objective was to quantify the changes in POP concentrations and patterns in grizzly bears following hibernation, while at the same time accounting for those differences in individual feeding ecology. To the authors' knowledge, changes in PBDEs following either hibernation or a fasting event in any wildlife species have not been previously documented.

The attributes associated with hibernation in grizzly bears provide a unique "closed system" for monitoring changes in POP concentration and patterns over time, namely a pharmacokinetic system which lacks two of its fundamental components - dietary intake and excretion via urine and feces.

Materials and Methods

Sample Collection. This study was conducted in collaboration with the BC Ministry of Water, Land and Air Protection (MWLAP), compulsory inspectors, and

conservation officers. Subcutaneous fat and hair samples for this study were obtained following a legal hunt of grizzly bears during the early spring of 2004 (n=14), and combined with data obtained from an expanded analysis from our earlier study of fall bears of 2003 (n=11) (144). Samples were collected from various locations on the body of the bears (mainly head, neck and thigh), placed in hexane-rinsed aluminum foil and shipped frozen to the lab for processing.

Stable Isotope Analysis. Hair is a metabolically inert tissue and so records dietary information chronologically along its length (115). Therefore, grizzly hair was plucked from the skin and subdivided into 1 cm segments commencing at the root towards the tip, with each segment reflecting approximately 20 days of growth (75). While the tip reflected the summer diet, the root reflected the most recent diet. All hair samples were processed and analyzed for carbon and nitrogen stable isotopes as detailed in Christensen et al. (144). Results are reported using standard isotope ratio notation (parts per thousand, ‰)

$$\delta X = [(R_{SAMPLE} / R_{STANDARD}) - 1] \times 1000 \quad (1)$$

where δX is $\delta^{13}C$ (‰ vs. PDB) or $\delta^{15}N$ (‰ vs. air N_2), and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, respectively (111).

Only the hair segments representing the estimated timeframe of August 10, 2003 to November 10, 2003 were used for each bear individual. Dates were determined by back-calculating from the sampling date for fall bears and estimated date of hibernation (early-November) for spring bears. Since bear hair stops growing at the commencement of hibernation, the stable isotope results from both fall and spring grizzly bears represented diets from the summer to late fall 2003 (Figure 7). However, most fall bear

samples were collected in October, while the spring bear samples would have experienced approximately four to six more weeks of feeding prior to their hibernation in November. This discrepancy was corrected for in the dietary index (DI) calculation.

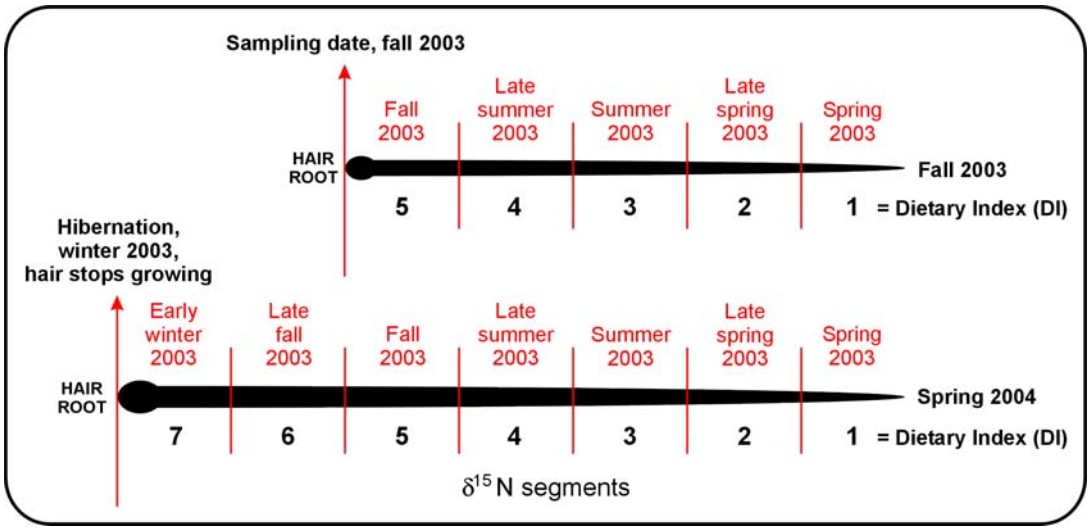

Figure 7. Illustration for the calculation of dietary index (DI) values using fall and spring grizzly bear hair. A summation of $\delta^{15}N$ values for each hair segment was necessary to incorporate, into the DI calculation, a temporal difference in time spent feeding by the bears prior to sampling. Fall grizzly bears were sampled approximately four weeks prior to hibernation, while spring grizzly bears were sampled after cessation of hair growth at the onset of hibernation. Therefore, spring grizzly bears experienced increased exposure duration to contaminants compared to fall grizzly bears that must be accounted for in the DI calculation. The tip of the hair represents spring 2003 diet, and the root of the hair represents the most recent diet prior to sampling (September-October 2003 for fall bears) or to hibernation (mid-November 2003 for spring bears). Figure taken from Christensen et al. (154), Supporting Information Figure S1.

Although both $\delta^{13}C$ and $\delta^{15}N$ were measured in the grizzly bear hair, only $\delta^{15}N$ was used for dietary interpretation, for reasons outlined in Christensen et al. (144). Stable isotope values for each bear ($\delta^{15}N$) were summed for sections of the hair that fell within the above-mentioned time-frame to obtain a DI value:

$$\text{Dietary Index (DI)} = \delta^{15}N_{SEG1} + \delta^{15}N_{SEG2} + ... + \delta^{15}N_{SEGn} \quad (2)$$

The DI calculated for each bear represented not only individual variations in diet, but also incorporated a temporal factor through the summation of stable isotopes, which was necessary for reasons outlined above.

Contaminant Analyses. For this study, approximately 3 g of fat from each spring sampled bear was analyzed for 159 PCB congeners, 39 PBDE congeners, and 28 organochlorine (OC) pesticides (Appendix II). Similarly, 3 g of fat from each fall bear was analyzed for 159 PCB congeners. PBDE and OC pesticide data for fall bears was extracted from Christensen et al. (144). Samples were analyzed using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) by AXYS Analytical Services, Sidney, BC, according to their laboratory procedures, as outlined elsewhere (144). Internal ^{13}C standards were included in the analyses to assess contaminant recovery, and a certified reference material was analyzed every 10 samples.

Included with each batch of samples was a procedural blank. For fall samples, specific lab blank information for PBDEs and OC pesticides can be found in Christensen et al. (144). All PCB congeners in the fall sample blank had concentrations <10 ng/kg. For the spring lab blank, all PCB congeners were <7 ng/kg, eight OC pesticides were in non-detectable ranges (NDR), and most PBDE congeners were detected at <5 ng/kg.

Methods for detection limit substitutions have been described elsewhere (144). Detection limits for fall and spring PCBs were generally <1 ng/kg. Detection limits in the spring samples were <1 ng/kg for both OC pesticides and PBDEs.

Percent lipid was assessed using gravimetric lipid determination by weight of extract method with dichloromethane. Results are expressed on a lipid weight (lw) basis

and expressed as mean ± 1 standard deviation (SD). Recoveries from internal standards were considered within acceptable limits set by AXYS, and sample wet weight concentrations were adjusted based on wet weight recoveries as well as wet weight concentrations found in the lab blank. Concentrations in samples were then lipid corrected.

Relative Contaminant Persistence versus PCB-153 during hibernation. Individual contaminant concentrations were plotted against the DI values of individual bears to produce fall (pre-hibernation) and spring (post-hibernation) "bioaccumulation slopes". To calculate relative persistence (RP) of these contaminants, first the observed fall slope ($FALL_{OBS}$) for a particular contaminant was multiplied by the value of the spring:fall slope ratio of CB-153 ($SPR_{CB153}/FALL_{CB153}$=2.25; Figure 8) to obtain a predicted spring slope (SPR_{PRED}) as follows:

$$SPR_{PRED} = FALL_{OBS} \times [SPR_{CB153} / FALL_{CB153}] \quad (3)$$

Then the observed spring slope (SPR_{OBS}) for that contaminant was divided by the respective SPR_{PRED} slope to obtain a persistence value relative to CB-153 (RP_i).

$$RP_i = SPR_{OBS} / SPR_{PRED} \times 100 \quad (4)$$

Any RP value <100% was considered to be less persistent in grizzly bears than CB-153 and values >100% were considered to be more persistent.

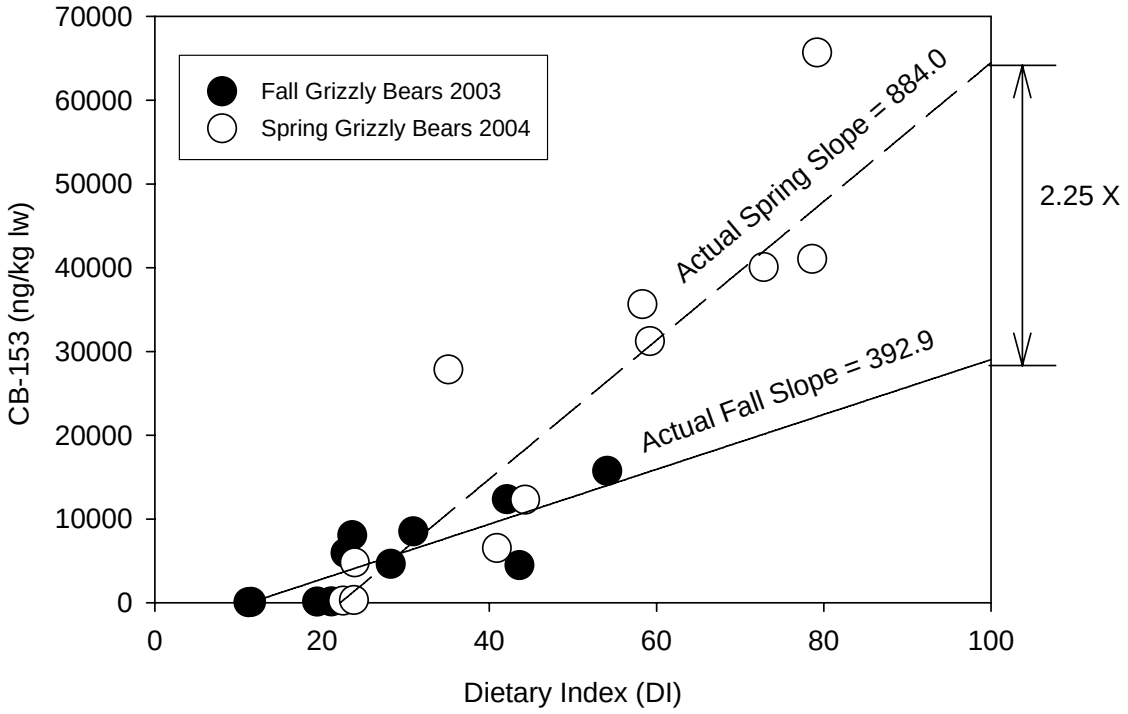

Figure 8. Relationships between individual grizzly bear dietary index (DI) values and the polychlorinated biphenyl (PCB) congener, CB-153, in fall 2003 (solid line; r^2=0.70) and early spring 2004 (dashed line; r^2=0.72). The ratio of spring:fall slopes equates to a 2.25 X increase in concentrations following grizzly bear hibernation for CB-153 after diet correction. The CB-153 ratio is the criterion to which all other individual contaminant slope ratios from fall to spring are compared to obtain relative persistence (RP) values. Figure taken from Christensen et al. (154), Supporting Information Figure S2.

To calculate a hibernation-associated, diet corrected concentration effect (CE), the following calculation was used:

$$CE_i = SPR_{OBS} \ / \ FALL_{OBS} \quad (5)$$

This value is the factor by which a particular congener or isomer increases (>1.0X) or decreases (<1.0X) following grizzly bear hibernation.

It was not possible to calculate RP or CE values for hepta- to deca-BDEs in this manner, as their relationship with grizzly bear diet did not follow that of CB-153, where

increasing DI resulted in increased POP concentrations. Therefore, in order to determine relative persistence for these congeners, the concentration of these congeners relative to BDE-203 was compared in fall and spring individual bears (Figure 9).

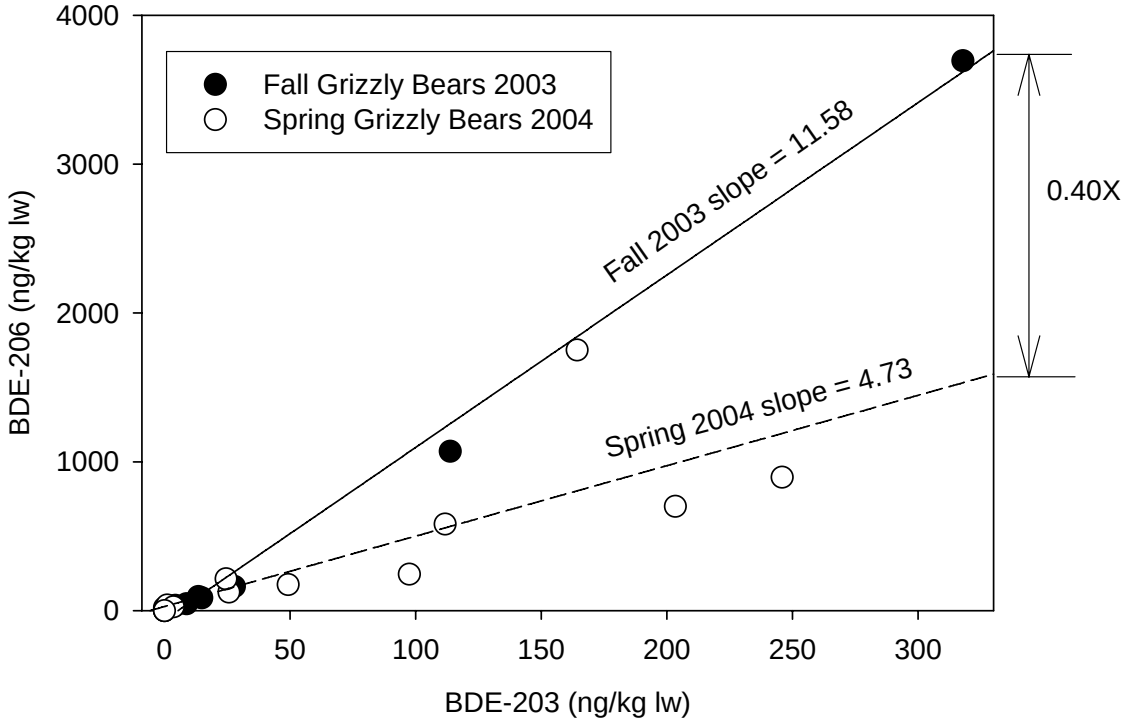

Figure 9. Determination of relative persistence values for polybrominated diphenyl ether (PBDE) congeners BDE-203 and -206. Concentrations of BDE-203 were compared to concentrations of BDE-206 in both fall (solid line; $r^2 = 0.99$) and spring (dotted line; $r^2 = 0.65$) grizzly bears. The decrease in slopes from 11.58 to 4.73 (fall to spring) suggest that during hibernation, BDE-206 has approximately 0.40X persistence (spring slope divided by fall slope) in the grizzly bears relative to BDE-203. This method was also used to assess relative persistence (to BDE-203) for BDE-183, -207, -208 and -209. Figure taken from Christensen et al. (154), Supporting Information Figure S3.

Statistical Analysis. Regression analyses were applied to 1) fall and spring bioaccumulation slopes, and 2) principal components (PCs) with contaminant log K_{ow} (octanol-water partition coefficient) and relative contaminant persistence (RP). Data points with standardized residuals of <-2 or >2 were considered outliers and removed.

Student's t-tests were conducted to test for contaminant persistence relative to CB-153 following the hibernation event. A one-way ANOVA was used to assess differences between structure-related PCB metabolic groups (155), followed by a *post-hoc* Tukey's test. The criterion for significance was α=0.05. Normality and constant variance were assessed and data were transformed if those tests resulted in α<0.05.

Principal Components Analysis (PCA). The stated concentration was used for analytes reported by the laboratory as NDR (peak detected but confirming-ion ratios outside of the specified range), while undetectable values were replaced by a random number between zero and the limit of detection before PCA (Appendix II). Each contaminant analyzed was evaluated for potential interferences, closeness to the limit of detection and the percentage of undetectable (random value estimated) values before inclusion in the PCA dataset. Samples were normalized to the concentration total before PCA to remove artifacts related to concentration differences between samples. The centered log ratio transformation (division by the geometric mean of the concentration-normalized sample followed by log transformation) was then applied to this compositional dataset to produce a dataset that was unaffected by negative bias or closure (17). This yielded a dataset where the average concentration and concentration total were identical for every sample. Data were then autoscaled and a Varimax rotation was applied to the first three principal components; this rotation maximized or minimized the loading of each variable on each principal component while preserving trends.

Results and Discussion

Hibernation represents a time of inactivity and fat utilization for grizzly bears, which could lead to potentially higher POP concentrations in residual fat tissues. The

wide ranges in POP concentrations within fall and spring grizzly bears, coupled with major differences in individual feeding preferences, necessitated an approach that would account for their omnivorous nature, and more accurately describe changes in POP concentrations during hibernation. Age and sex either did not exert any effect on contaminant concentrations and patterns (data not shown), or our data set was too limited to explore such effects (spring bears were all male).

Use of stable isotopes in segmented grizzly hair to calculate a dietary index (DI). Changes in $\delta^{13}C$ and $\delta^{15}N$ along the length of hair in spring-sampled grizzly bears reflect the temporal changes in their assimilated diets prior to hibernation, namely from summer to late fall 2003. Dietary shifts, as denoted by increases in both $\delta^{13}C$ and $\delta^{15}N$, are evident in the hair of some, but not all, spring grizzly bears. Enriched ratios of both $\delta^{13}C$ and $\delta^{15}N$ suggest an increasing diet of a high trophic-level marine species (i.e. salmon) for 6 out of the 14 spring (avg. DI=67.8±10.2) grizzly bears sampled ("maritime" bears). This dietary shift to salmon during late summer/early fall was noted in the stable isotope results from 5 out of 11 bears in our previous study (144) (recalculated here where DI=38.9±11.8).

Consistently low $\delta^{15}N$ and $\delta^{13}C$ values along the length of the hair in the remaining spring bears suggest the previous years' diets were both low trophically and within a terrestrial food web ("interior" bears). Both fall and spring interior bears have significantly lower overall DI values of 19.0 ± 6.6 and 31.9 ± 8.4, respectively, than the maritime bears, reflecting their lower position in the food web. As a result of the longer feeding bouts of the spring bears prior to sampling (see Methods), spring bear DI values are significantly higher (47.3 ± 20.4) compared to fall bear values (28.0 ± 13.6). It is the

wide range of DI values (fall DI range: 11.2 to 54.1; spring DI range: 22.5 to 79.2) and their correlations to POP concentrations ("bioaccumulation slopes") in both fall and spring grizzly bears that serve as the basis to explore congener- and contaminant-specific behavior during grizzly bear hibernation (Table 4).

Table 4. Summary of biological information and concentration ranges for major contaminant classes in fall 2003 and spring 2004 grizzly bears in British Columbia.

Variable	Fall grizzly bears	Spring grizzly bears
Sampling Date	September 9 – October	April 18 – May 18,
Sex	7 male, 4 female[b]	12 male
Age (years)	1 – 15[b]	3 – 21
Lipid (%)	26.8 – 101 %[b]	0.59 – 89.0 %
ΣPCBs[a]	571 – 65700	1710 - 248000
ΣPBDEs[a]	1120 - 53500[b]	636 – 40200
ΣDDT[a]	28.1 - 20300[b]	ND – 5130
ΣCHL[a]	213 - 27600[b]	116 – 65200
ΣHCH[a]	304 - 3780[b]	332 – 7450
ΣTEQ[c]	0.03 – 4.53	0.07 – 13.3

[a] Individual PCB and PBDE congeners, as well as individual OC pesticides used for calculations of totals can be viewed in the supplementary information. Totals included all contaminants or congeners detected in at least one sample. All concentrations are reported as ng/kg lipid weight.

[b] Data extracted from Christensen et al. (144)

[c] PCB congeners used to calculate ΣTEQ include: 77, 81, 105, 114, 118, 123, 126, 156/157, 167, 169, 170, 180/193, 189

Concentration effects vary by contaminant during hibernation. By comparing the predicted spring and observed spring bioaccumulation slopes (DI vs. [POP]), we aimed to approximate the concentration effects and relative persistence (RP) of contaminant classes, as well as individual POP congeners. Among contaminant classes, ΣPCBs elicit the greatest overall diet-corrected concentration effect (CE) of 2.21X (RP=98%), suggesting that post-hibernation ΣPCB concentrations are more than double those of pre-hibernation in the residual fat (Table 5). Male polar bears had a lesser

increase in ΣPCB concentrations (1.17X) following their seasonal fast (89), with differences likely due to their lack of true hibernation (in males) and/or differences in physiology. ΣCHL in grizzly bears increased 1.49X (RP=66%) following hibernation in this study, while male polar bears exhibited a decrease in ΣCHL (89). Surprisingly, ΣDDT decreased following hibernation in grizzly bears, and with a CE value of 0.16X, it is the least persistent of contaminant classes (RP=7%). Similar results were observed in male polar bears, which also exhibited significant decreases in ΣDDT (89). ΣPBDEs increased post-hibernation by 1.58X overall, and are thus considered moderately to highly persistent in grizzly bears (RP=70%). While comparisons made on a lipid weight basis provide the most defensible means of comparing fall and spring bears (different individuals), wet weight expression did not lead to appreciable differences in results (data not shown).

Table 5. Persistence relative to CB-153 (RP) and the associated "diet-corrected" concentration effect (CE) of pre-selected persistent organic pollutants (POPs) in British Columbia grizzly bears following hibernation.

Contaminant	Predicted Spring Slope[b]	Actual Spring Slope	"Diet-Corrected" Concentration Effect[c] (CE)	Relative Persistence (RP) to CB-153[d] (%)
ΣPCB[a]	3262	3211	2.21	98.4
Non-dioxin-like				
CB-28	38.45	38.43	2.25	100
CB-52	20.45	2.071	0.23	10.1*
CB-99	297.2	304.1	2.30	102
CB-101	37.18	5.409	0.33	14.5*
CB-138	316.0	280.6	2.02	88.8
CB-153	884.0	884.0	2.25	100
CB-190	21.63	19.77	2.06	91.4
Dioxin-like				
CB-77	0.241	0.098	0.91	40.7*
CB-81	0.196	0.060	0.69	30.6*
CB-105	111.8	115.6	2.33	103
CB-114	12.94	13.68	2.38	106
CB-118	419.2	425.6	2.28	102
CB-123	2.842	2.269	1.80	79.8
CB-126	0.736	0.373	1.14	50.7*
CB-156/157	111.4	126.8	2.56	114
CB-167	9.574	14.06	3.30	147
CB-169	0.369	0.000	0.00	0.00*
CB-170	167.9	138.08	1.85	82.2
CB-180	427.3	303.8	1.60	71.1*
CB-189	7.279	9.631	2.98	132
ΣTEQ	0.193	0.173	2.02	89.6
ΣPBDE[a]	211.1	148.3	1.58	70.3*
BDE-28	6.482	4.019	1.40	62.0*
BDE-47	146.5	118.2	1.82	80.7*
BDE-99	20.98	10.20	1.09	48.6*
BDE-100	9.538	7.332	1.73	76.9*
BDE-153	10.84	15.00	3.11	138
ΣDDT[a]	784.6	55.01	0.16	7.01*
4,4'-DDT	69.98	0.000	0.00	0.00*
4,4'-DDE	634.1	55.12	0.20	8.69*
4,4'-DDD	50.60	0.000	0.00	0.00*
ΣCHL[a]	1297	861.3	1.49	66.4*
Dieldrin	166.3	76.82	1.04	46.2*
βHCH	122.9	52.10	0.95	42.4*
HCB	586.4	202.8	0.78	34.6*
Mirex	9.902	4.612	1.05	46.6*
βEndosulfan	25.90	0.901	0.08	3.48*

[a] Individual PCB and PBDE congeners, as well as individual OC pesticides used for calculations of totals can be viewed in Appendix II.

[b] Predicted spring slope = actual fall slope x 2.25

[c] Diet-corrected concentration effect calculated by: CE = actual spring slope / actual fall slope

[d] Persistence relative to CB-153 (RP) = [actual spring slope/predicted spring slope] x 100.

*Significantly different persistence than predicted, calculated using a Student's t-Test.

While ΣPCBs appear highly persistent in grizzly bears, there was considerable variation in persistence and associated concentration effects among individual congeners. Surprisingly, many of the dioxin-like PCB congeners are also highly persistent relative to CB-153, with concentration increases from pre- to post-hibernation ranging from 1.80X to 3.30X. Accordingly, overall ΣTEQ in the bears elicits a concentration effect of over 2.00X (RP=90%). Of further toxicological interest is the observation that 14 PCB congeners have RP values that exceed that of the most recognized recalcitrant congener (CB-153): 162>189>167>111>194>156>206>205>114>146>105>133>99>118, with almost half of these congeners known to exhibit dioxin-like effects. While ΣPCB concentrations may be considered low in grizzly bears compared to those in polar bears and other marine mammals (136,153,156), spring salmon-eating grizzly bear ΣPCB TEQ values did attain levels that have been associated with altered circulating thyroid hormone (TH) concentrations and TH receptor α (TRα) expression levels in harbour seals (153).

Most OC pesticides are not persistent in the grizzly bears. One exception is oxychlordane, which increased in concentration by 2.24X (RP=99%). Heptachlor epoxide has the next highest increase at 1.29X (RP=57%). Concentration effects of individual OC pesticides are dominated by oxychlordane>heptachlor epoxide>αCHL>mirex>dieldrin>βHCH. Methoxychlor, β-endosulfan, δHCH, and DDT

and its metabolites exhibited CE values ranging from 0.00X to 0.25X, and were the least persistent OC pesticides with RP values <10% that of CB-153.

Overall, ΣPBDEs are moderately to highly persistent in grizzly bears following hibernation, with wide variation in concentration effects among individual congeners. While BDE-47, 79, 100, 119 and 153 were considered the most persistent in hibernating grizzly bears (CE range: 1.73X to 4.76X), only some of these congeners are considered dominant in the profiles of wildlife species (144,157-159). At the same time, BDE-99 which is usually a dominant congener in wildlife PBDE profiles (144,157-159) is only moderately persistent (RP=49%) in grizzly bears, with a concentration effect of only 1.09X. Three PBDE congeners are more persistent (79>119>153) than both BDE-47 and CB-153. Of the hepta- to deca-BDEs, BDE-183 is the most persistent, followed by 203>208>207>206>209, with persistence values relative to BDE-203 as follows: 3.13>1.00>0.48>0.45>0.40>0.26.

While we had anticipated that hibernation-associated fat loss would have consequences for lipid-based POP concentrations, the wide variation in congener-specific changes (CE and RP values) within the bears highlights a complex process, rather than a generalized "concentration effect". A number of factors can affect the preferential loss of contaminants relative to CB-153 in grizzly bears during hibernation, including excretion, placental and lactational transfer, contaminant mobilization and redistribution, differential binding to cellular receptors, as well as contaminant metabolism. Since grizzly bears do not urinate or defecate during hibernation, loss of POPs in this manner can be ruled out. Since the fall grizzly bears utilized in the study comprised either adult males or females below reproductive age, and spring grizzly bears were all male,

placental and lactational transfer to developing cubs during hibernation can also be ruled out. Fasting-associated increases of ΣPCBs in the blood serum have been observed in fasted mammals, reflecting lipid utilization and contaminant mobilization into circulation (160,161). Given the inability of hibernating grizzly bears to excrete these contaminants, the loss of circulating POPs is constrained, likely resulting in a redistribution of POPs based on lipid partioning among various body tissues.

Since POPs are differentially vulnerable to metabolic attack and subsequent elimination (93,162), metabolic enzymes may play the dominant role in the variations observed in RP values of individual contaminants in grizzly bears during hibernation. When we place our calculated PCB RP values from our bear data into structurally-related PCB metabolic groups (155,162,163), there are significant differences. PCB congeners which fall into Groups I (absence of vicinal H pairs) and II (>1 ortho-Cl) are known to be persistent in wildlife as a result of their resistance to enzymatic attack (162,163). This is consistent with our grizzly bear observations, where high values were observed in these metabolic groups (Group I: RP=77±40%; Group II: RP=67±26%). Groups IV and V are readily metabolized by CYP2B and CYP3A isozymes, and are therefore not considered to be as persistent in wildlife (162,163). This, too, is consistent with our observations, where significantly lower RP values were observed (Group IV: RP=27±28%; Group V: RP=20± 21%). Group III PCBs, however, comprised the planar PCB congeners which are not sterically hindered, and have vicinal *ortho-meta* H sites conducive to metabolism by CYP1A isozymes. This group is not known to be persistent in many wildlife species (155,163). However, the RP values for congeners from this metabolic group in our grizzly bears were as persistent as groups I and II (Group III: RP=70±32%), which

suggests that either 1) grizzly bears do not have CYP1A isozymes, 2) the activity of these isozymes is low during hibernation, or 3) CYP1A-inducible congeners in grizzly bear fat are less available for metabolic attack and elimination than in other species. In polar bears, CYP1A proteins were characterized and these correlated with PCBs and TEQ (164), which may indicate that true hibernation and appreciable fasting in grizzly bears may underlie the differences between the bear species.

An interwoven tale of diet, metabolism and POP-associated health risks. Following a fall gorging on food by grizzly bears, and the subsequent loss of fat reserves during their winter hibernation, we expected that concentrations of contaminants would increase in the residual adipose tissue. These concentration effects, however, varied from 0.00X to >4.00X among congeners and contaminants. Given this wide range in contaminant behavior, a Principal Components Analysis represented a more comprehensive approach for exploring the pharmacodynamics of POPs during a fasting event. Irrespective of their previous year's diets (ranging from wholly vegetarian to high trophic-level, salmon-eating), all post-hibernation grizzly bears had similar contaminant patterns (Figure 10). Some contaminants dominanting post-hibernation bears included oxychlordane, heptachlor epoxide, higher-chlorinated PCBs, and BDE-47, -119 and -153.

The clustering of all spring bears in one group sharply contrasts with fall bears, where we observed two distinct groups associated with two divergent feeding ecologies (salmon-eating vs. non-salmon-eating) (144). Fall interior bears were dominated by the volatile HCHs and lower-chlorinated PCBs, while fall maritime bears were dominated by DDT and its metabolites, as well as moderately-chlorinated PCBs. Since stable isotope signals reveal that our spring bears also comprised both maritime and interior grizzly

bears, PCA results indicate that adipose tissue POP patterns must converge during hibernation. This points to a single shared physiological process (e.g. metabolism), which drives POP patterns between feeding groups, and among individual bears.

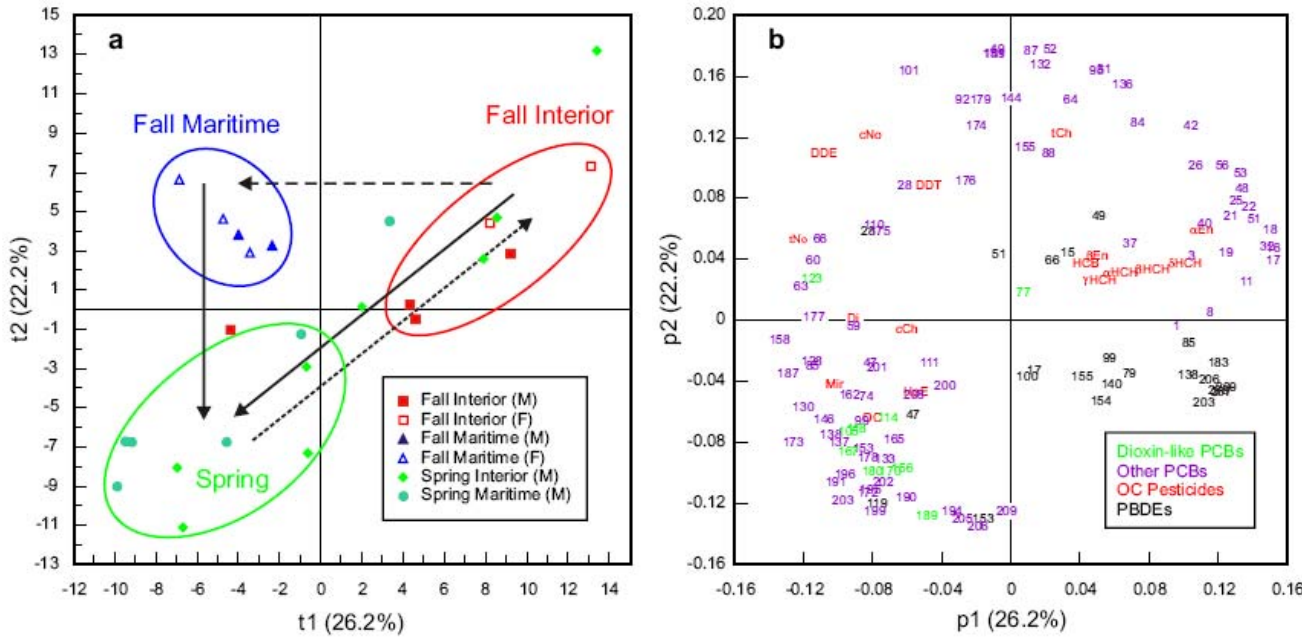

Figure 10. Principal components analysis (PCA) of a) fall and spring grizzly bears, and b) associated POP patterns. Principal components analysis (PCA) of a) a scores plot where individual fall and spring grizzly bears distinctly reveals three grizzly bear groupings: fall maritime bears (blue circle), fall interior bears (red circle) and all spring bears (green circle); and b) a loadings plot of polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides and polybrominated diphenyl ethers (PBDEs) in relation to the three grizzly bear groups. PCA demonstrates that during hibernation both "interior" and "maritime" contaminant patterns converge to a single "spring" contaminant pattern (along solid arrows), most likely reflecting common POP metabolic capacities in grizzly bears. The PCA and stable isotope results also demonstrate that upon commencement of spring/summer feeding on terrestrial food source, the contaminant patterns of all grizzly bears shift to an "interior" pattern (along dotted arrow). In the fall salmon-eating grizzly bears then shift their contaminant pattern back to a "maritime" pattern coincident with the arrival of returning salmon (along dashed arrow).

The important role that diet plays in driving contaminant patterns in feeding grizzly bears was evident in the previous study of fall grizzly bears (144). In this expanded fall dataset (i.e. adding PCBs for fall bears) and using all spring bear data, the PCA model revealed the importance of physicochemical properties in influencing POP patterns in bears, where PC1 correlated with contaminant log K_{ow} values (Figure 11). The PC1 values for both PCB congeners (r^2=0.51, ν=90) and OC pesticides (r^2=0.44, ν=17) were negatively correlated to their log K_{ow} values, while PC1 values for PBDE congeners were positively correlated to log K_{ow} (r^2=0.56, ν=22). The PC2 values for the grizzly bears were negatively correlated with the calculated RP values of individual contaminants, as dietary differences are less important for this second PC. In this case, all contaminants fell along one regression line (r^2=0.73, ν=73).

Contaminants in the upper left quadrant of the PCA variables plot represent non-persistent contaminants within the bears that were acquired from a marine food web, while those in the upper right quadrant represent non-persistent contaminants acquired through a terrestrial food web. Contaminants in the lower quadrants of the PCA variables plot, dominating spring bears, have the highest RP values and hence, demonstrate the greatest increases in concentrations following hibernation.

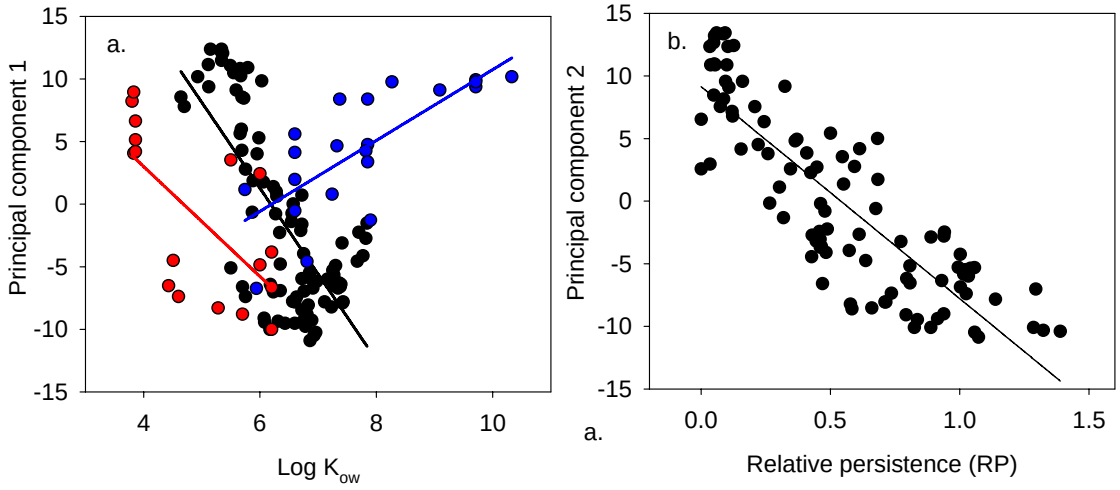

Figure 11. Relationship between a) PC1 and log K_{ow}, and b) PC2 and relative persistence of various POPs. a) Principal component axis 1 (PC1) describes the important role that log Kow plays in the behavior of POPs in grizzly bear food webs. Black circles: polychlorinated biphenyls (PCBs; r^2=0.51). Red circles: organochlorine (OC) pesticides (r^2=0.44). Blue circles: polybrominated diphenyl ethers (PBDEs; r^2=0.56). b) Principal component axis 2 (PC2) describes the role that metabolism (as measured by relative persistence; RP) in grizzly bears plays in the convergence of POP patterns observed in post-hibernation (spring) grizzly bears, irrespective of their fall diet (r^2=0.73).

The contaminant composition of the fall maritime bears thus represents a shift in contaminant composition from the fall interior pattern due to a substantial uptake of mid-range log K_{ow} contaminants from salmon (PC1) that overwhelm the terrestrial component. During hibernation of the fall maritime bears, metabolism proceeds roughly along PC2, with preferential removal of contaminants with lower RP values. For the fall interior bears, however, the PCA model suggests that metabolism follows from the upper right to the lower left quadrant, and contaminants with both lower RP values and log K_{ow} values falling outside an optimal uptake zone (i.e. <5.5 and >7.5) are removed. These linear relationships provide strong evidence of a common metabolic process among grizzly bears, regardless of their feeding ecology. Metabolism, therefore, appears to

represent the driving force behind the converging POP patterns observed in maritime and interior grizzly bears during hibernation.

Grizzly bear hibernation provides a unique opportunity to observe the changes in POP concentrations and patterns without the confounding effects of additional POP exposure and elimination through excretion. Despite a small sample size and use of different bears pre- and post-hibernation, our results strongly suggest that while food web accumulation (log K_{ow}) dictates POP concentrations and patterns during a feeding phase, metabolism ultimately governs the overall contaminant patterns in a fasting phase, irrespective of previous dietary choices by the bears. This study provides evidence of a duality of POP-associated health risks to grizzly bears during hibernation. First, increasing concentrations of recalcitrant POPs, including dioxin-like PCB congeners, may contribute to a disruption of endocrine processes. Second, the inability to excrete the metabolites resulting from the conversion of less persistent parent POPs may cause a prolonged build-up of water-soluble reactive species in the hibernating bears. We speculate that exposure during hibernation to increasing concentrations of some parent POPs, as well as POP metabolites, may increase risk of adverse health effects in grizzly bears and their cubs.

CHAPTER 3. SALMON-EATING GRIZZLY BEARS HIGHLIGHT THE IMPORTANCE OF METABOLISM VERSUS EXCRETION IN THE NET ACCUMULATION OF PCBS

Introduction

Top predators of aquatic food webs are particularly vulnerable to exposure to and accumulation of many persistent organic pollutants (POPs), particularly lipophilic and persistent, polychlorinated biphenyls (PCBs). PCBs have the propensity to biomagnify to apex predators (18,31), where concentrations reach levels that have deleterious health effects to the exposed individuals, their offspring and possibly, whole populations (165,166). It is therefore, paramount to characterize the biomagnification of PCBs, and processes behind the biomagnification. Biomagnification of PCBs in aquatic food webs is well documented, generally through calculations of biomagnification factors (BMFs) (82,131). BMFs represent increased uptake and/or decreased depuration of PCBs (and individual congeners) at each trophic level driven not only by availability of PCBs in the diet, but also by physico-chemical properties of individual PCB congeners and metabolic capacity of the predator (73,131,167,168).

Particularly, PCB metabolism, as a mechanism contributing to depuration in predators, has received considerable attention and is most often inferred by calculating a "metabolic index" (MI). The calculation is similar to BMF calculations, with the difference in that individual PCB concentrations are corrected to a recalcitrant congener (CB-153 or CB-180) prior to trophic level comparisons (163,169). It has become common practice, especially in mammals, to use calculated MIs to infer 1) presence or absence of cytochrome P450 (CYP) xenobiotic metabolizing enzymes, particularly with CYP1A- and CYP2B/3A-like catalytic activities and substrate selectivities, and 2) their magnitude of activity through MI comparisons with other species (162,170).

While calculation of MIs and BMFs have provided insight into inferred metabolic capacities of predators with respect to POPs (94,163) and net accumulation of POPs in food webs (171), a number of assumptions are required that may weaken the accuracy of conclusions and, hence, assessment of toxicological risk. First, MIs and BMFs assume a 'general' prey item for the predator, and individuals of the same predator species are grouped together, irrespective of possible unique dietary histories of individual animals (144,172). Different reliance on terrestrial and marine food webs had a profound influence on grizzly bear PCB patterns and concentrations (144). In a captive, semi-field study control West Greenland sled dog (*Canis familiaris*) adipose tissue tended to be distinguished by greater proportions of CB-170/-190, -180 and -194 to ΣPCB concentrations, whereas the exposed cohort (fed a diet of minke whale (*Balaenoptera acutorostrata*) blubber) accumulated higher concentrations of CB-153 (172).

MI interpretation of PCB congener patterns in tissues of e.g., fish-eating marine mammals includes assigning congeners to structure-activity groups (SAGs), and thus, classifies all congeners within each SAG as having similar MI values as a result of their similar molecular qualities governing their accumulation or loss (162,163). For example, all congeners within SAG 1 (no vicinal hydrogen substituted carbons) are assumed to be similarly persistent in the predator (MI = 1), since there are a lack of sites available on the molecule for optimal metabolic enzyme binding.

Another assumption is in regards to PCB concentration normalization for prey and predator by correction with the recalcitrant CB-153 (or CB-180) congeners. CB-153 is considered the most dominant congener in mammals (18,144,173), as well as the most recalcitrant of PCB congeners in mammals (163), although slow metabolism can still

occur (174). MI values thus provide a value representing depuration relative to CB-153 and not a measure of total depuration, creating an artifact of apparently high persistence in, for example, SAG I.

Potentially, the most erroneous assumption in the calculation of MI is that PCB pattern changes from prey to predator have been solely used as a means to assess metabolic capacity of predators, where no other modes of depuration (e.g. excretion), which could also contribute to low MI values, are considered. MIs, like BMFs, assess an amalgamation of pharmacokinetic processes, including uptake, elimination through excretion and respiration, as well as contaminant metabolism (94,100,162,175,176). Both uptake and excretion of PCBs can be influenced by the properties of the food item (e.g. fibre and lipid content) (177), feeding rate of the predator (177), as well as fugacity from/to the gastrointestinal tract (178). In air-breathing predators, respiratory loss of lower log K_{oa} PCBs may also contribute significantly to overall depuration (100). As well, tissue specific (or stratified, as in blubber) accumulation may produce artifacts of apparent "loss" of certain congeners, as a result of tissue-specific localization as a function of protein and lipid compositions (i.e. fatty acids), and/or presence of specialized cells (i.e. liver) - recently reported for PCBs in liver, fat, brain and blood tissues of East Greenland polar bears (*Ursus maritimus*) (168). In effect, neither the MI nor the BMF approach is able to differentiate or discern the true magnitude of individual factors and processes involved in PCB depuration/accumulation by the predator. The assessment and magnitude of these factors are important to examine, as each has a different consequence to the health of the exposed animal.

In this study we developed a model to assess the importance of individual pharmacokinetic processes influencing the net accumulation of PCBs in wild grizzly bears (*Ursus arctos horribilis*). The model attempts to minimize the aforementioned assumptions used in current BMF and MI approaches to strengthen our assessment of toxicological risk. Specifically, we 1) correct for dietary (thus, PCB concentration and pattern) variation within the predator term (BC grizzly bears) of the calculations, 2) characterize from salmon to bear the accumulation of 71 PCB congeners, both individually and within previously defined SAGs, 3) calculate and compare MIs for the grizzly bears using the most recalcitrant congener, rather than CB-153, and 4) differentiate between and establish the importance of metabolism and other pharmacokinetic processes that influence PCB depuration in grizzly bears.

Methods and Materials

Sample Collection. Information on individual grizzly bear traits, stable isotopes and PCB concentration and composition is thoroughly described in Christensen et al. (144,154). Briefly, subcutaneous fat and hair samples were obtained following a legal hunt of male and female grizzly bears during fall 2003 (n=10) and male bears during spring 2004 (n=13) (144,154) from various locations in BC (Table 6).

Eight spawned-out sockeye salmon (4 male, 4 female), *Oncorhynchus nerka*, were captured in Koeye River, BC. Another six pre-spawned sockeye salmon (3 male, 3 female) were captured in Fitz Hugh Sound. Although grizzly bears may consume any or all of the species of Pacific salmon available, we chose sockeye, as it is common and abundant in most coastal BC watersheds. Salmon were sacrificed and filleted. One ~25

g sample of filet from the left side immediately posterior the dorsal fin was placed in hexane-rinsed aluminum foil, and kept frozen at -20ºC. Three 1 g muscle samples from each fish were individually placed in single hexane-rinsed vials, freeze-dried at -50ºC for 48 hours, and ground to powder for stable isotope analysis.

Table 6. Summary of biological information on sampled bears in fall 2003 and spring 2004, as well as the percentage of sockeye salmon consumed as estimated using $\delta^{15}N$ stable isotope values in hair.

Bear ID	Age	Sex	Sockeye Equivalent (SE; %)
Fall Grizzly Bears[ab]			
1	3	F	0.00
2	Unknown	F	12.1
3	15	M	9.7
4	1	M	5.7
5	10	M	12.0
6	5	M	54.6
8	Unknown	M	13.1
9	5	F	32.5
10	5	F	43.0
11	Unknown	M	38.4
Spring Grizzly Bears			
12	10	M	81.2
13	3	M	75.5
14	3	M	58.3
15	6	M	55.8
16	13	M	83.9
17	Unknown	M	55.5
18	5	M	5.00
19	12	M	36.5
20	11	M	5.70
21	8	M	31.8
22	21	M	8.60
23	8	M	17.1
24	6	M	24.9

[a]Age and sex information taken from Christensen et al. (144)

[b]Bear #7 from Christensen et al. (144) was not used for this study, as it was a muscle sample

Stable Isotope Analysis. The determination and reporting of stable isotope data ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) for segmented grizzly bear hair can be found in Christensen et al. (144,154). Details of stable isotope analysis can also be found in Christensen et al. (144,154).

Contaminant Analysis. PCB data for the present grizzly bears has been reported elsewhere (154). Briefly, approximately 3 g of fat from each sampled bear and 25 g of salmon filet was analyzed for 159 PCB congeners (126 individual congeners, 33 homologue co-elutions). Samples were analyzed using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) by AXYS Analytical Services, Sidney, BC, according to their laboratory procedures, as outlined elsewhere (144). Twenty-nine internal ^{13}C standards were included in each sample analysis to assess contaminant recovery.

Also included with each batch of samples was a procedural blank. For grizzly bears, specific lab blank information can be found in Christensen et al. (154). For spawned-out sockeye salmon, all PCB congeners were <0.001 ng/g wet weight (wet wt.). Pre-spawned sockeye had sample blank concentrations <0.0032 ng/g wet wt. for all PCB congeners. Based on a criterion of the signal being greater than 3 times the standard deviation of the noise, the quantitative method detection limit (MDLs) for PCBs were all <0.001 ng/g wet wt for tissues of fall and spring grizzly bear as well as spawned and pre-spawned sockeye salmon. For statistical purposes, MDL substitutions were made for PCB analytes that were not detected in cases where >70% of samples had detectable values for that contaminant. When <70% had detectable concentrations, these congeners were not considered further.

PCB recovery efficiencies based on internal standards ranged from 89 to 107%, and were considered satisfactory based on limits set by AXYS. Despite consistently low lab blank concentrations, all sample concentrations were background corrected to the lab blank. Concentrations in samples were corrected for the extractable lipid content, and expressed as ng/g lipid weight (lw). The percent lipid was determined gravimetrically on a sample extract using dichloromethane .

Diet-Corrected Predator: a "Model" Salmon-Eating Grizzly Bear. Since proportion of salmon in grizzly bear diets (calculated using stable isotopes in hair) is correlated with many POPs in their fat (144,154), it is possible to establish the PCB composition of a grizzly bear that has a 100% salmon diet using data from bears that fall elsewhere along that dietary continuum. Percent salmon consumption for each bear was based on calculations from Christensen et al. (144), with an adjustment from using Chinook salmon (*O. tshawytscha*) as the salmon end-member to sockeye salmon (50:50, spawned:pre-spawned). This change was conducted so stable isotope results (i.e. proportion of sockeye salmon consumed) were consistent with contaminant data available for that salmon species. Hence, the two end-member model in this study was comprised of 100% vegetation and 100% sockeye salmon. Sockeye values were $\delta^{15}N = 10.9 \pm 0.6$ ‰.

The percentage salmon consumed for individual bears was then plotted against PCB congener concentrations from respective bear fat. This was done for fall and spring bears separately. Only significant regressions were used to calculate the PCB concentrations and patterns of the model grizzly bear. To create the model bear percent sockeye consumed (x variable) was considered 100% in each linear regression equation,

with the "y" value being the predicted concentration for that particular congener. For each calculated concentration, a 95% confidence interval (CI) was calculated. POP concentrations in the model bears are expressed as mean ± CI. In total, it was possible to assess 71 quantifiable PCB congeners using this method, and thus the sum-PCB is referred to as Σ_{71}PCB.

Biomagnification Factors and Metabolic Index Calculations: Salmon to Grizzly Bear. Biomagnification factors (BMFs) of PCBs from salmon to grizzly bears were determined by dividing the lipid corrected PCB congener concentration in the model grizzly bear by the lipid corrected concentration of that same congener in the salmon.

For the MI, lipid normalized PCB concentrations in grizzly bears and sockeye salmon were first normalized to concentrations of the congener with the greatest BMF in the sampled grizzly bears (in this case, CB-194). Normalized concentrations within each species were expressed as values R_{194}. Predator ratios for a particular congener were then divided by the prey ratio for that same congener to obtain a MI value, R_{total} (163).

While our focus is on behavior of individual congeners, for consistency with other studies PCB congeners were also placed in one of five structure-activity groups (SAGs) with regards to biotransformation (162,163). Group 1 is comprised of congeners without any vicinal hydrogen (H) atoms on carbons of either phenyl ring. Group 2 is comprised of congeners with vicinal H atoms exclusively on *ortho*- and *meta*-carbons in combination with ≥ 2 *ortho*-chlorine (Cl) atoms. Group 3 congeners have *ortho*- and *meta*- H pairs with < 2 *ortho*-Cl. Group 4 congeners have *meta*- and *para*-H pairs with ≤ 2 *ortho*-Cl. Group 5 congeners have *meta*- and *para*-H pairs with > 2 *ortho*-Cl.

Statistical analysis. Regression analyses were applied to 1) percentage of salmon consumed and PCB concentrations for both feeding and fasted grizzly bears, and 2) log R_{total} with log K_{ow} (octanol-water partition coefficient). Data points with standardized residuals of <-2 or >2 were considered outliers in the regression, removed, and the regression was recalculated. Student's t-tests were conducted to test for significant differences in regressions between sampling feeding phases. T-tests were used to compare log R_{total} within metabolic groups from feeding to fasted, as well as whether log R_{total} within SAGs were significantly different than 0. The criterion for significance was α=0.05.

Results and Discussion

PCB Delivery by Salmon versus PCB Retention in Bears. Using $\delta^{13}C$ or $\delta^{15}N$ measurements as food web/dietary tracers and PCB concentrations from 10 feeding and 13 fasted grizzly bears, we were able to predict PCB concentrations and patterns in bears that consumed 100% sockeye salmon. While this approach produces only one concentration value for each congener for each sampling season, it is based upon the data of numerous other grizzly bears that fall along that dietary (hence, contaminant) continuum, and reflects all physicochemical and environmental processes governing exposure and accumulation in the grizzly bears.

Via a sockeye salmon diet, grizzly bears were exposed to mean Σ_{71}PCB concentration pf 792 ± 1,020 ng/g lw. Σ_{71}PCB concentrations for individual pre-spawned and spawned salmon (data not shown) are consistent with that determined in other studies (82,179,180). The PCB pattern was dominated by CB-153 (10% of Σ_{71}PCBs): CB-153>-

129/-138>-101>-147/-149 >-70/-74>-118 with Σ_{71}PCB TEQ at 0.00535 ± 0.00657 ng/g lw (Figure 12). Grizzly bears in the feeding phase had an overall Σ_{71}PCB concentration of 117 ± 35.6 ng/g lw. The PCB profile of the feeding grizzly differed greatly from the sockeye and was dominated by CB-153>-180>-118>-129/-138>-99>-170. Σ_{71}PCB TEQ was 0.00459 ± 0. 000975 ng/g lw.

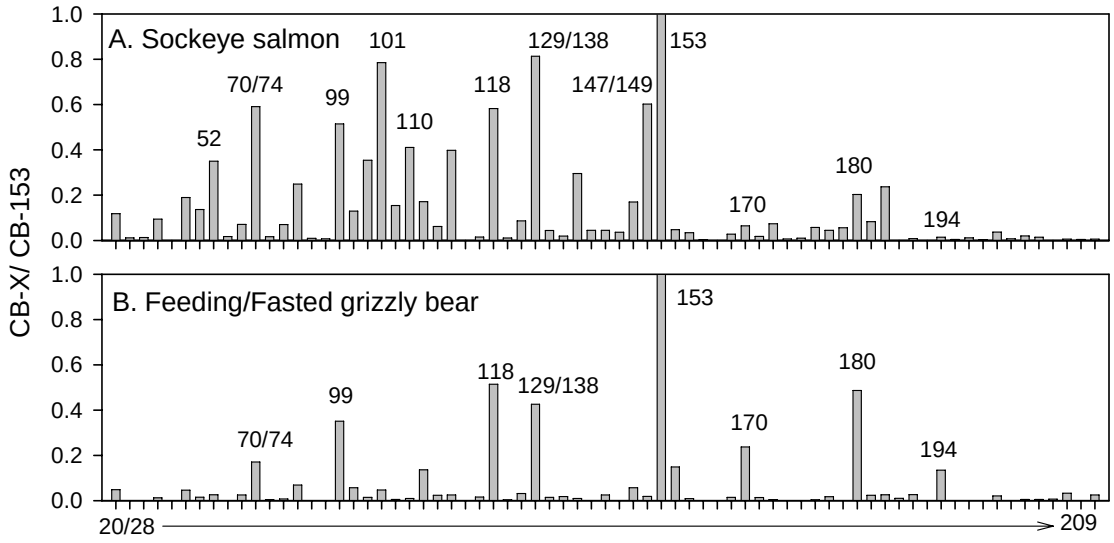

Figure 12. PCB congener patterns in A) sockeye salmon, and B) pre-hibernation (feeding) grizzly bear that hypothetically consumed 100% sockeye salmon. PCB pattern simplification reflects lack of PCB uptake and/or preferential depuration (i.e. loss through metabolism or excretion) of certain PCB congeners by the bear. Congeners are organized by increasing congener number. Concentrations are relative to CB-153, rather than CB-194, as the former congener dominated both salmon and grizzly bears.

The Σ_{71}PCB BMF of 0.147 for feeding grizzly bears (Appendix III) was low compared with the previously reported for other marine mammals (31,181). Only 8 PCB congeners had BMFs > 1.0 in the feeding grizzly bears: CB-194, the most biomagnified congener (BMF = 3.04), CB-205 (1.76), CB-206 (1.66), CB-189 (1.49), CB-209 (1.36),

CB-170 (1.19), CB-190 (1.06) and CB-156/157 (1.01). The CB-194 BMF was not only the highest, but increased from 0.14% of Σ_{71}PCBs in salmon to 3.0% in feeding bears. In contrast the BMF for CB-153 was <1 (0.324), and thus not biomagnified in grizzly bears. Comparatively, the ringed seal blubber to polar bear BMF for CB-194 was by far the highest at 75 relative to a BMF of 8 for CB-153 (172). Interestingly, the BMFs for Σ_{71}PCB in the actual grizzly bears ranged from 0.00064 and 0.082 when dietary variation was not considered, highlighting the importance of diet correction in individual predators prior to using a general prey term in the BMF calculation. Accordingly, BMF significantly increases with increasing salmon consumption by the bear ($y = 0.0015x - 0.003$, $p<0.0001$, $r^2 = 0.83$).

While our model for fasted grizzly bear had a similar PCB profile to the feeding bear, their Σ_{71}PCB concentration of 210 ± 61.3 ng/g lw ($p<0.05$) and Σ_{71}PCB TEQ concentration 0.00906 ± 0.00261 ng/g lw ($p<0.05$) were significantly greater than feeding bears. This reflects the fasting-associated utilization of lipids and the subsequent concentration effect for the most persistent PCBs in the residual fat, including the dioxin-like PCBs (89,154). CB-153 was again dominant: CB-153>-118>-180>-138>-170>-70/-74.

There was a simplification of PCB pattern from salmon to grizzly bear; however, it differed somewhat to the pattern simplification observed in a previous ringed seal blubber to polar bear fat food chain comparison (94). The high metabolic capacity towards PCBs of the polar bear is well documented (94,182), and although grizzly bears are closely related, their capacity to depurate several PCB congeners appears to be lower. Specifically, and as shown previously, PCB patterns in polar bears were overwhelmingly

dominated by CB-153, -180, -170, -138 and -99 (>95% of Σ_{71}PCB), all congeners classified in SAGs 1 and 2, and thus most slowly biotransformed, e.g., via CYP-related xenobiotic enzyme mediation (94). Grizzly bears, on the other hand, not only exhibit dominance by those same congeners (i.e. SAGs 1 and 2), but also CB-74, 105, 118, 156 and 194; four congeners thought to be suitable substrates and thus metabolizable via CYP1A isozyme mediation (SAG 3). Overall, while salmon deliver a plethora of PCB congeners, the grizzly bears appear to retain only selected congeners from SAGs 1, 2 and 3 resulting in a low Σ_{71}PCB BMF of 0.147 for feeding grizzly bears.

Differentiation between Metabolism and Other Depuration Processes. The altered PCB congener profile between salmon and grizzly bears (and resultant BMF) reflects a number of pharmacokinetic processes, such as gastrointestinal uptake, elimination through excretion and respiratory loss, as well as congener-specific metabolism. By calculating R_{total} for PCB congeners in feeding grizzlies, we are able to obtain a value that encompassed all active processes governing pharmacokinetically-driven changes, both metabolism or non-metabolic. Similarly, R_{total} values for fasted grizzlies capture both 1) processes occurring during their feeding phase the previous fall, and 2) the processes occurring during their extended fast (154). Since no uptake or excretion takes place during hibernation, PCB level and pattern changes during this time would apparently be restricted to metabolism alone. If no PCB differences between grizzly bear feeding and fasted phases exist, then we hypothesize that metabolism is not occurring and that other pharmacokinetic processes before hibernation determine the changes in PCBs derived from the salmon diet. Specifically, we define a metabolizable SAG using a two-tiered approach, and it must consider both 1) a log R_{total} value

significantly lower than zero during feeding phase, and 2) a log R_{total} value that significantly decreases during hibernation (from a feeding to a fasted state) in the grizzly bear.

Tier One: PCB Depuration in feeding grizzly bears. Although fat was opportunistically sampled from various locations on the body of the bear, all samples were subcutaneous fat. It was recently reported for polar bears PCB concentrations or patterns are not fat depot-specific (13). However, in bottlenose dolphins from the Southeast United States, fat has been shown to be stratified, in terms of cell types, cell sizes, infiltration of blood vessels and CYP activity (183). Hence, we suggest that subcutaneous fat samples from fall sampled grizzly bears consisted of multiple fat layers (outer, middle and possibly inner), while spring bear fat consisted solely of outer fat due to inner fat utilization during hibernation. Since outer fat (in bottlenose dolphins) is thought to be metabolically inactive (183), it represents longer term accumulation of PCBs and contains congeners that were likely not readily depurated through metabolism or other processes throughout the bear's life. The inner fat layers would, conversely, be metabolically active and represent those congeners recently taken up and retained following salmon consumption by the bear for that given year prior to hibernation, and would thus be more readily mobilized during fat utilization.

When bears are gorging on salmon and accumulating fat as they prepare their bodies for hibernation, the subcutaneous fat stores of the bear increase diluting the PCBs; concentrations appear low. However, lower PCB concentrations may also be explained by lack of uptake or considerable depuration prior to hibernation. In this instance, depuration is defined as a lack of retention either through means of increased metabolism,

excretion, respiratory loss or a combination of these factors. Assuming 100% uptake and retention of most highly biomagnified congener (CB-194) in the fat, feeding grizzly bears appear to depurate 89% of SAG 1, 93% of SAG 2, and 94% of SAG 3 obtained from salmon. Congeners from SAGs 4 and 5 were almost completely depurated prior to hibernation (>99% for both groups). Interestingly, normalizing to CB-153 suggests significant depuration of SAGs 3, 4 and 5 only, and hence using this MI approach we would conclude that both CYP1A- and CYP2B/3A-like xenobiotic-metabolizing enzymes are active in the bears, as well as that SAG 1 and 2 PCBs are extremely persistent in the bears with close to 100% retention. In fact, relative to CB-153, CB-194 is 937% retained, which is not realistic or possible.

Tier Two: PCB Depuration in fasting grizzly bears. When the fat from the inner layers is mobilized to the blood during hibernation, the PCBs within those layers are also mobilized and again processed through the liver, and thus subject to a second phase of metabolism by xenobiotic-metabolizing CYP enzymes. Since excretion is not possible during hibernation, those parent congeners that are not metabolized should partition into the residual fat of the outer layer. Since CB-194 is considered the most biomagnified congener in these bears, we assumed all of CB-194 mobilized during hibernation from the inner fat layers must partition back into the residual fat of the outer layer. Relative to CB-194, PCBs in SAGs 1, 2 and 3, on average, were not significantly depleted during hibernation (Figure 13). Conversely, significant decreases were observed in SAGs 4 ($p = 0.001$) and 5 ($p = 0.001$), with 78% and 74% loss, respectively. Almost all congeners from these SAGs individually experienced >50% loss during hibernation.

Based on our two-tiered approach, metabolism appears to only occur in SAGs 4 and 5, suggesting that only CYP2B/3A-like enzymes are actively catalyzing PCB metabolism in grizzly bears. Lack of change of SAG 3 PCB congeners during hibernation suggest that these congeners are not suitable substrates for CYP1A isoforms in grizzly bears. Thus, the lack of significant change in SAGs 1, 2 and 3 log R_{total} values during hibernation, suggested their low retention must be a result of a depuration process, other than metabolism, and the loss occurs prior to hibernation. Although metabolism occurs for all PCB congeners, albeit at highly variable rates, for simplicity we refer to SAGs 1, 2 and 3 as "non-metabolizable congeners", as the results suggest the rate of metabolism is slow (at least during hibernation) and other modes of depuration dominate their loss following exposure.

Role of Log K_{ow} in Depuration of Non-Metabolizable PCBs in Grizzly Bears. Non-metabolizable SAG 1, 2 and 3 PCB congeners have considerable variation and range in R_{total} values. The variation in SAGs 1 and 2 represents greater than two orders of magnitude (equating to 100% to <1% retention) within what are considered two "recalcitrant" metabolic groups in other wildlife species (163). SAG 3 had similarly large ranges in log R_{total}.

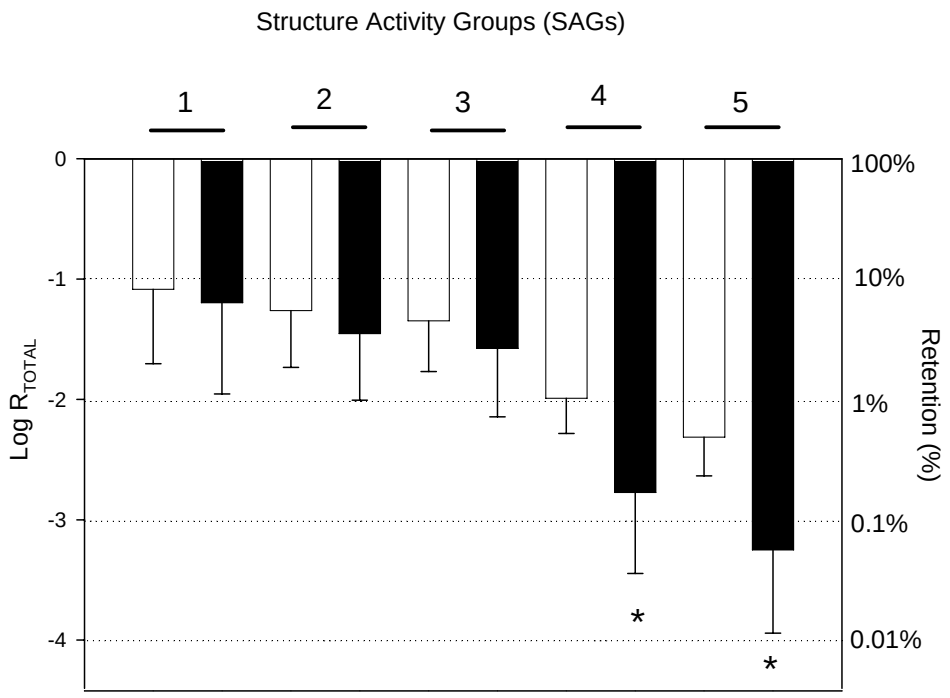

Figure 13. Calculated R_{TOTAL} values (predator/prey relative to CB-194) for PCB congeners in five structure-activity groups (SAGs) for feeding (white bars) and fasted (black bars) grizzly bears. In feeding bears log R_{TOTAL} values from all SAGs are significantly different from zero, suggesting significant depuration (>90% loss) of PCBs prior to hibernation. During hibernation, only SAGs 4 and 5 significantly decrease in the bears (denoted by asterisks). Since hibernating grizzly bears do not eat or excrete, changes during hibernation are likely a result of contaminant metabolism (i.e. CYP2B/3A-like enzymes). Conversely, while Log R_{TOTAL} values in SAGs 1, 2 and 3 are greater than 90% depurated, this loss cannot be attributed to metabolism, as the Rrel values do not significantly decrease during hibernation. Error bars denote 1 standard deviation of the mean. Group 1 = no vicinal hydrogen (H) atoms on carbons of either phenyl ring. Group 2 = vicinal H atoms exclusively on ortho- and meta-carbons with ≥ 2 ortho-chlorine (Cl) atoms. Group 3 = ortho- and meta- H pairs with < 2 ortho-Cl. Group 4 = meta- and para-H pairs with ≤ 2 ortho-Cl. Group 5 = meta- and para-H pairs with > 2 ortho-Cl.

For these non-metabolizable PCB congeners we measured a positive correlation between their log R_{total} and log K_{ow} (Figure 14). Log K_{ow} has significant relationships with non-metabolizable log R_{total} values for both feeding ($y = 0.460x - 3.32$, $r^2 = 0.32$, $p < 0.0001$) and fasted grizzly bears ($y = 0.588x - 4.26$, $r^2 = 0.40$, $p < 0.0001$). Since the only significant process of depuration during hibernation is metabolism, the lack of

significant difference in regressions between feeding and fasted grizzly bears supports our previous assertion that metabolism is not responsible for the depuration (low R_{total}) of congeners within these groups, and that most of the loss occurs prior to hibernation through another depuration process. Conversely, R_{total} values of metabolizable PCBs (SAGs 4 and 5) are not influenced by log K_{ow} during the feeding phase or the fasted phase, yet individual congeners show marked decreases in R_{total} from feeding to fasted phases.

The correlation between R_{rel} of non-metabolizable congeners and log K_{ow} is not surprising, as numerous pharmacokinetic processes are governed by log K_{ow}. Specifically for PCBs, although all congeners are readily absorbed by the gastrointestinal tract, highly hydrophobic congeners (i.e. high log K_{ow}) have lower absorption efficiencies as a result of either solubility limitations in the mixed micelle vesicles of the intestine, or diffusion limitations across the unstirred water layer (176,178). Alternatively, lower log K_{ow} contaminants, although potentially more susceptible to uptake, are also more water soluble and therefore may be more easily excreted by the predator (131,184). Since log K_{ow} and log K_{oa} (octanol-air partition coefficient) are linearly related for PCBs, lower log K_{oa} (low log K_{ow}) congeners may also be respired from the air-breathing predator to a greater extent than higher log K_{oa} congeners (178,185). Partitioning into lipid compartments is also driven by log K_{ow}, where more lipophilic contaminants are preferentially accumulated in fat.

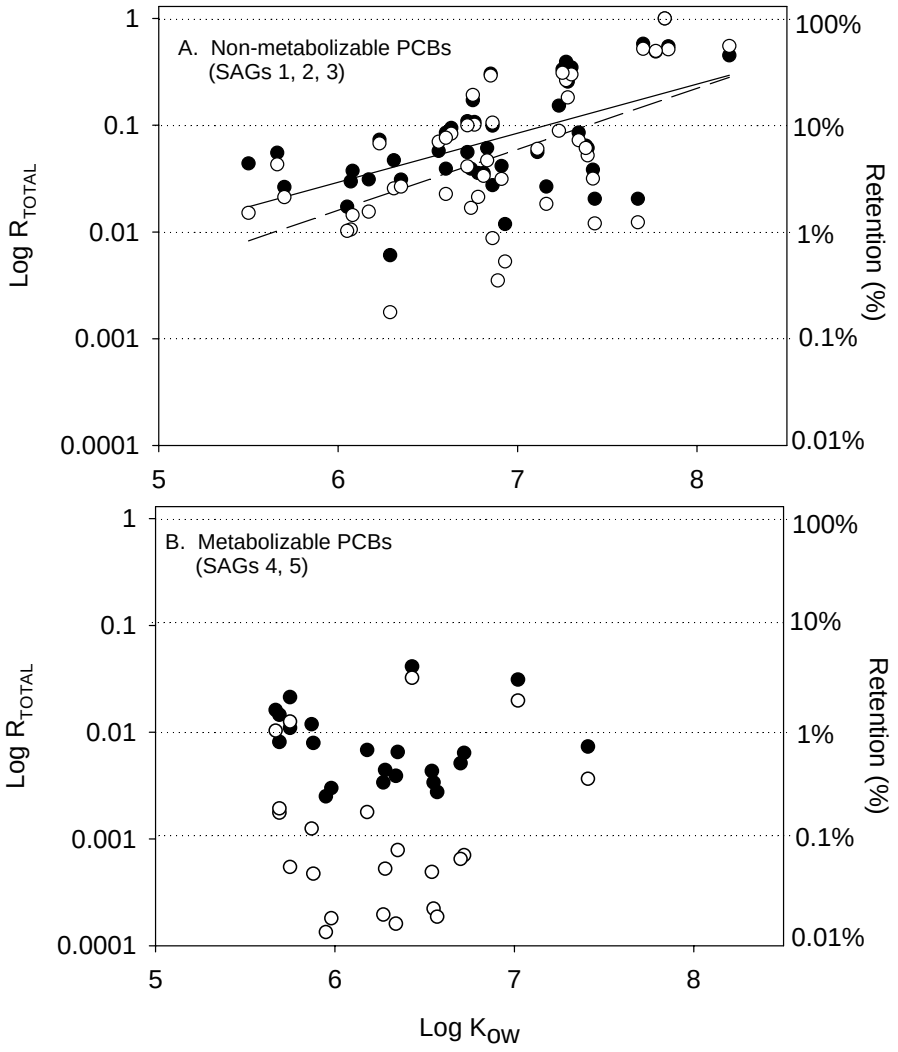

Figure 14. Relationship between log K_{ow} and Log R_{TOTAL} of PCB congeners from various structure activity groups (SAGs). A) Log K_{ow} is the driver behind the Log R_{TOTAL} values of non-metabolizable PCB congeners within Structure Activity Groups (SAGs) 1, 2 and 3 combined (feeding: $y = 0.460x - 3.32$, $r^2 = 0.32$; fasted: $y = 0.588x - 4.26$, $r^2 = 0.40$). The correlation suggests that depuration through excretion of lower log K_{ow} PCBs may be the major driving factors affecting non-metabolizable R_{TOTAL} values. The regressions are not significantly different from feeding to fasted phases, supporting that metabolism is not significantly influencing R_{TOTAL} values, since metabolism is the only pharmaco-kinetic depurative process at work during the hibernation period. B) Conversely, log K_{ow} plays no role in depuration of metabolized congeners from SAGs 4 and 5. As well, significant decreases in R_{TOTAL} from feeding to fasting supports metabolism, rather than excretion, as the main form of depuration for these congeners.

Increasing R_{total} with increasing log K_{ow} of non-metabolizable congeners suggests that depuration through excretion and/or respiration, rather than structure-related metabolism or differential uptake, explains the pharmacokinetic behavior of these PCBs within grizzly bears. However, excretion likely contributes to overall depuration of these congeners to a greater extent than respiration, as respiration (although lessened) does occur during hibernation, and we observed no significant loss of these congeners during that time. Loss through excretion here would be defined as 1) PCBs passing through the gastrointestinal tract unabsorbed, 2) PCBs absorbed and diffused back into the gastrointestinal tract, 3) PCBs partitioned into the bile and subsequently excreted, and 4) PCBs excreted in the urine.

Since we only used fat in this study, there may be tissue-specific accumulation occurring that is controlled by other factors (14), which may create an artifact of higher log K_{ow} PCBs in the fat tissue. For example, blood has a higher affinity for less lipophilic, more protein associated contaminants, such as hydroxylated (OH)-PCBs (168). However, as was shown for polar bears (14), blood-associated PCB congeners would make up a very small proportion of total PCB burden, and thus would not have such a strong influence on PCB patterns in fat tissue.

We thus hypothesize that non-metabolizable congeners with lower log K_{ow} are taken up through the gastrointestinal tract of the bear, and eventually removed via excretion (prior to hibernation). Conversely, the non-metabolizable, higher log K_{ow} contaminants absorbed by the bears become partitioned into their fat where they ultimately accumulate. Accordingly, we observed an increase in dominance of non-metabolizable congeners (SAGs 1, 2, and 3) from sockeye (57.7%) to feeding grizzly

(94.3%) to fasted grizzly (98.3%), with most notable increases in non-metabolizable congeners with higher log K_{ow}. It is important to note that metabolizable congeners have low log K_{ow} values, so may also be influenced by excretion prior to hibernation.

Kucklick et al. (186) found a similar relationship between log K_{ow} and accumulation of individual PCBs in Alaskan polar bears from seal prey, although they do not discuss its relevance. They included PCB congeners of SAG 4 and 5 in the regression, which increased the variation, as the accumulation of SAG 4 and 5 congeners was considerably lower than for other metabolic groups at similar log K_{ow} values (186). Nakata et al. (187) found in the Baikal seal (*Phoca sibirica)* that SAG 4 and 5 congeners also did not fit the regression between log K_{ow} and log BMF due to their extremely low BMF values.

Toxicological Implications. Our results with feeding and fasted salmon-eating grizzly bears clearly demonstrate the individual importance of both contaminant metabolism and excretion (via log K_{ow}) in PCB depuration. Feeding grizzly bears depurate >90% of Σ_{71}PCBs obtained from salmon prior to hibernation. Of this, we attribute ~60% of depuration to excretion and ~40% to metabolism.

Salmon-eating grizzly bears have a considerably lower BMF than observed in other marine mammals, including the polar bear, which may reflect a number of factors, such as different metabolic capacities, as well as decreased PCB uptake and/or increased excretory loss. Gobas et al. (80) suggests that predators that consume lipid-poor, organic-rich foods (i.e. vegetation), such as the omnivorous grizzly bear, will experience smaller increases in concentration relative to its prey. Hence, if the diet POP concentrations are low (i.e. vegetation) the diffusion gradient shifts and the result is *net excretion* of POPs

(80). While our model salmon-eating bears do not consume vegetation, the estimated concentration values are based on grizzlies that do rely on vegetation. Interestingly then, it appears as if the grizzly bear's reliance on vegetation may protect it from significant accumulation of PCBs obtained from the salmon portion of its diet. Thus, reliance on vegetation by other species may also be relevant for their calculated BMF and MI values.

Being able to differentiate between these depuration processes allows us to assess, more confidently, the toxicological implications to the salmon-eating grizzly bears. This degree of metabolism relates to an approximate upper-level concentration of 975 ng/g lw of PCB metabolites in the fat tissue available to a 100% salmon-eating grizzly prior to hibernation. Salmon-eating grizzly bears on the northern coast of BC consume upwards of 60% salmon (144), so upper-level concentrations of PCB metabolites would likely be closer to 500 ng/g lw. While metabolism of parent PCBs can increase the water solubility of parent PCBs and hence facilitate their loss through excretion, there would likely be some retention of the lipophilic metabolites produced through biotransformation. Metabolites would likely be in the form of $MeSO_2$-PCBs, as these are generally formed via CYP2B/3A-like enzymatic pathways via epoxide formation and conjugation (94). In polar bears $MeSO_2$-PCB concentrations varied from 95.9 to 699 ng/g lw in adipose tissue depending on geographic location (94,173,182). Since the inferred upper limit of available metabolites is appreciably high in grizzly bears, characterizing the actual PCB metabolite concentration in feeding and fasted grizzly bears would be a logical next step for an overall health risk assessment.

These results evoke some potentially important considerations when using either a "metabolic index" to infer metabolic capacity or BMF to assess net biomagnification in

predators. While calculation of BMFs and MIs have provided important insights into overall PCB biomagnification/ depuration in food webs, they are unable to differentiate between depuration processes. Our study using model grizzly bears and a two-tiered approach clearly demonstrates the important roles of excretion and metabolism leading to that net accumulation. This information strengthens the ability to assess toxicological risks by determining the magnitude of each depuration process. Despite the low BMF, which alone would signify low health risks to grizzlies, the magnitude of metabolism we were able to calculate infers that there is the potential for significant concentrations of toxic PCB metabolites that may or may not be excreted.

CHAPTER 4. PBDEs, PCBs, AND OC PESTICIDES ON A GRIZZLY BEAR DINNER PLATE: EXPOSURE, DEPURATION AND ACCUMULATION

Introduction

Grizzly bears (*Ursus arctos horribilis*) have large diversities in their individual feeding ecologies, consuming a variety of plants, roots, berries, terrestrial prey, carcasses, insects and, when available, Pacific salmon (7,8,144). Differences in feeding ecology result in varied dietary exposure to persistent organic pollutants (POPs), where increased Pacific salmon consumption by grizzly bears has been linked to elevated concentrations of many of the most bioaccumulative legacy POPs in grizzly fat tissues, such as dichlorodiphenyl ethylene (DDE) and polychlorinated biphenyls (PCBs) (154). Conversely, bear reliance on terrestrial food webs results in accumulation of volatile organochlorine (OC) pesticides, such as hexachlorocyclohexane (HCH) and hexachlorobenzene (HCB), as well as the higher brominated polybrominated diphenylether (PBDEs) flame retardants.

There is some information on POPs in wild Pacific salmon (79,83,179); however, there is a general lack of POP data in other grizzly bear foods, primarily within the terrestrial food web. Since dietary exposure to POPs is the main exposure pathway for mammals, the characterization of both diet and POP exposure through diet are vital to understanding and monitoring the overall risks posed to this threatened species.

Following dietary exposure to POPs numerous pharmacokinetic processes will have an effect on the overall POP concentrations and patterns observed in the exposed animal. POPs in the body tissues will depend upon exposure, gastrointestinal uptake, inter-tissue distribution, metabolic capabilities, as well as elimination and excretion (163,168,175,184). It has been suggested in Chapter 3 that following dietary exposure, only PCBs with no vicinal hydrogen (H) pairs, and those with vicinal H pairs in the

ortho- and *meta*- positions are the most persistent PCBs in grizzly bears. Other persistent contaminants in grizzly bears include BDE-47, 119, 153 and oxychlordane (154).

The depuration of contaminants is mainly through metabolic biotransformation and excretion. Since grizzly bears are suspected of having active cytochrome P450 2B (CYP2B)/3A-like enzymes, only PCB congeners with vicinal H pairs in *meta*- and *para*-positions appear readily metabolized by grizzly bears (154). The depuration of some non-metabolizable PCB congeners has been related to the octanol-water partitioning coefficient (log K_{ow}), where the lower log K_{ow} non-metabolizable congeners are readily depurated following dietary exposure (Chapter 3). This loss was presumably through fecal excretion, with possibly some minor loss through urination and respiration.

Fecal material represents the unabsorbed, waste fraction from the diet, and while it has its limitations, feces have provided dietary assessments of wild and elusive animals (188,189). Feces can also represent one of the main processes of parent POP depuration in an organism following exposure. Excretion of POPs results in their permanent loss from the exposed animal, lessening potential health effects associated with the original exposure. Therefore, this information coupled with original POP exposure data should be valuable for assessing overall risks in wild mammal populations. However, excreted POPs are under-characterized in wild animals. In fact, there is no information on contaminants in grizzly bear feces, and only a small handful of studies exist that examine contaminants in fecal material of wild populations of birds (190,191) and small mammals (192-194).

In this study the fate of 159 PCB congeners, 28 OC pesticides and 39 PBDE congeners in grizzly bears is assessed using food, fecal and fat samples. By

characterizing the "ins" and "outs" of grizzly bear exposure to POPs and then coupling this information with POP accumulation in grizzly bear fat, not only are we able to deduce what contaminants are absorbed from the various foods, but also which contaminants may be excreted, metabolized or accumulated, and potentially, how different diets (i.e. vegetation versus salmon) may impact POP absorption and elimination in bears. This array of contaminants was chosen to cover a wide range of physico-chemical properties, as well as to strengthen and compliment the existing knowledge of POP behavior in this species (144,154)(Chapter 3). The uptake, elimination and accumulation information gathered from this study, especially for PBDEs, will aid in drafting effective regulations.

The main objectives of this paper are to: 1) characterize dietary exposure to POPs through grizzly bear consumption of terrestrial and marine foods; 2) assess POP uptake, elimination and accumulation in grizzly bears consuming varied diets using a quantitative PCA-based model; and 3) assess the applicability of using grizzly bear feces as a non-invasive tool to monitor POP exposure.

Materials and Methods

Sample Collection. Food and fecal samples were collected in the Koeye River watershed located on the Central Coast of British Columbia (river mouth location: latitude: 51°46'00", longitude 127°53'00"); approximately 45 km south of Bella Bella and 7 km south of Namu River (Figure 15). The Koeye River is one of the few remaining intact coastal river valley ecosystems in Canada and is a part of the Heiltsuk First Nations Territory. It has a plethora of inter-tidal food species (i.e. crabs and mussels), wetlands,

estuaries and old growth forests with an abundance of nutritious plant species for grizzly bear consumption, as well as all five species of Pacific salmon: Chinook (*Oncorhynchus tshawytscha*), sockeye (*O. nerka*), pink (*O. gorbuscha*), chum (*O. keta*) and coho (*O. kisutch*). Plant and prey samples were chosen based on numerous observations of bear feeding, as well as bear signs (e.g., diggings) that facilitated an understanding of grizzly bear diet in the Koeye River watershed.

Figure 15. Location of Koeye River on the Central Coast of British Columbia.

In total, 15 fecal samples were collected from tidally influenced portions of the river (i.e. estuary), upper Koeye (not tidally influenced), and in the forested, riparian zones along the river. All fecal samples were less than two days old. Using gross visible

characteristics such as consistency, bones, color and presence of undigested plant species in the feces, as well as stable isotope analysis and fecal sample location we have divided the fecal samples into groups: pink salmon-rich (LK, n = 4); sockeye salmon-rich (UK, n = 5); and vegetation-rich (VG, n = 6). Feces were kept frozen until analysis. Each feces sample was homogenized and then sub-sampled for contaminant analysis (~50 g) and stable isotope analysis (3 replicate 1 g samples).

At the time of the study, pink salmon were the most abundant in the lower reaches. Sockeye salmon were abundant, and the only salmon species observed, in the upper reaches, upstream of Koeye Lake. Eight spawned-out pink salmon (4 male, 4 female) and eight spawned-out sockeye salmon (4 male, 4 female) were captured in the Koeye River using hand nets and fly-fishing rods, respectively. Six pre-spawned sockeye (3 male, 2 female, 1 unknown) muscle samples were captured in Fitz Hugh Sound using gill nets (generously provided by Heiltsuk First Nation). One 25 g sample of filet from the left side immediately posterior the dorsal fin was placed in hexane-rinsed aluminum foil, frozen at -20ºC and used for contaminant analysis. Three 1 g samples of filet, placed in hexane-rinsed vials, were used for stable isotope analysis.

Five male Dungeness crabs (*Cancer magister*) were captured using a crab trap submerged at the mouth of the Koeye River. Crabs were sacrificed and muscle tissues were removed from the carapace and claws, homogenized, and sub-sampled for contaminant analysis (n = 1; 10 g) and stable isotope analysis (1 g).

Fifty blue mussels, *Mytilus edulis*, were collected from inter-tidal areas at the mouth of the Koeye River. Mussels were kept frozen until shucked. Mussel somatic

tissue was removed from all individuals collected, homogenized and sub-sampled for contaminant analysis (n = 1; 10g) and stable isotope analysis (1 g).

Since moose (*Alces alces*) are not available in Koeye River, four moose muscle samples were sent frozen to our lab from Terrace, BC to represent terrestrial meat that may be available to grizzly bears. Samples (5g) from each moose were combined, homogenized and submitted for contaminant analysis, while 1g was allotted for stable isotope analysis.

Plant species available to grizzly bears were collected in the fall 2004 and spring 2005 from the Koeye watershed. In the fall, plant species chosen included salal berries (*Gaultheria shallon*), crab apples (*Malus fusca*), Lyngby's sedge (*Carex lyngbyei*), rice root (*Fritillaria camschatcensis*) and skunk cabbage (*Lysichiton americanum*). Spring plant species included sedge and skunk cabbage, as other species were not yet available. For each season an equal amount (by wet weight) of each plant species was homogenized in a blender. A 10 g sub-sample for each season was utilized for contaminant analysis. Three replicate samples (1 g) from each individual plant species and the homogenized plant samples were used for stable isotope analysis.

Stable Isotope Analysis. Stable isotope analysis of food web and fecal samples for $\delta^{13}C$ and $\delta^{15}N$ were carried out as described in Christensen et al. (144).

Contaminant Analyses. Salmon filets, other food items specified above and fecal samples were analyzed for 159 PCB congeners, 28 organochlorine pesticides, and 39 PBDE congeners. Salmon-eating grizzly bears from coastal BC were utilized as surrogates for Koeye River grizzlies, and non-salmon-eating grizzlies from interior BC were included as a terrestrial comparison (144,154). Samples were analyzed using high-

resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) by AXYS Analytical Services, Sidney, BC. Detailed methods and individual contaminants have been outlined in Christensen et al. (144).

Each batch of samples included a procedural blank. All PCB congeners and OC pesticides in the samples had concentrations <0.001 ng/g in the blank. BDE-47, -99, -203, -206 to -208 were generally found to be at concentrations of <0.010 ng/g, while BDE-209 ranged from 0.102 ng/g to 0.540 ng/g in 11 blanks analyzed. All other PBDE congeners were undetected in the blank.

Detection limit substitutions were made for contaminants that were not detected in cases where at least 70% of the samples had detectable values for that contaminant. Where less than 70% of the samples had detectable concentrations of an analyte, 0 ng/g was substituted for non-detect concentrations. Detection limits for PCBs were mainly <0.0005 ng/g, but all were <0.001 ng/g for all samples. For OC pesticides and PBDEs detection limits were mainly <0.001 ng/g, but were all <0.005 ng/g. Recoveries were considered within acceptable limits set by AXYS (PCBs: 84.2% to 115%; OCs: 95.3% to 116%; PBDEs: 72.9% to 118%). Reported concentrations were adjusted based on concentrations found in the lab blank. Results are expressed in lipid weight (lw) as mean ± 1 standard deviation (SD).

PCB congeners were placed in one of six structure-activity groups (SAGs) as extended from Boon et al. (162). Group 1 is comprised of congeners without any vicinal hydrogen (H) atoms. Group 2 is comprised of congeners with vicinal H atoms exclusively in *ortho*- and *meta*-positions in combination with ≥ 2 *ortho*-position chlorine (Cl) atoms. Group 3 congeners have *ortho*- and *meta*- vicinal H pairs with < 2 *ortho*-Cl.

Group 4 congeners have *meta*- and *para*- vicinal H pairs with ≤ 2 *ortho*-position Cl. Group 5 congeners have *meta*- and *para*-vicinal H pairs with > 2 *ortho*-position Cl. Group 6 congeners have both *meta*- and *para*- vicinal H pairs, and *ortho*- and *meta*-vicinal H pairs).

Principal Components Analysis (PCA). The stated concentration was used for analytes reported by the laboratory as NDR (non-detectable range; peak detected but confirming-ion ratios outside of the specified range), while undetectable values were replaced by a random number between zero and the limit of detection before PCA. Each contaminant analyzed was evaluated for potential interferences, closeness to the limit of detection and the percentage of undetectable (random value estimated) values before inclusion in the final PCA data set of 105 PCBs, 17 PBDEs and 11 OC pesticides. Samples were normalized to the concentration total before PCA to remove artifacts related to concentration differences between samples. The centered log ratio transformation (division by the geometric mean of the concentration-normalized sample followed by log transformation) was then applied to this compositional data set to produce a data set that was unaffected by negative bias or closure (17) and where the average concentration and concentration total were identical for every sample. Data were then auto-scaled before PCA to give every variable equal weight.

A coherent model of the retention of each contaminant in maritime grizzly bears was obtained by shifting the origin of the PCA variables plot to the position of PCB 170 (see discussion) and converting the reference frame from rectangular to polar coordinates. Rectangular coordinates for variables in the first two PCs (p1 and p2 on the *x*- and *y*-axes, respectively) were converted to polar coordinates relative to the positive *x*-axis by

calculating the radius vector $r = (x^2 + y^2)^{½}$ and the vectorial angle θ = arctan (y/x). For negative y the relation θ = 360 - arctan (y/x) was substituted to maintain a continuous counterclockwise trend in θ.

Results/Discussion

Diet is the cornerstone to POP exposure in any mammal, including grizzly bears. While interior bears rely mainly on a terrestrial food web, represented in this study by vegetation and moose meat samples, salmon-eating bears rely on a wider variety of foods encompassing both terrestrial and marine food webs; the latter of which is represented in this study by salmon, crab, and mussels. The use of stable isotope and contaminant analysis of these various foods, in combination with grizzly bear fecal material and fat provided insight into the exposure, depuration, and accumulation of POPs in a remote BC grizzly bear population.

POPs in Grizzly Bear Foods. Total PCBs and ΣOC pesticides are present in higher concentration in the marine foods of a grizzly bear diet than in the terrestrial foods (Table 7). ΣPCBs significantly correlate with the $\delta^{15}N$ of the food ($r^2 = 0.51$, $p < 0.0001$), although these grizzly bear prey and non-prey items exist within both terrestrial and marine food webs, so thus do not represent true trophic transfer (Figure 16). Similar patterns are observed for ΣOCs ($r^2 = 0.48$, $p<0.0001$), ΣDDT ($r^2 = 0.60$, $p<0.0001$), ΣCHL ($r^2 = 0.58$, $p<0.0001$) and HCB ($r^2 = 0.46$, $p=0.001$).

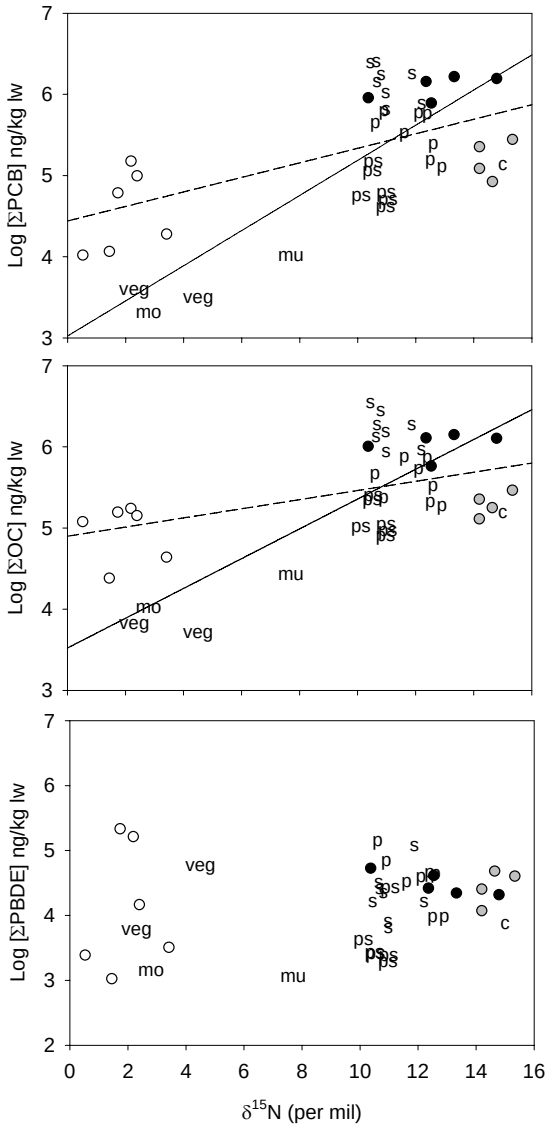

Figure 16. Relationship between log transformed concentrations of A) ΣPCBs, B) ΣOC pesticides, and C) ΣPBDEs and the $\delta^{15}N$ of various grizzly bear foods and fecal material. The significant increases in ΣPCBs ($y=0.217x+3.02$, $r^2 = 0.51$, $p<0.0001$) and ΣOC pesticide ($y =0.184x+3.52$, $r^2 = 0.48$, $p <0.0001$) concentrations with increasing $\delta^{15}N$ in various terrestrial and marine foods of grizzly bears (depicted by solid lines) clearly demonstrate the important role of salmon in the delivery of legacy contaminants to marine mammals. Conversely, since ΣPBDEs have no relationship with $\delta^{15}N$, it appears that PBDEs are provided to the bears through two food webs equally. Increasing ΣPCB ($y = 0.0895x + 4.44$, $r^2 = 0.51$, $p = 0.0026$) and ΣOC pesticide ($y = 0.0563x + 4.90$, $r^2 = 0.40$, $p = 0.0114$) concentrations with increasing $\delta^{15}N$ in grizzly bear feces (depicted by dashed lines), and the similarity in fecal and food suggest feces represent the unabsorbed fraction of POPs directly from the diet. Labels: veg – vegetation; mo – moose; mu – mussel; c – crab; p – spawned pink salmon; s – spawned sockeye salmon; ps – prespawned sockeye salmon; black circle – UK feces; gray circle – LK feces; white circle – VG feces.

In contrast, ΣPBDEs and individual PBDE congeners elicit no relationship with $\delta^{15}N$ of grizzly bear foods. This is surprising, as our previous work suggested that PBDEs are provided to the bears mainly through the terrestrial food web, especially for the higher brominated congeners. Our results show that vegetation and salmon are providing almost equal amounts of ΣPBDEs to the bears. Lack of significance between $\delta^{15}N$ of foods and PBDEs is also due to the high variability in salmon PBDE concentrations, which varied depending on species and reproductive condition. BDE-209 dominated the vegetation profile (~50% of ΣPBDEs), and more surprisingly, the salmon profile (37% to 70% of ΣPBDEs). BDE-209 is not detected in local marine prey (crabs and mussels). While concentrations may not be high, PBDEs dominated the vegetation profile. ΣPBDEs were 30X greater than ΣPCBs in vegetation, 100X greater than ΣDDT and 50X greater than ΣCHL.

Table 7. Summary of contaminant concentrations (ng/g lipid weight) in the major food items of grizzly bears in both terrestrial and marine food webs.

Food Item/ Contaminant	Spawned Pink (n = 8)	Spawned Sockeye (n = 8)	Pre-spawned Sockeye (n = 6)	Crab (n = 1)	Mussel (n = 1)	Moose (n = 1)	Vegetation (n = 2)
% Lipid	1.11 ± 0.81	0.79 ± 0.33	2.92 ±1.44	2.93	1.39	1.50	0.51 ± 0.04
ΣPCBs	403 ± 209	1,600 ± 739	81.3 ± 44.2	142	10.7	2.11	3.64 ± 0.621
CB-153	44.2 ± 25.9	153 ± 71.4	6.05 ± 3.27	13.6	1.00	0.0694	0.272 ± 0.078
CB-118	20.9 ± 11.7	88.8 ± 40.4	3.85 ± 2.14	10.1	0.670	0.0482	0.183 ± 0.0638
CB-99	19.3 ± 10.5	78.2 ± 36.8	3.64 ± 2.05	7.26	0.542	0.0354	0.116 ± 0.00612
CB-180	9.24 ± 5.26	31.2 ± 15.9	1.15 ± 0.597	2.59	0.0856	0.00440	0.086 ± 0.027
CB-190	0.387 ± 0.199	1.25 ± 0.659	0.0478 ± 0.0243	0.160	0.00942	0.000320	0.000775 ± 0.000213
ΣTEQ	0.012 ± 0.00726	0.0608 ± 0.0288	0.00266 ± 0.00204	0.00465	0.00014	0.0000010	0.000029 ± 0.000012
ΣOCs	444 ± 235	1,890 ± 938	148 ± 76.2	161	27.5	10.9	6.07 ± 0.978
ΣDDT	306 ± 181	1,423 ± 803	68.8 ± 4.04	79.3	4.86	1.12	0.319 ± 0.373
ΣCHL	95.5 ± 53.6	367 ± 131	35.2 ± 2.18	30.7	5.11	0.373	0.661 ± 0.375
ΣHCH	1.11 ± 0.638	0.187 ± 0.348	14.7 ± 1.03	34.9	9.78	2.13	1.54 ± 0.704
HCB	28.4 ± 16.5	77.8 ± 19.6	19.8 ± 11.7	14.3	2.09	3.60	2.16 ± 0.180
ΣENDO	4.21 ± 1.46	2.18 ± 0.980	0.375 ± 0.100	0.0410	2.23	2.67	0.880 ± 0.0655
ΣPBDEs	50.8 ± 44.3	31.9 ± 37.6	7.03 ± 10.3	7.73	1.19	1.48	33.3 ± 38.1
BDE-47	15.4 ± 7.43	6.16 ± 4.27	0.680 ± 0.270	5.59	0.712	0.473	4.46 ± 3.48
BDE-99	5.96 ± 2.82	0.466 ± 0.733	0.216 ± 0.0920	0.285	0.164	0.334	4.36± 3.48
BDE-100	3.09 ± 1.59	1.07 ± 0.495	0.110 ± 0.0419	0.316	0.133	0.066	0.927 ± 0.737
BDE-153	0.704 ± 0.359	0.237 ± 0.118	0.0373 ± 0.0125	0.0163	0.0116	0.0319	0.482 ± 0.326
BDE-209	18.7 ± 34.0	14.6 ± 37.6	4.90 ± 9.48	ND*	ND	0.200	15.5 ± 21.9

* ND = not detected

POP Excretion by Grizzly Bears. The stable isotope values in the various fecal groups are comparable to the major food items associated with them (Figure 17). Accordingly, the $\delta^{15}N$ and $\delta^{13}C$ values are higher for UK and LK feces relative to VG feces as are marine prey relative to vegetation food items. As with food, ΣPCBs and ΣOCs significantly increase with the $\delta^{15}N$ in the feces (Figure 16). Both ΣPCB and ΣOC pesticide concentrations are greatest in UK feces with decreasing amounts in LK feces, followed by VG feces (Table 8). Interestingly, no 4,4'-DDT or 2,4'-DDT is detected in any of the fecal types, despite being present in all food types, indicating either their complete uptake and retention by the grizzly bears or metabolic breakdown prior to excretion.

There is no relationship between $\delta^{15}N$ and ΣPBDEs in the fecal material, as ΣPBDE concentrations are equivalent, on average, among the fecal groups (UK: 9.57 ± 3.43 ng/g; LK: 10.9 ± 7.02 ng/g; VG: 9.20 ± 13.2 ng/g). While the salmon-dominated feces have little variability in either their $\delta^{15}N$ or ΣPBDEs, the VG feces have high variability in ΣPBDEs, especially BDE-209, which dominates the profile. In contrast, significant relationships are observed between some individual PBDE congener concentrations (BDE-28/33, -47, -49, -100, -154, and -155) and $\delta^{15}N$ of fecal material (r^2 values are 0.51, 0.35, 0.48, 0.41, 0.44, 0.46, respectively; data not shown). This demonstrates two important points: 1) the lack of significance between ΣPBDEs and $\delta^{15}N$ is likely a result of the high variation in BDE-209 (a dominant congener in vegetation and salmon), and 2) concentrations of PBDE congeners that are significantly related to $\delta^{15}N$ of fecal material, are likely salmon-delivered.

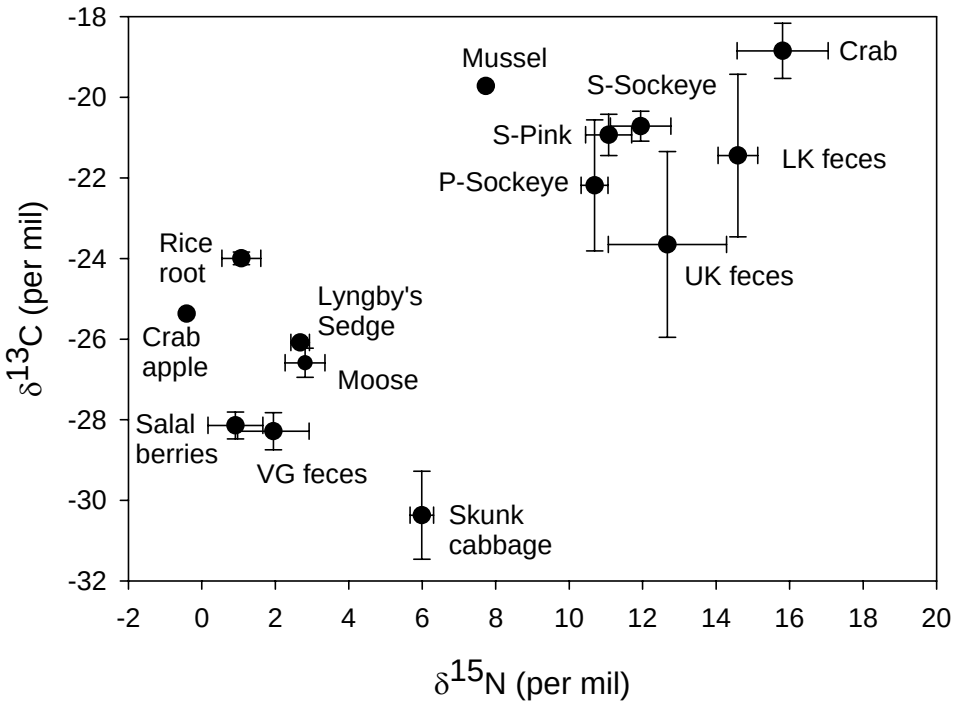

Figure 17. Dietary information for Koeye River grizzly bears extracted from comparisons of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in fecal material, as well as food items from both a terrestrial and marine food web. Both vegetation-dominated feces (VG) and Koeye River vegetation species have low $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures. Sockeye salmon-dominated feces (UK) and pink salmon-dominated feces (LK) have high $\delta^{13}C$ and $\delta^{15}N$ signatures, similar to available marine food items found in the Koeye watershed (S-pink = spawned pink salmon; S-sockeye = spawned sockeye salmon; P-sockeye = prespawned sockeye salmon).

The similarity in POP concentrations between salmon-dominated feces and salmon strongly suggests that most contaminants are passing through the bear unabsorbed. Since salmon is highly digestible for grizzly bears, lack of complete absorption may be a result of the increased fall feeding rate, which may limit digestion and absorption in the gastrointestinal tract.

Table 8. Summary of contaminant concentrations (ng/g lipid weight) in various grizzly bear fecal groups. Fecal groups are based on dominant food type and sample location (tidal or non-tidal).

Feces Type/ Contaminant	Pink-dominated feces (LK) (n = 4)	Sockeye-dominated feces (UK) (n = 5)	Vegetation-dominated feces (VG) (n = 6)
% Lipid	2.15 ± 0.90	1.14 ± 0.65	1.15 ± 0.36
ΣPCBs	53.4 ± 21.3	381 ± 136	8.42 ± 7.40
CB-153	6.26 ± 3.01	50.9 ± 15.5	1.03 ± 1.05
CB-118	2.71 ± 1.09	24.6 ± 6.27	0.646 ± 0.732
CB-99	2.32 ± 0.992	20.1 ± 6.04	0.357 ± 0.339
CB-180	2.46 ± 1.15	15.4 ± 4.70	0.429 ± 0.484
CB-190	0.148 ± 0.0698	0.645 ± 0.193	0.0494 ± 0.0546
ΣTEQ	0.00232 ± 0.000710	0.0198 ± 0.00712	0.000849 ± 0.000665
ΣOCs	61.0 ± 14.7	331 ± 108	16.8 ± 8.09
ΣDDT	28.2 ± 19.4	221 ± 106	0.559 ± 0.739
ΣCHL	13.1 ± 4.46	80.2 ± 16.4	7.27 ± 7.07
ΣHCH	3.65 ± 5.39	7.31 ± 12.2	3.94 ± 3.05
HCB	7.50 ± 0.408	10.3 ± 1.68	3.46 ± 2.21
ΣENDO	4.07 ± 6.88	1.19 ± 1.08	1.07 ± 0.331
ΣPBDEs	10.9 ± 7.02	9.57 ± 3.43	9.20 ± 13.2
BDE-47	3.23 ± 12.4	4.63 ± 3.67	0.894 ± 1.29
BDE-99	1.88 ± 0.771	1.71 ± 0.818	0.912 ± 1.64
BDE-100	0.839 ± 0.421	0.793 ± 0.406	0.198 ± 0.338
BDE-153	0.235 ± 0.0949	0.370 ± 0.106	0.125 ± 0.225
BDE-209	3.17 ± 6.34	ND*	5.44 ± 9.35

* ND = not detected

Interestingly, following vegetation consumption by the bears there appears to be increased excretion of ΣPCBs and ΣOC pesticides (represented by higher concentrations in VG feces, but at similar $\delta^{15}N$ values as vegetation), in addition to what is available in the vegetation that was consumed. Vegetation is highly indigestible to the grizzly bears carnivorous gastrointestinal tract (195,196), so absorption of POPs is expected to be low, resulting in a similarity between POPs in vegetation and in VG feces. Increased POP concentrations in VG feces could be a result of increased elimination of previously retained POPs. In humans, increasing the water-insoluble fiber content in the diet results in increased excretion of PCBs (197). Moreover, transit in the gastrointestinal tract is

influenced by fiber content, and thus, poorly fermented fiber sources (e.g. cellulose) that reduce residence time, could lower *in vivo* absorption of environmental contaminants (197). Also, Gobas et al. (80) suggested that animals that consume low contaminated foods (i.e. vegetation) will experience net excretion as a result of a fugacity from the more contaminated tissues into the residual food present in the gastrointestinal tract.

Grizzly bears are considered the gardeners of the forest, by digging up and turning over the soil, as well as providing nutrients through defecation and transport of salmon carcasses to the forest. Interestingly, these results suggest that grizzly bears are also delivering contaminants to the soil environment. Following a spawned sockeye salmon diet, one fecal deposit will contain upwards of 1,320 ng of ΣOCs, 1,540 ng of ΣPCBs and 20.9 ng of ΣPBDEs. However, one fecal deposit following a vegetation-rich diet will contain less legacy contaminants (up to 54.9 ng of ΣOCs and 47.4 ng of ΣPCBs), and up to 51.6 ng ΣPBDEs.

PCA Reveals Exposure and Fate of POPs in Grizzly Bears. The PCA model (Figure 18) clearly shows a dichotomy of POP dominance within food webs for grizzly bears: terrestrial and marine. PBDEs, volatile PCBs and OC pesticides (i.e. HCH) dominate terrestrial sources, while legacy, lipophilic contaminants (e.g. DDE, PCBs) are delivered to the grizzlies through their salmon consumption.

Contaminants and samples clearly separate into terrestrial (left side) and marine (right side) food webs in the PCA model. Within these two food webs, samples congregate into sub-groups following a top-to-bottom arrangement according to their POP content, with foods at the top, feces in the middle, and bears towards the bottom. Hence, six discreet groups are revealed in the PCA: 1) terrestrial foods, 2) marine foods,

3) vegetation-dominant (VG) feces, 4) pink and sockeye salmon-dominated (LK and UK) feces, 5) terrestrial (interior) grizzly bears, and 6) maritime (salmon-eating) grizzly bears. The moose sample projects very close to the vegetation samples, suggesting that if bears have consumed terrestrial animals, there would be little effect on the vegetation contaminant pattern. Note that the maritime grizzly bears cluster closely together, irrespective of percent salmon and/or salmon species consumed, confirming that they are appropriate surrogates for Koeye River salmon-eating grizzly bears.

The maritime grizzly bears and the terrestrial and marine food samples are end-members in the PCA and have the largest influence on the model, while the presence or absence of the terrestrial bears in PCA models has little effect on the PCA results. While the six sample types may contain any or all contaminants, the contaminant proportions in each group allow us to interpret the differences between samples in terms of their contaminant patterns.

The three trend lines in Figure 18 are valuable for visualizing and quantifying contaminant relationships between sample types and they clearly indicate that metabolism and excretion in the two food webs produce a single pattern of refractory contaminants in maritime bears. Nevertheless, it is difficult to combine the three regressions to derive a unified model of the relative retention of food-based contaminants by bears. To obtain a coherent model we take a new approach to the interpretation of PCA results, and change the reference frame from rectangular to polar coordinates with the origin shifted to PCB 170 (the contaminant closest to the intersection point of the two food chain trend lines). With this shift in origin and reference frame, the length of the radius vector r provides a measure relative to PCB 170 of the relative retention of each

contaminant in the PCA model by maritime bears and the vectorial angle θ provides a uniform measure of the relative contribution of terrestrial and marine food sources (Appendix IV). Furthermore, by using the contaminant retention values we can infer whether individual POPs are likely to be metabolized, excreted or accumulated, following terrestrial and marine dietary exposures.

The vastly different food groups have the highest proportions of PCB congeners in SAGs 4, 5 and 6 (i.e., congeners with *meta*- and *para*- vicinal H pairs). Along the trend line between terrestrial and marine foods, there is a significant correlation for the SAG 4, 5 and 6 PCB congeners between the angle θ in a polar coordinate reference frame and log K_{ow} ($r^2 = 0.594$, $v = 53$, $p < 0.0001$) due to a left to right progression from lower log K_{ow} congeners in terrestrial foods to higher log K_{ow} congeners in marine foods. Because the SAGs 4, 5 and 6 are metabolizable through CYP2B/3A-like enzymatic biotransformation, it is quite possible that their dominance in food relative to PCB congeners from SAGs 1, 2 and 3 (considered non-metabolizable in grizzly bears – see Chapter 3) may typify PCBs depurated by the grizzly bears through metabolism.

The close similarity in composition of the respective food and fecal components in the PCA model suggests that the bulk of the contaminant burden passes through the bear with relatively little alteration. This is supported by the results described above where relationships between $\delta^{15}N$ and ΣOC and ΣPCB concentrations are similar between salmon and salmon-dominated feces.

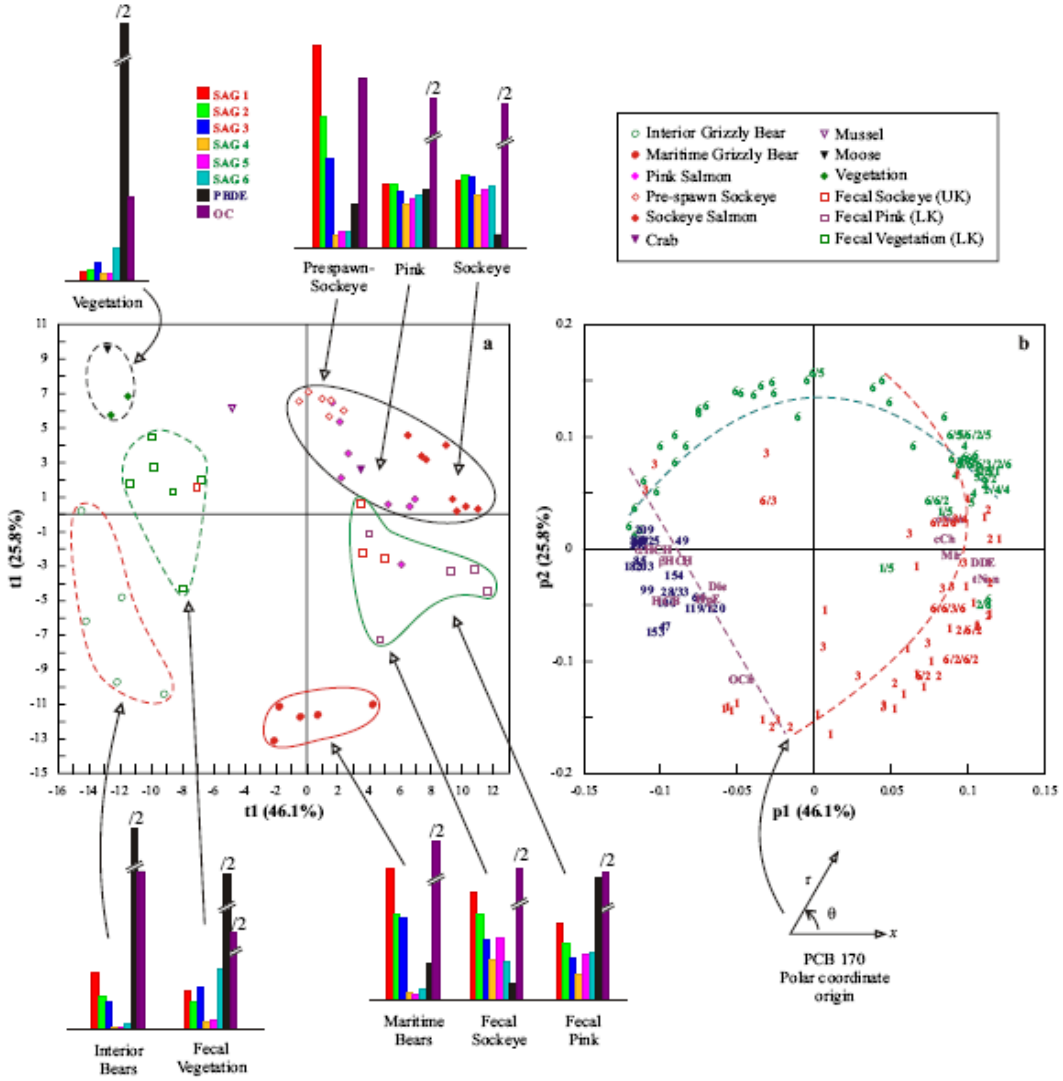

Figure 18. Principal Components Analysis (PCA) of grizzly bear foods, feces and fat samples in accordance with POP patterns. A) PCA defines six sample groups within two food webs (terrestrial and marine): terrestrial foods (black dotted), marine foods (black solid), vegetation-dominant feces (green dotted), salmon-dominated feces (green solid), interior grizzly bears (red dotted), and salmon-eating grizzly bears (red solid). B) In the PCA variables plot, PBDEs (in blue) clearly dominate in terrestrial food webs. PCB congeners from structure-activity groups (SAGs) 4, 5, and 6 (in green) dominate the food groups and the congener position along the terrestrial/marine food web quadratic trend line ($r^2 = 0.494$, $p = 0.00$; blue). Non-metabolizable contaminants from a marine food web are accumulated or excreted along a second quadratic trend line of OCs (in purple) and SAG 1-5 PCBs (in red; $r^2 = 0.697$, $p = 0.00$). POPs from terrestrial foods (PBDEs, volatile OCs and SAG 3 and 5 PCBs, and the most recalcitrant 1 and 2 PCB congeners) are accumulated or excreted along a linear (geometric mean regression) trend line towards the salmon-eating bear group ($r^2 = 0.347$; $p = 0.00$). Variable positions along the first two trend lines are primarily governed by log K_{OW} (see text). PCB congeners are depicted by their SAG and PBDEs use the congener number. Full names for OC pesticide labels can be found in Appendix IV. The polar coordinate reference frame with the origin at PCB 170 is depicted in the lower center. Bar plots show the concentration normalized, relative proportion of SAG 1-6 PCBs, PBDEs and OCs in the PCA data set for the major sample types. Bars with "/2" above the bar have a concentration proportion twice that shown.

Salmon-rich feces and salmon-eating grizzly bears both have a predominance of PCB congeners from SAGs 1, 2, and 3 over PCBs from SAGs 4, 5 and 6. While SAG 4,5, and 6 PCBs are easily metabolized by grizzly bears, SAG 1, 2, and 3 PCBs are not (154), suggesting that the contaminants defining the salmon fecal and maritime bear groups in the PCA are not readily metabolized. There is also a significant correlation along the salmon/salmon fecal/maritime bear trend line between the radius vector *r* for the SAG 1 – 3 PCBs and log K_{ow} (r^2 = 0.230, v = 48, p = 0.0004), suggesting that persistence of non-metabolizable PCBs in grizzly bears is related to log K_{ow}. This is supported by other work suggesting that lower log K_{ow} non-metabolizable PCBs would be depurated by the bear through excretion (Chapter 3). The relationship is also significant (r^2 = 0.323, v = 65, $p < 0.0001$) when PCBs from SAGs 1 to 5 are considered, suggesting that SAGs 4 and 5 congeners may also be depurated through excretion due to the low log K_{ow}. Hence, although salmon-dominated fecal material may represent unabsorbed POPs, the feces appear dominated by low log K_{ow} non-metabolizable contaminants as well. Conversely, the PCBs that dominate the salmon-eating bears are non-metabolizable congeners from SAGs 1, 2 and 3 that have short radius vectors (*r*) relative to PCB 170 in the PCA model, with high relative retention and log K_{ow} values. Principal among these congeners are SAG 1 PCBs 111, 180/193, 189, 191, 194, 205, 206, 209, SAG 2 PCBs 170 and 190 and SAG 3 PCB 156/157 (Appendix IV).

Interestingly, the OC pesticides provided to the bears through their consumption of salmon (*cis*- and *trans*-chlordane, DDE, *cis*-chlordane, and mirex) are also lost to fecal excretion. Their dominance in fecal material, rather than the food group, suggests these contaminants are not significantly metabolized. DDT may be an exception, as it is found

in all food groups, but is undetected in all fecal material. Oxychlordane is also an exception, as it is highly persistent in the bears as demonstrated by its dominance in fat tissue. No PBDEs dominate the salmon-rich feces, again stressing the fact that PCBs and OC pesticides dominate the marine food web, as well as the associated excretory loss following a meal of marine prey by the bears.

PBDEs dominate both the VG feces and terrestrial food in the PCA data set and analogous to a salmon-rich diet, the bears also are excreting the majority of the OC pesticides provided by the terrestrial food web: α-HCH, β–HCH and HCB. Again, this suggests that most of these POPs are not being absorbed by the bear. In fact, the vegetation-dominated feces project lower and slightly to the right towards the marine side of the PCA. This may support our suggestion above that vegetation could increase the excretion of previously absorbed and retained contaminants present in the tissues of the salmon-eating bear, in addition to the POPs the bear excretes directly with the undigested vegetation. The grizzly bear's consumption of vegetation may thus save this species from accumulating high concentrations of POPs measured in other salmon-eating marine mammals, and may explain why the biomagnification factor (BMF) from salmon to bear is so low for ΣPCBs at 0.247 (Chapter 3).

Despite significant loss of PBDEs through direct excretion with undigested vegetation, it is important to stress that there is some absorption of all PBDE congeners, as evidenced by the dominance of PBDEs, including BDE-209, in both moose and interior grizzly bears. However, the dominance of PBDEs on the terrestrial side of the PCA is due to the lower proportions of the lipophilic, legacy contaminants available within that food web, and not due to higher concentrations of PBDEs. Thus, the interior

grizzly bear group is distinct from the salmon-eating bears likely due to their dominant exposure to, and accumulation of PBDEs (and less PCBs and lipophilic OC pesticides) through the terrestrial food web.

From the terrestrial food web side of the PCA, the significant linear trend on the left in Figure 18 from the terrestrial food samples through the vegetation fecal samples to the salmon-eating bears is not strongly related to log K_{ow} as radius vectors from PCB 170 ($r^2 = 0.150$, $v = 44$, $p = 0.008$). This low correlation reflects the inclusion in the trend line of both terrestrial contaminants with a wide range of log K_{ow} values (mono- to trichloro PCBs, PBDEs, and the terrestrial source OCs heptachlor epoxide, dieldrin, HCB, αHCH, βHCH and oxychlordane) and the high log K_{ow} PCBs prevalent in maritime bears. This terrestrial food web to bear trend line represents the range of depuration to accumulation in salmon-eating bears from POPs dominating terrestrial foods.

Overall, this study bridges and supports our previous work on the fate of POPs in grizzly bears (144,154)(Chapter 3), as well as adds a new dimension of understanding to contaminant uptake and elimination dynamics (Figure 19). Relatively speaking, the grizzly bear terrestrial food web is supplying the bears with volatile (low log K_{ow}) POPs and PCBs, as well as current-use PBDEs, all of which have a low relative uptake in maritime bears. Conversely, while the Pacific salmon also deliver PBDEs, they provide higher concentrations of the higher log K_{ow} and more bioaccumulative, legacy OC pesticides and PCBs. Despite this plethora of contaminants on the omnivorous grizzly bear dinner plate, this study clearly demonstrates that for the grizzly bear, and for that matter, any mammal, the fate of POPs will depend upon: 1) food web exposure (terrestrial versus marine), 2) food digestibility, 3) metabolism (active CYP enzymes),

and 4) excretion vs. accumulation related to POP physicochemical properties (e.g., log K_{ow}). Ultimately, contaminants that land mammals are able to depurate through excretion will enter into the terrestrial environment and, thus, become available and integrated further into the terrestrial food web. Fecal material of grizzly bears could be used as an effective monitoring tool for diet (using stable isotopes), as well as POP dietary exposure (similarity in patterns between food and feces) in wild populations of grizzly bears; however, feces does not appear to be a good indicator of grizzly bear body burden.

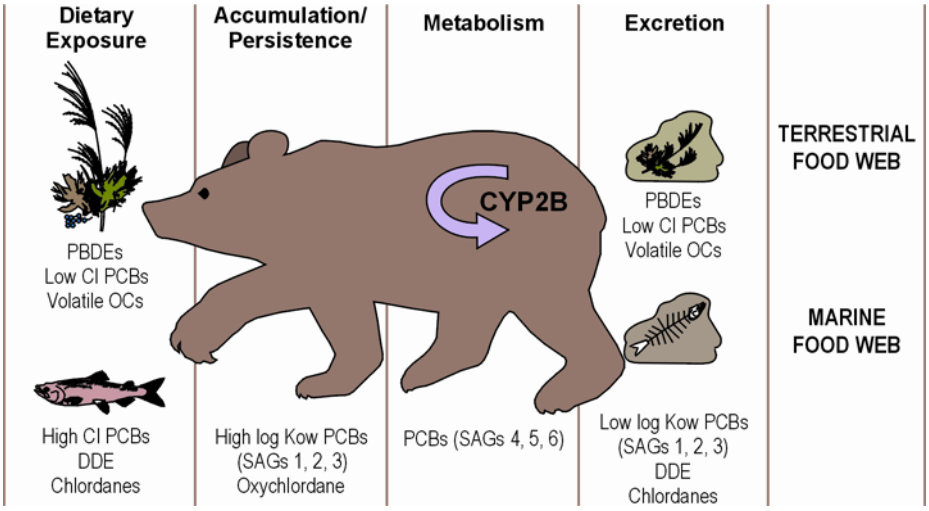

Figure 19. Dominant POPs (PBDEs, PCBs and OC pesticides) in grizzly bears as they pertain to dietary exposure (terrestrial and marine food webs), accumulation (fat tissue), as well as depuration through metabolism and excretion (fecal material). Following varied dietary exposures accumulation in grizzly bear fat of high log k_{ow} PCBs from structure-activity groups (SAGs) 1, 2 and 3, as well as oxychlordane occurs, is a result of metabolism of PCBs in SAGs 4, 5 and 6 by CYP2B/3A, and excretion of lower log k_{ow} PCBs in SAGs 1, 2, 3, PBDEs and most OCs.

This study exemplifies the elegance of applying PCA to understand the general concepts behind the fate of POPs in mammals, irrespective of which food web the mammal belongs or whether it may encompass a bit of both food webs. While food and

animal samples would suffice to understand the concepts of exposure and depuration versus accumulation, the addition of fecal samples can further help differentiate between contaminants that are more likely depurated through metabolism (dominance in food groups) or through excretion (dominance in fecal groups).

CHAPTER 5. REDEFINING CONTAMINANT-RELATED RISKS TO CANADIAN WILDLIFE: LESSONS LEARNED FROM GRIZZLY BEARS

Introduction

Persistent Organic Pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and polybrominated diphenylethers (PBDEs), are persistent compounds that bioaccumulate in wildlife and may have adverse toxicological effects, including endocrine disruption. Characterizing risks associated with wildlife exposure to these complex mixtures of chemicals, however, is a challenging task for risk assessors. In Canada, such assessments take the form of 1) water quality guidelines, 2) sediment quality guidelines, 3) tissue (i.e. prey) residue guidelines (198,199), and 4) tissue concentrations (i.e. predators) (200,201). Such approaches do not fully capture the nature of contaminant-related health risks in wildlife, in part reflecting limited consideration and understanding of the environmental and pharmacokinetic fate of contaminants in biota. Evidence that contaminant-associated health effects can arise at any point along the pharmacokinetic path is mounting, with effects measured following exposure (202-204), accumulation (166,205-212), metabolism (91,92,95,96,213), and excretion (i.e. costs associated with) (214) in a variety of wildlife species.

Without a controlled laboratory environment, however, risk assessors are restricted in their ability to accurately, realistically and conservatively assess POP-associated risks in wild populations of large mammals due to ethical, logistical, and monetary constraints. However, BC grizzly bears have unique characteristics that have provided an opportunity to help risk assessment move beyond those limitations. Firstly, the characteristics associated with grizzly bear hibernation provide a unique "closed system" for monitoring POP behaviour over an extended period of time (approx. 5 months), providing a pharmacokinetic system that lacks dietary intake and excretion.

Essentially, a wild grizzly bear during hibernation becomes a "controlled" environment, where POP behaviour in a wild animal can be studied outside a laboratory. Secondly, POP behaviour can be studied in both terrestrial and marine food webs, by virtue of the two feeding strategies of BC grizzly bears: salmon-eating and non-salmon-eating.

In this regard, these facets of the bears' unique ecology are utilized throughout this thesis for the characterization of POP behaviour and accumulation, and the results provide potential insight into issues of chemical fate in other wildlife species and the associated contaminant-related risks. Risk assessments for chemical regulations, environmental impact assessments, tissue residue (dietary) guidelines to protect wildlife, and tissue (predator) guidelines to protect wildlife, require insight into the factors affecting POP behaviour/fate. While studies of other wildlife species have revealed the influence of age, sex, diet and condition on body POP concentrations, our grizzly bear research has helped refine some of our ideas about how these factors influence POPs:

1) the importance of considering *individual* feeding behaviours (exposure) of sampled animals;
2) the importance of considering *integrated* dietary histories (temporal changes) of sampled animals;
3) the need to account for the influence of *unique biological or ecological traits* on POP fate and/or health assessment of the population or species being studied;
4) the contribution of modes of POP depuration/loss *other than metabolism* in studied animals;

5) the need to select the *most recalcitrant PCB congener* (using a full congener analysis) in support of a more robust evaluation of pattern changes from prey to predator;

6) the need to consider *non-invasive techniques* to document or characterize contaminant concentrations, exposures or feeding ecologies (e.g. feces) in endangered or difficult-to-study wildlife species;

7) since tissue residue guidelines only consider parent POPs, they may be insufficient to protect wildlife from by-products or metabolites.

Individual dietary histories of wildlife require consideration and correction. Terrestrial and marine food webs supply wildlife with distinct POP concentrations and patterns. Relative to each other, terrestrial food webs provide lower chlorinated PCBs, volatile OC pesticides and higher brominated PBDEs, while marine food webs (i.e. salmon) deliver higher chlorinated PCBs, lipophilic OC pesticides (e.g. DDT) and lower brominated PBDEs. Overall, it was deduced by comparing two bear feeding ecologies (salmon-eating and non-salmon-eating) that salmon deliver 70% of all OC pesticides, up to 85% of the lower brominated PBDE congeners, and 90% of PCBs found in salmon-eating grizzly bears. Correspondingly, when contaminants in actual grizzly bear foods were analyzed it was determined that ΣPBDE concentrations were over 30X greater than ΣPCBs in vegetation, 100X greater than ΣDDT and 50X greater than Chlordane (ΣCHL). Meanwhile, salmon had the greatest concentrations of ΣPCBs, ΣDDT, and ΣCHL.

It was discovered in Chapter 4 that even within each food web, POPs can vary depending upon the prey and the body condition of the prey. For example, pre-spawned salmon have lower lipid weight POP concentrations in their muscle and are dominated by relatively lower chlorinated PCBs, while spawned salmon have higher POP concentrations (due to fat utilization during spawning) and are dominated, rather, by higher chlorinated PCBs. It was also determined in Chapter 4 that digestibility, fiber content and/or lipid content of food may play a major role in the uptake and elimination of POPs, as evidenced by increased elimination of PCBs and OC pesticides following the consumption of indigestible vegetation by the carnivorous grizzly bears. Increased feeding rate by the bears, as they prepare their bodies for hibernation, may also negatively influence uptake of POPs.

It was shown that due to these dietary differences among grizzly bear individuals (i.e. percent salmon consumption), fat tissue POP patterns and concentrations were highly varied. Differences in dietary exposure in sledge dogs also resulted in different POP patterns, beyond what could be explained by similar metabolic capacity and excretion by the predator (215). In Chapter 3 it was also determined that individual dietary history can influence calculations of biomagnification factors (BMFs), where increased salmon consumption by the bears resulted in increased BMF values.

The integrated dietary histories (temporal changes) of sampled animals need to be considered. The use of grizzly bear hair segmentation stable isotope analysis was an extremely useful tool to characterize and quantify a clear dietary history of individual animals. It was determined that interior bears consume low trophic level terrestrial foods, as evidenced by both low $\delta^{15}N$ and $\delta^{13}C$ values along the length of their hair

(representing their entire feeding season) (144). Conversely, maritime grizzly bears that reside at the terrestrial-marine interface, diverged from a low trophic level terrestrial diet to salmon (high trophic level marine diet) in the fall, as evidenced by both high $\delta^{15}N$ and $\delta^{13}C$ closer to the root of the hair (most recent diet) (144). The common practice of using whole hair averages to understand the dietary history of the animal prior to assessment of contaminant exposure (through diet) fails to provide the pertinent information on dietary shifts or changes over shorter time periods. Further, segmented hair stable isotope analysis to obtain dietary information can be utilized to assess seasonal changes in contaminant exposure and to correct for individual differences in POP concentrations and patterns. It has become common practice in calculating BMF and metabolic indices (MI) to place POP information for all animals from one species together, irrespective of individual dietary differences. This has resulted in large variation in calculated BMFs for a variety of organisms, such as the polar bear (94,186). Correction for dietary differences becomes especially important for omnivorous wildlife species.

Not all animals are the same: unique biological features of a species must be considered when assessing contaminant-related health risks. Grizzly bears have unique features that may influence POP exposure, pharmacokinetics and associated health risks. These include their 1) annual fasting-associated hibernation, 2) tappen (plug) preventing urination and defecation during hibernation, 3) being one of the only carnivores to give birth and lactate during hibernation (with exception to black and polar bears), 4) seasonal gorging on fat-rich foods, and 5) their omnivorous nature in which individuals can be exposed to terrestrial and marine-dervided POPs.

Following a hibernation event, where fat stores are utilized for energy, thermogenesis and vital body processes, polychlorinated biphenyl (ΣPCB) concentrations increased by 2.21X, polybrominated diphenylethers (ΣPBDEs) by 1.58X and chlordanes (ΣCHL) by 1.49X in fat (154). Concentration effects as a result of fasting have also been measured in polar bears (89,90). However, this was the first study to demonstrate that individual POPs can elicit a wide range of fasting-associated concentration effects (e.g. CB-153, 2.25X vs. CB-169, 0.00X). This results in POP pattern convergence of the two distinct fall grizzly bear feeding groups (salmon-eating vs. non-salmon-eating) into a single spring (post-hibernation) group (154), suggesting that diet dictates contaminant patterns during a feeding phase, while metabolism drives patterns during a fasting phase. This work also concluded that there is thus a duality of POP-associated health risks to exposed adult grizzly bears: 1) increased concentrations of some POPs during hibernation; and 2) a potentially prolonged accumulation of water-soluble, highly reactive POP metabolites, since grizzly bears do not excrete during hibernation.

Bears are also the only carnivores to give birth and lactate during this fasting period. Parent POPs and metabolites are able to be transferred to cubs both placentally and lactationally (216-218). Hence, developing fetuses and nursing cubs may be exposed to exceptionally high concentrations of POPs, as well as their metabolites. Due to their sensitive nature, these are the grizzly bear life stages, where POP-associated health effects, through endocrine disruption, are most likely to be observed.

In other species, age and sex are considered important biological factors influencing POP concentrations and patterns (18). Due to the placental and lactational transfer of POPs to offspring, generally adult females have lower concentrations of POPs

relative to adult males (18,89). For long-lived mammals (especially males), which are continually exposed to POPs through their diet, POPs have been shown to increase with age due to their persistence in the organism (18). Due to our small sample size, it was difficult to assess the influence of these factors in grizzly bears; however, our results suggest that there appears to be no difference between sexes, and no significant relationship between age and POP concentrations. In polar bears, there is also no observed relationship between age and POP concentrations; however, there are some differences between sexes (89). The influence of these factors on POP concentrations and patterns should always be assessed in a species prior to risk assessment.

Contribution of modes of POP depuration other than metabolism must be determined. Current practices calculating BMF and MI values have numerous assumptions underlying their calculations which weaken their use in chemical risk assessment. These assumptions are thoroughly discussed in Chapter 3, and some are addressed in previous sections here (e.g. diet correction). The weakest assumption, particularly for MI calculations, is that the MI value has been utilized as a representation of the PCB portion lost by the predator through CYP-related metabolism. Both MIs and BMFs represent values that encompass the many pharmacokinetic processes governing the uptake, loss and accumulation of contaminants in predators, including metabolism, but neither calculation is able to distinguish between these processes. In Chapter 3, using both the ability to quantify dietary differences in individual bears and the closed pharmacokinetic loop during hibernation, we were able to differentiate between metabolism and other forms of POP depuration (i.e. excretion) in wild grizzly bears.

Specifically, we found that PCB congeners with *meta*- and *para*-vicinal hydrogen pairs were depurated before and during hibernation, suggesting that metabolism via CYP2B/3A-like enzymes dominates their depuration. Conversely, congeners structurally resistant to biotransformation, and congeners with *ortho*- and *meta*-vicinal H pairs ("non-metabolizable" congeners), exhibited large variation in depuration correlated with log K_{ow}. This positive correlation in combination with no significant depuration of these congeners during hibernation, suggests excretion dominates. Overall, contaminant metabolism through CYP biotransformation is an important factor driving PCB depuration, and therefore patterns, in grizzlies (responsible for 43% of total PCB loss), however, excretion (responsible for 57% of total PCB loss) may be equally or more important. The use of current MI assumptions could mischaracterize the metabolic capabilities of wildlife and hence, provide misinformation on overall chemical risk.

Select the most recalcitrant congener when characterizing POP pattern changes from prey to predator. Concurrently, for these calculations we corrected lipid weight concentrations in pre-hibernation (feeding) and post-hibernation (fasted) salmon-eating grizzly bears to CB-194, the most recalcitrant congener. Current MI and BMF calculations use the most dominant congener, CB-153, instead. However, corrections using CB-153 only provide a "relative" index for depuration and accumulation. Conversely, using CB-194 enables us to quantify, more precisely, "total" depuration and accumulation. In this way, we determined that grizzlies were able to depurate >90% of ΣPCBs from salmon, resulting in a very low biomagnification factor (BMF) of 0.147. We were also able to calculate the percent loss through metabolism and that which was lost through excretion, values mentioned in the preceding sections. These quantifications

are not possible with CB-153. As well, the use of CB-153, rather than CB-194, erroneously suggests that non-metabolizable PCBs are highly persistent in grizzly bears (and other predators), when in fact, we determined that approximately 90% of these "highly persistent" congeners were depurated.

Consider non-invasive techniques (e.g. feces) to characterize feeding ecology, POP exposure and tissue concentrations. Fecal material from the grizzly bear following consumption of various food items (salmon and vegetation) may also be a helpful tool during risk assessment for elusive and wild populations of some mammals. While animals can consume a variety of foods (e.g. numerous plant species), fecal material provides a homogenized representation of the compilation of that animal's diet. This can be helpful for omnivorous species, in which diets of individual animals can vary so greatly that they are hard to predict, and thus, POP exposure and potential health risks associated with diet are also hard to determine. The lack of digestion of vegetation-type foods in grizzly bears made it simplier to identify individual plant species. However, in other animals which show greater digestion of plants, or for diets (i.e. salmon) that are more digestible for carnivores, prey identification in feces may not be possible. In Chapter 4 we demonstrated that there was a strong similarity of stable isotope signatures between food and fecal material. Therefore, measuring carbon and nitrogen stable isotope signatures in fecal material may then allow an assessor to potentially utilize feces as an indication of diet, even when dietary items are unrecognizable in the feces.

The POP pattern of fecal material, relative to food and the bear, also allowed differentiation between POP processes of excretion, metabolism, and accumulation, respectively, in governing observed POP patterns. The results suggest that relative to

food and grizzly bear fat, the fecal material was dominated by lower log K_{ow} non-metabolizable PCBs and most OC pesticides, and indicates that these are preferentially excreted relative to other contaminants (Chapter 4). Following vegetation consumption, fecal material was also dominated by PBDEs, illustrating the importance of vegetation in PBDE exposure for grizzly bears and other terrestrial species, including humans.

Tissue residue guidelines may not adequately protect wildlife. A common practice to assess risk is to characterize accumulation or biomagnification of parent compounds in animal tissues (particularly, fat tissue). Chapter 3 highlighed that bioaccumulation of PCBs was extremely low in grizzly bears, with only 10% of the most recalcitrant congeners retained by the bears following salmon consumption, and less than 1% of metabolizable congeners were retained. This lack of retention translates to low fat concentrations and biomagnification factors (BMFs) in the grizzly bears relative to other wildlife (Tables 9 to 12). Does this lack of POP accumulation suggest that grizzly bears are not at risk to POP-associated health effects? Not necessarily. Depuration, and more specifically, metabolism, of POPs can create metabolites that may be more toxic than the parent compounds (see previous section on differentiation between modes of POP depuration). And while these metabolites are more water soluble than their parents, and thus are more easily excreted by the organism, they are still lipophilic, especially $MeSO_2$-PCBs, MeO-PBDEs and oxychlordane, as well as highly toxic (93,159,174).

Table 9. PCB concentrations in British Columbia grizzly bears relative to other wildlife.

Mammal	Concentration*	Tissue	Reference
Grizzly bear (pre-hibernation)	0.571 to 65.7 ng/g lw (feeding)	Fat	(154)
Grizzly bear (post-hibernation)	1.71 to 248 ng/g lw (fasted)	Fat	(154)
Polar bears (Svalbard)	6,200 to 33,000 ng/g lw	Fat	(84,85)
	2,200 to 33,000 ng/g lw	Blood	
Polar bears (East Greenland)	2,708 to 18,148 ng/g lw	Fat	(182)
Dall's porpoise	1,000 to 18,000 ng/g ww	Blubber	(86)
Baird's beaked whale	1,800 to 2,800 ng/g ww	Blubber	(86)
Killer whale	350,000 to 410,000 ng/g ww	Blubber	(86)
Finless porpoise	320,000 ng/g ww	Blubber	(86)
Pacific white-sided dolphin	40,000 to 71,000 ng/g ww	Blubber	(219)
Gray seal pup	700 to 1,300 ng/g lw	Blubber	(220)

* lw = lipid weight, ww = wet weight

Table 10. OC pesticide concentrations in British Columbia grizzly bears relative to other wildlife.

Mammal	Concentration*	Tissue	Reference
Grizzly bears	0.281 to 20.3 ng/g lw (ΣDDT)	Fat	(144)
(pre-hibernation)	0.214 to 27.6 ng/g lw (ΣCHL)		
	0.304 to 3.78 ng/g lw (ΣHCH)		
Grizzly bears	ND to 5.13 ng/g lw (ΣDDT)	Fat	(154)
(post-hibernation)	0.116 to 65.2 ng/g lw (ΣCHL)		
	0.332 to 7.45 ng/g lw (ΣHCH)		
Polar bears (East Greenland)	73 to 1,113 ng/g lw (ΣDDT)	Fat	(182)
	446 to 3,699 ng/g lw (ΣCHL)		
	128 to 818 ng/g lw (ΣHCH)		
Gray seals	160 to 3,800 ng/g lw (DDE)	Blubber	(221)
	30 to 670 ng/g lw (DDT)		
	4 to 210 ng/g lw (ΣHCH)		
Gray seals	1,800 ng/g lw (ΣDDT)	Blubber	(222)
Ringed seals	950 to 1,240 ng/g lw (DDE)	Blubber	(223)

* ND = not detected, lw = lipid weight

Table 11. PBDE concentrations in British Columbia grizzly bears relative to other wildlife.

Mammal	Concentration*	Tissue	Reference
Grizzly bear	1.12 to 53.5 ng/g lw (ΣPBDEs)	Fat	(144)
(pre-hibernation)	0.124 to 4.35 ng/g lw (BDE-47)		
	0.0286 to 41.5 ng/g lw (BDE-209)		
Grizzly bear	0.636 to 40.2 ng/g lw (ΣPBDEs)	Fat	(154)
(post-hibernation)	0.0909 to 9.66 ng/g lw (BDE-47)		
	ND to 20.9 ng/g lw (BDE-209)		
Gray seals	45 to 1,500 ng/g lw (ΣPBDEs)	Blubber	(221)
	3.3 to 1,200 ng/g lw (BDE-47)		
Pacific white-sided dolphin	2,410 ng/g lw (BDE-47)	Blubber	(224)
Harbour porpoise	52 to 6,110 ng/g lw (BDE-47)	Blubber	(225)
Beluga whale	11 ng/g lw (BDE-47)	Blubber	(226)

*ND = not detected, lw = lipid weight

Table 12. Biomagnification Factors (BMFs) for PCBs, OC pesticides and PBDEs in British Columbia grizzly bears relative to other wildlife.

Mammal	PCB BMF*	OC Pesticide BMF	PBDE BMF*	Reference
Grizzly bear	0.147	0.027 (ΣDDT) 0.16 (ΣCHL) 0.290 (HCB) 0.38 (ΣHCH)	0.587	Chapter 3 (PCBs) and calculated here (OC pesticides and PBDEs)
Ringed seal	5.5	2.4 (ΣCHL) 4.7 (ΣDDT)	n/a	(73)
Bowhead whale	10.9	4.3 (ΣHCH) 12.8 (ΣCHL) 14.3 (ΣDDT)	n/a	(227)
Ringed seal	6.8	2.8 (ΣHCH) 4.6 (ΣCHL) 2.4 (ΣDDT)	n/a	(227)
Bearded seal	4.0	0.9 (ΣHCH) 2.2 (ΣCHL) 2.1 (ΣDDT)	n/a	(227)
Beluga whale	24.4	3.7 (ΣHCH) 13.5 (ΣCHL) 23.0 (ΣDDT)	n/a	(227)
Polar bear	13.0 (CB-153)	n/a	3.9 (BDE-47) 5.8 (BDE-99)	(158)

* value represents ΣPCBs or ΣPBDEs, unless otherwise stated in brackets

Using grizzly bears, as demonstrated in Chapter 3, it was determined that low BMF was a result of PCB depuration, namely metabolism and excretion. Relative to the low concentrations of parent PCBs in bear fat pre-hibernation as a result of significant PCB congener excretion, potential concentrations of metabolites are considerably higher

(Figure 20). During hibernation, when further excretion of parent PCBs and their metabolites is not possible, we estimate that the concentrations of metabolites double. The parent PCB contribution to overall risk becomes even less relative to metabolites. Our results clearly suggest that metabolites, rather than parent compounds, are of greater risk to grizzly bears partly as a result of their unique biological traits, something that may also be relevant to other wildlife. Recent studies have reported that endocrine-related activities associated with PCBs are mediated, in part, through the formation of hydroxylated and methylsulfonyl PCB metabolites (91).

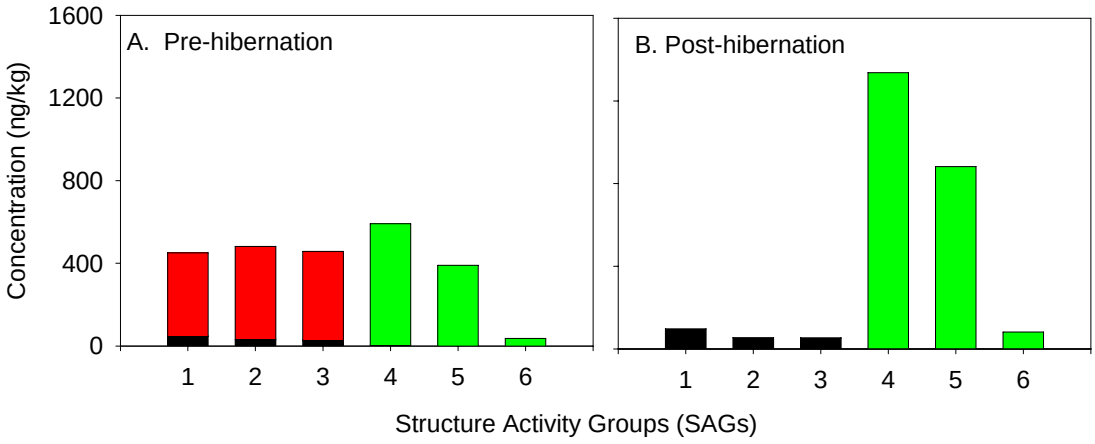

Figure 20. Predicted concentrations of parent PCBs accumulated in fat (black bars), excreted (red bars) and metabolized (green bars) in A) pre-hibernation (feeding) and B) post-hibernation (fasted) grizzly bears. Potential formation of toxic metabolites from PCBs in SAGs 4, 5 and 6 increases the potential for health risks to feeding grizzly bears, despite low parent PCB concentrations in fat tissues. Lack of excretion during hibernation more than doubles the potential concentration of toxic metabolites in fasted grizzly bears.

PBDE flame retardants are POPs: classification and regulation. The Stockholm Convention is a global treaty to protect human health and the environment from POPs. POPs are contaminants that are persistent in the environment for long

periods of time becoming widely distributed geographically, accumulate in the fatty tissue of organisms and are highly toxic. Currently, of the contaminants studied within this thesis, PCBs, aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, HCB, and mirex are considered POPs under the Stockholm Convention. Despite growing evidence of their persistence in the environment and wildlife, circulation around the globe, as well as their toxic, endocrine-disrupting properties, PBDEs are not currently considered POPs. Rather, PBDEs are under consideration for POP classification under the Stockholm Convention due to evidence of their POP-like qualities.

It was determined during this work that PBDEs were present in both terrestrial and marine food webs, with PBDEs most dominant in interior (non-salmon-eating) grizzly bears. PBDEs are more dominant in the terrestrial food web than any of the other "dirty dozen" POPs outlined in the Stockholm Convention. The BMF of PBDEs is calculated here for the salmon-grizzly bear food chain at 0.587. Albeit low, this is approximately 3X that of the BMF for ΣPCBs (Chapter 3) and almost 30X that of the BMF for ΣDDT, suggesting greater persistence of ΣPBDEs in the grizzly bears relative to these other persistent contaminants.

In June 2006, Environment Canada produced an Ecological Screening Assessment Report on PBDEs (228) concluding that, of tetra- to deca-BDE, only tetra-, penta- and hexa-BDE satisfied the criteria outlined in the Persistence and Bioaccumulation Regulations of CEPA 1999. Our results clearly show that deca-BDE (BDE-209) is also bioavailable, persistent and bioaccumulated. Not only was BDE-209 found in the fat of all grizzly bears, moose, and salmon, but interior grizzly bears relying on terrestrial food webs had concentrations of BDE-209 approximately 25X higher than

salmon-eating grizzly bears, suggesting diet has profound influence on exposure, accumulation and potential health risks associated with PBDEs. Interestingly, only aquatic food webs were considered in the Ecological Screening Assessment to characterize the bioaccumulative nature of PBDEs. It is becoming increasingly clear that the higher brominated PBDEs, such as decaBDE, are rather dominating the terrestrial food webs (47,48).

During hibernation there is almost no change in proportion of BDE-203, -206, -207 and -208 in grizzly bears (Figure 21). Since metabolic loss would account for any changes to these congeners during this time (since hibernating grizzly bears do not excrete), these results suggest that these congeners are not readily metabolized and thus, are highly persistent in grizzly bears. Conversely, the percentage of BDE-209 decreases during hibernation, concurrently with an increase in other PBDE congeners. This may suggest that BDE-209 is debrominated during hibernation to lower-brominated congeners, or is metabolized.

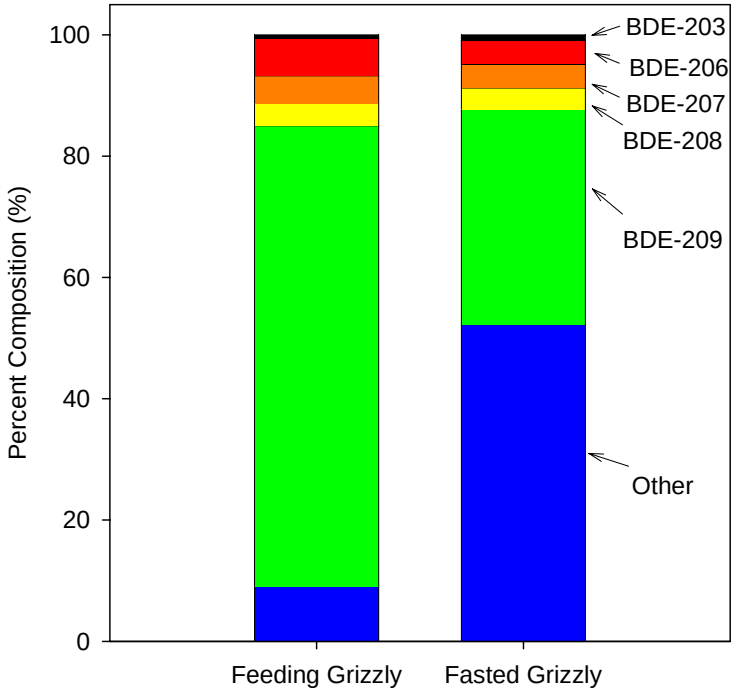

Figure 21. The percent composition of PBDEs in feeding (fall) and fasted (spring) grizzly bears. A significant compositional change from fall to spring grizzly bears suggests metabolic transformation of BDE-209 to less brominated PBDE congeners.

While PBDEs clearly demonstrate qualities characteristic of POPs, some of our results suggest that PBDEs behave differently than POPs. In Chapter 1, while salmon delivery of PCB and OC pesticides increased with increasing log K_{ow} values, salmon delivery of PBDEs decreased indicating a more terrestrial influence for these contaminants. The terrestrial and marine dichotomy in exposure to PBDEs and other POPs, respectively, was again highlighted in Chapter 2. Another difference between PBDEs and the other POPs was that many of the higher-brominated PBDE congeners did not increase in concentration in the bears with increasing salmon consumption. Rather, their predominance was elevated in bears which relied more heavily on terrestrial foods (i.e. vegetation). These same PBDE congeners also did not increase in concentration in

bear food or fecal material with increased trophic position of foods and salmon consumption by the bear (as indicated by increased $\delta^{15}N$), respectively, while OC pesticide and PCB concentrations did increase significantly in both these sample matrices.

There are two main factors dictating the difference in behaviour/fate between PBDEs and other POPs, such as PCBs and OC pesticides. The first factor is molecular size and weight. PBDEs are considerably larger and heavier than volatile OC pesticides and most PCBs. This characteristic of these flame retardants will limit their atmospheric transport to the North Pacific Ocean and the uptake into the base of the grizzly bear marine food web (67). If transported to the North Pacific, heavier PBDEs may be subject to sedimentation, and thus not available for biological uptake (72). The second factor is that PBDEs are currently used in North America, whereas OC pesticides and PCBs have been, for the most part, regulated for 30 to 40 years. Therefore, two things are going on: 1) heavy PBDEs will be concentrated closer to the source, which in the case for Canada, would be terrestrial environments relative to marine environments, and 2) PBDEs are not at equilibrium with the environment due to their continued use and breakdown (e.g. debromination). Over the coming decades, lower brominated PBDEs (either originating from continued use of Penta-BDE formulations or from the debromination of Octa- and Deca-BDE formulations) will become increasingly incorporated into the North Pacific Ocean, and thus the marine food web. However, as long as PBDEs (especially Deca-BDE) continues to be used in Canada and worldwide, the terrestrial environment will be the dominant source of these contaminants to terrestrial wildlife and humans.

Despite the different behaviour demonstrated by PBDEs relative to other legacy POPs, our results clearly highlight the need to classify PBDEs as POPs under the Stockholm Convention to ensure global regulation of their production and use. The results from this thesis, specifically on BDE-209 and other higher-brominated congeners dominating deca-BDE formulations, clearly suggest that deca-BDE falls under the POP classification in both the Stockholm Convention and CEPA (1999). Thus, it is highly recommended that deca-BDE become regulated in Canada, alongside the pending regulation of penta- and octa-BDE formulations.

Conclusions

Using current risk assessment protocols, both salmon-eating and non-salmon-eating grizzly bears do not appear to have POP concentrations in food or tissues associated with appreciable health risk. Tissue concentrations are well below those in which toxic effects have been described (59,137,229,230), and food samples are below critical guidelines set out by the Canadian Council of Ministers of the Environment (CCME) (198). However, these guidelines are not adequate to address all risks posed to exposed wildlife. The results from this thesis strongly suggest grizzly bears may have increased health risks associated with:

- continued and unregulated PBDE (particularly BDE-209) exposure mainly through the terrestrial foods it consumes,
- PBDE and PCB metabolites through CYP biotransformation of parent contaminants, particularly PCBs from SAGs 4, 5 and 6,
- elevated POP concentrations during hibernation as a result of fat utilization,

- prolonged exposure to parent POPs and their metabolites during the hibernation period, as a result of lack of excretion (tappen plug), and
- potential lactational and placental transfer of POPs and metabolites to developing cubs.

While BC's salmon-eating and non-salmon-eating grizzly bears have low tissue POP concentrations and their food POP concentrations are below guidelines, the work included here to redefine the risk assessment process in Canada cautions that grizzly bears may not be out of the woods yet.

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APPENDIX I

Example of how we estimated the proportion of a contaminant concentration in a grizzly bear that is attributed to salmon consumption. SDDT and stable isotope data from bear #6 (5 year old, maritime, male grizzly bear) are used as a model. Bear #6 contained 11,100 ng/kg ΣDDT ($[\Sigma DDT]_{TOTAL}$), while our baseline herbivore bear (#1) contained 31.727 ng/kg ($[\Sigma DDT]_{BASELINE}$). See text for equations. Table taken from Christensen et al. (144), Supporting Information Table S1.

Equation (Eq.#) and variable	Solving	Calculated Values	Meaning of value obtained
(3) $\Delta\delta^{15}N_{SEG}$	(11.6–3.5), (14.1–3.5), (14.4–3.5), (14.0–3.5), (13.2–3.5), (9.3–3.5)	=8.1, 10.6, 10.9, 10.5, 9.7 and 5.8 ‰	Deviation from an herbivorous (100% plant) diet for the bear, using $\delta^{15}N$ values in each segment of hair
(4) $\Sigma\Delta\delta^{15}N_{SEG}$	8.1+10.6+10.9+ 10.5+9.7+5.8	=55.6 ‰	Cumulative deviation from an herbivorous diet over a four month period
(7) P_{MEAT}	55.6/91.8	=0.61	Proportion of diet consisting of salmon (based on Chinook Equivalency index)
(8) P_{VEG}	1.0–0.61	=0.39	Proportion of diet consisting of vegetation
(9) $[\Sigma DDT]_{VEG}$	0.39(31.727)	=12 ng/kg	Concentration of ΣDDT attributed to vegetation
(10) $[\Sigma DDT]_{MEAT}$	11,100–12.374	=11,088 ng/kg	Concentration of ΣDDT attributed to salmon
(11) $P_{[\Sigma DDT]}$	11,088/11,100	=0.99	Proportion of ΣDDT attributed to salmon

APPENDIX II

Contaminants measured, number and percent non-detectable and NDR values, and observed concentration ranges. Table taken from Christensen et al. (154), Supporting Information Table S1.

Contaminant	Number (%) Non-detectable	Number (%) NDR	Range, ng/kg wet (detectable only)
ΣPCB			
CB-1	1 (4%)	4 (16%)	0.9-43.2
CB-2	9 (36%)	4 (16%)	1.0-21.6
CB-3	3 (12%)	10 (40%)	2.2-23.6
CB-4	8 (32%)	-	3.2-47.9
CB-5	25 (100%)	-	-
CB-6	16 (64%)	2 (8%)	1.3-14.6
CB-7	15 (60%)	-	1.8-56.4
CB-8	6 (24%)	-	4.7-72.2
CB-9	23 (92%)	1 (4%)	1.3-1.3
CB-10	25 (100%)	-	-
CB-11	-	3 (12%)	8.0-57.5
CB-12/13	20 (80%)	1 (4%)	1.3-6.4
CB-14	25 (100%)	-	-
CB-15	10 (40%)	-	1.9-20.7
CB-16	1 (4%)	6 (24%)	0.9-24.0
CB-17	-	5 (20%)	1.1-30.6
CB-18/30	-	1 (4%)	2.0-53.1
CB-19	5 (20%)	9 (36%)	0.7-11.2
CB-20/28	-	-	6.4-3460
CB-21/33	-	3 (12%)	2.3-38.6
CB-22	1 (4%)	2 (8%)	1.7-21.9
CB-23	25 (100%)	-	-
CB-24	21 (84%)	-	0.2-0.8
CB-25	7 (28%)	3 (12%)	0.4-4.4
CB-26/29	2 (8%)	2 (8%)	1.2-15.0
CB-27	11 (44%)	4 (16%)	0.3-3.8
CB-31	-	-	3.7-271
CB-32	2 (8%)	6 (24%)	1.0-14.9

Contaminant	Number (%) Non-detectable	Number (%) NDR	Range, ng/kg wet (detectable only)
CB-34	24 (96%)	1 (4%)	-
CB-35	14 (56%)	2 (8%)	0.3-1.3
CB-36	24 (96%)	-	0.6-0.6
CB-37	1 (4%)	6 (24%)	1.5-33.3
CB-38	24 (96%)	-	0.2-0.2
CB-39	24 (96%)	1 (4%)	-
CB-40/41/71	1 (4%)	3 (12%)	1.2-29.3
CB-42	1 (4%)	8 (32%)	1.1-21.6
CB-43	11 (44%)	5 (20%)	0.2-2.2
CB-44/47/65	-	-	3.0-949
CB-45/51	-	6 (24%)	0.6-9.6
CB-46	10 (40%)	5 (20%)	0.2-3.5
CB-48	2 (8%)	6 (24%)	0.8-10.9
CB-49/69	-	2 (8%)	2.2-316
CB-50/53	1 (4%)	6 (24%)	0.4-10.7
CB-52	-	1 (4%)	3.6-525
CB-54	17 (68%)	4 (16%)	0.3-0.8
CB-55	21 (84%)	2 (8%)	0.6-2.4
CB-56	2 (8%)	2 (8%)	1.1-16.9
CB-57	25 (100%)	-	-
CB-58	25 (100%)	-	-
CB-59/62/75	1 (4%)	4 (16%)	0.6-65.9
CB-60	-	3 (12%)	2.7-1900
CB-61/70/74/76	-	-	5.9-6190
CB-63	6 (24%)	-	0.3-104
CB-64	1 (4%)	4 (16%)	1.3-163
CB-66	-	-	3.7-5390
CB-67	21 (84%)	2 (8%)	0.6-4.2
CB-68	9 (36%)	2 (8%)	0.5-50.2
CB-72	17 (68%)	5 (20%)	0.5-10.1
CB-73	23 (92%)	1 (4%)	1.2-1.2
CB-77	3 (12%)	2 (8%)	0.6-162

Contaminant	Number (%) Non-detectable	Number (%) NDR	Range, ng/kg wet (detectable only)
CB-78	22 (88%)	-	1.2-3.7
CB-79	20 (80%)	1 (4%)	1.2-5.9
CB-80	24 (96%)	-	3.8-3.8
CB-81	13 (52%)	2 (8%)	0.5-4.0
CB-82	8 (32%)	4 (16%)	0.3-25.8
CB-83/99	-	-	3.7-10900
CB-84	3 (12%)	5 (20%)	0.8-62.5
CB-85/116/117	-	1 (4%)	1.9-1090
CB-86/87/97/108/119/125	-	-	1.9-322
CB-88/91	4 (16%)	4 (16%)	0.5-34.9
CB-89	23 (92%)	-	0.4-1.6
CB-90/101/113	-	-	2.1-959
CB-92	2 (8%)	2 (8%)	0.9-122
CB-93/95/98/100/102	-	2 (8%)	1.2-211
CB-94	24 (96%)	-	0.8-0.8
CB-96	23 (92%)	-	0.3-1.8
CB-103	19 (76%)	3 (12%)	0.2-3.2
CB-104	16 (64%)	6 (24%)	0.7-0.7
CB-105	-	1 (4%)	1.7-4600
CB-106	25 (100%)	-	-
CB-107/124	12 (48%)	2 (8%)	0.7-33.7
CB-109	3 (12%)	3 (12%)	0.8-868
CB-110/115	-	1 (4%)	2.9-510
CB-111	5 (20%)	2 (8%)	0.8-73.5
CB-112	24 (96%)	-	8.8-8.8
CB-114	2 (8%)	1 (4%)	3.8-557
CB-118	-	-	4.3-19300
CB-120	13 (52%)	2 (8%)	0.2-15.6
CB-121	16 (64%)	2 (8%)	0.2-5.3
CB-122	24 (96%)	1 (4%)	-
CB-123	3 (12%)	1 (4%)	0.5-152
CB-126	8 (32%)	3 (12%)	0.4-33.2

Contaminant	Number (%) Non-detectable	Number (%) NDR	Range, ng/kg wet (detectable only)
CB-127	8 (32%)	3 (12%)	1.3-20.3
CB-128/166	1 (4%)	2 (8%)	2.5-525
CB-129/138/160/163	-	-	7.0-9710
CB-130	2 (8%)	2 (8%)	0.7-339
CB-131	25 (100%)	-	-
CB-132	5 (20%)	4 (16%)	0.5-103
CB-133	2 (8%)	1 (4%)	1.2-485
CB-134/143	16 (64%)	-	0.4-19.3
CB-135/151/154	-	2 (8%)	0.7-189
CB-136	-	7 (28%)	0.3-28.4
CB-137	1 (4%)	2 (8%)	2.5-661
CB-139/140	8 (32%)	3 (12%)	0.8-21.2
CB-141	10 (40%)	2 (8%)	0.9-129
CB-142	25 (100%)	-	-
CB-144	4 (16%)	7 (28%)	0.2-23.1
CB-145	25 (100%)	-	-
CB-146	1 (4%)	3 (12%)	2.6-1720
CB-147/149	-	3 (12%)	1.2-413
CB-148	20 (80%)	4 (16%)	0.2-0.2
CB-150	24 (96%)	-	1.1-1.1
CB-152	25 (100%)	-	-
CB-153/168	-	-	7.9-30900
CB-155	7 (28%)	5 (20%)	0.2-8.3
CB-156/157	-	-	1.1-4160
CB-158	1 (4%)	3 (12%)	0.8-128
CB-159	21 (84%)	1 (4%)	1.5-10.0
CB-161	25 (100%)	-	-
CB-162	6 (24%)	2 (8%)	0.8-134
CB-164	17 (68%)	1 (4%)	2.1-26.2
CB-165	4 (16%)	2 (8%)	0.3-43.5
CB-167	2 (8%)	-	1.2-926
CB-169	24 (96%)	1 (4%)	-

Contaminant	Number (%) Non-detectable	Number (%) NDR	Range, ng/kg wet (detectable only)
CB-172	-	3 (12%)	1.8-909
CB-174	-	3 (12%)	0.3-80.5
CB-175	6 (24%)	7 (28%)	1.0-8.5
CB-176	6 (24%)	3 (12%)	0.2-12.2
CB-177	1 (4%)	2 (8%)	0.7-114
CB-170	-	1 (4%)	18.4-7220
CB-171/173	-	5 (20%)	0.9-133
CB-178	-	2 (8%)	1.0-681
CB-179	1 (4%)	3 (12%)	0.7-43.0
CB-180/193	-	-	3.3-16100
CB-181	14 (56%)	5 (20%)	0.5-2.3
CB-182	10 (40%)	7 (28%)	0.7-3.9
CB-183/185	4 (16%)	2 (8%)	2.4-773
CB-184	11 (44%)	4 (16%)	0.6-3.9
CB-186	25 (100%)	-	-
CB-187	-	1 (4%)	1.7-462
CB-188	12 (48%)	5 (20%)	0.3-1.6
CB-189	-	3 (12%)	1.9-1290
CB-190	-	1 (4%)	0.4-1580
CB-191	-	5 (20%)	0.6-123
CB-192	14 (56%)	5 (20%)	0.4-11.2
CB-194	-	1 (4%)	1.6-27700
CB-195	1 (4%)	3 (12%)	0.2-223
CB-196	2 (8%)	3 (12%)	0.7-124
CB-197/200	6 (24%)	2 (8%)	0.6-7.8
CB-198/199	-	4 (16%)	3.2-1810
CB-201	5 (20%)	6 (24%)	1.0-12.0
CB-202	2 (8%)	4 (16%)	0.7-281
CB-203	-	3 (12%)	0.2-433
CB-204	19 (76%)	4 (16%)	0.2-0.7
CB-205	1 (4%)	2 (8%)	4.3-957
CB-206	1 (4%)	1 (4%)	15.3-5450

Contaminant	Number (%) Non-detectable	Number (%) NDR	Range, ng/kg wet (detectable only)
CB-207	11 (44%)	-	0.7-21.6
CB-208	7 (28%)	-	0.6-60.2
CB-209	-	2 (8%)	9.6-1100
ΣPBDE			
BDE-7	21 (84%)	1 (4%)	0.5-5.3
BDE-8/11	21 (84%)	1 (4%)	0.5-38.3
BDE-10	25 (100%)	-	-
BDE-12/13	10 (40%)	15 (60%)	-
BDE-15	7 (28%)	10 (40%)	1.6-6.6
BDE-17/25	-	1 (4%)	0.7-18.8
BDE-28/33	-	3 (12%)	3.6-207
BDE-30	21 (84%)	4 (16%)	-
BDE-32	20 (80%)	-	0.9-12.0
BDE-35	10 (40%)	10 (40%)	0.8-49.2
BDE-37	8 (32%)	6 (24%)	0.6-2.8
BDE-47	-	-	47.8-10000
BDE-49	1 (4%)	-	1.4-55.6
BDE-51	5 (20%)	2 (8%)	0.4-61.1
BDE-66	-	-	1.5-70.2
BDE-71	11 (44%)	2 (8%)	0.5-8.4
BDE-75	18 (72%)	2 (8%)	0.7-2.7
BDE-77	11 (44%)	4 (16%)	0.5-10.2
BDE-79	-	8 (32%)	4.4-59.1
BDE-85	1 (4%)	-	2.2-111
BDE-99	-	-	41.8-4400
BDE-100	-	-	9.8-1600
BDE-105	25 (100%)	-	-
BDE-116	24 (96%)	-	1.5-1.5
BDE-119/120	4 (16%)	2 (8%)	0.7-408
BDE-126	23 (92%)	-	0.4-3.3
BDE-128	23 (92%)	1 (4%)	21.6-21.6
BDE-138/166	3 (12%)	2 (8%)	1.1-31.0

Contaminant	Number (%) Non-detectable	Number (%) NDR	Range, ng/kg wet (detectable only)
BDE-140	7 (28%)	5 (20%)	0.4-27.5
BDE-153	-	-	10.8-8700
BDE-154	-	-	4.3-619
BDE-155	-	4 (16%)	1.2-41.5
BDE-181	24 (96%)	-	2.4-2.4
BDE-183	-	-	2.4-762
BDE-190	21 (84%)	-	3.1-16.4
BDE-203	-	-	2.4-230
BDE-206	-	2 (8%)	16.4-2663
BDE-207	-	-	20.7-1917
BDE-208	-	-	15.1-1554
BDE-209	-	-	173-29905
ΣHCH			
α-HCH	-	1 (4%)	8.0-2050
β-HCH	1 (4%)	3 (12%)	56.0-4150
γ-HCH	3 (12%)	3 (12%)	6.0-3190
δ-HCH	5 (20%)	19 (76%)	16.0-16.0
ΣCHL			
Heptachlor-Epoxide	-	1 (4%)	2.0-1320
Methoxychlor	13 (52%)	5 (20%)	10.0-57.0
Heptachlor	8 (32%)	14 (56%)	4.0-8.0
Oxychlordane	-	3 (12%)	144-31300
trans-Chlordane	-	9 (36%)	8.0-442
cis-Chlordane	1 (4%)	2 (8%)	5.0-3860
trans-Nonachlor	-	4 (16%)	12.0-7480
cis-Nonachlor	1 (4%)	7 (28%)	7.0-1110
ΣDDT			
o,p-DDD	19 (76%)	2 (8%)	6.0-122
p,p-DDD	17 (68%)	1 (4%)	15.0-1290
o,p-DDE	21 (84%)	2 (8%)	19.0-112
p,p-DDE	-	1 (4%)	14.0-16600

Contaminant	Number (%) Non-detectable	Number (%) NDR	Range, ng/kg wet (detectable only)
o,p-DDT	14 (56%)	4 (16%)	9.0-445
p,p-DDT	6 (24%)	-	9.0-1140
Other OC Pesticides			
HCB	-	-	42.0-21200
α-Endosulphan	2 (8%)	22 (88%)	29.0-29.0
Aldrin	20 (80%)	3 (12%)	4.0-5.0
Dieldrin	-	1 (4%)	8.0-3260
Endrin	16 (64%)	3 (12%)	2.0-63.0
β-Endosulphan	3 (12%)	22 (88%)	-
Endosulphan-Sulphate	12 (48%)	5 (20%)	11.0-136
Endrin-Aldehyde	25 (100%)	-	-
Endrin-Ketone	25 (100%)	-	-
Mirex	1 (4%)	7 (28%)	3.0-239

APPENDIX III

Individual PCB congener concentrations (ng/g lipid weight) in sockeye salmon muscle, fall (feeding) grizzly bear fat and spring (fasted) grizzly bear fat, as well as resultant biomagnification factors (BMFs) between a feeding grizzly bear and salmon.

PCB Congener	Salmon	Fall bear	Spring bear	BMF
CL3-PCB-20/28	9.43	1.25	0.974	0.133
CL3-PCB-21/33	0.919	0.0450	0.0652	0.0490
CL3-PCB-26/29	1.04	0.0256	0.0138	0.0246
CL3-PCB-31	7.48	0.330	0.0902	0.0441
CL3-PCB-37	0.151	0.0253	0.0444	0.168
CL4-PCB-44/47/65	15.1	1.21	2.19	0.0799
CL4-PCB-49/69	10.9	0.392	0.0932	0.0360
CL4-PCB-52	27.8	0.669	0.0903	0.0240
CL4-PCB-59/62/75	1.39	0.0901	0.120	0.0647
CL4-PCB-60	5.69	0.647	0.561	0.114
CL4-PCB-61/70/74/76	47.0	4.41	8.58	0.0938
CL4-PCB-63	1.34	0.126	0.143	0.0941
CL4-PCB-64	5.61	0.187	0.0210	0.0333
CL4-PCB-66	19.8	1.78	1.43	0.0901
CL4-PCB-68	0.755	0.0396	0.0531	0.0524
CL4-PCB-77	0.593	0.0109	0.00720	0.0184
CL5-PCB-83/99	40.9	9.06	18.9	0.222
CL5-PCB-85/116/117	10.3	1.47	1.80	0.143
CB-86/87/97/108/119/125	28.2	0.378	0.102	0.0134
CL5-PCB-90/101/113	62.4	1.24	0.336	0.0198
CL5-PCB-92	12.3	0.146	0.0136	0.0118
CL5-PCB-93/95/98/100/102	32.6	0.249	0.0300	0.00762
CL5-PCB-105	13.6	3.51	7.12	0.258
CL5-PCB-109	4.91	0.616	1.09	0.125
CL5-PCB-110/115	31.6	0.652	0.386	0.0206
CL5-PCB-111	0.0890	0.0462	0.117	0.520

CL5-PCB-114	1.25	0.414	0.854	0.331
CL5-PCB-118	46.3	13.3	26.5	0.286
CL5-PCB-123	0.892	0.106	0.139	0.119
CL6-PCB-128/166	6.86	0.813	0.791	0.119
CL6-PCB-129/138/160/163	64. 7	11.0	18.2	0.170
CL6-PCB-130	3.50	0.378	0.510	0.108
CL6-PCB-132	10.1	0.114	0.0143	0.0113
CL6-PCB-133	1.54	0.462	1.11	0.301
CL6-PCB-135/151/154	23.5	0.241	0.0314	0.0103
CL6-PCB-136	3.56	0.0323	0.00441	0.00908
CL6-PCB-137	3.55	0.658	1.14	0.185
CL6-PCB-144	2.90	0.0242	0.00371	0.00835
CL6-PCB-146	13.6	1.47	3.12	0.108
CL6-PCB-147/149	47.9	0.491	0.0729	0.0102
CL6-PCB-153/168	79.5	25.8	55.1	0.324
CL6-PCB-156/157	3.80	3.85	8.06	1.01
CL6-PCB-158	2.70	0.225	0.162	0.0831
CL6-PCB-162	0.321	0.0559	0.154	0.174
CL6-PCB-165	0.0514	0.0474	0.103	0.922
CL6-PCB-167	2.18	0.371	0.899	0.170
CL7-PCB-170	5.13	6.12	9.27	1.19
CL7-PCB-171/173	1.76	0.139	0.149	0.0792
CL7-PCB-172	1.43	0.368	0.707	0.258
CL7-PCB-174	5.82	0.113	0.0281	0.0194
CL7-PCB-175	0.546	0.0131	0.00472	0.0239
CL7-PCB-176	0.819	0.0127	0.00365	0.0155
CL7-PCB-177	4.56	0.0974	0.110	0.0213
CL7-PCB-178	3.58	0.451	0.769	0.126
CL7-PCB-179	4.48	0.0588	0.0151	0.0131
CL7-PCB-180/193	16.2	12.5	20.1	0.777
CL7-PCB-183/185	6.56	0.619	0.889	0.0943

CL7-PCB-187	18.8	0.679	0.682	0.0360
CL7-PCB-189	0.186	0.277	0.632	1.49
CL7-PCB-190	0.650	0.688	1.33	1.06
CL7-PCB-191	0.177	0.0819	0.108	0.462
CL8-PCB-194	1.14	3.47	7.85	3.04
CL8-PCB-195	0.377	0.0737	0.158	0.196
CL8-PCB-196	0.931	0.0578	0.0766	0.0621
CL8-PCB-197/200	0.337	0.00749	0.00846	0.0222
CL8-PCB-198/199	2.95	0.549	1.06	0.186
CL8-PCB-201	0.601	0.0120	0.0120	0.0200
CL8-PCB-202	1.65	0.134	0.207	0.0808
CL8-PCB-203	1.17	0.136	0.252	0.116
CL8-PCB-205	0.0953	0.167	0.339	1.76
CL9-PCB-206	0.512	0.851	1.80	1.66
CL9-PCB-208	0.382	0.0237	0.0322	0.0620
CL10-PCB-209	0.477	0.651	1.80	1.36
Total PCBs	792	117	210	0.147

APPENDIX IV

PCA variables, their abbreviations, halogen numbers and SAG, number and percent undetectable, radius vector *r* and vectorial angle θ in polar coordinates, the percent uptake in maritime grizzly bears relative to PCB 170 and the log K_{ow} values.

PCA parameter	Congener abbreviation	Halogen No.	SAG	SAG (major congener)	ortho Halogen No. (major congener)	No. (%) undetectable	radius *r*	angle θ	Uptake relative to PCB 170, %	log K_{OW}
PCB-1	1	1	6	6	1	3.0 (4.9%)	0.202	118.9	36.2	4.70
PCB-2	2	1	6	6	0	11.0 (18.0%)	0.227	112.4	28.4	4.67
PCB-3	3	1	6	6	0	3.0 (4.9%)	0.198	120.5	37.5	4.64
PCB-4	4	2	6	6	2	9.0 (14.8%)	0.220	117.2	30.6	4.76
PCB-8	8	2	6	6	1	6.0 (9.8%)	0.248	107.5	21.8	5.12
PCB-11	11	2	6	6	0	-	0.208	120.2	34.3	5.11
PCB-15	15	2	3	3	0	10.0 (16.4%)	0.232	113.7	26.6	5.05
PCB-16	16	3	6	6	2	1.0 (1.6%)	0.271	105.8	14.3	5.15
PCB-17	17	3	6	6	2	-	0.290	101.7	8.4	5.34
PCB-18/30	18/30	3	6	6	2	-	0.291	100.8	8.0	5.33
PCB-19	19	3	6	6	3	5.0 (8.2%)	0.240	113.2	24.3	4.93
PCB-20/28	20/28	3	6/3	3	1	-	0.203	94.0	35.9	5.50
PCB-21/33	21/33	3	6	6	1	-	0.259	105.0	18.1	5.67
PCB-22	22	3	6	6	1	1.0 (1.6%)	0.302	96.7	4.6	5.49
PCB-25	25	3	6	6	1	7.0 (11.5%)	0.299	96.0	5.6	5.55
PCB-26/29	26/29	3	6	6	1	2.0 (3.3%)	0.297	94.5	6.2	5.69
PCB-27	27	3	6	6	2	11.0 (18.0%)	0.286	101.9	9.6	5.35
PCB-31	31	3	6	6	1	-	0.277	88.8	12.5	5.69
PCB-32	32	3	6	6	2	2.0 (3.3%)	0.263	108.5	16.8	5.36
PCB-37	37	3	3	3	0	1.0 (1.6%)	0.251	110.7	20.8	5.66
PCB-40/41/71	40/41/71	4	6	6	2	1.0 (1.6%)	0.309	87.8	2.3	5.59
PCB-42	42	4	6	6	2	1.0 (1.6%)	0.315	78.9	0.5	5.72
PCB-43	43	4	6	6	2	16.0 (26.2%)	0.264	72.2	16.5	5.80
PCB-44/47/65	44/47/65	4	6/2/6	2	2	-	0.208	61.4	34.2	5.70

PCB-45/51	45/51	4	6	6	3	-	0.308	92.1	2.6	5.34
PCB-46	46	4	6	6	3	10.0 (16.4%)	0.306	93.5	3.4	5.21
PCB-48	48	4	6	6	2	2.0 (3.3%)	0.308	79.7	2.7	5.79
PCB-49/69	49/69	4	6	6	2	-	0.266	64.1	15.8	5.87
PCB-50/53	50/53	4	6/5	5	3	1.0 (1.6%)	0.316	86.8	0.0	5.66
PCB-52	52	4	4	4	2	-	0.276	65.7	12.9	5.88
PCB-56	56	4	6	6	1	2.0 (3.3%)	0.297	91.9	6.1	6.03
PCB-59/62/75	59/62/75	4	6/6/2	6	2	1.0 (1.6%)	0.224	64.3	29.2	5.75
PCB-60	60	4	3	3	1	-	0.159	51.2	49.8	6.08
PCB-61/70/74/76	61/70/74/76	4	6/6/3/6	3	1	-	0.149	46.3	53.0	6.35
PCB-63	63	4	3	3	1	9.0 (14.8%)	0.165	50.1	47.9	6.17
PCB-64	64	4	6	6	2	1.0 (1.6%)	0.266	64.8	16.0	5.75
PCB-66	66	4	3	3	1	-	0.186	52.4	41.1	6.07
PCB-68	68	4	3	3	1	14.0 (23.0%)	0.191	65.9	39.7	6.05
PCB-77	77	4	3	3	0	3.0 (4.9%)	0.245	93.5	22.6	6.29
PCB-82	82	5	6	6	2	9.0 (14.8%)	0.297	77.4	6.1	6.16
PCB-83/99	83/99	5	6/2	2	2	-	0.098	28.0	69.0	6.23
PCB-84	84	5	6	6	3	3.0 (4.9%)	0.294	69.9	7.1	5.68
PCB-85/116/117	85/116/117	5	2/6/2	2	2	-	0.146	37.0	53.9	6.31
PCB-86/87/97/108/119/125	86/87/97/108/119/125	5	6/6/6/3/2/6	6	2	-	0.268	61.3	15.4	6.28
PCB-88/91	88/91	5	6	6	3	4.0 (6.6%)	0.262	66.1	17.4	6.06
PCB-90/101/113	90/101/113	5	2/4/4	4	2	-	0.251	57.6	20.7	6.35
PCB-92	92	5	4	4	2	2.0 (3.3%)	0.250	59.6	21.0	6.34
PCB-93/95/98/100/102	93/95/98/100/102	5	6/5/6/2/5	5	3	-	0.285	65.9	10.0	5.95
PCB-105	105	5	3	3	1	-	0.064	18.1	79.7	6.60
PCB-107/124	107/124	5	3/4	3	1	14.0 (23.0%)	0.216	59.4	31.7	6.72
PCB-109	109	5	6	6	2	3.0 (4.9%)	0.173	41.4	45.5	6.43
PCB-110/115	110/115	5	6/2	6	2	-	0.258	59.6	18.6	6.18
PCB-111	111	5	1	1	1	11.0 (18.0%)	0.107	77.8	66.3	6.75

PCB-114	114	5	3	3	1	3.0 (4.9%)	0.064	47.0	79.7	6.72
PCB-118	118	5	3	3	1	-	0.065	18.9	79.6	6.63
PCB-123	123	5	3	3	1	6.0 (9.8%)	0.117	40.5	62.9	6.60
PCB-126	126	5	3	3	0	17.0 (27.9%)	0.075	73.0	76.2	6.93
PCB-128/166	128/166	6	2	2	2	1.0 (1.6%)	0.152	36.9	52.0	6.74
PCB-129/138/160/163	129/138/160/163	6	6/2/6/2	2	2	-	0.126	28.6	60.0	6.72
PCB-130	130	6	2	2	2	2.0 (3.3%)	0.163	38.1	48.4	6.78
PCB-132	132	6	6	6	3	5.0 (8.2%)	0.271	63.6	14.4	6.23
PCB-133	133	6	1	1	2	5.0 (8.2%)	0.081	22.6	74.5	6.86
PCB-134/143	134/143	6	6/5	6	3	17.0 (27.9%)	0.264	64.0	16.6	6.36
PCB-135/151/154	135/151/154	6	5/5/1	5	3	-	0.262	60.6	17.2	6.27
PCB-136	136	6	5	5	4	-	0.285	67.1	10.1	5.98
PCB-137	137	6	2	2	2	1.0 (1.6%)	0.107	25.4	66.1	6.83
PCB-139/140	139/140	6	2	2	3	10.0 (16.4%)	0.233	56.6	26.3	6.66
PCB-141	141	6	4	4	2	10.0 (16.4%)	0.250	64.5	20.9	6.81
PCB-144	144	6	5	5	3	4.0 (6.6%)	0.266	63.0	16.1	6.57
PCB-146	146	6	1	1	2	1.0 (1.6%)	0.165	38.4	47.8	6.81
PCB-147/149	147/149	6	2/5	5	3	-	0.262	60.9	17.2	6.55
PCB-153/168	153/168	6	1	1	2	-	0.070	14.0	77.9	6.76
PCB-155	155	6	1	1	4	14.0 (23.0%)	0.165	60.0	47.8	6.72
PCB-156/157	156/157	6	3	3	1	-	0.011	139.5	96.6	7.25
PCB-158	158	6	2	2	2	1.0 (1.6%)	0.184	44.8	41.9	6.86
PCB-162	162	6	1	1	1	14.0 (23.0%)	0.104	42.7	67.2	6.56
PCB-164	164	6	4	4	2	18.0 (29.5%)	0.241	60.3	23.9	6.56
PCB-167	167	6	1	1	1	3.0 (4.9%)	0.095	30.3	69.9	7.11
PCB-170	170	7	2	2	2	-	0.000	0.0	100.0	7.27
PCB-171/173	171/173	7	2/6	6	3	1.0 (1.6%)	0.168	41.0	47.0	6.96
PCB-172	172	7	1	1	2	2.0 (3.3%)	0.095	23.0	70.1	7.34
PCB-174	174	7	5	5	3	-	0.233	59.4	26.4	6.72
PCB-175	175	7	1	1	3	11.0 (18.0%)	0.226	56.0	28.5	6.98
PCB-176	176	7	5	5	4	9.0 (14.8%)	0.259	64.7	18.0	6.70

PCB-177	177	7	2	2	3	1.0 (1.6%)	0.214	52.3	32.5	6.89
PCB-178	178	7	1	1	3	-	0.152	37.2	51.8	6.91
PCB-179	179	7	5	5	4	1.0 (1.6%)	0.255	61.6	19.4	6.54
PCB-180/193	180/193	7	1	1	2	-	0.027	349.5	91.3	7.28
PCB-183/185	183/185	7	1/5	5	3	7.0 (11.5%)	0.155	66.3	50.9	7.02
PCB-184	184	7	1	1	4	17.0 (27.9%)	0.250	64.2	20.9	7.06
PCB-187	187	7	1	1	3	-	0.217	51.1	31.3	6.93
PCB-189	189	7	1	1	1	3.0 (4.9%)	0.041	157.2	87.0	7.77
PCB-190	190	7	2	2	2	1.0 (1.6%)	0.012	175.2	96.3	7.30
PCB-191	191	7	1	1	2	5.0 (8.2%)	0.022	33.6	93.1	7.23
PCB-194	194	8	1	1	2	1.0 (1.6%)	0.020	159.1	93.8	7.82
PCB-195	195	8	2	2	3	5.0 (8.2%)	0.079	30.2	74.9	7.38
PCB-196	196	8	1	1	3	4.0 (6.6%)	0.171	47.7	46.0	7.43
PCB-197/200	197/200	8	1/5	5	4	10.0 (16.4%)	0.219	62.5	30.9	7.41
PCB-198/199	198/199	8	1	1	3	-	0.110	32.6	65.4	7.39
PCB-201	201	8	1	1	4	8.0 (13.1%)	0.235	60.5	25.8	7.36
PCB-202	202	8	1	1	4	4.0 (6.6%)	0.163	43.0	48.4	7.16
PCB-203	203	8	1	1	3	-	0.137	40.7	56.8	7.42
PCB-205	205	8	1	1	2	5.0 (8.2%)	0.045	158.1	85.6	7.70
PCB-206	206	9	1	1	3	5.0 (8.2%)	0.045	154.8	85.9	7.84
PCB-209	209	10	1	1	4	1.0 (1.6%)	0.041	146.2	86.9	8.18
DPE-17/25	17/25	3	6	6	2	-	0.190	119.1	39.9	5.74
DPE-28/33	28/33	3	3/6	3	1	-	0.143	121.4	54.9	5.94
DPE-47	47	4	2	2	2	-	0.122	131.9	61.6	6.81
DPE-49	49	4	6	6	2	1.0 (1.6%)	0.181	112.7	42.8	6.60
DPE-66	66	4	3	3	1	3.0 (4.9%)	0.131	117.0	58.6	6.60
DPE-85	85	5	2	2	2	6.0 (9.8%)	0.178	123.2	43.8	7.37
DPE-99	99	5	2	2	2	-	0.154	126.8	51.2	7.32
DPE-100	100	5	2	2	3	-	0.137	125.9	56.6	7.24
DPE-119/120	119/120	5	2/1	2	2	12.0 (19.7%)	0.119	117.4	62.3	7.22
DPE-153	153	6	1	1	2	-	0.123	135.5	61.2	7.90
DPE-154	154	6	1	1	3	-	0.155	118.9	51.0	7.82
DPE-183	183	7	1	1	3	2.0 (3.3%)	0.177	125.1	44.2	8.27

DPE-203	203	8	1	1	3	9.0 (14.8%)	0.173	123.1	45.4	9.09
DPE-206	206	9	1	1	3	-	0.191	121.1	39.8	9.71
DPE-207	207	9	1	1	4	-	0.192	120.5	39.3	9.71
DPE-208	208	9	1	1	4	-	0.194	120.6	38.8	9.71
DPE-209	209	10	1	1	4	-	0.201	118.1	36.6	10.33
Heptachlor epoxide	HpE	7				2.0 (3.3%)	0.126	114.6	60.3	5.40
Dieldrin	Die	6				1.0 (1.6%)	0.134	110.0	57.8	5.40
HCB	HCB	6				-	0.137	125.5	56.6	5.73
α-HCH	αHCH	6				5.0 (8.2%)	0.180	119.0	43.1	3.80
β-HCH	βHCH	6				14.0 (23.0%)	0.165	116.3	47.7	3.78
Oxychlordane	OCh	8				2.0 (3.3%)	0.053	126.0	83.2	6.16
c-Chlordane	cCh	8				2.0 (3.3%)	0.196	59.0	38.1	6.16
t-Nonachlor	tNon	9				-	0.184	45.6	42.0	6.20
c-Nonachlor	cNon	9				4.0 (6.6%)	0.214	60.5	32.4	6.20
p,p-DDE	DDE	4				2.0 (3.3%)	0.194	49.4	38.6	6.51
Mirex	Mir	12				3.0 (4.9%)	0.187	55.7	41.0	5.28