Hibernation-Associated Changes in Persistent Organic Pollutant (POP) Levels and Patterns in British Columbia Grizzly Bears (*Ursus arctos horribilis*)

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We hypothesized that depleted fat reserves in grizzly bears (*Ursus arctos horribilis*) following annual hibernation would reveal increases in persistent organic pollutant (POP) concentrations compared to those present in the fall. We obtained fat and hair from British Columbia grizzly bears in early spring 2004 to compare with those collected in fall 2003, with the two tissue types providing contaminant and dietary information, respectively. By correcting for the individual feeding habits of grizzlies using a stable isotope-based approach, we found that polychlorinated biphenyls (ΣPCBs) increased by 2.21×, polybrominated diphenylethers (ΣPBDEs) increased by 1.58×, and chlordanes (ΣCHL) by 1.49× in fat following hibernation. Interestingly, individual POPs elicited a wide range of hibernation-associated concentration effects (e.g., CB-153, 2.25× vs CB-169, 0.00×), resulting in POP pattern convergence in a PCA model of two distinct fall feeding groups (salmon-eating vs non-salmon-eating) into a single spring (post-hibernation) group. Our results suggest that diet dictates contaminant patterns during a feeding phase, while metabolism drives patterns during a fasting phase. This work suggests a duality of POP-associated health risks to hibernating 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.

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) (*1*), 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 (*2*). While fall grizzly bears had moderate concentrations of contaminants relative to other top aquatic predators (*1*, *3*), we speculated that fat loss associated with their approximate 5-month hibernation period would result in a concentration of fat-soluble POPs (*3*).

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 (*4*, *5*). There is a 75% reduction in heart rate (*6*), with a corresponding 50−60% reduction in the basal metabolic rate of the bears (*5*), a depression thought to be much less than that of other hibernators. Hibernating bears form a plug in their rectum ("tappen") preventing defecation from occurring during the hibernation period (*7*), and they also do not urinate during this time (*7*, *8*). Of additional interest is the fact that bears are the only carnivores in which pregnancy and lactation coincide with hibernation (*9*). 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 (*10*−*14*).

Given the highly variable feeding habits among individual grizzly bears, even within the same feeding group (i.e., salmon-eating or non-salmon-eating) (*2*), 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) (*2*). Samples were collected from various locations on the body of the bears (mainly head,

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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, so dietary information is recorded chronologically along its length (*15*). Therefore, grizzly hair was plucked from the skin and subdivided into 1 cm segments commencing at the root toward the tip, with each segment reflecting approximately 20 days of growth (*16*). 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. (*2*). 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 (*17*).

Only the hair segments representing the estimated time frame 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 (Supporting Information, Figure S1). 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.

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. (*2*). 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 (Supporting Information, Table S1). 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. (*2*). 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 (*2*). 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. (*2*). 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 nondetectable ranges (NDR), and most PBDE congeners were detected at <5 ng/kg.

Methods for detection limit substitutions have been described elsewhere (*2*). 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 ($SPR_{CB153}/FALL_{CB153} = 2.25$; Supporting Information, Figure S2) 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.

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 (>1.0×) or decreases (<1.0×) 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 (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 (*18*), followed by a *post-hoc* Tukey's test. The criterion for significance was $\alpha = 0.05$. Normality and constant variance were assessed and data were transformed if those tests resulted in $\alpha < 0.05$.

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. Each contaminant analyzed was evaluated for potential interferences, closeness to the limit of detection, and the percentage of undetectable (random value estimated)

TABLE 1. 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 25, 2003[b]	April 18 − May 18, 2004
sex	7 male, 4 female[b]	14 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 Supporting 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. (*2*) [c] PCB congeners used to calculate ΣTEQ include: 77, 81, 105, 114, 118, 123, 126, 156/157, 167, 169, 170, 180/193, 189.

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 (*19*). 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 (Table 1), 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 δ^{13}C and δ^{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 δ^{13}C and δ^{15}N, are evident in the hair of some, but not all, spring grizzly bears. Enriched ratios of both δ^{13}C and δ^{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 (*2*) (recalculated here where DI = 38.9 ± 11.8).

Consistently low δ^{15}N and δ^{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 Materials and Methods), spring bear DI values are significantly higher (47.3 ± 20.4) compared to those of fall bear values (28.0 ± 13.6). It is the wide range of DI values (fall DI range, 11.2−54.1; spring DI range, 22.5−79.2) 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.

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 2.21× (RP = 98%), suggesting that post-hibernation ΣPCB concentrations are more than double those of pre-hibernation in the residual fat (Table 2). Male polar bears had a lesser increase in ΣPCB concentrations (1.17×) following their seasonal fast (*3*), 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 (*3*). Surprisingly, ΣDDT decreased following hibernation in grizzly bears, and with a CE value of 0.16×, 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 (*3*). ΣPBDEs increased post-hibernation by 1.58× 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).

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 1.80× to 3.30×. Accordingly, overall ΣTEQ in the bears elicits a concentration effect of over 2.00× (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 (*12*−*14*), 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 (*14*).

Most OC pesticides are not persistent in the grizzly bears. One exception is oxychlordane, which increased in concentration by 2.24× (RP = 99%). Heptachlor epoxide has the next highest increase at 1.29× (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 0.00× to 0.25×, 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

TABLE 2. 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*[e]
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*
DE-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 Supporting Information. [b] Predicted spring slope = actual fall slope × 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] × 100. [e] *Significantly different persistence than predicted, calculated using a Student's *t*-test.

persistent in hibernating grizzly bears (CE range: 1.73×−4.76×), only some of these congeners are considered dominant in the profiles of wildlife species (*2*, *20*−*22*). At the same time, BDE-99 which is usually a dominant congener in wildlife PBDE profiles (*2*, *20*−*22*) is only moderately persistent (RP = 49%) in grizzly bears, with a concentration effect of only 1.09×. 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, and 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 male 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 (*23*, *24*). 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 (*25*, *26*), 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 (*18*, *25*, *27*), 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 (*25*, *27*). 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 (*25*, *27*). 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 (*18*, *27*). 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 (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 (*28*), 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 0.00× to >4.00× 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 1a). Some contaminants dominanting post-hibernation bears included oxychlordane, heptachlor epoxide, higher-chlorinated PCBs, and BDE-47, -119 and -153 (Figure 1b).

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) (*2*). Fall interior bears

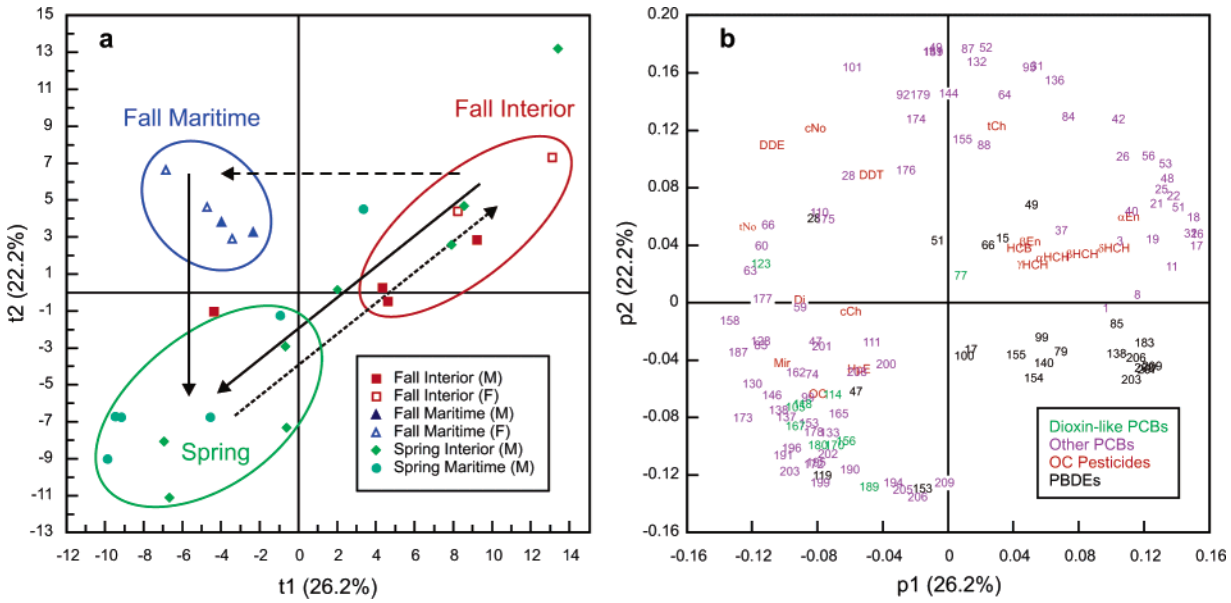

FIGURE 1. Principal components analysis (PCA) of (a) a scores plot where individual fall and spring grizzly bears distinctly reveals three grizzly bear groupings: fall maritime bears (blue circle), fall interior bears (red circle), and all spring bears (green circle); and (b) a loadings plot of polychlorinated 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 co-incident with the arrival of returning salmon (along dashed arrow).

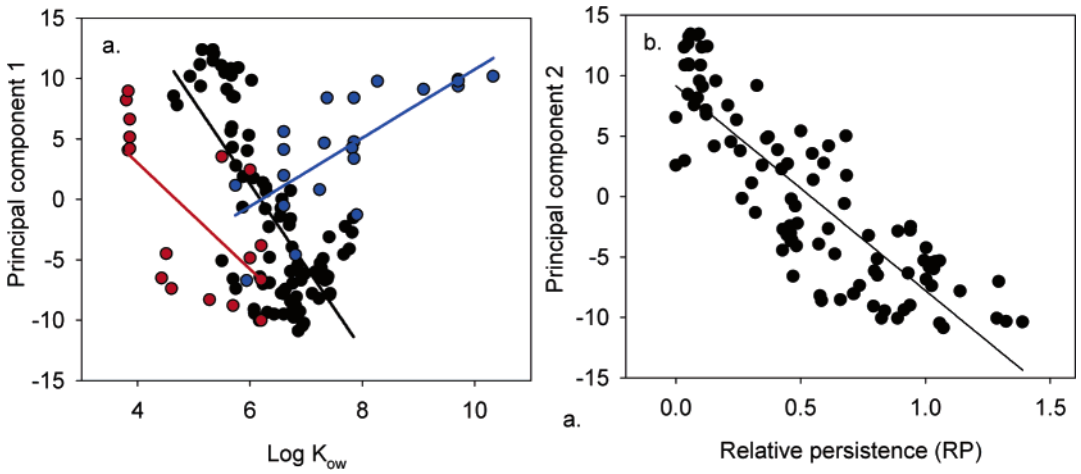

FIGURE 2. (a) Principal component axis 1 (PC1) describes the important role that log K_{ow} plays in the behavior of POPs in grizzly bear food webs. Black circles: polychlorinated biphenyls (PCBs; $r^2 = 0.51$). Red circles: organochlorine (OC) pesticides ($r^2 = 0.44$). Blue circles: polybrominated 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 ($r^2 = 0.73$).

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.

The important role that diet plays in driving contaminant patterns in feeding grizzly bears was evident in the previous study of fall grizzly bears (*2*). 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 physico-chemical properties in influencing POP patterns in bears, where PC1 correlated with contaminant log K_{ow} values (Figure 2a). The PC1 values for both PCB congeners ($r^2 = 0.51$, $\nu = 90$) and OC pesticides ($r^2 = 0.44$, $\nu = 17$) were negatively correlated to their log K_{ow} values, while PC1 values for PBDE congeners were positively correlated to log K_{ow} ($r^2 = 0.56$, $\nu = 22$). 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 (Figure 2b). In this case, all contaminants fell along one regression line ($r^2 = 0.73$, $\nu = 73$).

Contaminants in the upper left quadrant of the PCA variables plot represent nonpersistent contaminants within the bears that were acquired from a marine food web, while those in the upper right quadrant represent nonpersistent

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.

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 midrange 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.

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Supporting Information Available

Table S1 lists the contaminants measured, number and percent nondetectable and NDR values, observed concentration ranges, and PCA variable abbreviations. Figure S1 illustrates how the dietary index (DI) was calculated for fall and spring grizzly bear hair samples. Figure S2 shows the changes in bioaccumulation slopes from fall to spring for CB-153. Figure S3 shows changes in the relationships between BDE-203 and -206 from fall to spring. This material is available free of charge via the Internet at http://pubs.acs.org.

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