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## Supporting Information: Influence of Carbon and Lipid Sources on Variation of Mercury and Other Trace Elements in Polar Bears (*Ursus maritimus*)

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## **Supporting Information**

### **Influence of Carbon and Lipid Sources on Variation of Mercury and Other Trace Elements in Polar Bears (*Ursus maritimus*)**

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## MATERIALS AND METHODS

### *Stable Carbon Isotope Analysis and Quality Control*

All stable carbon isotope (SI) analysis were carried out by the Environmental Isotope Laboratory (EIL), University of Waterloo (Waterloo, ON, Canada), and have been described for the present polar bear muscle and other quality control samples [1,2]. Polar bear muscle tissues were homogenized, lipid-removed and prepared for analysis by standard protocols (e.g., ref [3]).

Solid polar bear muscle and necessary quality control samples were run for carbon analysis on one of 2 systems. The systems were either, 1) A Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan / Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy), or 2) an Isochrom Continuous Flow Stable Isotope Ratio Mass Spectrometer (GVInstruments / Micromass-UK) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy). The Micromass Isochrom EA-IRMS system was checked each day before analyzing samples for SIs by running the peak shape program. With the standard gas flowing, the mass spectrometer scanned from peak 43 to 47. The operator checked to make sure the peak shape was correct and that the collectors were set in the center of the peaks. The standard was then introduced 10 times to check stability and reproducibility. If the reproducibility was less than 0.01, the peak shape was adjusted and the standard check was repeated. The mass spectrometer sample run program performed an analysis on the gas standard with each sample to confirm stability. The performance of the complete sample was checked by the reproducibility of this analysis.

For each group of 100 samples, standards were run to check and allow for bias and linearity corrections. Four replicates of different weights were placed at the beginning and end of each run. This set allowed for linearity corrections. Two sets of three or four replicates of NIST or EIL carbon standards were analyzed in each group. Two sets of replicates of element standards were also run in each group. EIL has accumulated a set of data on a number of NIST elemental standards with respect to their isotope content. This set of control standards was used to confirm that the EA is combusting and transporting gases with the correct isotope ratio to the mass spectrometer. A sample replicate is run at least every eight samples and duplicates were run from previous groups. The overall average number of repeats was about one every eight samples. A few sample replicates of different weights were analyzed to check linearity of the sample matrix. EIL recommended to establish a statistical population of 30 or more analysis of one sample to confirm the error associated with the natural variation of that sample type.

SI results were generally corrected to carbon standards IAEA-CH6 (sugar), EIL-72 (cellulose) and EIL-32 (graphite). The error for clean ball-milled standard material was  $\pm 0.2\%$ . This error was expected to increase depending on the homogeneity, type and amount of sample used in analysis. A truer representation of sample reproducibility was gained through sample repeats. Mean deviation of duplicate SI analysis on 10% of the polar bear samples was  $0.07\%$  for  $\delta^{13}\text{C}$ . Standards were placed throughout each run at a range of weights to allow for an additional linearity correction, when necessary, due to machine fluctuations or samples of varying signal peak areas. Carbon compositions were calculated based on Carlo Erba Elemental Standards B2005, B2035 and B2036 with an error of  $\pm 1\%$ . Other NIST organic materials are run at EIL and cross-checked with other labs in hopes of producing a collection of organic reference

materials for use in SI research. At present these materials were not used in correction but rather as monitoring material.

### *Fatty Acids Analysis and Quality Control*

All fatty acid (FA) analysis for the polar bear fat and quality control samples were carried out by the Organic Contaminants Research Laboratory (OCRL) at NWRC and are described elsewhere [1,2]. Briefly, extraction and analysis of FAs was from 10-20 mg of inner adipose tissue of a collected polar bear sample, to avoid potentially oxidized outer tissue [4]. Lipids were extracted thrice using 2:1  $\text{CHCl}_3$ :MeOH [4] containing 0.01% 2,6-di-*t*-butyl-4-methylphenol as antioxidant. 5- $\alpha$ -Cholestane was used as internal standard. The extract was evaporated to dryness under  $\text{N}_2$ , and lipid was redissolved in toluene. FAs were methylated via the Hilditch reagent [5]. After addition of 2%  $\text{KHCO}_3$ , the organic layer was collected and fatty acid methyl esters (FAMES) were completely collected by two further extractions with hexane. FAMES were analyzed by GC-FID equipped with a Supelco- 2560 bis-cyanopropyl column and quantified against a Supelco 37 component FAME external standard [6]. Here, we report only on the “dietary” FAs (i.e., those that are incorporated relatively unchanged from prey to predator adipose tissues for a monogastric predator) that were available for quantification based on the external standard. Each FAME was calculated as the % of total dietary FAME.

Typical of FA analysis at NWRC (carried out by Lab Services and OCRL) Group personnel and students), a blank, duplicate and two reference materials, Great Lakes herring gull (*Larus argentatus*) egg pool and the NIST Pilot Whale blubber SRM1945, were extracted with every batch of 20 FA samples. Relative differences in duplicate analyses of polar bear and ringed seal samples were on average 6% and 7%, respectively, for dietary FAs. The relative standard

deviation (RSD) of dietary FAs averaged 6% for the herring gull egg pool. SRM1945 dietary FA values were on average within 15% RSD of our laboratory results from the 2007 NIST/NOAA Interlaboratory Comparison Exercise Program for Organic Contaminants in Marine Mammal Tissues. Recovery of 5- $\alpha$ -cholestanol was  $100 \pm 10\%$ .

#### *Mercury and Other Trace Metal and Element Analysis and Quality Control*

All trace element analysis of the present polar bear liver samples was carried out at the NWRC, Environment Canada, Carleton University and are described elsewhere [7]. Liver samples were thawed, homogenized and then refrozen immediately at -20 °C. A thoroughly validated method used extensively at NWRC was used for total Hg determination in the liver samples. Briefly, freeze-dried and homogenized liver samples were analyzed for total Hg using DMA-80 Direct Mercury Analyzer (Milestone, Shelton, CT). The certified reference materials (CRMs) used for total Hg were Dolt-3 and Tort-2 (lobster hepatopancreas) from the National Research Council (NRC) of Canada and Oyster Tissue 1566b from National Institute of Standards Technology (NIST). To check for the homogeneity of total Hg in the samples, 22 random liver samples were analyzed in replicate. Replicates of the CRMs, Oyster Tissue 1566b, Dolt-3 and Tort-2 were also analyzed to check calibration of the instrument, the within-run precision and the reproducibility of the method. Recoveries of Total Hg for the daily calibration check standards (CRMs Dolt-3, Tort-2 and Oyster Tissue 1566b) ranged from 83.0 to 111.4%. Values obtained were within the acceptable limits. Standard deviations for all replicate readings for CRM samples were below



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Figure S1. Relationships between liver total Hg, Se and As, and muscle  $\delta^{15}\text{N}$  values in polar bears from ten subpopulations. Pearson correlations coefficients are shown.

