The following supplement accompanies the article

Predicted polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) accumulation in southern resident killer whales

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Supplement. Chemical analyses and accumulation model descriptions

Chemical Analyses of Killer Whale Blubber and Their Prey

Killer whale blubber samples and whole body Chinook salmon were analyzed for ΣPCBs and ΣPBDEs (previously reported in O'Neill et al. 2006, Krahn et al. 2007, 2009) using a gas chromatography/mass spectrometry method described in Sloan et al. (2005). Briefly, each whale blubber sample (0.3–0.5 g) or salmon whole body sample (2 g) was mixed with drying agents (sodium sulfate and magnesium sulfate), transferred to a 33-mL accelerated solvent extraction (ASE) cell, and the surrogate standard (PCB 103) was added to the top of each sample cell. Using the ASE, the PCBs, PBDEs and lipids were sequentially extracted at 2000 psi and 100°C with two cell volumes using dichloromethane and the combined extract (~50 mL) was collected in a 60-mL collection tube. For blubber samples, approximately 1.0 mL of each extract was transferred to a pre-weighed vial for percent lipid determination using a thin layer chromatography/flame ionization detection method (Ylitalo et al. 2005). The remaining non-lipid sample extract was filtered through a column of silica gel and alumina, and concentrated for further cleanup to remove polar compounds. The POPs were separated from the bulk lipid and other biogenic material present in each sample using high-performance size exclusion liquid chromatography, and the cleaned extract was analyzed for POPs using a low-resolution quadrupole GC/MS system equipped with a 60 m DB-5 GC capillary column and a electron impact mass spectrometer in selected ion monitoring mode. The instrument was calibrated using sets of up to ten multi-level calibration standards of known concentrations. Percent lipid and lipid class percentages were determined in the blubber samples using thin-layer chromatography with flame ionization detection (Ylitalo et al. 2005). In this method, each lipid extract sample was spotted on a Type SIII Chromarod and developed in a chromatography tank containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v). The lipid classes were separated based on polarity

and measured using flame ionization detection. Percent lipid values were calculated by summing the concentrations of five lipid classes (i.e., sterol esters/wax esters, triglycerides, free fatty acids, cholesterol, phospholipids) for each sample, using the mean of two measurements.

All blubber tissue contaminant concentrations are reported in ng g^{-1} lipid weight (lw) and salmon samples were reported in ng g^{-1} wet weight (ww). Sum PCBs (∑PCBs) includes the sum of congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, and 209. Sum PBDEs (∑PBDEs) is the sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, and 183. As part of a performance-based quality assurance program (Sloan et al. 2006), a method blank and National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM 1945 or SRM 1974b) were analyzed with each killer whale sample batch containing 10-14 field samples. Concentrations of individual analytes measured in NIST SRMs were in excellent agreement with the reference values published by NIST. Other quality control samples met established laboratory criteria.

Total PBDE and PCB Accumulation ($A_{c,i,y}$)

The individual-based model was constructed in the R language (R Development Core Team 2006). The total contaminant accumulation ($A_{c,i,y}$; reported in *ng*) where *c* = ΣPCBs or ΣPBDEs for an individual *i*, in year *y*, equals the total contaminant accumulation in year *y*-1 ($A_{c,i,y-1}$) plus the contaminant accumulation via prey intake ($PI_{c,y,p,x,g}$; equation 2) minus the elimination ($E_{i,y}$):

$$A_{c,i,y} = \begin{cases} 0.8(A_{c,m_i,y} TT_{m_i}) & \text{if } x_i = 0 \\ 0.8(A_{c,i,y-1} + A_{c,m_i,y} LO_{m_i}) & \text{if } x_i = 1 \\ 0.8(A_{c,i,y-1}(1 - TT_i) + PI_{c,y,p,x,g} - E_{i,y}) & \text{if gave birth in year } y \\ 0.8(A_{c,i,y-1}(1 - LO_i) + PI_{c,y,p,x,g} - E_{i,y}) & \text{if gave birth in year } y-1 \\ 0.8(A_{c,i,y-1} + PI_{c,y,p,x,g} - E_{i,y}) & \text{otherwise} \end{cases} \quad (1)$$

where p is the pod, x is the age, g is the gender, m_i is the individual's mother, TT_{m_i} is the proportion of transplacental transfer from the individual's mother, LO_{m_i} is the proportion of offload via lactation from the individual's mother, and $E_{i,y}$ is the total contaminant accumulation multiplied by a uniform random variable with lower and upper bounds of 0.016 and 0.024, respectively (Hickie et al. 2007). Approximately 80% of the total contamination is assumed to be in the blubber (Ross et al. 2000, Hickie et al. 2007). Therefore, the total contamination is reduced by 20% at the end of each year. The 20% reduction of the total contamination is assumed to be in the body core (e.g., brain, liver, and kidneys) and is no longer tracked by the model.

The contaminant accumulation via prey intake ($PI_{c,y,p,x,g}$) is the sum of the products of the contaminant concentration in the prey ($PC_{c,y,p}$), and the annual biomass of the prey ($BIO_{y,x,g}$) consumed by an individual:

$$PI_{c,y,p,x,g} = \sum (PC_{c,y,p} BIO_{y,x,g}) \quad (2)$$

Parameterization

Killer Whale Mass and Energetic Requirements ($M_{x,g}, DPER_{x,g}$)

In this section, the calculations behind the mass of an individual in age-class *x* and gender *g* ($M_{x,g}$) and the daily prey energetic requirements for a male or female in age-class *x* ($DPER_{x,g}$) are given.

The energetic requirement of an individual ($DPER_{x,g}$) depends on its mass as well as its activity or behavior states (Kleiber 1975, Costa 2002). To obtain an estimate of minimum and maximum DPERs for free-ranging killer whales, Noren (2011) used published field metabolic rates (FMRs)—or the metabolic rates of active free-ranging individuals—of marine mammals and estimated that daily energy expenditure in killer whales is between five (minimum) and six (maximum) times Kleiber (1975) predicted basal metabolic rate (BMR). BMR is the amount of energy required by a post-absorptive, non-reproductive adult at rest in its thermal neutral zone. Kleiber (1975) determined that BMR for terrestrial mammals can be predicted by the equation:

$$BMR = 70M_{x,g}^{.75} \tag{3}$$

where $M_{x,g}$ is the total body weight in *kg* of an individual in age-class *x* and of gender *g*.

Following Noren (2011), the estimates of minimum and maximum daily energy expenditure (i.e., five to six times Kleiber [1975] predicted BMR) were converted to minimum and maximum daily prey energetic requirements ($DPER_{x,g}$, reported in kcal day^{-1}) by assuming a digestive efficiency of 84.7% for killer whales (Williams et al. 2004).

$$\min DPER_{x,g} = 413.2M_{x,g}^{.75} \tag{4}$$

$$\max DPER_{x,g} = 495.9M_{x,g}^{.75} \tag{5}$$

The DPER equations (equations 4 and 5) require an estimate of the mass of an individual of age-class *x* and gender *g*. Clark et al. (2000) used the Gompertz model to compute mass in captive female killer whales in age-class *x* using measured lengths and weights of known-aged individuals:

$$M_x = W[\exp(-b\exp(-k \cdot age))] \tag{6}$$

where age is in days and mass for age-class *x* (M_x) is in kg. *W* is the asymptotic weight, *b* is the integration constant, and *k* is the growth rate constant. Noren (2011) used the parameters developed by Clark et al. (2000) to predict total body mass for male and female killer whales aged 1–12 years old (W = 2763 kg, b = 2.3, k = 0.0007 year^{-1}). Females aged 13 through 20 years were assumed to grow at a constant rate of 107 kg year^{-1} and females $\geq$20 years were assumed to maintain a body mass of 3338 kg (following Noren 2011). A pregnant female's weight was calculated as the weight of a sexually mature female in age-class *x*, plus the weight of a newborn calf ($M_0 = 155$ kg; Clark et al. 2000). The result of this assumption is that the predicted DPER of pregnant females is 3–5% greater than those of non-pregnant females of the same age for the entire gestation period. This is because the model assumed that the mass of a pregnant female would be equivalent to that at full-term for the entire year. A more accurate approach to predict the energetic requirements of pregnant females might be to

track the monthly growth of individuals and their calves *in utero*. However, the growth curve of calves *in utero* is currently unknown and the sensitivity analysis revealed the model output is robust to small changes in the DPER. Thus, the simpler approach of an annual time step was used. For males aged 13 through 20 years, Noren (2011) assumed a constant rate of growth of 244 kg year^{-1}, and that males ≥ 20 years maintained a total mass of 4434 kg. The individual age data from the annual surveys (data courtesy of the Center for Whale Research, Friday Harbor, WA.) with the mass-at-age estimates from Noren (2011) were used to estimate the mass for each individual in the population at each year during its life. The estimated mass for each individual was then used to estimate the energetic requirements for each individual at age x.

There is annual variability in the energetic requirements of an individual killer whale as well as variability among individuals in similar age- and sex-classes. For example, a juvenile female may need more energy due to growth than an adult female during senescence. A pregnant or lactating female will also have higher energy demands than another female in the same age-class that is not pregnant or lactating. However, it is assumed that pregnant and lactating females have a DPER that is within the range of five to six times Kleiber (1975) predicted BMR (Noren 2011). Therefore, the model randomly selected values from a uniform distribution between the minimum and maximum DPER values for each individual i in each year y regardless of reproductive status:

$$DPER_{x,g} = U(\min DPER_{x,g}, \max DPER_{x,g}) \tag{7}$$

Caloric Content and Consumption of the Prey ($CC_y, BIO_{y,x,g}$)

In general, Chinook salmon (*Oncorhynchus tshawytscha*) are thought to be the primary summer diet of SRKWs based on available data (Ford et al. 1998, Ford & Ellis 2006, Hanson et al. 2010). For simplicity, it was assumed that the SRKWs consume solely Chinook salmon for the prey caloric content estimates. J pod is seen in the semi-enclosed "inland" marine waters throughout the year, with winter observations less often than summer observations (Osborne 1999). Therefore one model scenario ("uniform" diet scenario) assumed 100% of their prey consists of inland Chinook salmon. In our model contaminant concentrations in inland Chinook salmon were estimated as the combined average values (WDFW & NMFS, unpublished data) of Chinook salmon from the Fraser, Duwamish, Nooksack, Nisqually, and Deschutes River watersheds. K and L pods, however, have been seen along the outer coast from California to Vancouver Island during the non-summer months, or approximately 2/3 of the year, and are observed approximately 1/3 of the year in the inland marine waters (Krahn et al. 2004). Therefore, another model scenario ("mixed" diet scenario) assumed K and L pod diets included outer coast Chinook salmon for 2/3 of their diet and included inland Chinook salmon for 1/3 of their diet. Contaminant concentrations in outer coast Chinook salmon were estimated as the average values (WDFW & NMFS, unpublished data) of Chinook salmon from the Sacramento, Columbia (fall and spring runs), and Skeena Rivers.

Using the DPERs for killer whales (equation 7) and the caloric content (kcal kg^{-1}) of Chinook salmon in year y (CC_y), the average annual biomass of Chinook salmon consumed by each individual in age-class x and gender-class g was estimated as:

$$BIO_{y,x,g} = (DPER_{x,g} / CC_y)365 \tag{8}$$

The caloric content (CC_y) used in the simulations was based on the caloric content of Chinook salmon sampled in the inland waters and along the outer coast (WDFW & NMFS, unpublished data). It is reasonable to assume that the caloric content of fish varies seasonally as well as annually and therefore the average caloric content of Chinook salmon consumed by a killer whale will vary among years. Indeed, the killer whales are consuming different salmon stocks and the caloric content of those stocks vary between years depending on factors such as the productivity of salmon prey at sea. Due to this variability, the model randomly drew from a uniform random variable for CC_y for each year, with an upper bound of 1804 kcal kg^{-1} and a lower bound of 1643 kcal kg^{-1}:

$$CC_y = U(1643,1804) \tag{9}$$

The range from 1643 to 1804 kcal kg^{-1} reflects the 95% CI for the average Chinook salmon sampled in years 2000 and 2004 from the inland waters and along the outer coast from western Vancouver Island to California (WDFW & NMFS, unpublished data).

Blubber Mass and Total Lipid Blubber Mass ($B_{i,y}, L_{i,y}$)

The standard unit of contaminant concentration is reported in $ng\ g^{-1}$ total lipid blubber weight (*lw*), the determination of which requires an estimate of total blubber mass ($B_{i,y}$) and total lipid mass ($L_{i,y}$) in the blubber of an individual. A blubber mass range (equations 10 and 11) was estimated for each age group, where age x is ≤ 20, using an adapted relationship established from the blubber and age relationship found in bottlenose dolphins sampled in the winter (Noren & Wells 2009). Individuals aged ≥ 20 are assumed to be physically mature and maintained total blubber mass within the range of a 20 year old. The adapted relationship encompassed the estimated blubber mass of 30% total body mass found in adult killer whales (Christensen 1982):

$$B\%_{lower} = 39x^{-.10} \tag{10}$$

$$B\%_{upper} = 44x^{-.10} \tag{11}$$

The model then drew from a uniform distribution between the lower and upper bounds to estimate the blubber mass as a proportion of total body mass in the individual:

$$B\% = U(B\%_{lower}, B\%_{upper})/100 \tag{12}$$

The blubber mass of individual i during year y ($B_{i,y}$) is the product of the mass of the individual of age-class x and gender g ($M_{x,g}$) and the estimated proportion of blubber ($B\%_{i,y}$):

$$B_{i,y} = M_{x,g} B\%_{i,y} \tag{13}$$

The percentage lipid in the blubber was measured for a limited number of killer whale samples (Krahn et al. 2004, 2007, 2009, Herman et al. 2005, Koopman 2007). Krahn et al. (2004) collected samples from an L pod individual via both biopsy (post-mortem) and necropsy. They found that the

percentage lipid in the biopsy sample (post-mortem) ranged from 8-10% whereas the percent lipid from the necropsy ranged from 28–40% at a similar depth (0–2 cm). Biopsy samples from free-ranging resident killer whales in the eastern North Pacific had an average of 10% lipid in the blubber (Herman et al. 2005). Lower lipid levels found in biopsy samples may result from leaching of lipid from the sample during ejection of the biopsy dart from the animal, or when the dart enters the water (Krahn et al. 2004). Therefore, the model generated the percent lipid for every individual *i* in each year *y* ($L\%_{i,y}$) using the range of lipid percentages established from the necropsy (28–40%; Krahn et al. 2004) where leaching was not a concern:

$$L\%_{i,y} = U(28\%,40\%) \tag{14}$$

The proportion of lipid ($L\%_{i,y}$) was multiplied by the estimated blubber mass ($B_{i,y}$) to calculate the reported total lipid mass in the blubber ($L_{i,y}$) for each individual in the population in each year:

$$L_{i,y} = L\%_{i,y} B_{i,y} / 100 \tag{15}$$

Total concentration values were predicted by dividing the total contaminant accumulation, $A_{c,i,y}$, by the estimated total lipid mass in the blubber ($L_{i,y}$).

Transplacental Transfer and Lactation Offloading (TT_i and LO_i)

Currently, the amount of contaminants typically offloaded from mother to calf in killer whales is unknown and may vary among individuals. However, PCB offload data are available for other delphinids. Female bottlenose dolphins off South Africa offload almost 80% of their total PCB body burden to their first-born offspring via transfer during gestation and then lactation (Cockcroft et al. 1989). Cockcroft et al. (1989) calculated that approximately 4% of a female's total body burden can be transferred daily during lactation, indicating the mother's full load would be transferred after approximately 7 weeks of lactation. Salata et al. (1995) estimated a transplacental transfer of 3.7% of the total body burden in female bottlenose dolphins (*Tursiops truncatus*) off the Gulf of Mexico. Fukushima and Kawai (1980) found a maximum transfer of the total body burden to be approximately 88% from lactation in striped dolphins (*Stenella coeruleoalba*), while the percent of total load transferred during gestation were estimated to be approximately 3.8%. Tanabe et al. (1981) also reported a transplacental transfer percentage of 3.7% and lactation offload percentage up to 90% of the total body burden for striped dolphins.

The amount of contaminants offloaded due to lactation varies within a year (Cockcroft et al. 1989). For simplicity, offloading was assumed to occur in one time step at the end of the first year. The calf was assumed not to consume fish until the beginning of the second year (Heyning 1988) although weaning can be variable among individuals within a species. It is possible that the calf will begin to consume fish prior to turning age one. However, the amount of contaminants acquired from fish compared to the amount acquired from the mother in the first year is probably negligible.

Based on information in the published literature, it is reasonable to assume that the proportion of a mother's total contaminant body burden offloaded to a fetus during gestation is between 3% and 5% of the mother's total load, while the amount offloaded to a dependent calf during lactation is between 70% and 90% of the mother's load. Offloaded amounts probably vary among individual females. However, it is unknown if these amounts change over time as females age and their contaminant loads change.

The model also assumes that the ∑PBDE offload parameters have the same range as the ∑PCB offload parameters with the caveat that little is currently known about the transfer rate of ∑PBDEs in killer whales or in other delphinids. However, comparable ∑PCB and ∑PBDE transfer rates are a reasonable assumption because of the similar chemical structure and mode of action of these two classes of contaminants.

For each female in each simulation, the model randomly drew the transplacental transfer percentage for individual *i* (TT_i) from a uniform distribution with a lower bound of 3% and an upper bound of 5%. The transfer proportion for individual *i* was constant throughout the female's life, but varied among individuals and simulations:

$$TT_i = U(0.03,0.05) \tag{16}$$

The model also randomly drew the lactation offload percentage for female *i* (LO_i) from a uniform distribution with lower and upper bounds of 70% to 90%, respectively, for each female in each simulation.

$$LO_i = U(0.7,0.9) \tag{17}$$

∑PCB Concentration Levels in Killer Whale Prey ($PC_{c=PCB,y}$)

∑PCB accumulation via prey intake ($PI_{c=PCB,y,p_i,x,g}$; equation 2) is the sum of the products of the ∑PCB concentration in the prey types consumed during year *y* (for inland Chinook salmon, $PC_{c=PCB,y,in}$; for outer coast Chinook salmon, $PC_{c=PCB,y,out}$) and the annual biomass of prey consumed ($BIO_{x,g}$, equation 8). Thus, to calculate the contaminant intake for an individual older than 1 year in a given year *y*, estimates of contaminant concentrations in the prey during year *y* were needed. In the calculations described below, prey is assumed to be Chinook salmon exclusively.

To estimate the ∑PCB trend in inland Chinook salmon, a time series for the proxies for the period 1931 to 2007 was developed, using piecewise linear function divided into 3 time periods. The first time period (1931:1959 or years 1:29) showed an increasing trend, the second time period (1960:1988 or years 30:58) showed a decreasing trend, and the third time period (1989:2007 or years 59:77) had no trend. Negative concentrations, such as those predicted for 1931 and in 1932, were set to equal zero. Proxy values were multiplied by the scaling factors (4.2 and 5.8 for equations 18 and 19, respectively) to estimate the 1931 to 2007 average Chinook salmon ∑PCB contaminant concentrations.

The outer coast Chinook salmon were assumed to have a different contaminant history. For simplicity, outer coast Chinook salmon were assumed to have ∑PCB levels that increased linearly from 1930 (no PCBs) to current levels. Although this is a simplified general linear trend in outer coast contamination, sensitivity analyses indicated that this trend did not significantly affect the model results, and was found to be a reasonable assumption during model validation. Scaling factors for outer coast Chinook salmon trends were 2.0 and 2.8 for equations 21 and 22, respectively.

To allow yearly variability in the contaminant concentrations of inland Chinook salmon ($PC_{c=PCB,y,in}$; reported in μg kg^{-1} ww), the annual inland Chinook salmon concentration level was selected from a uniform distribution with lower and upper bounds that equaled the lower and upper 95% confidence limits ($CI_{in,low}, CI_{in,high}$), respectively, for the average ∑PCB levels found in inland Chinook salmon (WDFW & NMFS, unpublished data).

$$CI_{in,low} = \begin{cases} 4.4y - 9 \\ -3.2y + 217 \\ 30 \end{cases} \quad (18)$$

$$CI_{in,high} = \begin{cases} 6y - 13 \\ -4.4y + 300 \\ 41 \end{cases} \quad (19)$$

$$PC_{c=PCB,y,in} = U(CI_{in,low}, CI_{in,high}) \quad (20)$$

The ∑PCB concentration in outer coast Chinook salmon was selected from a uniform distribution with lower and upper bounds equal to the lower and upper 95% confidence limits, respectively, from outer coast Chinook salmon ($PC_{c=PCB,y,out}$ reported in µg kg^{-1} ww; WDFW & NMFS, unpublished data).

$$CI_{out,low} = .18y - .18 \quad (21)$$

$$CI_{out,high} = .25y - .25 \quad (22)$$

$$PC_{c=PCB,y,out} = U(CI_{out,low}, CI_{out,high}) \quad (23)$$

∑PBDE Concentration Levels in Killer Whale Prey ($PC_{c=PBDE,y,p_i}$)

Following the PBDE trends seen locally and worldwide (Ikonomou et al. 2002, 2006, Rayne et al. 2003), the model assumed that the ∑PBDE concentration accumulation from the prey ($PC_{c=PBDE,y,p_i}$) is exponential with a doubling time (T) ranging from 3.2 to 4.0 years (with variations among the pods and from year to year). These doubling times were also found to be a reasonable assumption during model validation. ∑PBDE intake via prey consumption for an individual is the product of the concentration of ∑PBDEs in the prey consumed by pod p ($PC_{c=PBDE,y,p_i}$; reported in $\mu g \ kg^{-1} ww$) and the annual biomass of prey consumed ($BIO_{x,g}$). To allow for annual variability, the model drew the doubling time parameter (T_y) from a uniform distribution for each year. The first year ($y = 0$) was 1970 and the initial ∑PBDE concentration was $PC_{c=PBDE,y=0,p_i} = .01$.

$$PC_{c=PBDE,y,p_i} = PC_{c=PBDE,y=0,p_i} \exp(ry) \quad (24)$$

$$r = \ln(2) / T_y \quad (25)$$

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