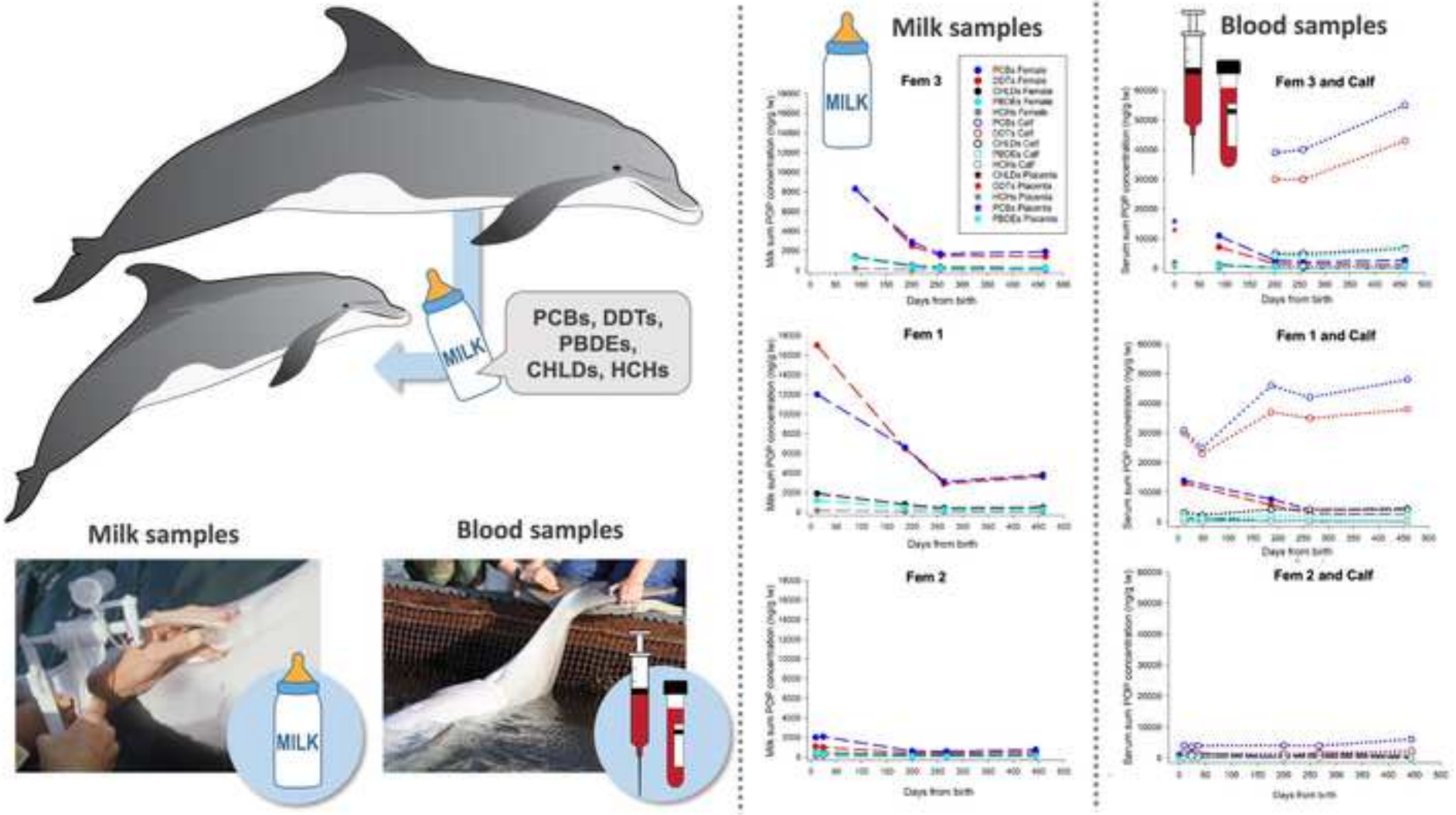
Graphical Abstract



Highlights

1. Studies on contaminant transfer from female delphinids to calves are limited.
2. This is the first longitudinal study of contaminant transfer in live dolphins.
3. Female milk and blood toxicant levels decline while calf blood levels increase.
4. Female reproductive history and chemical structure influence contaminant transfer.
5. Data are useful for predictive models and evaluating exposure risk to calves.

The dynamics of persistent organic pollutant (POP) transfer from female bottlenose dolphins (*Tursiops truncatus*) to their calves during lactation

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Abstract

Persistent organic pollutants (POPs) are lipophilic compounds that can accumulate in high concentrations in the blubber of marine mammals, which are long-lived, top-level predators in their ecosystems. These compounds, which include DDTs, PCBs, PBDEs, HCHs, and CHLDs, impact mammalian health, including neurological effects, reduced immune system efficiency, and reproductive failure. POPs are transferred from females to their offspring during gestation and lactation, which have implications for the health of newborn marine mammals, particularly first-born offspring who receive higher concentrations. The dynamics of POP transfer during lactation have been studied in a few pinniped species, but there are no comparable studies on living cetaceans. Because life history strategies and behavior of lactating phocids differ from dolphins, a study on delphinid maternal transfer is warranted. To accomplish this, placenta and longitudinally collected blood and milk samples were taken concurrently from trained bottlenose dolphin, *Tursiops truncatus*, mother/calf pairs to assess the dynamics of maternal contaminant transfer. Initial POP levels in placenta, blood serum, and milk varied by individual and were related to the age and reproductive history of the females. Regardless of initial POP levels, maternal serum and milk concentrations decreased while calf serum POP levels increased over time. Pollutant transfer varied by POP class and by congener. Contaminant transfer efficiency to calves was most apparent for 4- to 6-chlorine PCBs, DDT isomers *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDD, and *o,p'*-DDE, *trans*-nonachlor, *cis*-nonachlor, heptachlor epoxide, nonachlor III, and oxychlordane. By the end of the lactation period, calf serum POP levels were considerably greater than those of their mothers, particularly for compounds with fewer chlorines. POP levels were most biomagnified in the calf born to the primiparous female. These results provide critical

information on one component of contaminant transfer in the marine ecosystem and for understanding potential risks of POP exposure to developing odontocete calves.

Key Words: blood, DDT (dichloro-diphenyl-trichloroethane), marine mammal, milk, PBDE (polybrominated diphenyl ethers), PCB (polychlorinated biphenyls)

1. Introduction

Top predators, such as marine mammals, accumulate high levels of persistent organic pollutants (POPs), primarily through prey consumption due to their high trophic level status (Ross et al., 2000; Ylitalo et al., 2001). While these contaminants are detected in several tissues, POPs are lipophilic and mainly accrue in the blubber, which is the main lipid storage site for marine mammals (e.g., Martineau et al., 1987; Yordy et al. 2010a). POP levels in odontocete blood are also highly correlated to levels in blubber, thus individuals with relatively greater blubber POP levels will also have higher levels of POPs circulating throughout the body (McCormley et al., 2021; Reddy et al., 1998; Yordy et al., 2010c). Elevated tissue concentrations of DDTs, PCBs, and PBDEs have been linked to a range of health effects, including immunosuppression, thyroid disruption, cancer, and reproductive failure in marine mammals (e.g., de Swart et al., 1994; Hall et al., 2003; Hammond et al., 2005; O'Hara and O'Shea, 2001; Ross et al., 1996; Ross et al., 1995; Schwacke et al., 2002; Tabuchi et al., 2006; Ylitalo et al., 2005a). Some investigations have proposed that exposure to contaminants can severely impact cetaceans, potentially leading to extirpation of some populations (Desforges et al., 2018; Desforges et al., 2016; Hall et al., 2018; Schwacke et al., 2002). Because population growth may be limited by exposure to contaminants, it is important to understand the dynamics of maternal transfer of POPs and POP exposure to the most vulnerable members of the population, the neonates. This is also important to understanding the fate of organic pollutants in the ecosystem.

POPs are transferred from female marine mammals to their offspring via transplacental transfer during gestation and via milk ingestion during the suckling period, when transfer rates are greater (e.g., Addison and Brodie, 1987; Aguilar and Borrell, 1994; Barbosa et al., 2018; Borrell et al., 1995; Debier et al., 2003a; Debier et al., 2003b; Desforges et al., 2012; Donkin et

al., 1981; Duinker and Hillebrand, 1979; Frouin et al., 2012; Greig et al., 2007; Mongillo et al., 2016; Pomeroy et al., 1996; Ridgway and Reddy, 1995; Schweigert and Stobo, 1994; Tanabe et al., 1981; Tanabe et al., 1982). First-born odontocetes typically receive greater contaminant loads from their mothers compared to subsequent offspring (Wells et al., 2005; Ylitalo et al., 2001) because primiparous females generally have greater organocholorine contaminant body burdens loads and consequently higher levels in their milk compared to multiparous females (Mongillo et al., 2012; Ridgway and Reddy, 1995). This influx of contaminants at such a young age is concerning because contaminants may interfere with developmental processes, which could have life-long impacts. Studies on lab mice have shown that postnatal exposure to PCBs and PBDEs during a critical stage of neonatal brain development caused developmental neurotoxic effects (Eriksson et al., 2006; Eriksson et al., 2002), and exposure to specific combinations of contaminants can exacerbate these detrimental effects (Eriksson et al., 2006). Furthermore, bottlenose dolphin calf survival rates may also be influenced by maternal blubber contaminant burdens. Mothers of stillborn calves and calves that did not survive beyond 12 days had pre-parturition blubber ΣDDT and ΣPCB that were more than 3 and 2.5 times, respectively, greater than the blubber levels of females whose calves survived beyond 6 months (Reddy et al., 2001). Because of these serious impacts on neonatal health, it is important to elucidate temporal changes in contaminant exposure during gestation and lactation.

Assessing POP transfer from female marine mammals to their young is difficult, given their aquatic lifestyle. Thus, it is not surprising that the few studies that have directly sampled marine mammal mother-offspring pairs to assess POP transfer dynamics over the lactation period focused on pinnipeds (Debier et al., 2003a; Debier et al., 2003b; Frouin et al., 2012; Schweigert and Stobo, 1994), which haul out on land or ice to give birth and nurse their young. The fully

aquatic lifestyle of cetaceans precludes obtaining necessary samples to replicate these studies in free-ranging porpoises, dolphins, and whales. One study on trained bottlenose dolphins measured changes in milk organochlorine levels over the lactation period, yet female and calf body burdens were not investigated simultaneously (Ridgway and Reddy, 1995). Furthermore, PBDEs, which can impact neonatal development (Eriksson et al., 2006; Eriksson et al., 2002), were not quantified in the study. Due to their inaccessibility, many studies have primarily relied on samples collected from deceased individuals to assess contaminant transfer from cetacean females to their offspring (e.g., Aguilar and Borrell, 1994; Borrell et al., 1995; Cadieux et al., 2016; Cockcroft et al., 1989; Duinker and Hillebrand, 1979; Fukushima and Kawai, 1981; Kajiwara et al., 2008; Tanabe et al., 1981; Tanabe et al., 1982; van den Heuvel-Greve et al., 2021). However, this constrained methodology provides potentially sub-optimal tissues for analysis and only provides a snapshot of relative contaminant concentrations in female/calf pairs at the time that the individuals died. As such, these studies provide limited data necessary to understand the dynamic contaminant transfer process during the entire gestation and/or lactation periods. Similarly, some results from the earlier studies conducted on pinnipeds (Debier et al., 2003a; Debier et al., 2003b; Frouin et al., 2012; Schweigert and Stobo, 1994; Vanden Berghe et al., 2012) may have limited relevance to the dynamics of contaminant transfer from female delphinids to their offspring. Although POPs are primarily stored in the blubber of marine mammals, which are then transferred via milk during lactation, life history patterns and behavior of species within this specialized mammal group vary substantially during the lactation period. For example, unlike delphinids, female phocid seals fast during lactation, which reduces their blubber stores and consequently influences circulating POP levels (Debier et al., 2006; Debier et al., 2003a; Debier et al., 2003b; Peterson et al., 2014). Therefore, contaminant transfer patterns

are likely to vary across marine mammal groups, and studies across several taxa are needed. The direct quantification of contaminants transferred during gestation and lactation can elucidate potential high risk periods to females and their offspring as well as provide parameters for individual-based and ecosystem wide contaminant transfer and impact models (Desforges et al., 2018; Hall et al., 2018; Hickie et al., 2013; Mongillo et al., 2012).

In this study we aimed to understand the dynamics of persistent organic pollutant transfer from female delphinids to their calves. We quantified concentrations of many persistent organic pollutants (polychlorinated biphenyl (PCB) congeners, dichlorodiphenyltrichloroethane (DDT) compounds, chlordanes, polybrominated diphenyl ether (PBDE) congeners, hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), aldrin, dieldrin, mirex and endosulfan I) in placenta collected after birth as well as milk and blood serum samples collected longitudinally from trained bottlenose dolphin (*Tursiops truncatus*) mother/calf pairs from birth to approximately 15 months post-partum. This time period is comparable to the obligate nursing period in wild bottlenose dolphins (Oftedal 1997; Noren and Edwards 2007). Blubber thicknesses were also measured at three sites to ascertain how blubber stores in both the females and their calves changed during the lactation period. The influence of female age and reproductive history on contaminant transfer dynamics were also investigated in this novel study.

2. Methods

2.1. Subjects

Six trained female Atlantic bottlenose dolphins, *Tursiops truncatus*, and three of their calves living in open water enclosures at the U.S. Navy Marine Mammal Program (MMP) facility in San Diego, CA were sampled for this study (Table 1). The MMP houses and cares for a

141 population of dolphins in San Diego Bay, CA. Before 1990, most MMP dolphins originated from
142 the Gulf of Mexico, especially the Mississippi Sound. Since the early 1990s, MMP dolphins
143 have been born at the MMP facility in San Diego Bay. The Secretary of Navy Instruction
144 3900.41H directs that Navy marine mammals be provided the highest quality care. The MMP is
145 accredited by AAALAC International and adheres to the national standards of the U.S. Public
146 Health Service Policy on the Humane Care and Use of Laboratory Animals and the Animal
147 Welfare Act. The animal care and use protocol for MMP dolphins in support of this study was
148 approved by the Navy Marine Mammal Program's Institutional Animal Care and Use Committee
149 (IACUC No. 73-2007) and the Navy Bureau of Medicine and Surgery (NRD-449). Due to issues
150 with reproduction (one stillborn calf) and lactation (one calf suckling from two females), the
151 study primarily focused on three mother/calf pairs (Table 1). Three of the six females were born
152 at the facility and are of known age. The ages of the three females that were captured from the
153 wild are estimated. These individuals were captured when sexually immature, thus full
154 reproductive histories of all six females are known (Table 1).
155 Each adult dolphin received a varied daily diet, including capelin (*Mallotus villosus*),
156 Pacific herring (*Clupea pallasii*), and squid (*Loligo opalescens*). Although small fish could swim
157 through the netted enclosures in San Diego Bay, these fish are not considered to significantly
158 contribute to the dolphins' diets. The dolphins are trained for the collection of biological samples
159 (milk and blood) and measurements (body mass and ultrasound measured blubber thicknesses)
160 that were required for this study.
161
162 2.2 Sample collection
163 Following methods from earlier studies on this population (Reddy et al., 1998; Reddy et al.,

2001; Ridgway et al., 1995; Ridgway and Reddy, 1995), milk (15 ml) and blood serum (10 ml) samples were collected from each mother/calf pair as soon as feasible after birth (0-12 days post-partum, with the exception of one mother/calf pair) and at three intervals (~ 6-7 months, ~ 8-9 months, and ~15 months post-partum) during the lactation period (Table 1). Additional serum and milk samples were provided to the study when excess material was collected for other husbandry purposes. Collecting blood from fasting animals was preferred, however, this study was constrained to sampling adult females when staff were available. As such, not all samples were collected from fasted animals. Furthermore, it is not feasible to collect fasting blood samples from calves that nurse on demand. Although blubber biopsy samples are typically collected to assess POP levels in free-ranging cetaceans, this sampling technique is more invasive than collecting blood samples from trained dolphins and was consequently not performed. Because POP levels in blood are positively correlated with blubber levels in bottlenose dolphins (Reddy et al., 1998; Yordy et al., 2010c) and killer whales (McCormley et al., 2021), serum POP levels in serially collected samples should reflect how blubber POP levels change during the lactation period. When available, placentas were also collected after birth for POP analysis. The timing of initial sample collection was based on maternal experience and female/calf pair behavior in order to minimize impacts to the dolphins. Consequently, with the exception of collecting the placenta following birth, the first samples from the primiparous 5-year old female and her calf were collected later than the other female/calf pairs (primiparous female and her calf first sampled at 89 and 201 days post-partum, respectively, Table 1).

For husbandry purposes, small quantities of fish and squid were offered to calves, beginning around 3-6 months post-partum. These first feedings were not expected to affect the results of the study. The maximum total combined mass of prey items consumed reached ~1.25-

187 2.75 kg per calf/day by the end of the study period, which only represents 7.2%-16.9% of the
188 total kcals/day consumed when the calves were fully weaned. Furthermore, POP concentrations
189 in prey items provided to calves were quite low (Supplemental Table 1), compared to levels in
190 dolphin milk. Therefore, it is likely that during the ~15-month study period, calves received the
191 bulk of their contaminants via ingestion of their mother's milk.
192
193 2.3 Blubber thickness measurements
194 Ultrasound measured blubber thicknesses were recorded from the three focal mother/calf pairs at
195 three time points during the study. Blubber thicknesses was measured with a Voluson i portable
196 ultrasound machine with a 2 to 5 MHz 4D transducer (RAB2-5-RS; General Electric Healthcare)
197 at three diagnostic sites (site B1 [midline of the lateral surface, in line with the cranial insertion
198 of the dorsal fin], site B2 [midline of the dorsal surface, in line with the caudal insertion of the
199 pectoral fin], and site B3 [roughly ½ distance from the dorsal surface to the midline of the lateral
200 surface, approximately 10 cm behind the caudal insertion of the dorsal fin]) on the thorax, where
201 blubber thickness is primarily reduced during periods of starvation in porpoises (Koopman et al.,
202 2002). These measurements are indicative of individuals' overall fat stores. For adult females,
203 these measurements were taken voluntarily while individuals stationed, floating at the water
204 surface. Calves were held at the water surface by husbandry staff while blubber thicknesses were
205 measured.
206
207 2.4 Chemical Analysis
208 Prior to chemical contaminant and lipid analyses of dolphin food items, individual fish and squid
209 samples were composited (capelin n = 5; herring n = 5; squid = 3) based on species and were

210 homogenized using a food grinder. The homogenized samples were placed in pre-cleaned glass
211 jars and were stored at -80°C until analyses.
212 Concentrations of POPs in the dolphin tissue and food samples were determined using a
213 gas chromatography-mass spectrometry (GC-MS) method (Sloan et al., 2014; Sloan et al., 2005).
214 Briefly, samples were weighed (milk ~ 0.5 – 1.0 g, placenta ~ 2.0 g, serum ~ 2.0 g, food ~ 2.0 g),
215 mixed with drying agents (sodium sulfate and magnesium sulfate), and packed into 33-mL
216 stainless steel accelerated solvent extraction cells. Each milk and serum sample was mixed
217 thoroughly using a clean glass rod prior to the weighing step. The samples were extracted with
218 dichloromethane using an accelerated solvent extractor after the addition of a surrogate standard
219 (CB 103; 75 ng). Prior to the sample extract cleanup steps, a 1.5 mL portion of extract was
220 removed for lipid class and percent lipid determinations using a thin-layer chromatography-
221 flame ionization detection (TLC-FID) method (Sloan et al., 2014; Ylitalo et al., 2005b).
222 Following the extraction step, the remaining sample extract was cleaned up on a single stacked,
223 gravity flow silica gel/alumina column to remove highly polar compounds present in the sample
224 extract. The POPs were then separated from lipids and other biogenic material present in each
225 sample extract using a high-performance size exclusion liquid chromatography cleanup step. The
226 cleaned extract was analyzed for POPs using a low-resolution quadrupole GC-MS system
227 equipped with a 60-meter DB-5 GC capillary column and an electron impact mass spectrometer
228 in selected ion monitoring mode (Sloan et al., 2014; Ylitalo et al., 2005b). Standard solutions
229 containing POPs were prepared commercially or in-house from commercial stock solutions using
230 isooctane as the solvent.
231 The instrument was calibrated using sets of up to ten multi-level calibration standards of
232 known concentrations. The analysis included 46 polychlorinated biphenyl (PCB) 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, 209), six dichlorodiphenyltrichloroethane (DDT) compounds (*p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, *o,p'*-DDD, *o,p'*-DDE, *o,p'*-DDT), eight chlordanes (CHLD; *cis*-chlordane, *cis*-nonachlor, *trans*-chlordane, *trans*-nonachlor, nonachlor III, heptachlor, heptachlor epoxide, oxychlordane), and 10 polybrominated diphenyl ether (PBDE) congeners (28, 47, 49, 66, 85, 99, 100, 153, 154, and 183). Additional organochlorine compounds analyzed included hexachlorocyclohexanes (HCHs; including lindane (γHCH), α-HCH, and β-HCH), hexachlorobenzene (HCB), aldrin, dieldrin, mirex and endosulfan I. All sum contaminants include all measured congeners in their specific POP classes.

Percent lipid and lipid class profiles were determined in the dolphin and food samples using TLC-FID. A 1µL aliquot of each lipid extract was spotted onto a Type SIII Chromarod (silica-based), and the Chromarods were developed in a chromatography tank containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v) for 24 minutes. The Chromarods were removed from the solvent tank and placed in a 60°C oven for two minutes to evaporate the development solvent. Lipid classes in each sample were separated based on polarity and were measured using flame ionization detection. The lipid TLC-FID calibration standards were prepared in-house using dichloromethane. The 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.

A solvent (dichloromethane) method blank and a National Institute of Standards and Technology (NIST) Standard Reference Material (i.e., human serum SRM 1589a, fish tissue SRM 1947, and human serum SRM 1958) were analyzed with each sample batch as part of a

performance-based quality assurance program and the results met our laboratory criteria (Sloan et al., 2006). The percent recovery of the surrogate standard ranged from 61 – 109%. For the TLC-FID lipid analyses, a method blank, a solvent blank, and a NIST SRM were analyzed as part of our QA protocols. In the current study, we met our laboratory criteria for the solvent and method blanks (each lipid class must not be detected in a method blank or solvent blank). We met our laboratory criteria for the NIST SRMS (the percent lipid values are to be within 35% of either end of the 95% confidence interval of the NIST reference value), with the exception of the serum SRMs. For these SRMs, we were within 45 to 65% of either end of the 95% confidence interval of the NIST reference percent lipid value.

2.5 Data Analysis

Due to constraints of working with trained cetaceans, a small number of samples were collected from the three focal mother/calf pairs, and consequently statistical analyses are somewhat limited. Unless otherwise specified, data were lipid-normalized prior to performing calculations and statistical analyses because lipid content greatly influences lipophilic compound concentrations (O'Brien, 2015). This is particularly important for minimizing the effect of sample lipid content on POP concentrations in samples with relatively low, but highly variable lipid content (e.g., serum samples, especially from Calf 1B). To assess the contaminant loads of calves relative to their mothers' loads and relative to the milk the calves consumed, biomagnification factors (BMFs) were calculated for each sampling interval. Two BMFs were calculated for every POP that was above the lower limit of quantitation for all time points in which both the female and her calf were sampled on the same day (note that the final milk

sample from Fem 3 was collected 5 days after the serum samples were collected, but was considered suitable for the final BMF calculations):

$$\text{BMF 1} = \frac{[POP]\ Calf\ serum}{[POP] Maternal\ serum} \quad \text{equation 1}$$

$$\text{BMF 2} = \frac{[POP]\ Calf\ serum}{[POP] Milk} \quad \text{equation 2}$$

Each of the two BMFs was calculated two ways: using lipid corrected values and using R153 values. R153 values were calculated by dividing the wet weight of each congener by the wet weight of CB153 (one of the most abundant PCB congeners found in biological tissue) in the sample. This method of standardization has been used to calculate BMF from serum and milk samples collected from marine mammal female/offspring pairs (Beckmen et al., 1999; Debier et al., 2003a; Wolkers, 2004) and is ideal for comparing biological samples that have vastly different lipid levels, especially when lipid content of some samples (e.g., serum) are particularly low. However, this approach is problematic when the concentration of CB153 changes during the lactation period at a rate that differs from those of other compounds within a sample type and when the rate of change in CB153 also differs across sample types. Therefore, because calculations using R153 values produced somewhat counterintuitive results, we only present them in the supplemental information for comparison to results from earlier studies.

The proportional change in POP concentrations across lactation were calculated for both serum and milk, using lipid corrected POP values, according to the following equation:

$$\text{Proportional change} = \frac{(Final\ [POP] - Inital\ [POP])}{Initial\ [POP]} \quad \text{equation 3}$$

2.6 Statistical Analysis

Lipid content of the first milk, first serum, and placenta samples were compared by Kruskal-Wallis One Way Analysis of Variance on Ranks with the Holm-Sidak method for all pairwise multiple comparison procedures because the Shapiro-Wilk normality test failed (SigmaPlot 14, Systat Software, Inc., San Jose, CA, USA). Changes in milk lipid content, serum lipid content, and blubber thickness at each of the three sites during the lactation period were assessed for the three focal mother/calf pairs using ANCOVA (using SigmaPlot 14, Systat Software Inc., San Jose, CA, USA). If ANCOVA was not warranted, Two Way ANOVA with all pairwise multiple comparison procedures (Holm-Sidak method, SigmaPlot 14, Systat Software, Inc., San Jose, CA, USA) were utilized to analyze a sub-set of the parameters.

Additional statistical analyses were used to evaluate the profile of contaminant concentrations across the matrices collected as well as changes in the profile of compounds over the course of the lactation period. Specifically, Principal Component Analysis (PCA) was performed on the congener- and metabolite-specific data obtained from placenta, maternal serum, calf serum, and milk collected after birth using a correlation matrix (prcomp R package). PCAs were also performed on individual sample types (maternal serum, calf serum, and milk) and for all sample types combined (including placenta samples). The input included log-transformed POPs (PCB homologues; DDT metabolites; BDEs 47, 99, and 100; individual CHLDs; and individual HCHs as ng/g lipid adjusted values) in each sample. In total, 72 compounds/compound combinations (e.g., CB138/163/164, see above) were analyzed in each sample. Three compounds (endosulfan I, BDE85, and BDE183) were below the lower limit of quantitation (<LOQ) in all samples and were excluded from the dataset. When more than 80% of the samples analyzed for each matrix were below the lower limit of quantitation for an individual compound, that compound was given a value of zero for all samples of that sample

type. These included heptachlor, aldrin, and CB33 for all sample types; and *trans*-chlordane; CBs 70, 191, 205, and 209; and BDEs 28, 49, 66, 153, and 154 for all sample types except milk (maternal serum, calf serum, and placenta). Alpha-HCH; lindane; heptachlor epoxide; nonachlor III; oxychlordane; CBs 18, 49, 74, 82, 156, 195, 206, and 208; *o,p'*-DDD; and BDEs 99 and 100 were assigned a value of zero for maternal serum samples. Alpha-HCH; lindane; mirex; and CBs 17, 18, 28, 31, 49, 82, 156, 195, 206, and 208 were assigned a value of zero for placenta samples. With the exception of specific compounds mentioned previously, measured values were retained for milk and calf serum samples. Additionally, *trans*-chlordane; CBs 70, 191, 205, and 209; and BDEs 28, 49, 66, 153, and 154 in milk samples were not included as variables in the PCAs for consistency with the other matrices. For all sample types, remaining analytes with values below the LOQ were assigned a value of half the LOQ. PCBs were summed by homologues (tri-chlorinated through nona-chlorinated; octa- and nona-chlorinated PCBs were combined). The final full dataset for the PCA analysis contained 27 variables. The retained compounds were analyzed by isomer (DDTs), grouped homologues (PCBs), or by individual compound. All input variables were mean-centered and scaled to variance. Output was rotated to show component loading values. Components needed to have an eigenvalue >1.0, account for >10% of the variance, and have loadings different from random expectation (tested using a broken-stick model (Peres-Neto et al., 2003) to be retained. Change in the profile of measured POP compounds represented by PC1 from each sample type was analyzed across the lactation period (i.e., number of collection days after birth) with a mixed effects model (nlme R package) where individual dolphin was included as a random effect. These models used the output from the PCA by sample type, and separate regression models were run for each sample type (calf serum, maternal serum, and milk).

We considered a more conservative threshold of <50% of compounds being <LOQ for inclusion in the PCA, but that resulted in a considerable reduction of female serum compounds included in the analysis. As expected, female serum POP concentrations declined considerably during lactation, and thus several concentrations were <LOQ later in the lactation period, despite being >LOQ soon after birth. The PCA using a 50% threshold was not substantively different, but did result in an underreporting of which compounds are transferred from females to calves. As such, we only present the results for the more inclusive threshold.

To evaluate clustering of the compounds across all four sample types, we used the mclust R package (Scrucca et al., 2016) on lipid-adjusted contaminant values to perform unsupervised clustering by fitting Gaussian mixture models (GMM) using expectation maximization (EM). Models were estimated by EM algorithm that is initialized by hierarchical model-based agglomerative clustering, with the optimal model selected according to Bayesian information criterion. PC1 and PC2 scores from the PCA of all sample types combined was used to visualize the data. The PCA plot markers were overlayed with sample type designations (milk, calf serum, maternal serum, and placenta) and cluster numbers.

Due to the small sample size and high variability in data across individuals, p-values <0.05 were considered to be significant while p-values <0.1 were considered to be a trend. Means are presented ± Std. Dev., unless otherwise noted. For illustrative purposes, data from all compounds that were above the LOQ for each individual sample were included in figures and tables depicting ΣPCBs, ΣDDTs, ΣCHLDs, ΣPBDEs, and ΣHCHs. Biomagnification factors and proportional changes in compounds are reported when compounds used to calculate these variables were above the LOQ. Sigma Plot Software (San Jose, CA, USA) was used to construct figures, except for figure 2, which was constructed in R version 4.1.0 (R Core Team 2015).

3. Results

3.1 Blubber thickness

Blubber thickness ranged from 1.2-2.7 cm (mean: 1.8 ± 0.4 cm) and from 1.0-2.3 cm (mean: 1.6 ± 0.4 cm) for adult females and calves, respectively. Blubber thickness varied by individual, by measurement site, and for calves, by number of days post-partum (Figure 1). Adult female blubber thickness did not change over time at any of the three sites (Figure 1). Overall, the oldest female, Fem 1, had significantly greater blubber thickness than the youngest female, Fem 3, (T=3.5, P=0.009), and moderately greater blubber thickness than Fem 2 (T=1.8, P=0.09). For all females, blubber thickness at site 2 was significantly greater than blubber thickness at site 1 (T=4.3, P=0.002) and site 3 (T=3.7, P=0.004). In contrast, calf blubber thickness at all sites increased with days post-partum (site 1: F=5.2, P=0.07, df=1; site 2: F=4.6, P=0.08, df=1; site 3: F=15.5, P=0.01, df=1), but did not vary by individual (Figure 1). Similar to their mothers, calf blubber thickness at site 2 was significantly greater than blubber thickness at site 3 (T=3.0, P=0.02) and moderately greater than blubber thickness at site 1 (T=2.0, P=0.10).

3.2 Lipid content and contaminant profiles in placenta, serum, and milk

Lipid content and contaminant profiles varied by sample type and by individual (Supplemental Tables 2, 3). For all females and calves sampled, the lipid content of the first milk sample (range: 11.0-26.0%, mean: 17.0±6.0%, n=6) was much greater than the lipid content of the first maternal serum sample (range: 0.19-0.43%, mean: 0.33±0.10%, n=4),the lipid content of the first calf serum sample (range: 0.24-0.44%, mean: 0.32±0.11%, n=3), and the lipid content of placenta (range: 0.16-0.59%, mean: 0.40±0.17%, n=5, Supplemental Table 3; H=11.248, P=0.010, df=3). Lipid content of placenta, maternal serum, and calf serum did not differ (Supplemental Table 3).

Because lipid content is significantly greater in milk, compared to serum and placenta, both wet weight and lipid corrected data are presented.

There were distinct differences in ΣPOP classes across samples, and for adult females, the differences were particularly evident in the first samples collected (Supplemental Tables 2, 3). However, despite differences in absolute concentrations, the relative concentrations of ΣPOP classes were similar across sample types. For the most part, concentrations of contaminants in placenta, milk, and serum followed the order: ∑PCBs > ∑DDTs > ∑CHLDs > ∑PBDEs > ∑HCHs, though there was some minor variability among a few samples. Regardless, ∑PCBs and ∑DDTs were always found in the greatest concentrations while ∑HCHs were nearly always found in the lowest concentrations (Supplemental Tables 2 and 3).

Cluster analysis that incorporated all samples collected from the three longitudinally sampled mother/calf pairs revealed that contaminant composition in placenta, milk, maternal serum, and calf serum separated into 5 distinctive clusters based on POP concentrations (Figure 2). This is presented using the PC1 and PC2 scores from the PCA using all sample types (Supplemental Table 4). PCA results are discussed in further detail below. Separation into clusters appeared to be dictated by a combination of individual, age-class (adult female or calf), female reproductive history, sample type, and days post-partum (Supplemental Table 5). The grouping (cluster 1) of placenta and all maternal serum samples reflects the similarity of these two matrices. The composition of POPs in milk and serum samples collected from females and their calves was also influenced by female reproductive history. For example, cluster 2 was comprised of all serum samples collected from the two calves born to the females with the lower reproductive outputs (Fem 3 and Fem 1) while serum from the calf born to the female with the greatest reproductive output (Fem 2) clustered separately (cluster 3) with the early lactation (≤89

415 days post-partum) milk samples from all females. Furthermore, the remaining milk samples
416 collected from the females with the lower reproductive outputs (Fem 3 and Fem 1) clustered
417 together (cluster 4), while the remaining milk samples collected from the female with the
418 greatest reproductive output and shorter calving intervals (Fem 2) grouped together in an
419 exclusive cluster (cluster 5). Changes in milk POP composition over time as pollutants were
420 transferred to calves are also reflected by the results of the cluster analysis. Specifically, cluster 3
421 consisted of the early (≤89 days post-partum) lactation milk samples from all females, cluster 4
422 consisted of the remaining milk samples collected from the two females with the lower
423 reproductive outputs, and cluster 5 exclusively consisted of the later milk samples collected from
424 the female with the greatest reproductive output (Figure 2, Supplemental Table 5). This cluster
425 model did not account for time, which could clarify some additional temporal relationships.
426
427 3.3 Changes in lipid content in milk and serum over time
428 Data from the three mother/calf pairs that were sampled longitudinally over the entire 15-month
429 sampling period were used to evaluate changes in milk and serum lipid content during the
430 lactation period. There were no significant effects of individual or the interaction between days
431 post-partum and individual on milk lipid content (range: 5.2-20.4%, mean: 13.7 ± 3.7%, n=13).
432 There was a trend for milk lipid content to increase linearly over time (r^2=0.2, P=0.08, n=13;
433 power of the test (0.4) was low, which indicates that negative results should be interpreted
434 cautiously). For maternal serum lipid content, there was no interaction between days post-partum
435 and individual (range: 0.30-0.61%, mean: 0.41 ± 0.092%, n=13), but there was a significant
436 effect of days post-partum as well as individual. Similar to milk lipid content, maternal serum
437 lipid content increased linearly over time for all females (r^2=0.5, P=0.012, n=13); and serum

lipid content was greater in Fem 2, compared to Fem 1 (T=3.06, P=0.04). Despite increasing trends in both female serum and milk lipid content over the course of lactation, there was no significant relationship between milk and serum lipid content. Unlike maternal serum lipid content, there was no effect of individual or days-postpartum on calf serum lipid content (range: 0.20-0.51%, mean: 0.35 ± 0.027%, n=12).

3.4 Changes in contaminant profiles in milk and serum over time

Data from the three mother/calf pairs that were sampled longitudinally over the entire 15-month sampling period were used to evaluate changes in contaminant levels during the lactation period. Because POPs are associated with lipid and lipid content varied over time in both milk and maternal serum, both wet weight and lipid corrected POP levels are presented.

Contaminant concentrations in milk varied by POP class and by individual, yet overall, milk POP levels decreased over time (Figure 3). In general, milk contaminant profiles were dominated by PCBs and DDTs (Figure 3, Table 2), and milk from the multiparous female (Fem 2) that birthed the greatest number of calves in a shorter period of time (Table 1) had substantially lower POP concentrations, with many more compounds being at levels <LOQ, compared to the other two females. The difference in milk POP concentrations across the three females was particularly striking soon after parturition. Duration of time in which females produced milk with higher contaminant levels also varied by reproductive history. Milk POP concentrations plateaued at low levels, well before 200 days post-partum for the multiparous female with the greatest reproductive output (Fem 2; Figures 3E, 3F). Meanwhile, milk POP concentrations for the other two females plateaued at somewhat higher levels, particularly for ΣPCBs and ΣDDTs, sometime between 200-250 days post-partum (Figures 3A-3D). POP

concentrations in maternal serum sampled soon after birth were similar to those found in the placenta (Figures 4A, B and 4E, F, see Supplemental Table 2 for data from all females in the study). Thus, despite being unable to collect a serum sample from the primiparous female soon after parturition, it is likely that the POP concentrations in her placenta reflect this female's serum POP levels at birth. Soon after birth, maternal serum POP concentrations decreased concomitantly with milk POP concentrations (Figure 4). Trends in relative serum POP concentrations across the three females and the time when POP levels plateaued (Figure 4) were similar to those reported for milk POP concentrations (Figure 3). Thus, changes in milk POP levels reflect changes in maternal serum POP levels.

During the lactation period, the percent reduction in ΣPOP class levels (relative to initial concentrations) in milk and maternal serum varied by POP class and by female. Specifically, ΣPOP class concentrations declined by 43% to 83% in milk and by 68% to 89% in maternal serum (Supplemental Table 6). The range for declines in individual compounds was even greater (Table 2). For all adult females the relative proportional reduction in maternal serum was as follows: ΣHCHs (non-detectable in the last samples) > ΣPBDEs (non-detectable in Fem 2's last sample) > ΣCHLDs > ΣDDTs > ΣPCBs. Meanwhile ΣPBDEs and/or ΣDDTs demonstrated the greatest proportional reduction in milk over time and the relative order of reduction in milk varied widely across the three females (Supplemental Table 6). The proportional declines in maternal serum and milk ΣPOP class levels were the greatest and most consistent across POP classes for the primiparous female, compared to the multiparous females (Supplemental Table 6). It is important to note, however, that the overall reduction in Fem 3's serum and milk POPs is undoubtedly more significant than our data show since we were not able to sample this

primiparous female until 89 days post-partum, thereby missing the first 3 months of contaminant transfer.

Samples collected from Calf 2C at 9 days post-partum suggest that calf serum POP levels at birth are similar to POP levels in the placenta (Figures 4E, F). Calf serum POP levels continued to increase as their mother's serum and milk POP levels decreased (Figure 4, Table 2, and Supplemental Table 6). The relative increase in calf serum ΣPOP class levels differed across individuals (Supplemental Table 6). This disparate pattern could be due to differences in POP transfer dynamics related to initial maternal POP body burden as well as an artifact of our sampling design. Due to logistical constraints, Fem 3 and her calf were sampled later (89 days and 201 days post-partum, respectively) than the two other calves and their mothers (9 and 12 days post-partum). Consequently, the proportional changes in POP levels for Calf 3A are only indicative of changes from approximately 6.5 months post-partum onward.

The relative final calf serum POP concentrations corresponded to their mother's relative serum POP concentrations. Thus, Calf 2C, born to the female with the greatest reproductive output (Fem 2), had the lowest serum POP levels throughout the lactation period (Figure 4). As a result, at the end of the sampling period, Calf 3A, born to the primiparous female, had serum ΣDDTs and ΣPCBs that were 18X and 9X, respectively, greater than that of Calf 2C. Calf 1B, born to the older multiparous female with fewer calves, had similarly high serum POP levels. Calf 1B had serum ΣDDTs and ΣPCBs that were 16X and 8X, respectively, greater than that of Calf 2C. This demonstrates that calves born to older multiparous females that do not regularly produce calves can have relatively high contaminant loads that are comparable to calves born to primiparous females.

505 Dominant POPs and changes in concentration varied by sample type and compounds,
506 respectively. For milk, the dominant variables included 4- to 5-chlorine PCBs; BDEs 47, 99, and
507 100; *p,p'*-DDT and *p,p'*-DDD; *o,p'*-DDT and *o,p'*-DDD; *cis*-nonachlor; heptachlor epoxide;
508 nonachlor III; and oxychlordane. These compounds had high loading weights (absolute value of
509 ≥0.2) for PC1 in the milk specific principal components analyses (Supplemental Table 4). The
510 compounds represented by PC1 decreased with increasing days post-partum ($p=0.002$;
511 Supplemental Table 7). Two of these compounds (*p,p'*-DDD and BDE47) were among the
512 fifteen compounds found in the highest concentrations across all samples. The proportional
513 decline, relative to the initial lipid corrected concentration in milk, of these dominant compounds
514 ranged from 0.53 to 0.88 (Table 2). The greatest relative reductions in milk POP levels were
515 observed in the primiparous female while the lowest reductions were observed in the multiparous
516 female with the greatest reproductive output (Table 2). It is likely that the proportional reduction
517 in milk POP levels over the entire lactation period was even greater than presented here for the
518 primiparous female since we were not able to collect samples from her until 89 days post-
519 partum, compared to 9 and 12 days post-partum for the other two females.
520 The calf serum specific PCA was dominated by a somewhat similar profile of
521 contaminants (i.e., the compounds with absolute value of ≥0.2 for PC1). Specifically, these
522 included 4- to 6-chlorine PCBs, all DDT isomers except for *o,p'*-DDT and *p,p'*-DDE, *trans*-
523 nonachlor, *cis*-nonachlor, heptachlor epoxide, nonachlor III, and oxychlordane). The compounds
524 represented by PC1 increased with increasing days after birth ($p<0.001$; Supplemental Table 7).
525 The proportional increase, relative to the initial lipid corrected concentration in calf serum, of the
526 two dominant compounds (*p,p'*-DDD, *trans*-nonachlor) ranged from 0.15 to 0.62 (Table 2).
527 Although the relative increase in serum POP levels in the calf born to the primiparous mother

was often greater than the other two calves, it was not the case for all compounds (Table 2). However, this result is likely due to the deferred sampling of the first-born calf, which began at 201 days post-partum, compared to 9 and 12 days post-partum for the other two calves. Increases in serum POP levels after birth for the first-born calf are undoubtedly greater than presented here.

The maternal serum specific PCA was dominated by nearly all maternal serum compounds included in the PCA, except hexachlorobenzene and *cis*-chlordane. The compounds represented by PC1 decreased with increasing days post-partum ($p=0.005$, Supplemental Table 7). Five of the maternal serum compounds that had high loading weights (absolute value of ≥ 0.2) for PC1 (*p,p'*-DDE, *p,p'*-DDD, BDE47, *trans*-nonachlor, and dieldrin) were among the fifteen compounds found in the highest concentrations across all samples. The proportional decline, relative to the initial lipid corrected concentration in maternal serum, of these dominant compounds ranged from 0.65 to 0.86 (Table 2). Similar to the results for milk, the greatest relative reductions in maternal serum POP levels were observed in the primiparous female while the lowest reductions were observed in the multiparous female with greatest reproductive output (Table 2). Though, as mentioned above, reductions in the primiparous female maternal serum POP levels are likely to be greater than presented here due to deferred initial sampling. It is also worth noting that concentrations of *p,p'*-DDD and BDE47, as well as concentrations of several other compounds, were <LOQ in the final serum sample collected from Fem 2, the female with the greatest reproductive output, which indicates that this female had nearly eliminated those compounds from her system.

3.5 Biomagnification of POPs in calf serum relative to milk and maternal serum.

Biomagnification factors (BMFs) of compounds in calf serum relative to milk and relative to maternal serum varied by mother/calf pair and increased with days post-partum (Table 3, Supplemental Table 8). We present BMFs of ∑POP classes calculated using R153 values (Supplemental Table 8) for comparison to other marine mammal studies (Beckmen et al., 1999; Debier et al., 2003a; Wolkers, 2004). BMFs calculated from lipid corrected data are much greater than BMFs calculated from R153 data, and for each mother/calf pair, the relative order of increasing BMF values calculated from lipid corrected data are not always identical to that of BMF values calculated from R153 data (Supplemental Table 8). This is likely due to the differential transfer of CB153 relative to the transfer of other compounds, which can make interpretation of these BMF calculations difficult in longitudinal studies on contaminant transfer dynamics (Debier et al., 2003a). Because of this complexity, we focus on BMFs calculated from lipid corrected data.

The relative biomagnification of PCB compounds in calf serum relative to maternal serum (for compounds that were >LOQ) varied by female reproductive history, lactation stage, log P (n-Octanol/Water Partition Coefficient (Kow); the ratio of the concentration of a chemical in n-octanol and water at equilibrium; generally inversely related to water solubility and directly proportional to molecular weight of a substance), and number of chlorines (Figure 5). At the end of the study period, POPs were most biomagnified in the calf born to the primiparous female and least biomagnified in the calf born to the female with the greatest reproductive output and relatively short calving intervals (Fig 5). The influence of lactation stage, log P, and the number of chlorines was most obvious for the primiparous female/calf pair (Figure 5A) and least apparent for the greatest output multiparous female/calf pair (Figure 5C). Note that the elevated early lactation BMFs for the primiparous mom/calf pair are likely due to the later collection of

574 the first sample (201 days, compared to 9 and 12 days for the other two female/calf pairs, Fig 5).
575 Comparable to results from the PCA, and complimentary to the relative reduction of compounds
576 in maternal serum and milk over the lactation period (Table 2), 3- to 6-chlorine PCB compounds
577 with relatively low log P values were more biomagnified in calf serum at the end of the lactation
578 period (Fig 5). This includes dioxin-like congeners, CB105 and CB118, which ranged from 8 to
579 28.5 times and 5.8 to 26.7 times greater in calf serum compared to maternal serum, respectively,
580 for the three mother calf pairs at the end of the lactation period (Table 3).
581
582 **4. Discussion**
583 This is the first study to evaluate the dynamic transfer of persistent organic pollutants (POPs)
584 from live female cetaceans to their calves, and the most extensive study on marine mammal
585 maternal transfer during lactation in terms of the number of compounds analyzed. Unlike phocid
586 seal females that fast while nursing their young (Debier et al., 2003a; Debier et al., 2003b;
587 Oftedal, 2000), bottlenose dolphin females continue to feed regularly while nursing (Oftedal,
588 1997; West et al., 2007). Thus, despite mobilizing fat and POPs from their blubber (inferred
589 from reductions in serum POP concentrations, which correlate with blubber POP concentrations
590 (McCormley et al., 2021; Reddy et al., 1998; Yordy et al., 2010c) to produce milk, female
591 dolphin blubber thickness remained relatively stable over the course of the ~15-month lactation
592 period. This contrasts with fasting and lactating phocid seal females, that lose significant fat
593 stores while lactating (Gales and Burton, 1987). Similar to phocid pups (Debier et al., 2003a;
594 Debier et al., 2003b; Stewart and Lavigne, 1980), delphinid calves (Dunkin et al., 2005, present
595 study) develop thicker blubber and accumulate higher POP loads while nursing. We also found
596 that bottlenose dolphin blubber thickness varied by body site, which has been reported

previously for killer whales (Raverty et al., 2020), the largest delphinid. These findings are important to consider for future studies that monitor changes in blubber thickness with other physiological processes.

Relative concentrations of POPs in bottlenose dolphin milk, maternal serum, and calf serum typically followed the order: ΣPCBs > ΣDDTs > ΣCHLDs > ΣPBDEs > ΣHCHs. This pattern is similar to concentrations in milk, blood plasma, and blubber collected from free-ranging female bottlenose dolphins in Sarasota Bay, FL, USA (Yordy et al., 2010 b, c). Furthermore, in general, the range of lipid-corrected values for ΣPCBs, ΣDDTs, ΣCHLDs, ΣPBDEs, HCB, Mirex, and Dieldrin in captive female dolphin milk and blood serum overlap with corresponding values reported for milk (Yordy et al., 2010 b) and blood plasma (Yordy et al., 2010 c) collected from free-ranging female bottlenose dolphins. These findings likely reflect both the availability of contaminants in the environment as well as toxicokinetics in bottlenose dolphins and suggest that results from the present study can be extrapolated to free-ranging populations.

Milk lipid content and POP concentrations in milk, maternal serum, and calf serum varied by days post-partum. Over the first 460 days of lactation, milk lipid content and calf POP serum concentrations increased while both milk and maternal serum POP concentrations decreased. These results are consistent with findings from previous studies on changes in dolphin milk lipid content (Ridgway and Reddy, 1995; West et al., 2007) and PCB and DDT concentrations (Ridgway and Reddy, 1995). In contrast, milk lipid content as well as POP levels in both milk and maternal serum increase during the relatively short lactation period of gray seals, which were sampled shortly after birth and at approximately 2-2.5 weeks after birth (Debier et al., 2003b; Vanden Berghe et al., 2012). Similar to dolphin calves, seal pup serum

POP levels (Debier et al., 2003a; Debier et al., 2003b; Vanden Berghe et al., 2012), and presumably blubber POP levels (McCormley et al., 2021; Reddy et al., 1998; Yordy et al., 2010c), increase during the suckling period and are also positively correlated with maternal levels (Debier et al., 2003b). Similar to gray seals, harp seal milk and maternal serum POP levels increase, yet distinctly different from gray seals and bottlenose dolphins, harp seal pup serum POP levels generally decrease during the lactation period (sampled at 1-6 days post-partum and resampled at 6-10 days post-partum (Frouin et al., 2012)). This decrease was potentially attributed to growth dilution (Frouin et al., 2012). However, if growth dilution caused the decline in harp seal pup serum POP levels in such a short time period, we would expect to see similar results for neonatal bottlenose dolphins and gray seals. Another explanation could be related to differences in sampling and data analysis. Unlike the present study on dolphins and the earlier studies on gray seals, the harp seals were only sampled twice during the lactation period, and not necessarily at the very beginning and end of the lactation period. Linear regressions were subsequently used to interpolate POP concentrations over the entire lactation period (Frouin et al., 2012), which may have introduced error since the present study demonstrates that milk and serum POP concentrations do not change linearly in dolphins. Future contaminant transfer studies with higher sampling rates are needed to compare delphinid POP transfer dynamics during the first month post-partum to phocid POP transfer dynamics. Regardless, because the delphinid lactation period is much longer than phocid lactation periods, the full sampling period in the present study is biologically relevant as bottlenose dolphins typically wean at 1-3 years post-partum (Noren and Edwards, 2007; Oftedal, 1997).

Differences in maternal POP transfer dynamics across species are likely related to ecological, behavioral, and physiological differences. Unlike female bottlenose dolphins, female

643 gray and harp seals fast while lactating, which could explain why phocid maternal serum and
644 milk POP concentrations increase, rather than decrease, during the lactation period. Similarly,
645 serum POP concentrations increase in fasting weaned northern elephant seal pups (Debier et al.,
646 2006) as the animals rely on energy from blubber lipid stores to meet metabolic demands (Noren
647 et al., 2003). This illustrates the importance of investigating contaminant transfer dynamics for
648 several marine mammal species, which have distinct ecological, behavioral, and physiological
649 traits.

650 The transfer of POPs during marine mammal reproduction varies by chemical class.
651 Previous studies on a cross-section of deceased delphinids and pinnipeds reported that the most
652 readily transferred organochlorines (OCs) are HCHs and HCB, followed by DDTs and then
653 finally PCBs (Addison and Brodie, 1987; Aguilar, 1987; Borrell et al., 1995; Fukushima and
654 Kawai, 1981; Tanabe et al., 1982). While individual compounds within each group of chemicals
655 have variable transfer efficiencies, similar to earlier studies (which reported fewer POP classes),
656 we found that the relative reduction in maternal POP serum concentrations followed the pattern:
657 ΣHCHs (non-detectable in the last samples) > ∑PBDEs (non-detectable in Fem 2's last sample)
658 > ΣCHLDs > ΣDDTs > ΣPCBs (Supplemental Table 6). Meanwhile ΣPBDEs and/or ΣDDTs
659 demonstrated the greatest proportional reduction in milk over time (Supplemental Table 6). An
660 earlier review suggested that it might be reasonable to assume that the transfer of PCBs and
661 PBDEs during gestation and lactation are comparable due to similarities in the chemical
662 structures of these compounds (Mongillo et al., 2016), but the present study suggests that might
663 not be the case. Maternal serum ΣPBDEs were significantly more reduced than ΣPCBs during
664 the lactation period such that in some cases individual PBDEs were below quantitation levels by
665 the end of the lactation period. This could be due to differences in transfer dynamics related to

chemical properties and/or differences in the initial levels (initial maternal serum ΣPBDEs were quite low), particularly for the multiparous female with the greatest reproductive output. It is also possible that these differences are related to other physiological processes, such as the potential for adult females to metabolize PBDEs more readily than PCBs (Fair *et al.* 2007; Houde *et al.* 2009).

Molecular weight and degree of lipophilicity also influence transfer rates of specific PCB compounds from mother to calf. Studies on stranded delphinids have suggested that PCBs with higher molecular weight, or higher degree of chlorination, have lower placental transfer (Salata et al., 1995; Zhang et al., 2021) and are therefore less mobilized from mother to offspring during gestation (Tanabe et al., 1981; Tanabe et al., 1982; Zhang et al., 2021). The results of the PCA as well as the change in PCB biomagnification factors calculated for calf serum concentrations relative to maternal serum concentrations from early lactation to very late lactation (Figure 5) demonstrate that preferential maternal transfer of PCB compounds with lower chlorination and log P values occurs during lactation in bottlenose dolphins. This has also been reported in seals (Frouin et al., 2012; Miranda Filho et al., 2009), wild bottlenose dolphins (Yordy et al., 2010b), and other cetacean species (Cadieux et al., 2016; Haraguchi et al., 2009; Hayes et al., 2022; Park et al., 2010).

Identifying individual compounds transferred from females to their calves is also important to evaluate potential risks to neonates. For example, BDE99 and CB52 (a 4-chlorine compound) were both readily transferred in milk. This is concerning, given the potential neurotoxic effects of neonatal exposure to PCBs and PBDEs (Eriksson et al., 2006; Eriksson et al., 2002), especially since the neurobehavioral defects worsen with age when mice are neonatally exposed to both BDE99 and CB52 (Eriksson et al., 2006).

689 Female reproductive history influences contaminant transfer during lactation. Compared
690 to the multiparous females, the primiparous female delivered the most contaminated milk and
691 had the highest proportional reduction in milk and maternal serum POP levels over the lactation
692 period. Earlier studies that quantified POP levels in bottlenose dolphin tissues also noted striking
693 differences in exposure to first-born calves, compared to calves that were born to multiparous
694 females. Fukushima and Kawai (1981) proposed that the transfer rate of PCBs and DDTs to first-
695 born dolphin calves is 4X greater than to subsequent calves, and Cockcroft et al. (1989)
696 suggested that nearly 80% of the PCB and DDT load of a female bottlenose dolphin is passed to
697 her first-born calf. Although we were unable to sample the primiparous female until 89 days
698 post-partum, results from our study suggest that primiparous female transfer rates calculated
699 from carcass tissue analysis by earlier studies may be underestimated. Lipid-normalized levels of
700 ΣPCBs and ΣDDTs in milk from the primiparous female collected at 89 days post-partum, after
701 nearly 3 months of transfer, with presumable reductions in milk POP concentrations over time,
702 were still 4.2X greater and 7.5X greater, respectively, than the concentrations found in milk from
703 the female with the greatest reproductive output collected soon after birth, at 9 days post-partum
704 (Supplemental Table 3). Meanwhile, wet weight concentrations of ΣPCBs and ΣDDTs in milk
705 were 6.3X and 11.5X greater, respectively, for the primiparous female (Supplemental Table 2).
706 Furthermore, despite not being able to quantify POP transfer for the first 89 days after birth,
707 which means that the actual proportional reduction in maternal serum levels (and body burden) is
708 greater than what we calculated, it is evident that the primiparous female reduced her PCB and
709 DDT body burdens by more than 80% for most compounds (Table 2, Supplemental Table 6).
710 Additionally, inter-birth interval also influences milk POP concentrations; longer intervals allow
711 for increased accrual of contaminants in the mother between birthing events. As a consequence,

712 Fem 1, the older multiparous female with extended birthing intervals, also delivered milk with
713 significantly higher POP concentrations to her calf, compared to the younger multiparous female
714 with shorter inter-birth intervals. For example, concentrations of ΣPCBs and ΣDDTs (lipid-
715 normalized) in milk were 6X and 15.5X greater, respectively, for the older multiparous female
716 sampled at 12 days-post-partum compared to the younger multiparous female comparably
717 sampled at 9 days post-partum (Supplemental Table 3). The wet weight concentrations of ΣPCBs
718 and ΣDDTs in milk were 7.1X and 18.5X greater, respectively, for the older multiparous female
719 (Supplemental Table 2).
720 Due to constraints on sampling intervals, this study was unable to precisely determine
721 how long the highest POP levels were transferred from mothers to their calves. Despite these
722 limitations, however, it is evident that temporal changes in milk and maternal serum POP
723 concentrations are related to initial female POP burdens. Serum and milk POP levels of the least
724 contaminated female plateaued earlier than more contaminated females. . Data from the 25 yr-
725 old multiparous female with the greatest reproductive rate suggests that some multiparous
726 females could eliminate the majority of their POP loads within the first 24 days after birth. Milk
727 and maternal serum POP levels from the other two females plateaued by at least 200-250 days
728 post-partum, though additional sampling earlier in the lactation period is required to identify
729 when the plateau actually occurs. A previous study that sampled deceased dolphins postulated
730 that primiparous females eliminate the majority of their load by 7 weeks post-partum (Cockcroft
731 et al., 1989). While that might be true for multiparous females with short inter-birth intervals,
732 data from the placenta and serum collected from the primiparous female in the present study
733 suggest that at 89 days (~13 weeks) post-partum this female still had not decreased her body
734 ΣPCBs, ΣDDTs, ΣPBDEs, or ΣCHLDs burdens by 50% (Figure 4, Supplemental Table 3).

735 Concomitantly with the decrease in maternal POP serum levels, calf serum POP levels increased.
736 As expected, the calf born to the primiparous female had the highest calf serum POP levels at the
737 end of the lactation period (Figure 4) and the greatest BMFs (Figure 5, Supplemental Table 8).
738 Interestingly, the calf born to the oldest multiparous female with a longer calving interval also
739 had relatively high calf serum levels at the end of the lactation period (Figure 4) and intermediate
740 BMFs (Figure 5, Supplemental Table 8). This is because both the primiparous young mother
741 and the oldest mother with a low lifetime reproductive output had greater POP body burdens and
742 transferred larger contaminant loads to their calves than the middle-aged mother with the greatest
743 lifetime reproductive output. This demonstrates the need for longitudinal, rather than cross-
744 sectional, studies that include females with disparate reproductive histories to thoroughly
745 investigate contaminant transfer in delphinids.
746
747 **5. Conclusion**
748 In conclusion, the current study demonstrated that POP transfer dynamics in marine mammals
749 are species-specific, and in particular, changes in milk and maternal serum POP levels during
750 lactation in delphinids differ from that of phocids. Furthermore, several factors, including the
751 chemical structure and hydrophilicity of specific compounds, as well as maternal age and
752 reproductive history, dictate the rate of POP transfer in milk and the subsequent declines in
753 maternal serum POP concentrations and the increases in calf serum POP concentrations. It is
754 important to note that data on rates of POP transfer and relative changes in female and calf POP
755 body burdens during lactation are only obtainable from longitudinal studies. Thus, this study
756 provides important new information that builds on results from previous studies on contaminant
757 transfer in odontocetes. Additional longitudinal studies with higher sampling rates are needed to

inform assessments of risk to cetacean calves from POP exposure as well as better parameterize models that aim to predict changes in cetacean POP levels over time (Desforges et al., 2018; Hall et al., 2006; Hall et al., 2018; Mongillo et al., 2012).

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References:

781 Addison R, Brodie P. Transfer of organochlorine residues from blubber through the circulatory
782 system to milk in the lactating grey seal *Halichoerus grypus*. Canadian Journal of
783 Fisheries Aquatic and Sciences 1987; 44: 782-786.
784 Aguilar A. Using organochlorine pollutants to discriminate marine mammal populations: A
785 review and critique of the methods. Marine Mammal Science 1987; 3: 242-262.
786 Aguilar A, Borrell A. Reproductive transfer and variation of body load of organochlorine
787 pollutants with age in fin whales (*Balaenoptera physalus*). Archives of Environmental
788 Contamination Toxicology 1994; 27: 546-554.
789 Barbosa APM, Méndez-Fernandez P, Dias PS, Santos MCO, Taniguchi S, Montone RC.
790 Transplacental transfer of persistent organic pollutants in La Plata dolphins (*Pontoporia*
791 *blainvillei*; Cetartiodactyla, Pontoporiidae). Science of The Total Environment 2018;
792 631-632: 239-245.
793 Beckmen KB, Ylitalo GM, Towell RG, Krahn MM, O'Hara TM, Blake JE. Factors affecting
794 organochlorine contaminant concentrations in milk and blood of northern fur seal
795 (*Callorhinus ursinus*) dams and pups from St. George Island, Alaska. Science of the
796 Total Environment 1999; 231: 183-200.
797 Borrell A, Bloch D, Desportes G. Age trends and reproductive transfer of organochlorine
798 compounds in long-finned pilot whales from the Faroe Islands. Environmental Pollution
799 1995; 88: 283-292.
800 Cadieux MA, Muir DCG, Béland P, Hickie BE. Lactational transfer of polychlorinated-
801 biphenyls (PCBs) and other organochlorines in St. Lawrence beluga whales
802 (*Delphinapterus leucas*). Archives of Environmental Contamination and Toxicology
803 2016; 70:169-179.
804 Cockcroft V, De Kock A, Lord D, Ross G. Organochlorines in bottlenose dolphins *Tursiops*
805 *truncatus* from the east coast of South Africa. South African Journal of Marine Science
806 1989; 8: 207-217.
807 de Swart RR, Ross PP, Vedder LE, Timmerman HH, Heisterkamp SS, van Loveren HH, et al.
808 Impairment of immune function in harbor seals (*Phoca vitulina*) feeding on fish from
809 polluted waters. Ambio: A Journal of the Human Environment 1994; 23: 155-159.
810 Debier C, Chalon C, Le Bœuf BJ, de Tillesse T, Larondelle Y, Thomé J-P. Mobilization of PCBs
811 from blubber to blood in northern elephant seals (*Mirounga angustirostris*) during the
812 post-weaning fast. Aquatic Toxicology 2006; 80: 149-157.
813 Debier C, Pomeroy PP, Dupont C, Joiris C, Comblin V, Le Boulengé E, et al. Dynamics of PCB
814 transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*:
815 differences in PCB profile between compartments of transfer and changes during the
816 lactation period. Marine Ecology Progress Series 2003a; 247: 249-256.
817 Debier C, Pomeroy PP, Dupont C, Joiris C, Comblin V, Le Boulengé E, et al. Quantitative
818 dynamics of PCB transfer from mother to pup during lactation in UK grey seals
819 *Halichoerus grypus*. Marine Ecology Progress Series 2003b; 247: 237-248.
820 Desforges J-P, Hall A, McConnell B, Rosing-Asvid A, Barber JL, Brownlow A, et al. Predicting
821 global killer whale population collapse from PCB pollution. Science 2018; 361: 1373-
822 1376.
823 Desforges J-PW, Sonne C, Levin M, Siebert U, De Guise S, Dietz R. Immunotoxic effects of
824 environmental pollutants in marine mammals. Environment International 2016; 86: 126-
825 139.

826 Desforges JPW, Ross PS, Loseto LL. Transplacental transfer of polychlorinated biphenyls and
827 polybrominated diphenyl ethers in arctic beluga whales (*Delphinapterus leucas*).
828 Environmental Toxicology and Chemistry 2012; 31: 296-300.
829 Donkin P, Mann SV, Hamilton EI. Polychlorinated biphenyl, DDT and dieldrin residues in grey
830 seal (*Halichoerus grypus*) males, females and mother-foetus pairs sampled at the Farne
831 Islands, England, during the breeding season. Science of the Total Environment 1981; 19:
832 121-142.
833 Duinker J, Hillebrand MTJ. Mobilization of organochlorines from female lipid tissue and
834 transplacental transfer to fetus in a harbour porpoise (*Phocoena phocoena*) in a
835 contaminated area. Bulletin of Environmental Contamination Toxicology 1979; 23: 728-
836 732.
837 Dunkin RC, McLellan WA, Blum JE, Pabst DA. The ontogenetic changes in the thermal
838 properties of blubber from Atlantic bottlenose dolphin *Tursiops truncatus*. Journal of
839 Experimental Biology 2005; 208: 1469-1480.
840 Eriksson P, Fischer C, Fredriksson A. Polybrominated diphenyl ethers, a group of brominated
841 flame retardants, can interact with polychlorinated biphenyls in enhancing developmental
842 neurobehavioral defects. Toxicological Sciences 2006; 94: 302-309.
843 Eriksson P, Viberg H, Jakobsson E, Örn U, Fredriksson A. A brominated flame retardant, 2, 2, 4,
844 4, 5-pentabromodiphenyl ether: uptake, retention, and induction of neurobehavioral
845 alterations in mice during a critical phase of neonatal brain development. Toxicological
846 Sciences 2002; 67: 98-103.
847 Fair PA, Mitchum G, Hulsey TC, Adams J, Zolman E, McFee W, Wirth E, Bossart GD.
848 Polybrominated diphenyl ethers (PBDEs) in blubber of free-ranging bottlenose dolphins
849 (*Tursiops truncatus*) from two southeast Atlantic estuarine areas. Archives of
850 Environmental and Contaminant Toxicology 2007; 53: 483-94.
851 Frouin H, Lebeuf M, Hammill M, Fournier M. Transfer of PBDEs and chlorinated POPs from
852 mother to pup during lactation in harp seals *Phoca groenlandica*. Science of The Total
853 Environment 2012; 417-418: 98-107.
854 Fukushima M, Kawai S. Variation of organochlorine residue concentration and burden in striped
855 dolphin (*Stenella coeruleoalba*) with growth. Studies on the levels of organochlorine
856 compounds heavy metals in the marine organisms 1981; University of the Ryukyus,
857 Okinawa: 97-114.
858 Gales NJ, Burton HR. Ultrasonic Measurement of Blubber Thickness of the Southern Elephant
859 Seal, *Mirounga-Leonina* (Linn). Australian Journal of Zoology 1987; 35: 207-217.
860 Greig DJ, Ylitalo GM, Hall AJ, Fauquier DA, Gulland FMD. Transplacental transfer of
861 organochlorines in california sea lions (*Zalophus californianus*). Environmental
862 Toxicology and Chemistry 2007; 26: 37-44.
863 Hall AJ, Kalantzi OI, Thomas GO. Polybrominated diphenyl ethers (PBDEs) in grey seals during
864 their first year of life—are they thyroid hormone endocrine disrupters? Environmental
865 Pollution 2003; 126: 29-37.
866 Hall AJ, McConnell BJ, Rowles TK, Aguilar A, Borrell A, Schwacke L, et al. Individual-based
867 model framework to assess population consequences of polychlorinated biphenyl
868 exposure in bottlenose dolphins. Environmental Health Perspectives 2006; 114: 60-64.
869 Hall AJ, McConnell BJ, Schwacke LH, Ylitalo GM, Williams R, Rowles TK. Predicting the
870 effects of polychlorinated biphenyls on cetacean populations through impacts on
871 immunity and calf survival. Environmental Pollution 2018; 233: 407-418.

Hammond JA, Hall AJ, Dyrynda EA. Comparison of polychlorinated biphenyl (PCB) induced effects on innate immune functions in harbour and grey seals. Aquatic Toxicology 2005; 74: 126-138.

Haraguchi K, Hisamichi Y, Endo T. Accumulation and mother-to-calf transfer of anthropogenic and natural organohalogens in killer whales (*Orcinus orca*) stranded on the Pacific coast of Japan. Science of the Total Environment 2009; 407: 2853-2859.

Hayes KRR, Ylitalo GM, Anderson TA, Urbán R J, Jacobsen JK, Scordino JJ, et al. Influence of life-history parameters on persistent organic pollutant concentrations in blubber of Eastern North Pacific Gray Whales (*Eschrichtius robustus*). Environmental Science & Technology 2022; 56:17119-17130.

Hickie BE, Cadieux MA, Riehl KN, Bossart GD, Alava JJ, Fair PA. Modeling PCB-Bioaccumulation in the Bottlenose Dolphin (*Tursiops truncatus*): Estimating a Dietary Threshold Concentration. Environmental Science & Technology 2013; 47: 12314-12324.

Houde M, Pacepavicius G, Darling C, Fair PA, Alaee M, Bossart GD, Solomon KR, Letcher RJ, Bergman A, Marsh G, Muir DC. Polybrominated diphenyl ethers and their hydroxylated analogs in plasma of bottlenose dolphins (*Tursiops truncatus*) from the United States east coast. Environmental Toxicology and Chemisty 2009; 28: 2061-2068.

Kajiwara N, Kamikawa S, Amano M, Hayano A, Yamada TK, Miyazaki N, et al. Polybrominated diphenyl ethers (PBDEs) and organochlorines in melon-headed whales, *Peponocephala electra*, mass stranded along the Japanese coasts: maternal transfer and temporal trend. Environmental Pollution 2008; 156: 106-114.

Koopman HN, Pabst DA, McLellan WA, Dillaman RM, Read AJ. Changes in blubber distribution and morphology associated with starvation in the Harbor porpoise (*Phocoena phocoena*): evidence for regional differences in blubber structure and function. Physiological and Biochemical Zoology 2002; 75: 498-512.

Martineau D, Béland P, Desjardins C, Legace A. Levels of organochlorine chemicals in tissues of beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Québec, Canada. Archives of Environmental Contamination and Toxicology 1987; 16:137–147.

McCormley MC, Noren DP, Ylitalo GM, St. Leger J. Partitioning of persistent organic pollutants between blubber and blood in killer whales (*Orcinus orca*). Marine Mammal Science 2021; 37: 1531-1543.

Miranda Filho KC, Metcalfe CD, Metcalfe TL, Muelbert MM, Robaldo RB, Martinez PE, et al. Lactational transfer of PCBs and chlorinated pesticides in pups of southern elephant seals (*Mirounga leonina*) from Antarctica. Chemosphere 2009; 75: 610-616.

Mongillo TM, Holmes EE, Noren DP, VanBlaricom GR, Punt AE, Neill SM, et al. Predicted polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) accumulation in southern resident killer whales. Marine Ecology Progress Series 2012; 453: 263-277.

Mongillo TM, Ylitalo GM, Rhodes LD, O'Neill SM, Noren DP, Hanson MB. Exposure to a mixture of toxic chemicals: Implications for the health of endangered Southern Resident killer whales. U.S. Dept. Commer., NOAA Tech. Memo. NMFSNWFSC-135, 2016, pp. 107.

Noren D, Crocker D, Williams T, Costa D. Energy reserve utilization in northern elephant seal (*Mirounga angustirostris*) pups during the postweaning fast: size does matter. Journal Comparative Physiology B 2003; 173: 443-454.

Noren SR, Edwards EF. Physiological and behavioral development in delphinid calves: implications for calf separation and mortality due to tuna purse-seine sets. 2007; 23: 15-29.

O'Hara TM, O'Shea TJ. Toxicology. In: Dierauf L, Gulland FM, editors. CRC handbook of marine mammal medicine: health, disease, and rehabilitation. CRC press, Boca Raton, FL, 2001, pp. 471-520.

O'Brien KM, Upson, K., Cookm N.R., Weinbergm C.R. Environmental chemicals in urine and blood: improving methods for creatinine and lipid adjustment. Environmental health perspectives. Environmental Health Perspectives 2015; 124: 220-227.

Oftedal OT. Lactation in whales and dolphins: evidence of divergence between baleen- and toothed-species. . Journal of Mammary Gland Biology and Neoplasia 1997; 2: 205-230.

Oftedal OT. Use of maternal reserves as a lactation strategy in large mammals. Proceedings of the Nutrition Society 2000; 59: 99-106.

Park B-K, Park G-J, An Y-R, Choi H-G, Kim GB, Moon H-B. Organohalogen contaminants in finless porpoises (*Neophocaena phocaenoides*) from Korean coastal waters: contamination status, maternal transfer and ecotoxicological implications. Marine Pollution Bulletin 2010; 60: 768-774.

Peres-Neto PR, Jackson DA, Somers KM. Giving Meaningful Interpretation to ordination axes: assessing loading significance in principal component analysis. Ecology 2003; 84: 2347-2363.

Peterson SH, Hassrick JL, Lafontaine A, Thomé J-P, Crocker DE, Debier C, et al. Effects of age, adipose percent, and reproduction on PCB concentrations and profiles in an extreme fasting North Pacific marine mammal. PloS ONE 2014; 9: e96191.

Pomeroy P, Green N, Hall A, Walton M, Jones K, Harwood J. Congener-specific exposure of grey seal (*Halichoerus grypus*) pups to chlorinated biphenyls during lactation. Canadian Journal of Fisheries Aquatic Sciences 1996; 53: 1526-1534.

Raverty S, St. Leger J, Noren DP, Burek Huntington K, Rotstein DS, Gulland FMD, et al. Pathology findings and correlation with body condition index in stranded killer whales (*Orcinus orca*) in the northeastern Pacific and Hawaii from 2004 to 2013. PLOS ONE 2020; 15: e0242505.

Reddy M, Echols S, Finklea B, Busbee D, Reif J, Ridgway S. PCBs and chlorinated pesticides in clinically healthy *Tursiops truncatus*: Relationships between levels in blubber and blood. Marine Pollution Bulletin 1998; 36: 892-903.

Reddy ML, Reif JS, Bachand A, Ridgway SH. Opportunities for using Navy marine mammals to explore associations between organochlorine contaminants and unfavorable effects on reproduction. Science of the Total Environment 2001; 274: 171-182.

Ridgway S, Kamolnick T, Reddy M, Curry C, Tarpley RJ. Orphan-induced lactation in Tursiops and analysis of collected milk. Marine Mammal Science 1995; 11: 172-182.

Ridgway S, Reddy M. Residue levels of several organochlorines in Tursiops truncatus milk collected at varied stages of lactation. Marine Pollution Bulletin 1995; 30: 609-614.

Ross P, De Swart R, Addison R, Van Loveren H, Vos J, Osterhaus A. Contaminant-induced immunotoxicity in harbour seals: wildlife at risk? Toxicology 1996; 112: 157-169.

Ross PS, De Swart RL, Reijnders P, Van Loveren H, Vos JG, Osterhaus A. Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. Environmental Health Perspectives 1995; 103: 162-167.

Ross PS, Ellis G, Ikonomou M, Barrett-Lennard L, Addison R. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: effects of age, sex and dietary preference. Marine Pollution Bulletin 2000; 40: 504-515.

Salata G, Wade T, Sericano J, Davis J, Brooks J. Analysis of Gulf of Mexico bottlenose dolphins for organochlorine pesticides and PCBs. Environmental Pollution 1995; 88: 167-175.

Schwacke LH, Voit EO, Hansen LJ, Wells RS, Mitchum GB, Hohn AA, et al. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the Southeast United States Coast. Environmental Toxicology and Chemistry 2002; 21: 2752-2764.

Schweigert FJ, Stobo WT. Transfer of fat-soluble vitamins and PCBs from mother to pups in grey seals (*Halichoerus grypus*). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 1994; 109: 111-117.

Scrucca L, Fop M, Murphy TB, Raftery AE. mclust 5: Clustering, Classification and Density Estimation Using Gaussian Finite Mixture Models. The R Journal 2016; 8: 289-317.

Sloan C, Anulacion BF, Baugh K, Bolton JL, Boyd D, Boyer R, et al. Northwest Fisheries Science Center's analyses of tissue, sediment, and water samples for organic compounds by gas chromatography/mass spectrometry and analyses of tissue for lipid classes by thin layer chromatography/flame ionization detection. U.S. Deparment of Commerce, NOAA Technical Memorandum, NMFS-NWSFC-125 2014: 61.

Sloan C, Brown D, Pearce R, Boyer R, Bolton J, Burrows D, et al. Determining aromatic hydrocarbons and chlorinated hydrocarbons in sediments and tissues using accelerated solvent extraction and gas chromatography/mass spectrometry. Techniques in Aquatic Toxicology, volume 2. CRC Press, 2005, pp. 651-672.

Sloan CA, Brown DW, Ylitalo GM, Buzitis J, Herman DP, Burrows DG, et al. Quality assurance plan for analyses of environmental samples for polycyclic aromatic compounds, persistent organic pollutants, fatty acids, stable isotope ratios, lipid classes, and metabolites of polycyclic aromatic compounds. US Department of Comerce, NOAA Tech. Memo. NMFS-NWFSC-147, Seattle WA 2006.

Stewart REA, Lavigne DM. Neonatal Growth of Northwest Atlantic Harp Seals, *Pagophilus Groenlandicus*. Journal of Mammalogy 1980; 61: 670-680.

Tabuchi M, Veldhoen N, Dangerfield N, Jeffries S, Helbing CC, Ross PS. PCB-related alteration of thyroid hormones and thyroid hormone receptor gene expression in free-ranging harbor seals (*Phoca vitulina*). Environmental Health Perspectives 2006; 114: 1024-1031.

Tanabe S, Tanaka H, Maruyama K, Tatsukawa R. Ecology and bioaccumulation of *Stenella coeruleoalba*. Elimination of chlorinated hydrocarbons from mother striped dolphins (*Stenella coeruleoalba*) through partruition and lactation. Studies on the levels of organochlorine compounds heavy metals in the marine organisms 1981; University of Ryuskyus: 115-121.

Tanabe S, Tatsukawa R, Maruyama K, Miyazaki N. Trans-placental transfer of OCBs and chlorinated-hydrocarbon pesticides from the pregnant striped dolphin (*Stenella coeruleoalba*) to her fetus. Agricultural and Biological Chemistry 1982; 46: 1249-1254.

van den Heuvel-Greve MJ, van den Brink AM, Kotterman MJJ, Kwadijk CJAF, Geelhoed SCV, Murphy S, et al. Polluted porpoises: Generational transfer of organic contaminants in harbour porpoises from the southern North Sea. Science of The Total Environment 2021; 796: 148936.

Vanden Berghe M, Weijs L, Habran S, Das K, Bugli C, Rees J-F, et al. Selective transfer of persistent organic pollutants and their metabolites in grey seals during lactation. Environment International 2012; Oct 1: 1-15.

Wells RS, Tornero V, Borrell A, Aguilar A, Rowles TK, Rhinehart HL, et al. Integrating life-history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Science of the Total Environment 2005; 349: 106-119.

West K, Oftedal O, Carpenter J, Krames B, Campbell M, Sweeney J. Effect of lactation stage and concurrent pregnancy on milk composition in the bottlenose dolphin. Journal of Zoology 2007; 273: 148-160.

Wolkers H, Lydersen, C., Kovaks, K.M. Accumulation and lactational transfer of PCBs and pesticides in harbor seals (*Phoca vitulina*) from Svalbard, Norway. The Science of the Total Environment 2004; 319: 137-146.

Ylitalo GM, Matkin CO, Buzitis J, Krahn MM, Jones LL, Rowles T, et al. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK. Science of the Total Environment 2001; 281: 183-203.

Ylitalo GM, Stein JE, Hom T, Johnson LL, Tilbury KL, Hall AJ, et al. The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*). Marine Pollution Bulletin 2005a; 50: 30-39.

Ylitalo GM, Yanagida G, Hufnagle GK, Krahn MM. Determination of lipid classes and lipid content in tissues of aquatic organisms using a thin layer chromatography/flame ionization detection (TLC/FID) microlipid method. Techniques in Aquatic Toxicology 2. CRC Press, Boca Raton, FL, USA, 2005b, pp. 449-464.

Yordy JE, Pabst DA, McLellan WA, Wells RS, Rowles TK, Kucklick JR. Tissue-specific distribution and whole-body burden estimates of persistent organic pollutants in the bottlenose dolphin (Tursiops truncatus). Environmental Toxicology and Chemistry. 2010a; 29: 1263-73.

Yordy JE, Wells RS, Balmer BC, Schwacke LH, Rowles TK, Kucklick JR. Life history as a source of variation for persistent organic pollutant (POP) patterns in a community of common bottlenose dolphins (*Tursiops truncatus*) resident to Sarasota Bay, FL. Science of the Total Environment 2010b; 408: 2163-2172.

Yordy JE, Wells RS, Balmer BC, Schwacke LH, Rowles TK, Kucklick JR. Partitioning of persistent organic pollutants between blubber and blood of wild bottlenose dolphins: implications for biomonitoring and health. Environmental Science & Technology 2010c; 44: 4789-4795.

Zhang X, Zhan F, Yu R-Q, Sun X, Wu Y. Bioaccumulation of legacy organic contaminants in pregnant Indo-Pacific humpback dolphins (*Sousa chinensis*): Unique features on the transplacental transfer. Science of The Total Environment 2021; 785: 147287.

Tables

Table 1. Bottlenose dolphin study subjects and samples collected. The females are identified by distinct numbers and the calves are identified by their mother's number plus a letter indicating their birth order (e.g., first calf = A, second calf = B, etc.). The three focal mother/calf pairs are designated by *.

Animal ID (sex)	Age at parturition	Body length (cm)	Viable calf birth order (reproductive history)	Placenta collection days post-partum	Milk sample collection days post-partum	Serum sample collection days post-partum
Fem 1 (F)*	42 (est.)[1]	252	2 (2 live births)	NS	12, 186, 263, 458	12, 186, 263, 458
Calf 1B (M)*	Neonate	-		NA	NA	12, 48, 186[2], 263[2], 458[2]
Fem 2[3] (F)	22 (est.)[1]	-	2 (2 live births)	0	NS	NS
Fem 2 (F)*	25 (est.)[1]	254	3 (3 live births)	0	9, 24, 200, 268, 444	9, 24, 200, 268, 444
Calf 2C (M)*	Neonate	-		NA	NA	9, 24, 36, 200[2], 268[2], 444[2]
Fem 3 (F)*	5	225	1 (primiparous)	0	89, 201, 257, 465	89, 201, 257, 460
Calf 3A (M)*	Neonate	-		NA	NA	201[2], 257[2], 460[2]
Fem 4[4] (F)	28	-	6 (6 live births, 1 late-term abortion)	0	0	0
Fem 5[5] (F)	25 (est.)[1]	-	1 (primiparous, surrogate for Fem 4's Calf)	NS	846(10)	846(10), 864(28)
Fem 6 (F)	14	-	0 (stillborn)	0	1	1

[1]Caught from a wild population at approximately 6 yrs (Fem 2, Fem 5) and 7 yrs (Fem 1) of age; age is estimated.
[2]Calf serum samples collected after solid food was introduced.
[3]Placenta from Fem 2's second birth provided for comparison to samples collected from her third birth.
[4]Fem 4 gave birth to a calf that also received milk from Fem 5. Because contaminant influx was from two sources, Fem 4 and her calf were not sampled after the parturition date.
[5]Fem 5 had given birth to her first calf about 2 yrs prior to the study and was a surrogate to Fem 4's calf. Fem 5's initial milk and serum samples were collected 846 days after the birth of her calf, which was 10 days after the birth of Fem 4's calf.
NA = not applicable
NS = not sampled

Table 2. Proportional change over the lactation period for the 15 POP compounds found in the highest concentrations. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Females are presented in order of increasing female reproductive output. POPs are presented in order of increasing log P. Changes in milk, maternal serum, and calf serum for each compound was calculated as a proportional change in lipid corrected concentration relative to the initial lipid corrected concentration. Negative values indicate reductions, while positive values indicate gains over the lactation period. Log P values are also presented (see Supplemental Table 9 for additional chemical properties of compounds).

				Proportional change over lactation period		
				Adult Female		Calf
Dolphin	Days between first and last samples	POPs	log P	Serum	Milk	Serum
				Lipid corr.	Lipid corr.	Lipid corr.
Fem 3[1]	371 (Fem serum)	Dieldrin	4.97	-0.84	-0.84	+0.25
	376 (Fem milk)	Hexachlorobenzene	5.73	-0.77	-0.74	+0.08
	259 (Calf serum)	CB101/90	6.07	-0.83	-0.84	+0.29
		trans-nonachlor	6.09	-0.77	-0.81	+0.43
		p, p'-DDD	6.12	-0.86	-0.84	+0.29
		CB153/132	6.53	-0.74	-0.75	+0.54
		p, p'-DDE	6.73	-0.81	-0.83	+0.56
		BDE47	6.81	-0.82	-0.88	+0.52
		CB187/159/182	7.00	-0.67	-0.72	+0.62
		CB118[2]	7.12	-0.80	-0.84	+0.35
		CB99	7.21	-0.83	-0.85	+0.39
		CB149	7.28	-0.81	-0.82	+0.41
		CB138/163/164	7.35	-0.75	-0.77	+0.46
		CB180	7.72	-0.64	-0.72	+0.62
		CB199	7.94	-0.68	-0.59	+0.64
Fem 1	446	Dieldrin	4.97	-0.84	-0.84	+0.35
		Hexachlorobenzene	5.73	-0.84	-0.81	+0.42
		CB101/90	6.07	-0.81	-0.80	+0.27
		trans-nonachlor	6.09	-0.77	-0.72	+0.24
		p, p'-DDD	6.12	-0.85	-0.75	+0.15
		CB153/132	6.53	-0.70	-0.67	+0.54
		p, p'-DDE	6.73	-0.78	-0.80	+0.30
		BDE47	6.81	-0.81	-0.81	+0.46
		CB187/159/182	7.00	-0.55	-0.54	+0.68
		CB118[2]	7.12	-0.81	-0.80	+0.25
		CB99	7.21	-0.81	-0.79	+0.29
		CB149	7.28	-0.79	-0.73	+0.39
		CB138/163/164	7.35	-0.72	-0.71	+0.44
		CB180	7.72	-0.54	-0.52	+0.85
		CB199	7.94	-0.29	-0.41	+1.58
Fem 2	435	Dieldrin	4.97	-0.74	-0.64	+0.68
		Hexachlorobenzene	5.73	-0.56	-0.56	+1.38
		CB101/90	6.07	-0.65	-0.63	+0.39
		trans-nonachlor	6.09	-0.66	-0.55	+0.62
		p, p'-DDD	6.12	<LOQ[3]	-0.53	+0.59
		CB153/132	6.53	-0.67	-0.63	+0.39
		p, p'-DDE	6.73	-0.65	-0.56	+0.33
		BDE47	6.81	<LOQ[3]	-0.73	+0.25
		CB187/159/182	7.00	-0.56	-0.52	+0.63
		CB118[2]	7.12	-0.53	-0.62	+0.18
		CB99	7.21	<LOQ[3]	-0.63	+0.22
		CB149	7.28	-0.65	-0.64	+0.30
		CB138/163/164	7.35	-0.64	-0.63	+0.31
		CB180	7.72	-0.53	-0.50	+0.43
		CB199	7.94	<LOQ[3]	-0.46	+0.55

[1]Due to the young age of Fem 3 and that she was a primparous female, sample collection from both Fem 3 and her calf were delayed. Maternal serum and milk were collected 89 days post-partum while calf serum was collected 201 days post-partum. The days between initial and final collections also differed, so they are indicated separately. The other two female/calf pairs were sampled on the same days.

[2]dioxin-like PCB

[3]<LOQ = not determined because the compound was below the lower limit of quantitation in at least one of the samples required for the calculation

Table 3. Biomagnification factors (BMFs; ng/g lipid weight) for 15 contaminants found in the highest concentrations during early and late lactation determined for three calves. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Females are presented in order of increasing female reproductive output. POPs are presented in order of increasing log P. BMFs were determined between calf (C) serum and adult (A) serum as well as calf serum and adult milk. Note that the BMFs were not calculated under typical steady state conditions. Log P values are also presented (see Supplemental Table 9 for additional chemical properties of compounds).

			Biomagnification Factor			
			Early Lactation		Late Lactation	
Dolphin	**POPs**	**log P**	**C Serum/A Serum**	**C Serum/A Milk**	**C Serum/A Serum**	**C Serum/A Milk**
Fem 3[1]	Dieldrin	4.97	16.0	15.4	38.5	35.7
	Hexachlorobenzene	5.73	21.4	16.2	38.2	28.9
	CB101/90	6.07	18.1	15.5	31.4	38.6
	trans-nonachlor	6.09	15.0	10.0	23.9	25.3
	p, p'-DDD	6.12	22.7	10.6	37.3	23.9
	CB153/132	6.53	13.2	12.5	20.0	29.2
	p, p'-DDE	6.73	16.7	11.4	32.5	32.5
	BDE47	6.81	22.5	11.7	41.0	37.3
	CB187/159/182	7.00	11.1	11.1	12.6	22.7
	CB118[4]	7.12	15.5	15.5	26.7	37.7
	CB99	7.21	17.7	13.5	32.7	38.6
	CB149	7.28	16.8	14.5	28.1	37.5
	CB138/163/164	7.35	12.6	11.8	20.0	26.9
	CB180	7.72	9.1	12.4	11.3	26.2
	CB199	7.94	7.4	11.5	9.4	20.0
Fem 1[2]	Dieldrin	4.97	2.3	1.7	19.2	14.3
	Hexachlorobenzene	5.73	2.1	1.8	19.0	13.6
	CB101/90	6.07	2.4	2.4	16.3	15.6
	trans-nonachlor	6.09	2.1	1.8	11.3	7.6
	p, p'-DDD	6.12	2.3	1.6	17.4	7.5
	CB153/132	6.53	2.4	3.0	12.6	14.1
	p, p'-DDE	6.73	2.3	1.7	13.5	10.9
	BDE47	6.81	2.3	1.2	17.7	9.3
	CB187/159/182	7.00	2.0	2.7	7.4	10.0
	CB118[4]	7.12	2.2	2.4	15.0	15.0
	CB99	7.21	2.3	2.1	15.0	12.9
	CB149	7.28	2.3	2.7	15.2	13.9
	CB138/163/164	7.35	2.3	2.5	11.7	12.5
	CB180	7.72	1.9	3.4	7.8	13.2
	CB199	7.94	1.4	3.0	5.0	13.1
Fem 2[3]	Dieldrin	4.97	2.4	1.9	15.2	8.9
	Hexachlorobenzene	5.73	2.9	1.6	15.8	8.6
	CB101/90	6.07	2.5	2.2	10.0	8.1
	trans-nonachlor	6.09	2.1	1.6	9.8	5.8
	p, p'-DDD	6.12	2.2	1.4	<LOQ[5]	4.7
	CB153/132	6.53	2.4	2.5	10.0	9.2
	p, p'-DDE	6.73	2.4	1.7	8.9	5.0
	BDE47	6.81	2.6	1.4	<LOQ[5]	6.5
	CB187/159/182	7.00	2.2	2.2	8.1	7.4
	CB118[4]	7.12	2.3	2.5	5.8	7.9
	CB99	7.21	2.3	2.0	<LOQ[5]	6.5
	CB149	7.28	2.5	2.3	9.2	8.1
	CB138/163/164	7.35	2.3	2.5	8.7	8.8
	CB180	7.72	2.3	3.0	7.0	8.6
	CB199	7.94	2.2	2.5	<LOQ[5]	7.3

[1]Fem 3 early BMF calculated from samples collected 201 days post-partum, late BMF calculated for samples collected 460 days (adult and calf serum) and 465 days (milk) post-partum

[2]Fem 1 early BMF calculated from samples collected 12 days post-partum, late BMF calculated for samples collected at 458 days post-partum

[3]Fem 2 early BMF calculated from samples collected 9 days post-partum, late BMF calculated for samples collected at 444 days post-partum

[4]dioxin-like PCB

[5]<LOQ = not determined because the compound was below the lower limit of quantitation in at least one of the samples required for the calculation

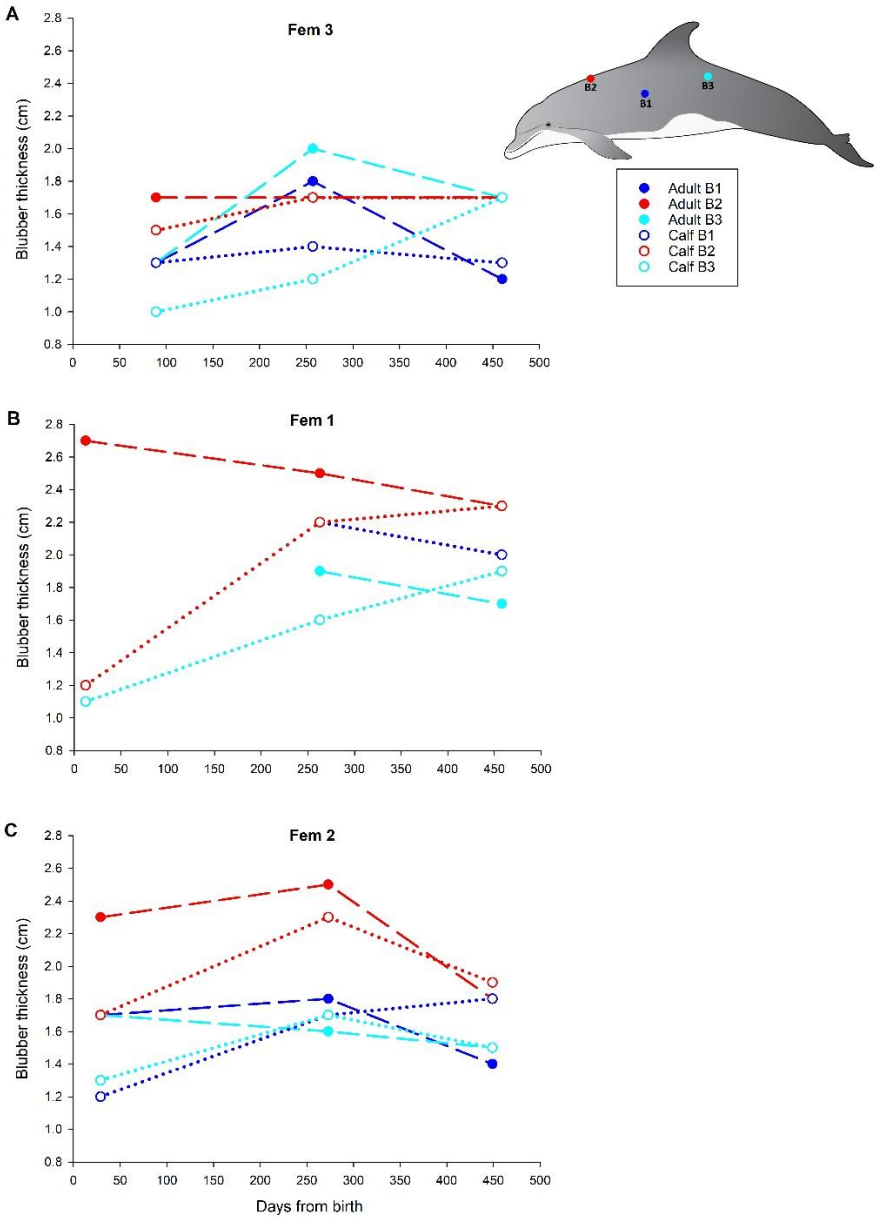

Figure 1. Blubber thicknesses at three sites on bottlenose dolphins during the lactation period. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Figures are presented in order of increasing female reproductive output. Blubber thicknesses at three sites on adult females (A) Fem 3, (B) Fem 1, and (C) Fem 2 (closed circles, dashed lines) and their calves (open circles, dotted lines) are presented in relation to days after birth. Lines simply connect sequential data points for clarification. Measurement locations are site B1 (blue, midline of the lateral surface, in line with the cranial insertion of the dorsal fin, site B2 (red, midline of the dorsal surface, in line with the caudal insertion of the pectoral fin), and site B3 (cyan, roughly ½ distance from the dorsal surface to the midline of the lateral surface, approximately 10 cm behind the caudal insertion of the dorsal fin). In panel B), Sites B1 and B3 were not measured on Fem 1 during the first measurement period, and blubber thicknesses for these two sites are identical for the later measurement periods (B3 closed circles and line overlap and hide those of B1). Blubber

thicknesses at sites B1 and B2 on Calf 1B are identical for the first two measurements (B2 open circles and line overlap and hide those of B1). In panels A) and C), all three sites were measured three times for all dolphins. Some measurements were identical, and thus, some data points overlap.

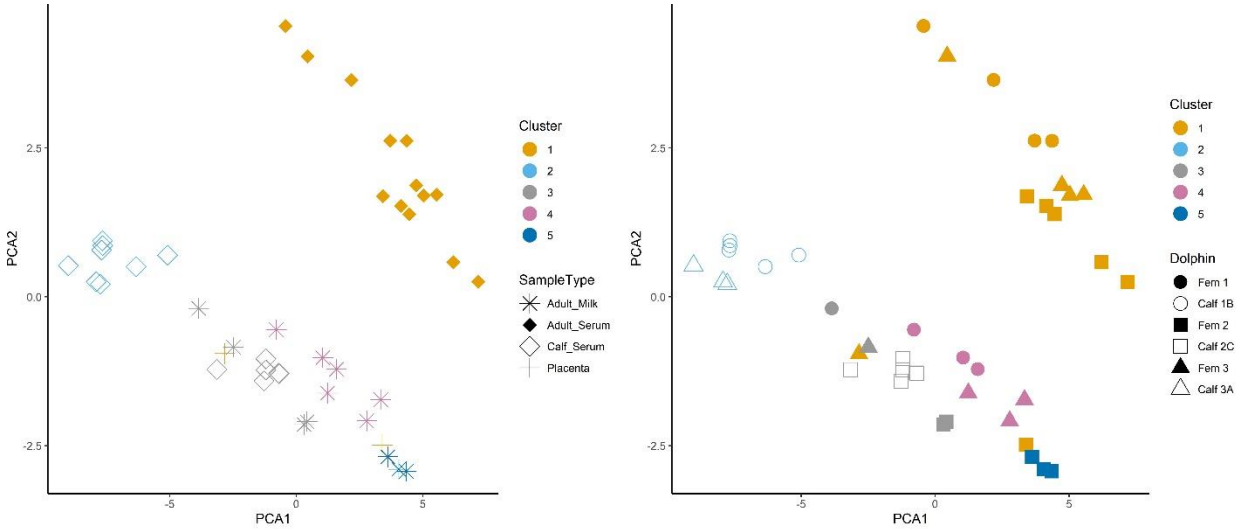

Figure 2. PCA plots of the log-transformed lipid adjusted contaminant data with all sample types (milk, calf serum, maternal serum, and placenta) from the three focal mother/calf pairs. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Shapes indicate the sample type (left panel) and individual dolphin (right panel). The Cluster colors indicate cluster designation from the Gaussian mixture model using expectation maximization.

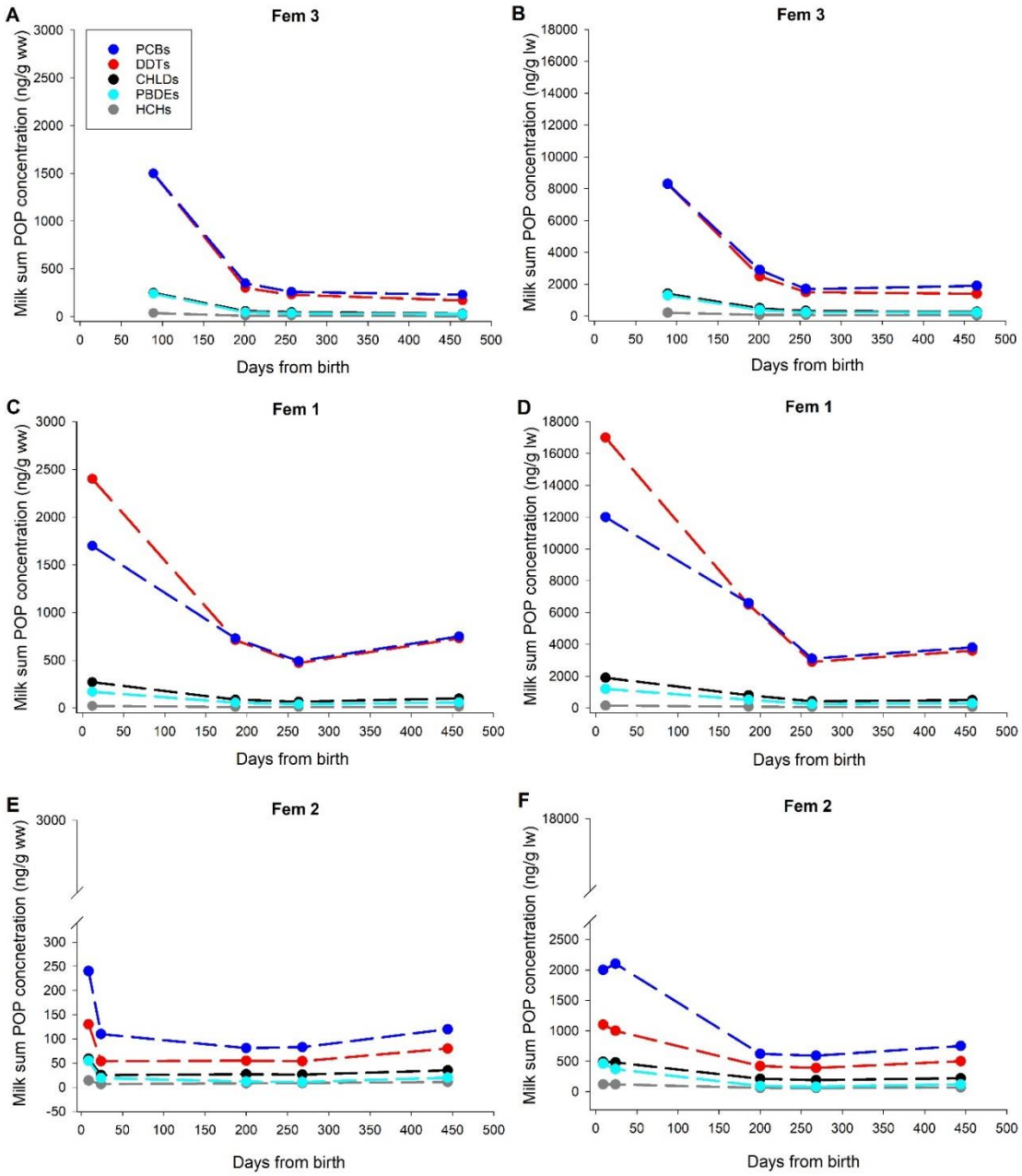

Figure 3. Milk sum POP concentrations in adult female bottlenose dolphins during the lactation period. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Figures are presented in order of increasing female reproductive output. Wet weight (ng/g wet weight, left panel) and lipid corrected (ng/g lw, right panel) sum POPs (calculated from all compounds within detectable limits) are presented for adult females [Fem 3 (A, B), Fem 1 (C, D), and Fem 2 (E, F)] in relation to days after birth. Dashed lines simply connect sequential data points for clarification.

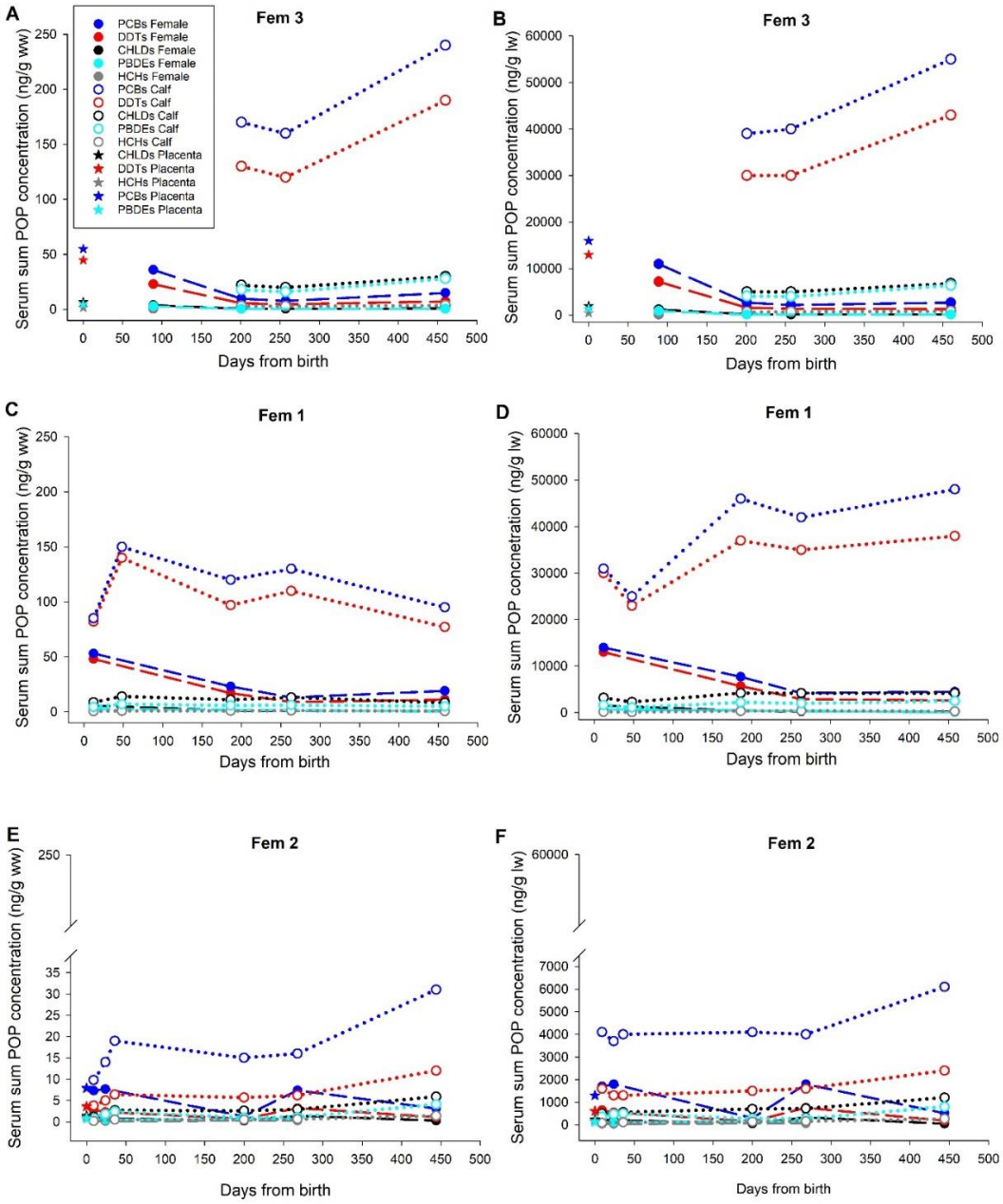

Figure 4. Placental and serum sum POP concentrations in adult female bottlenose dolphins and their calves during the lactation period. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Figures are presented in order of increasing female reproductive output. Wet weight (ng/g wet weight, left panel) and lipid corrected (ng/g lw, right panel) sum POPs (calculated from all compounds within detectable limits) are presented for adult females [Fem 3 (A, B), Fem 1 (C, D), and Fem 2 (E, F); closed circles, dashed lines)] and their calves (open circles, dotted lines) in relation to days after birth. Lines simply connect sequential data points for clarification. Placental POP concentrations (Fem 3 and Fem 2 only) are denoted by stars at day 0.

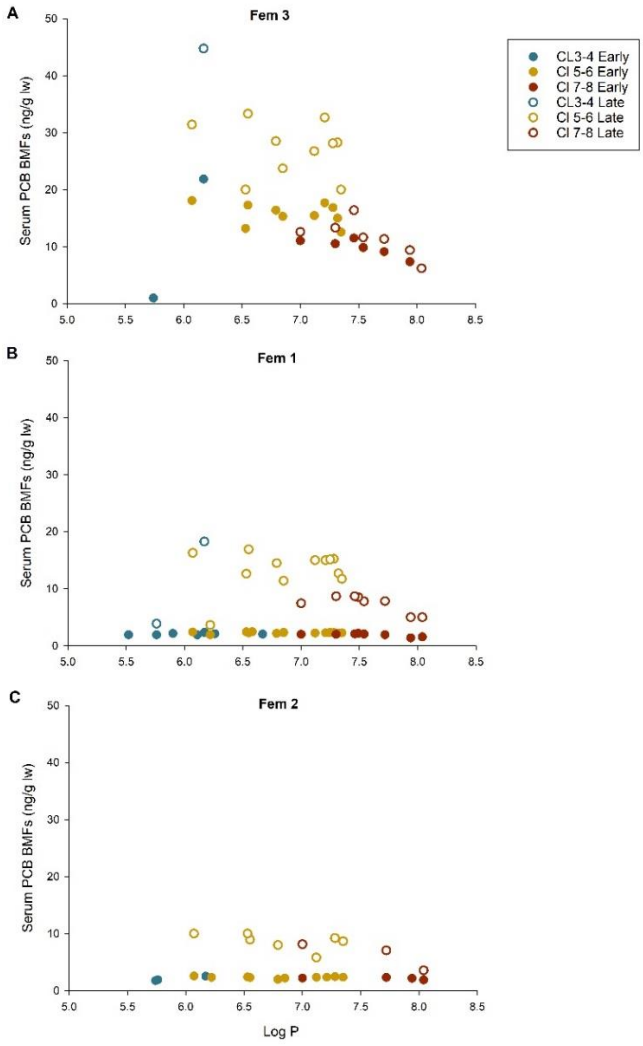

Figure 5. Serum PCB biomagnification factors (BMFs; calf serum/mother serum; ng/g lw) during early (closed circles) and late (open circles) lactation in relation to log P values of PCBs. Note that the BMFs were not calculated under typical steady state conditions. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Figures are presented in order of increasing female reproductive output. Early and late lactation serum samples were collected on the same day for each mother/calf pair, but collection day varied across mother/calf pairs. Early and late paired serum samples were taken after birth at 201 and 460 days for Fem 3 (A), 12 and 458 days for Fem 1 (B), and 9 and 444 days for Fem 2 (C), respectively. PCB congeners are delineated by degree of chlorination [(number of hydrogen atoms in the biphenyl that are replaced by chlorine atoms: 3-4 (blue), 5-6 (yellow), 7-8 (red)]. BMFs were only calculated when a compound was detectable in samples from both the female and her calf. Consequently, if a female eliminated a compound during lactation to levels below the LOQ, no value for late BMF is presented for that compound. The early BMF value is still presented for that compound, however. Average experimental (Exp) log P values were preferentially used, however in some cases, only average predicted (Pred) values were available (see Supplemental Table 9, data obtained from the United States Environmental Protection Agency (EPA) CompTox Chemicals Dashboard; https://comptox.epa.gov/dashboard/; accessed March 21, 2022).

Supplemental Table 1. Sum POP concentrations [ng/g lipid weight (lw) and wet weight (ww)] for each prey type fed to dolphin calves, beginning at approximately 3 months post-partum (87-106 days, depending on the calf).

Prey Type	∑PCBs lw	∑PCBs ww	∑PBDEs lw	∑PBDEs ww	∑DDTs lw	∑DDTs ww	∑CHLDs lw	∑CHLDs ww	∑HCHs lw	∑HCHs ww
Capelin	300	4.2	<LOQ	<LOQ	340	4.7	250	3.5	<LOQ	<LOQ
Herring	31	3.7	<LOQ	<LOQ	32	3.9	18	2.1	28	3.3
Squid	360	5	43	0.6	110	1.6	<LOQ	<LOQ	<LOQ	<LOQ

Supplemental Table 2. Sum POP concentrations (ng/g wet weight) for samples collected from all adult females near the time of parturition.

Subject	Age	Viable calf birth order (reproductive history)	Sample type	Days post-partum	∑PCBs	∑PBDEs	∑DDTs	∑CHLDs	∑HCHs
Fem 1	42[1]	2 (2 live births)	Placenta	NS	-	-	-	-	-
			Serum	12	53	2.6	48	5.6	0.35
			Milk	12	1700	170	2400	270	21
Fem 2[2]	22[1]	2 (2 live births)	Placenta	0	2.1	<LOQ	1.3	0.26	0.36
			Serum	NS	-	-	-	-	-
			Milk	NS	-	-	-	-	-
Fem 2	25[1]	3 (3 live births)	Placenta	0	7.9	0.71	3.6	1.4	0.99
			Serum	9	7.3	0.62	2.8	1.0	0.18
			Milk	9	240	55	130	59	14
Fem 3	5	1 (primiparous)	Placenta	0	55	4.6	45	6.8	1.7
			Serum	89[3]	36	2.9	23	3.9	0.43
			Milk	89[3]	1500	240	1500	250	37
Fem 4[4]	28	6 (6 live births, 1 late-term abortion)	Placenta	0	22	3.4	21	3.9	1.3
			Serum	0	23	4.5	19	1.9	<LOQ
			Milk	0	1800	360	1800	410	46
Fem 5[5]	25[1]	1 (primiparous, surrogate for Fem 4's Calf)	Placenta	NS	-	-	-	-	-
			Serum	846(10)[6]	8.9	<LOQ	3.2	<LOQ	<LOQ
			Milk	846(10)[6]	420	31	180	46	4.4
Fem 6	14	0 (stillborn)	Placenta	0	9.5	0.54	8.3	1.2	0.26
			Serum	1	37	3.3	25	3.6	<LOQ
			Milk	1	2600	320	2200	490	41

[1]Caught from a wild population at approximately 6 yrs (Fem 2, Fem 5) and 7 yrs (Fem 1) of age; age is estimated.
[2]Placenta from Fem 2's second birth provided for comparison to samples collected from her third birth.
[3]Due to Fem 3's young age and that she was a primiparous female, initial milk and serum collection was delayed.
[4]Fem 4 gave birth to a calf that also received milk from another female (Fem 5). Because contaminant influx was from two sources, Fem 4 was not sampled after the parturition date, and Fem 4's calf was never sampled.
[5]Fem 5, having given birth to her first calf about 2 yrs prior to the study, was a surrogate to Fem 4's calf.
[6]Fem 5 had given birth to her calf 846 days prior to when the initial milk and serum samples were collected. The samples were collected 10 days after Fem 4's calf was born. Fem 5, along with Fem 4, nursed Fem 4's calf.
NS = not sampled
<LOQ = the compound was below the lower limit of quantitation

Supplemental Table 3. Sum POP concentrations (ng/g lipid weight) for samples collected from all adult females near the time of parturition.

Subject	Age	Viable calf birth order (reproductive history)	Sample type	Days post-partum	Lipid (%)	∑PCBs	∑PBDEs	∑DDTs	∑CHLDs	∑HCHs
Fem 1	42[1]	2 (2 live births)	Placenta	NS	-	-	-	-	-	-
			Serum	12	0.37	14000	700	13000	1500	95
			Milk	12	13.69	12000	1200	17000	1900	150
Fem 2[2]	22[1]	2 (2 live births)	Placenta	0	0.37	570	<LOQ	350	70	97
			Serum	NS	-	-	-	-	-	-
			Milk	NS	-	-	-	-	-	-
Fem 2	25[1]	3 (3 live births)	Placenta	0	0.59	1300	120	610	240	170
			Serum	9	0.43	1700	140	650	230	42
			Milk	9	11.80	2000	460	1100	490	120
Fem 3	5	1 (primiparous)	Placenta	0	0.34	16000	1400	13000	2000	500
			Serum	89[3]	0.32	11000	910	7200	1200	130
			Milk	89[3]	17.79	8300	1300	8300	1400	210
Fem 4[4]	28	6 (6 live births, 1 late-term abortion)	Placenta	0	0.54	4100	630	3900	720	240
			Serum	0	0.19	12000	2400	10000	1000	<LOQ
			Milk	0	26.16	6900	1400	6900	1600	180
Fem 5[5]	25[1]	1 (primiparous, surrogate for Fem 4's Calf)	Placenta	NS	-	-	-	-	-	-
			Serum	846(10)[6]	0.00[7]	-	-	-	-	-
			Milk	846(10)[6]	11.18	3800	280	1600	420	40
Fem 6	14	0 (stillborn)	Placenta	0	0.16	5900	340	5200	750	160
			Serum	1	0.00[7]	-	-	-	-	-
			Milk	1	21.75	12000	1500	10000	2200	190

[1]Caught from a wild population at approximately 6 yrs (Fem 2, Fem 5) and 7 yrs (Fem 1) old, so age is estimated.
[2]Placenta from Fem 2's second birth provided for comparison to samples collected from her third birth.
[3]Due to Fem 3's young age and that she was a primiparous female, initial milk and serum collection was delayed.
[4]Fem 4 gave birth to a calf that also received milk from another female (Fem 5). Because contaminant influx was from two sources, Fem 4 was not sampled after the parturition date, and Fem 4's calf was never sampled.
[5]Fem 5, having given birth to her first calf about 2 yrs prior to the study, was a surrogate to Fem 4's calf.
[6]Fem 5 had given birth to her calf 846 days prior to when the initial milk and serum samples were collected. The samples were collected 10 days after Fem 4's calf was born. Fem 5, along with Fem 4, nursed Fem 4's calf.
[7]Lipid content was underestimated to be 0.00, which precluded lipid correction.
NS = not sampled
<LOQ = the compound was below the lower limit of quantitation

Supplemental Table 4 Principal Components Analysis (PCA) loading weights. This analysis only includes the three focal females and their calves (Milk, n=13; Maternal Serum, n=13; Calf Serum, n=14; Placenta, n=2).

	CS (n=14)	MS (n=13)	Milk (n=13)	All sample types (n=42)	All sample types (n=42)
	PC1	PC1	PC1	PC1[a]	PC2[a]
Eigenvalue, Percent variance	23.5, 87.2%	14.0, 73.9%	21.2, 78.4%	20.3, 75.0%	3.6, 13.4%
PCB3	-0.152	-0.204	-0.164	-0.202	0.109
PCB4	-0.205	-0.260	-0.213	-0.223	x
PCB5	-0.204	-0.263	-0.214	-0.26	x
PCB6	-0.201	-0.236	-0.186	-0.278	x
PCB7	-0.197	-0.219	-0.183	-0.287	x
PCB8/9	-0.185	-0.201	-0.183	-0.279	x
BDE47	-0.197	-0.230	-0.209	-0.206	x
BDE99	-0.192	NA	-0.209	x	-0.344
BDE100	-0.199	NA	-0.200	x	-0.333
p,p'-DDD	-0.205	-0.262	-0.212	-0.215	x
p,p'-DDE	-0.198	-0.225	-0.180	-0.265	x
p,p'-DDT	-0.204	-0.248	-0.212	-0.201	x
o,p'-DDD	-0.204	NA	-0.208	x	-0.338
o,p'-DDE	-0.201	-0.242	-0.194	-0.254	x
o,p'-DDT	-0.196	-0.233	-0.204	-0.239	x
hexachlorobenzene	-0.191	-0.185	-0.175	-0.162	-0.117
α-HCH	-0.114	NA	-0.135	x	-0.336
β-HCH	-0.191	-0.228	-0.190	-0.164	-0.108
lindane	-0.150	NA	-0.108	x	-0.289
trans-nonachlor	-0.204	-0.252	-0.199	-0.256	x
dieldrin	-0.198	-0.221	-0.191	-0.191	x
cis-chlordane	-0.189	-0.199	-0.161	-0.15	-0.139
cis-nonachlor	-0.200	-0.220	-0.205	-0.183	-0.109
heptachlor epoxide	-0.201	NA	-0.206	x	-0.335
nonachlor III	-0.202	NA	-0.211	x	-0.317
oxychlordane	-0.202	NA	-0.215	x	-0.325
mirex	-0.185	-0.206	-0.183	-0.264	0.150

a: loadings varimax rotated

NA = greater than 80% of the samples were below the lower limit of quantitation so the compound was not included in the analysis.

x = loading value less than the absolute value of 0.1

Supplemental Table 5. Attributes of samples in each cluster

Mclust cluster	Total samples in the cluster	Predominant samples in the cluster Number of each sample type (individual and days collected post-partum)
1	15	13 Maternal serum (Fem 1 days 12, 186, 263, 458; Fem 3 days 89, 201, 257, 460; Fem 2 days 9, 24, 200, 268, 444), 2 Placenta (Fem 3, Fem 2)
2	8	8 Calf serum (Calf 1B days 12, 48, 186, 263, 458; Calf 3A days 201, 257, 460)
3	10	4 Milk (Fem 1 day 12; Fem 3 day 89; Fem 2 days 9, 24) 6 Calf serum (Calf 2C days 9, 24, 36, 200, 268, 444)
4	6	6 Milk (Fem 1 days 186, 263, 458; Fem 3 days 201, 257, 465)
5	3	3 Milk (Fem 2 days 200, 268, 444)

Supplemental Table 6. Proportional change over the lactation period for each sum POP class. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Females are presented in order of increasing female reproductive output. Proportional changes in milk, maternal serum, and calf serum were calculated two ways: using R153 values (each [∑POP] was divided by [CB153] prior to performing calculations) and using lipid corrected values (each [∑POP] was corrected for lipid content prior to performing calculations). Negative values indicate reductions, while positive values indicate gains over the lactation period.

Subject	Days from birth to first sample	Days between first and last samples	POPs	Proportional change over lactation period					
				Adult Female				Calf	
				Serum		Milk		Serum	
				R153	Lipid corr.	R153	Lipid corr.	R153	Lipid corr.
Fem 3[1]	89 (Fem serum) 89 (Fem milk) 201 (Calf serum)	371 (Fem serum) 376 (Fem milk) 259 (Calf serum)	∑PCBs	-0.08	-0.75	-0.09	-0.77	-0.06	+0.41
			∑CHLDs	-0.45	-0.85	-0.26	-0.81	-0.09	+0.36
			∑DDTs	-0.30	-0.82	-0.32	-0.83	-0.03	+0.43
			∑PBDEs	-0.57	-0.89	-0.35	-0.83	+0.04	+0.56
			∑HCHs	<LOQ[2]	<LOQ[2]	-0.21	-0.80	-0.20	+0.20
Fem 1	12	446	∑PCBs	+0.08	-0.68	-0.04	-0.68	-0.02	+0.55
			∑CHLDs	-0.49	-0.85	-0.19	-0.74	-0.17	+0.31
			∑DDTs	-0.31	-0.80	-0.34	-0.79	-0.18	+0.27
			∑PBDEs	-0.62	-0.89	-0.25	-0.75	-0.01	+0.56
			∑HCHs	<LOQ[2]	<LOQ[2]	+0.24	-0.60	+0.21	+0.81
Fem 2	9	435	∑PCBs	-0.14	-0.70	0.000	-0.63	+0.11	+0.49
			∑CHLDs	-0.41	-0.79	+0.19	-0.55	+0.73	+1.40
			∑DDTs	-0.20	-0.72	+0.23	-0.55	+0.11	+0.50
			∑PBDEs	<LOQ[2]	<LOQ[2]	-0.27	-0.74	+0.66	+1.22
			∑HCHs	<LOQ[2]	<LOQ[2]	+0.57	-0.43	+1.46	+2.25

[1]Because Fem 3 was a young, primparous female, initial sample collection was delayed. Maternal serum, milk, and calf serum were initially collected on different days, and the days between initial and final collections also differed, so they are indicated separately. Matched - samples from the other two female/calf pairs were collected on the same day.

[2]<LOQ = not determined because the compound was below the lower limit of quantitation for at least one of the samples required for the calculation

Supplemental Table 7. Mixed effects model results. This analysis only includes the three focal females and their calves. Estimates reflect change in vector loading over time (i.e., collection days post-partum)

Sample type	Model type	Estimate (Std Err)	p-value
Milk, PC1	Collection day	-0.011 (0.002)	0.002
Maternal serum, PC1	Collection day	-0.009 (0.002)	0.007
Calf serum, PC1	Collection day	+0.005 (0.001)	0.001

Supplemental Table 8. Biomagnification factors (BMFs; ng/g lipid weight and R153 wet weight) during early and late lactation. Female numbers are based on age, from oldest (Fem 1) to youngest (Fem 3). Females are presented in order of increasing female reproductive output. BMFs were determined between calf (C) serum and adult (A) serum as well as calf serum and adult milk. Note that the BMFs were not calculated under typical steady state conditions.

		Biomagnification Factor							
		Early Lactation				Late Lactation			
Dolphin	POPs	C Serum/A Serum		C Serum/Milk		C Serum/A Serum		C Serum/Milk	
		lw	R153	lw	R153	lw	R153	lw	R153
Fem 3[1]	∑PCBs	14.4	1.08	13.4	1.07	20.4	1.04	28.9	0.99
	∑CHLDs	25.0	1.94	10.2	0.82	37.8	1.99	26.2	0.92
	∑DDTs	18.8	1.43	12.0	0.95	33.1	1.69	30.7	1.06
	∑PBDEs	34.2	2.50	10.8	0.86	64.0	3.19	29.1	1.02
	∑HCHs	<LOQ[4]	<LOQ[4]	8.4	0.68	<LOQ[4]	<LOQ[4]	20.5	0.72
Fem 1[2]	∑PCBs	2.2	0.92	2.6	0.88	10.7	0.83	12.6	0.90
	∑CHLDs	2.1	0.89	1.7	0.57	18.3	1.46	8.4	0.59
	∑DDTs	2.3	0.98	1.8	0.60	14.6	1.17	10.6	0.75
	∑PBDEs	2.3	0.97	1.3	0.46	31.6	2.53	8.3	0.60
	∑HCHs	2.1	0.69	1.1	0.35	<LOQ[4]	<LOQ[4]	4.8	0.34
Fem 2[3]	∑PCBs	2.4	0.99	2.1	0.82	12.0	1.28	8.1	0.91
	∑CHLDs	2.2	0.88	1.0	0.41	25.0	2.60	5.5	0.59
	∑DDTs	2.5	1.00	1.5	0.58	13.3	1.39	4.8	0.53
	∑PBDEs	2.6	1.03	0.8	0.3	<LOQ[4]	<LOQ[4]	6.7	0.72
	∑HCHs	2.0	0.82	0.7	0.29	<LOQ[4]	<LOQ[4]	3.9	0.45

[1]Fem 3 early BMF calculated from samples collected 201 days post-partum, late BMF calculated for samples collected 460 days (A and C serum) and 465 days (milk) post-partum.

[2]Fem 1 early and late BMFs calculated from samples collected 12 and 458 days post-partum, respectively.

[3]Fem 2 early and late BMFs calculated from samples collected 9 and 444 days post-partum, respectively.

[4]<LOQ = not determined because the compound was below the lower limit of quantitation for at least one of the samples required for the calculation

Supplemental Table 9. Chemical properties of compounds quantified in samples from bottlenose dolphins. Average molecular mass (mol mass, g mol^{-1}) and log P (Exp or Pred) as well as the chlorination (Cl) of PCBs and organochlorine compounds and the bromination (Br) of PBDEs are reported. Data were obtained from the United States Environmental Protection Agency (EPA) CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/; accessed March 25, 2022). Average experimental (Exp) log P values were preferred. However in some cases, only average predicted (Pred) log P values were available, and in one case, there were no log P data available (NA).

POP (mol mass)	Exp	Pred	Cl	POP (mol mass)	Exp	Pred	Cl	POP (mol mass)	Exp	Pred	Cl or Br
CB17 (257.54)	5.76		3	CB156 (360.86)	7.58		6	*cis*-chlordane (409.76)	6.16		8
CB18 (257.54)	5.52		3	CB158 (360.86)	7.25		6	*trans*-chlordane (409.76)		5.87	8
CB28 (257.54)	5.62		3	CB170 (395.31)		7.54	7	oxychlordane (423.74)		5.09	8
CB31 (257.54)	5.74		3	CB171 (395.31)		7.49	7	*trans*-nonachlor (444.2)		6.09	9
CB33 (257.54)	5.87		3	CB177 (395.31)		7.46	7	*cis*-nonachlor (444.20)		6.09	9
CB44 (291.98)	5.90		4	CB180 (395.31)	7.72		7	nonachlor III (444.20)	NA	NA	9
CB49 (291.98)	6.26		4	CB183 (395.31)	7.30		7	BDE28 (406.90)	5.94		3
CB52 (291.98)	6.17		4	CB187/159/182 (395.31)	7.00		7	BDE47 (485.79)	6.81		4
CB66 (291.98)	6.11		4	CB191 (395.31)		7.52	7	BDE49 (485.79)		6.85	4
CB70 (291.98)	6.31		4	CB194 (429.75)	8.04		8	BDE66 (485.79)		6.83	4
CB74 (291.8)	6.67		4	CB195 (429.75)		7.98	8	BDE85 (564.69)	7.37		5
CB82 (326.42)		6.58	5	CB199 (429.75)		7.94	8	BDE99 (564.69)	7.32		5
CB87 (326.42)	6.85		5	CB205 (429.75)		8.01	8	BDE100 (564.69)	7.24		5
CB95 (326.42)	6.55		5	CB206 (464.19)	8.92		9	BDE153 (643.58)	7.90		6
CB99 (326.42)	7.21		5	CB208 (464.19)	8.16		9	BDE154 (643.58)	7.82		6
CB101/90 (326.42)	6.07		5	CB209 (498.63)	8.38		10	BDE183 (722.48)	8.27		7
CB105 (326.42)	6.79		5	*o,p*'-DDD (320.03)		5.97	4	lindane (γ-HCH) (290.81)	3.72		6
CB110 (326.42)	6.22		5	*p,p*'-DDD (320.03)	6.12		4	α-HCH (290.81)	3.72		6
CB118 (326.42)	7.12		5	*o,p*'-DDE (318.02)		6.21	4	β-HCH (290.81)	3.72		6
CB128 (360.86)	7.32		6	*p,p'*-DDE (318.02)	6.73		4	hexachlorobenzene (284.77)	5.73		6

CB138/163/164 (360.86)	7.35	6	*o*,*p*'-DDT (354.48)		6.46	5	Aldrin (364.90)		6.50	6
CB149 (360.86)	7.28	6	*p*,*p*'-DDT (354.48)	6.91		5	Dieldrin (380.90)	4.97		6
CB151 (360.86)	6.85	6	heptachlor epoxide (389.30)	4.98		7	Endosulfan I (406.90)		3.83	6
CB153/132 (360.86)	6.53	6	heptachlor (373.30)	6.10		7	Mirex (545.51)		6.89	12

CRediT author statement

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