

Emerging Contaminants and New POPs (PFAS and HBCDD) in Endangered Southern Resident and Bigg's (Transient) Killer Whales (*Orcinus orca*): In Utero Maternal Transfer and Pollution Management Implications

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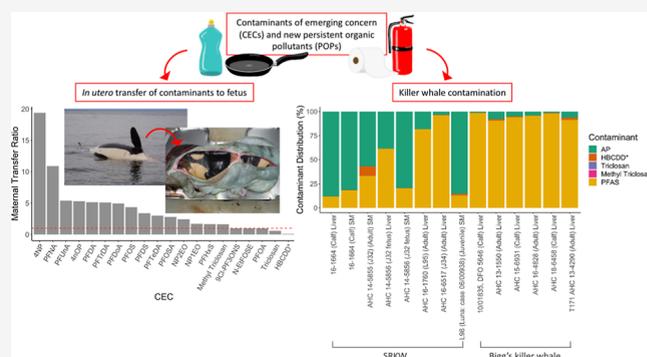
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Supporting Information

ABSTRACT: Killer whales (*Orcinus orca*) have been deemed one of the most contaminated cetacean species in the world. However, concentrations and potential health implications of selected ‘contaminants of emerging concern’ (CECs) and new persistent organic pollutants (POPs) in endangered Southern Resident and threatened Bigg’s (Transient) killer whales in the Northeastern Pacific (NEP) have not yet been documented. Here, we quantify CECs [alkylphenols (APs), triclosan, methyl triclosan, and per- and polyfluoroalkyl substances (PFAS)] and new POPs [hexabromocyclododecane (HBCDD), PFOS, PFOA, and PFHxS] in skeletal muscle and liver samples of these sentinel species and investigate *in utero* transfer of these contaminants. Samples were collected from necropsied individuals from 2006 to 2018 and analyzed by LC–MS/MS or HRBC/HRMS. AP and PFAS contaminants were the most prevalent compounds; 4-nonylphenol (4NP) was the predominant AP (median 40.84 ng/g ww), and interestingly, 7:3-fluorotelomer carboxylic acid (7:3 FTCA) was the primary PFAS (median 66.35 ng/g ww). Maternal transfer ratios indicated 4NP as the most transferred contaminant from the dam to the fetus, with maternal transfer rates as high as 95.1%. Although too few killer whales have been screened for CECs and new POPs to infer the magnitude of contamination impact, these results raise concerns regarding pathological implications and potential impacts on fetal development and production of a viable neonate. This study outlines CEC and new POP concentrations in killer whales of the NEP and provides scientifically derived evidence to support and inform regulation to mitigate pollutant sources and contamination of Southern Resident killer whale critical habitat and other marine ecosystems.

KEYWORDS: marine ecotoxicology, contaminants of emerging concern, endangered killer whales, alkylphenols, per- and polyfluoroalkyl substances, maternal transfer



INTRODUCTION

The ubiquity of anthropogenic chemical contaminants and ocean pollution is a significant concern to human health, and marine ecosystems and biodiversity.^{1–3} Bioaccumulative and toxic pollutants such as PFOS, PFOA, PFHxS [all classified as per- and polyfluoroalkyl substances (PFAS)], and hexabromocyclododecane (HBCDD) have recently been added to the Stockholm convention on persistent organic pollutants (POPs).⁴ Although regulated under this organization, such new POPs can still be manufactured in several countries and can be produced as byproducts of certain chemicals;⁴ for example, HBCDD is still used as a flame retardant in polystyrene materials. Another group of chemicals termed ‘contaminants of emerging concern’ (CECs) including alkylphenols (AP), triclosan, methyl triclosan, and other PFAS compounds have been detected in the marine environ-

ment; however, they are not well understood in this context and, consequently, are not well regulated. Both CECs and new POPs can be found in everyday products such as pesticides, surfactants, flame retardants, antibacterial consumer items, and water-repellant materials.^{5–8} They may be inefficiently removed in wastewater treatment plants and may be poorly monitored in industrial, agricultural, and residential leaks and runoffs.^{9,10}

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Table 1. Biometrics and Descriptions of Analyzed Tissue in the 12 Stranded Killer Whales (*O. orca*) Collected in British Columbia, Canada, 2006–2018

killer whale identification (ID)	recovery date	location	age category	age estimate (year)	sex	ecotype	sample(s) analyzed (SM = skeletal muscle)	carcass condition code	body condition index (BCI)	percent (%) lipid	pathological findings (Raverty et al.; ³⁸ unpublished data)	total contaminant concentration (ng/g in ww, lw)	dominant contaminant
L98 (Luna: case 06/00938)	2006-03-10	Nootka Sound, Gold River, BC	Juvenile	7	Male	SRKW	SM	2	NA	7.76	Trauma (boat strike).	136.3, 1756.5	4NP
10/01835, DFO 5646	2010-05-04	Sooke, BC	Neonate	0.1 ^a	Male	Bigg's	Liver	3	NA	3.65	Failure of passive transfer of maternal antibodies/live strand.	554.3, 15185	7:3 FTCA
AHC 13-1550	2013-04-13	Carmanah Beach, BC	Adult	NA	Female	Bigg's	Liver	4	NA	13.4	Autolysis, possible subcutaneous hematoma around blow-hole.	186.7, 1393.2	7:3 FTCA
T171 AHC 13-4290	2013-10-18	Prince Rupert, BC	Adult	29	Female	Bigg's	Liver	Late 3	0.56	10.4	Vertebral bridging spondylosis with emaciation.	1028.3, 9887.9	7:3 FTCA
AHC 14-5855 (J32 Mother)	2014-12-06	Comox, BC	Adult	18	Female	SRKW	SM	3	0.65	2.76	Dystocia and endometrial perforation.	25.6, 927.8	NP1EO
AHC 14-5856 (J32 Fetus)	2014-12-06	Comox, BC	Fetus	NA	Female	SRKW	SM + Liver	3	0.73	5.9 (SM) + 4.48 (Liver)	Fetal breech presentation.	125.7, 2130.2 (SM) + 306.5, 6840.7 (Liver)	4NP
AHC 15-6931	2015-12-25	Tofino, BC	Neonate	0.1 ^a	Female	Bigg's	Liver	3	0.05	3.83	Presumptive metabolic derangements, hypoglycemia, possible dystocia.	516.9, 13495.1	7:3 FTCA
16-1664	2016-03-25	Sooke	Neonate	0.1 ^a	Female	SRKW	SM + Liver	3	NA	1.3 (SM) + 16.3 (Liver)	Trauma, inadvertent or deliberate aggression from pod mates, con-specifics, or mother (mis-mothering).	197.2, 15171.9 (SM) + 3799.0, 23306.7 (Liver)	4NP
AHC 16-1760 (L95)	2016-03-31	Esperanza Inlet, BC	Adult	20	Male	SRKW	Liver	4	NA	11.3	Mucormycosis secondary to satellite tag implant	423.1, 3744	PFOSA
AHC 16-4828	2016-09-15	Pachena Bay, BC	Adult	34	Male	Bigg's	Liver	3	0.6	9.36	Trauma, possible vessel strike and aggression by conspecifics.	476.5, 5091	7:3 FTCA
AHC 16-6517 (J34)	2016-12-20	Sechelt, BC	Adult	18	Male	SRKW	Liver	3	NA	4.32	Trauma, possible vessel strike, left thorax.	175.3, 4057.2	PFOSA
AHC 18-6458	2018-11-14	Nootka Island, BC	Neonate	0.1 ^a	Unknown	Bigg's	Liver	3	NA	3.96	Failure to thrive, possible fetal distress, hypoglycemia and emaciation.	591.7, 14942.5	7:3 FTCA

^aAll neonates were considered to be 1 month old (~0.1 year). Note: SRKW = southern resident killer whale, NA = not available.

Little is known about the fate and consequences of many CECs (hereafter referring to AP, triclosan, methyl triclosan, and selected PFAS compounds) and new POPs (hereafter referring to PFOS, PFOA, PFHxS, and HBCDD) in killer whales. Some of these chemicals are persistent and have a strong propensity to be present in these mammals due to constant exposure or accumulate in various tissues. Their concentrations can biomagnify throughout the food web, threatening the health of higher trophic-level marine mammals.^{11–14} Further background information on the contaminants targeted in this study can be found in the [Supporting Information](#).

In the northeastern Pacific (NEP) ocean, there are three recognized killer whale (*Orcinus orca*) ecotypes: Bigg's (Transient), Resident, and Offshore killer whales, each being genetically distinct with different behaviors, culture, socialization, and feeding ecology.^{15,16} Facing a population extinction probability of 26%,¹⁴ the Southern Resident killer whale (SRKW) is considered one of the most endangered marine mammals in the world. With a small population of approximately 73 to 74 individuals, its main threats include scarce food availability (i.e., quantity and quality of its main prey, Chinook salmon), anthropogenic disturbances (i.e., maritime traffic, acoustic pollution), and chemical pollution.^{17,18}

Recently, CECs and new POPs have been detected in killer whales stranded in Greenland and Norway and other odontocetes in New Zealand, with maternal transfer and feeding ecology playing an important role in contamination burdens.^{19–21} Recent research on legacy contaminant exposure in SRKW critical habitat and POP modulation in SRKWs showed effects on lipid-remobilization, reproductive hormones, pregnancy failure, and viable calf production based on primary prey (Chinook salmon, *Oncorhynchus tshawytscha*) availability, fecal contaminant loads, and reproductive status.^{11,18,22,23} As free-ranging killer whales are long lived, top predators, and have large amounts of fat storage, they are at higher risk of pollution accumulation and adverse health effects including population decline; recent research claims these species are among the most contaminated cetaceans in the world.^{24–29}

Screening of selected CECs and new POPs has yet to be undertaken in any of the three ecotypes of known killer whale populations frequenting the coast of British Columbia (BC), Canada.³⁰ While our scientific understanding of the state of marine pollution in the NEP is limited,¹¹ the ubiquity of CECs and other legacy chemical contaminants suggests exposure and possible biomagnification in marine mammals inhabiting BC coastal waters.^{31–33} A major objective of the resident killer whale recovery strategy outlined by Fisheries and Oceans Canada is to “ensure that chemical and biological pollutants do not prevent the recovery of resident killer whale populations”.¹⁸ Therefore, the aim of the present study is as follows: (1) conduct the first assessment of selected CECs and new POPs in liver and skeletal muscle (SM) samples collected from SRKWs and Biggs killer whales stranded from 2006 to 2018 in BC and (2) investigate *in utero* transfer of these pollutants in a pregnant SRKW. These data will provide regulatory policy new information to support risk management and control of specific contaminant sources and enhance the conservation of killer whales both in the NEP and globally.

MATERIALS AND METHODS

Ethics Declaration. The study involved the post mortem examination of dead and stranded killer whales under permit from the Department of Fisheries and Oceans Canada (DFO licence number XMMS 2 2021) with no live animal capture or sampling.

Tissue Sampling and Additional Data Collection. SM ($n = 4$) and liver ($n = 10$) samples were collected from twelve stranded individuals along the coast of BC from 2006–2018 (Figure S1) according to standardized necropsy protocols.³⁴ Morphometrics were compiled and the stranding location, date, age category and estimates (six adults, one juvenile, four neonates, one fetus), sex (five males, six females, one unknown), ecotype (six SRKWs, six Bigg’s killer whales), class, and carcass condition code were recorded, following protocols by Raverty et al.³⁵ (Table 1). Individual animals were identified, and age classes were assigned according to morphometrics, photo-identification, and comparison with individual distinguishing features detailed in population catalogues.³⁶ The three ecotypes of killer whales described in the NEP were determined based on morphology via long-term photo-identification, dietary preference, genetics, social organization/culture, behavioral traits, vocal habits, and geographic range.^{15,16,37} Further sample collection protocols can be found in the Supporting Information.

A carcass condition code and body condition index (BCI) for killer whales were obtained through published data and

case reports.³⁸ Carcass condition code criteria are based on Geraci & Loundsbury:³⁹ 1 is a live animal; 2 is freshly dead; 3 is fair condition (early stage of decomposition, but organs essentially intact); 4 is in poor condition (advanced decomposition); and 5 is a mummified carcass or skeletal remains. BCI was calculated as a function of the individual killer whale’s girth and length (i.e., $BCI = \text{girth}/\text{length}$) and ranges from poor ($BCI = 0.5–0.6$) to good ($BCI = 0.6–0.7$) values.³⁸ Individuals that may have been artificially inflated to mimic bloating or pregnancy have a BCI ranging from 0.7–0.8. BCI data were obtained for five individuals (Table 1).

Analytical Methods. The signalment, tissue sample inventory, and ecotypes of archived killer whale samples were transported to SGS AXYS Analytical Services Ltd. (Sidney, BC) for organic chemical analysis. Tissue samples were processed for a total of 49 contaminants, including four AP [4-nonylphenol (4NP), 4-*n*-octylphenol (4nOP), nonylphenol mono-ethoxylate (NP1EO), and nonylphenol di-ethoxylate (NP2EO)], three HBCDD (alpha, beta, and gamma HBCDD), triclosan, methyl triclosan, and 40 PFAS (see Table S1 for a full list of analytes and raw data for each contaminant). The description of the analytical procedures for the target contaminants and quality assurance/quality control details can be found in the Supporting Information.

Data Treatment and Statistical Analysis. Data treatment and statistical analyses were performed using RStudio version 4.0.2. Contaminant concentrations were blank-corrected by subtracting the concentration in the associated method blank [method detection limit (MDL)] for each analyte from the sample concentration to account for background contamination throughout laboratory analyses (see Table S1 for MDL values). Samples with no contaminant detection were substituted with either 1/2 half of the blank concentration ($[\text{blank}]/2$) or blank reporting limit (RL) divided by the square-root of two ($RL/\sqrt{2}$) if no blank contaminant concentration was detected.^{20,40,41} These adjusted samples and those analytes with contaminant concentrations below the associated blank were not blank corrected. Contaminants that were equal in concentration to the blank sample were replaced with values derived from the $[\text{blank}]/2$ calculation. Sample RLs for AP data ranged from 0.478 to 12 ng/g wet weight (ww); HBCDD from 0.0933 to 0.177 ng/g ww; triclosan from 0.0002 to 0.0005 ng/g ww; methyl triclosan from 0.0004 to 0.0025 ng/g ww; and PFAS from 0.093 to 3.36 ng/g ww (Table S1). If more than 50% of samples were reported as not detected (ND) for a given contaminant, the contaminant was no longer considered in the present study.^{20,42} Taking this into account, only 21 out of the 49 total contaminants were analyzed in this study.

Contaminants are reported in wet weight (ng/g ww) with the exception of HBCDD, which is reported in lipid weight (ng/g lw) unless otherwise stated (Table S2). Wet weight reporting is the most rational and frequent type of reporting in the literature for most CECs and protein binding PFAS, while lipid weight is generally used in conjunction with wet weight when reporting concentrations for lipophilic and hydrophobic compounds. For example, PFAS contaminants are normally reported in wet weight as these substances preferentially associate with proteins, while HBCDD compounds primarily associate with lipids (lipophilic). Therefore, in the present study, HBCDD was reported on a lipid-weight basis.

All statistical data analyses used a significance level of 0.05 ($\alpha = 0.05$). The contaminant data were tested for normal

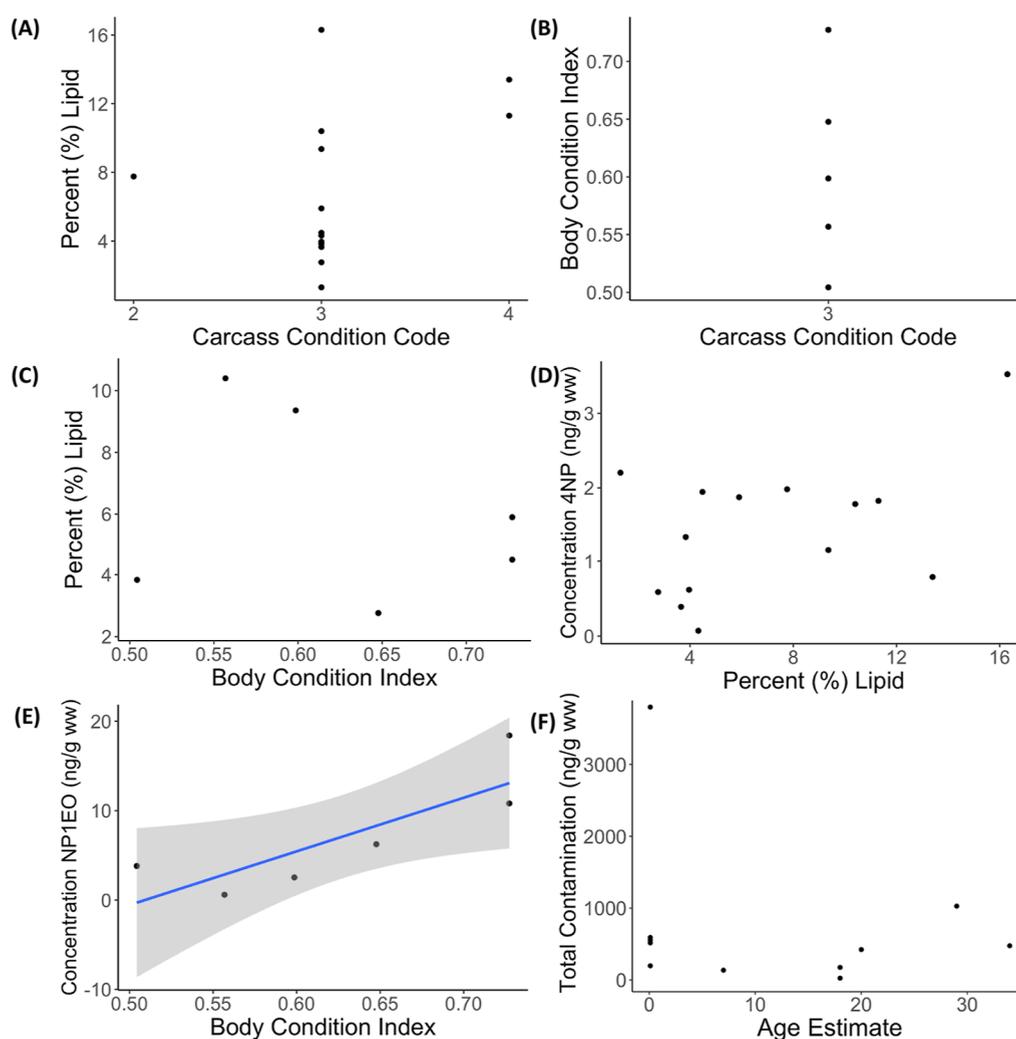


Figure 1. Confounding variable correlation analyses. (A) Relationship between carcass condition code and percent (%) lipid in each killer whale sample ($p = 0.21$). (B) Relationship between carcass condition code and body condition index (BCI). (C) Correlation of BCI with percent (%) lipid ($p = 0.62$). (D) Relationship between percent (%) lipid and contaminant concentration (4NP as an example, $p = 0.26$). (E) Significant positive relationship between BCI and contaminant concentration (NP1EO as an example, $p = 0.041$, $r = 0.83$). (F) Correlation between age estimate and total contamination concentration in each sample ($p = 0.46$). Note in (F): the age of two killer whales are unknown (Table 1) and were thus not included, and HBCDD is included as wet weight. Carcass condition code criteria are based on Geraci & Loundsbury:³⁹ 1 is a live animal; 2 is freshly dead; 3 is fair condition (decomposed but organs basically intact); 4 is in poor condition (advanced decomposition); and 5 is a mummified carcass or skeletal remains. BCI is a function of the individual killer whale's girth and length ($BCI = \text{girth}/\text{length}$) and ranges from poor values ($BCI = 0.5\text{--}0.6$) to good ($BCI = 0.6\text{--}0.7$).³⁸ Individuals that may have been artificially inflated to mimic bloating or pregnancy have a BCI ranging from $0.7\text{--}0.8$. Best-fit lines are denoted in blue with 95% confidence levels shown in dark gray for significant correlations only. CEC and New POP Exposure in SRKW and Bigg's Killer Whales.

distribution using the Shapiro Wilk test, and for homogeneity of variance using the Brown–Forsythe test. Correlation analyses (Pearson or Spearman) were used to examine correlations between carcass condition codes, lipid content, BCI, and contaminant concentrations. For comparisons between variables, the non-parametric Wilcoxon Rank Sum Exact test was applied to non-normal data, while the parametric Welch's Two Sample t -test was used for normally distributed data.

Maternal Transfer Assessment. For assessing *in utero* transfer of CECs and new POPs, a mother-fetus SM sample pair, AHC 14–5855 (J32 Mother) and AHC 14–5856 (J32 Fetus) (Table 1), was available. Maternal transfer ratios (MTRs) were based on SM contaminant concentrations and calculated for each contaminant [i.e., (contaminant concentration in J32 Fetus SM)/(contaminant concentration in J32

Mother SM)] to assess the proportion of contaminant concentrations observed in the fetus relative to concentrations in the mother. Any resulting values above one (i.e., $MTR > 1$) indicate efficient and preferential exposure of the contaminant from the mother to the fetus through the placenta, while $MTR < 1$ for a given contaminant is indicative of scarce or lack of maternal transfer. Any contamination detected in J32 Fetus demonstrates maternal transfer. These ratios were then correlated with $\log K_{ow}$ values corresponding to the respective contaminant to explore whether K_{ow} (a criterion of contaminant lipophilicity and bioaccumulation potential) influenced transplacental maternal transfer of contaminants. Maternal transfer rates (%) were also calculated based on SM mass contaminant concentrations by applying the following formula reported in Gebbink et al.:²⁰ [contaminant concentration (ng/g) in J32 Fetus]/[contaminant concentration (ng/g

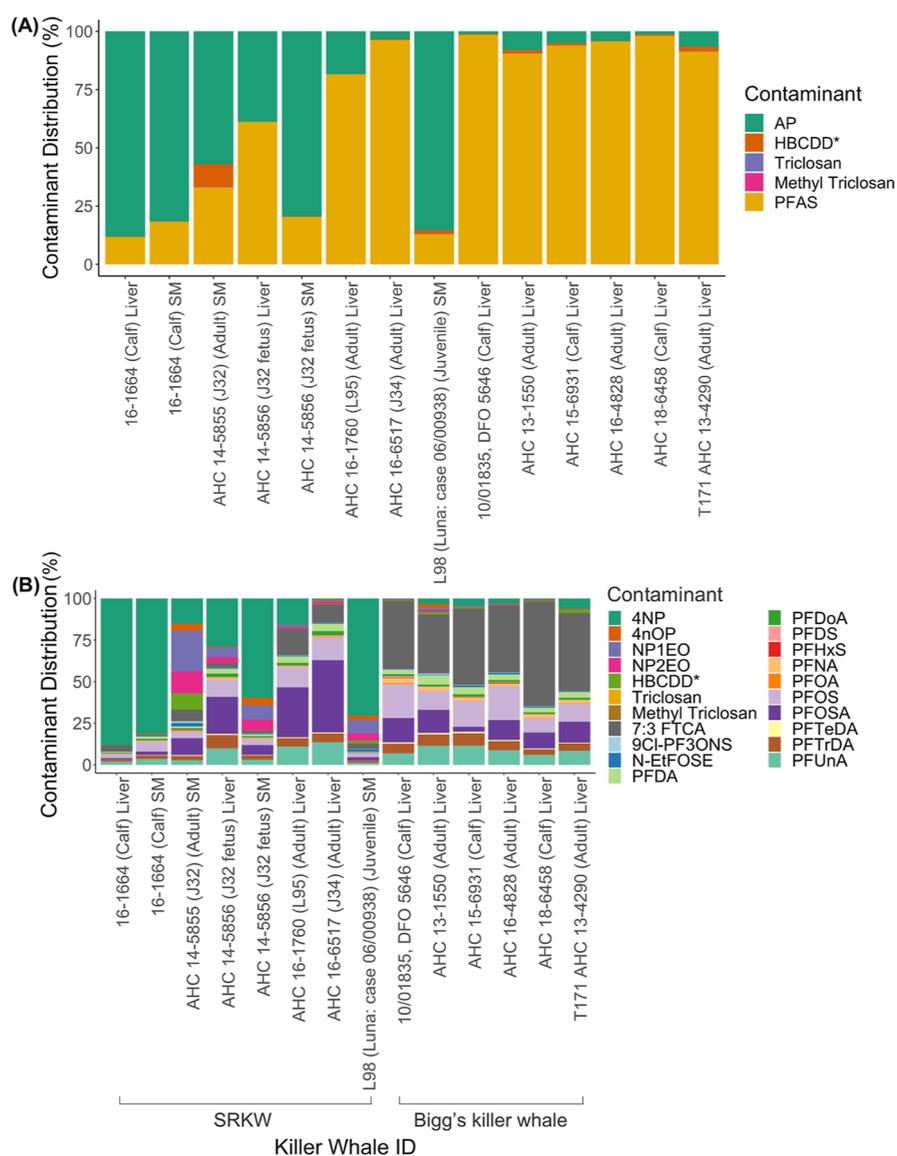


Figure 2. Patterns of contaminants (wet weight) in SRKW and Bigg's killer whale (*O. orca*) samples ($n = 14$, SM and liver) analyzed in this study. (A) Overall summary distribution (%) of CECs (i.e., AP, HBCDD*, methyl-triclosan, PFAS, and triclosan) analyzed in liver and SM tissue samples. (B) Detailed analyte-specific composition and distribution (%) analyzed in liver and SM tissue samples. *HBCDD is included as wet weight.

g) in J32 Mother + J32 Fetus] $\times 100$. Note that contaminant burdens could not be calculated as total SM mass measurements were not available.

RESULTS AND DISCUSSION

Controlling for Confounding Variables. Correlations between lipid, BCI, carcass condition codes, and age were evaluated to determine if these variables influenced contaminant concentrations. Carcass condition codes and lipid content across all samples were not significantly correlated (Figure 1A), indicating that carcass condition did not influence lipid content. Similarly, carcass condition codes and BCI were not significantly correlated (Figure 1B), suggesting that the stage of carcass decomposition did not affect BCI. No significant correlation was found between BCI and lipid content (Figure 1C), indicating that BCI did not affect lipid content in the killer whale samples despite a positive trend (higher lipid content and BCI values) being indicative of relatively healthier animals. Nonetheless, some individuals may

have been inflated by bloating due to carcass decomposition which may have artificially increased BCI values (>0.7 or 0.8). Contaminant concentrations showed no significant correlations with sample lipid content (4NP presented in Figure 1D as an example).

BCI showed a significant correlation with four contaminants (Figure S2): NP1EO ($p = 0.041$, $r = 0.83$), NP2EO ($p = 0.032$, $r = 0.89$), 9Cl-PF3ONS ($p = 0.049$, $r = -0.83$), and N-EtFOSE ($p = 0.036$, $r = -0.84$), suggesting a plausible association between BCI and contaminant concentration observed in these samples (NP1EO presented in Figure 1E as an example). In the case of NP1EO and NP2EO, a good BCI value (BCI = 0.6 – 0.7) was related to a higher contaminant concentration. Conversely, 9Cl-PF3ONS and N-EtFOSE showed a negative correlation with BCI.

Discrepancies found between relationships of carcass condition codes and BCI with contaminant loads may be due to carcass condition values and/or states of decomposition. For example, PFAS contamination has been shown to

be dependent on the contaminant type, the organism in which it is found (i.e., size), and the recovery time of the carcass (i.e., duration of decomposition).^{43–45} Interestingly, some PFAS substances such as PFOS can be formed in the process of degradation. Therefore, such extraneous variables may be impacting the contaminant concentrations in these killer whales.

The present study did not show significant differences in contaminant concentrations between males ($n = 5$) and females ($n = 8$) nor between age and total contamination in these killer whale samples (Figure 1F). Nonetheless, it was interesting to observe the trends of these correlations. Four neonates (one SRKW and three Bigg's killer whales with an estimated age of ~ 1 month or ~ 0.1 year) exhibited higher total contaminant concentrations compared to the concentrations measured in a juvenile (7 years) and adult animals (ages ranging from 18 to 34 years; Table 1) with the exception of T171 AHC 13–4290, a 29 year old female. Neonate contaminant concentrations exceeded those observed in the eldest individual (Bigg's killer whale; Table 1). High contaminant concentration levels observed in neonates relative to the juvenile and adult individuals (Figure 1F) may be due to the onset of sexual maturity and reproduction in subadult/adult individuals of these killer whale ecotype populations,^{26,46,47} as well as the influence of contaminant maternal transfer processes (e.g., *in utero* transfer to fetus, neonate lactation), which is further discussed hereafter. Overall, few significant correlations were identified between these confounding variables in relation to contaminant concentration and did not impact contaminant prevalence in the studied samples.

The relationships discussed here may be influenced by sex, age, ecotype, tissue sample type, and underlying pathologies and health status of the animals.^{21,38,46,47} Nutritional stress may also influence these relationships; SRKWs face scarce availability of their main prey, Chinook salmon, which may ultimately modulate lipid reserves and contaminant burdens through processes such as lipid mobilization.^{18,22,31,48,49} Along with a small sample size, these aspects may reduce statistical power of these results.

Alkylphenol. Four alkylphenol contaminants were screened in each SM and liver sample. Both 4NP and NP2EO were detected above RL ($>RL$) in all samples, whereas 4nOP and NP1EO were not detected at RL in two samples (Table S1). 4NP sample concentrations ranged from 1.8 to 3344.94 ng/g ww (median 40.84 ng/g ww); 4nOP from 0.035 to 5.69 ng/g ww (median 1.21 ng/g ww); NP1EO from 0.07 to 18.41 ng/g ww (median 2.66 ng/g ww); and NP2EO from 0.21 to 10.9 ng/g ww (median 2.058 ng/g ww) across all samples (Table S2). The sum of the total AP concentration in each sample accounted for 47.76% of the total contaminant concentrations (Figure 2A), with 4NP accounting for 96.67% of the total AP concentration (Figure 2B).

Past studies have focused on testing AP presence in bivalves, gastropods, and fish, and there is a paucity of AP screening and detection in marine mammals.^{50–53} Klosterhaus et al.⁵⁰ reported concentrations of APs detected in mussels found in San Francisco (California, USA) that were 1–2 orders of magnitude lower than the concentration expected to elicit toxic effects in marine organisms. Although risk management strategies for these compounds were established in 1999, results from David et al.⁵¹ indicated that even after institution of contamination regulation, biomagnification of APs con-

tinues to accrue in higher trophic levels. An enhanced understanding of how these compounds enter into and are distributed throughout critical killer whale habitat may better inform or refine mitigation strategies.

Compared to other AP contaminants screened in this study, 4NP had the highest concentration and was the most prevalent contaminant in several tissue samples [i.e., 16–1664 liver and SM, J32 Fetus SM, and L98 (Luna: case 06/00938) SM; Figure 2B]. Alkylphenol ethoxylates (APEOs) are primarily incorporated into herbicides, pesticides, lubricating oils, and surfactants, and can biodegrade to nonylphenols (NPs), including 4NP.^{7,51} Similar to other compounds in this contaminant class that are released to the environment through sewage treatment plants and industrial runoffs, sources of 4NP in seawater are derived primarily from the degradation of commercial and industrial products and sewage. High concentrations of this contaminant have been reported in toilet paper, especially those products high in recycled-paper content.^{54,55} According to assessments by Diehl et al.,⁵⁴ Morro Bay (California, USA) had the highest 4NP levels measured in septic sludge (3750 mg/kg dry weight), followed by Canada at 4.6–1230 mg/kg dry weight. Killer whale contaminant concentrations in this study (751.78 ± 422.32 ng/g lw) were lower compared to those in organisms of Morro Bay, where levels ranged from 14000 ± 5600 ng/g lw in harbor porpoise (*Phocoena phocoena*) liver and 138000 ± 55000 ng/g lw in sea otter (*Enhydra lutris*) liver samples.⁵⁴

Due to reproductive, developmental, and endocrine health implication of AP contaminants, as well as 4NP's specific ability to interact with the nervous system and influence cognitive function,^{51,56} it imperative to better define the prevalence and potential impacts of these compounds in marine mammals.^{57,58} Under the European Chemical Agency (ECHA), 4NP manufacture in or imported to the European Union (EU) is restricted based on weight, and NPs and their ethoxylates have also been added under the List of Toxic Substances by the Canadian Environmental Protection Act (CEPA). These compounds are also regulated in many Asian countries such as Singapore and China. Although regulation proposals are under consideration, there are currently no specific restriction in place for NPs under the United States Environmental Protection Agency (EPA).

Hexabromocyclododecane. All tissue samples were screened for alpha-, beta-, and gamma-HBCDD. Beta- and gamma-isomers were not detected at RL in any sample (Table S1). Alpha-HBCDD was not detected at RL in three samples (J32 Fetus liver, AHC 16–1760 (L95) liver, and 16–1664 SM). Lipid normalized concentrations of HBCDD ranged from 0.63 to 226.92 ng/g lw (median 23.89 ng/g lw; Figure S3) and accounted for 0.61% of the total contaminant lipid weight concentrations across all samples (Table S2). For reference of HBCDD wet weight distribution in each sample, see Figure 2A,B.

HBCDD concentrations found here were consistent with HBCDD levels in prior marine mammal research.⁵⁹ In a recent study, analyzing this contaminant in killer whales off the coast of Norway, concentrations ranged from not detected (ND) to 196 ng/g lw in blubber and 50 to 360 ng/g lw in muscle samples.¹⁹ Likewise, Lam et al.⁵⁹ reported HBCDD concentrations ranging from 32 to 519 ng/g lw and 4.1 to 501 ng/g lw for Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*), respectively. Cetacean species from the Northern Pacific Ocean also

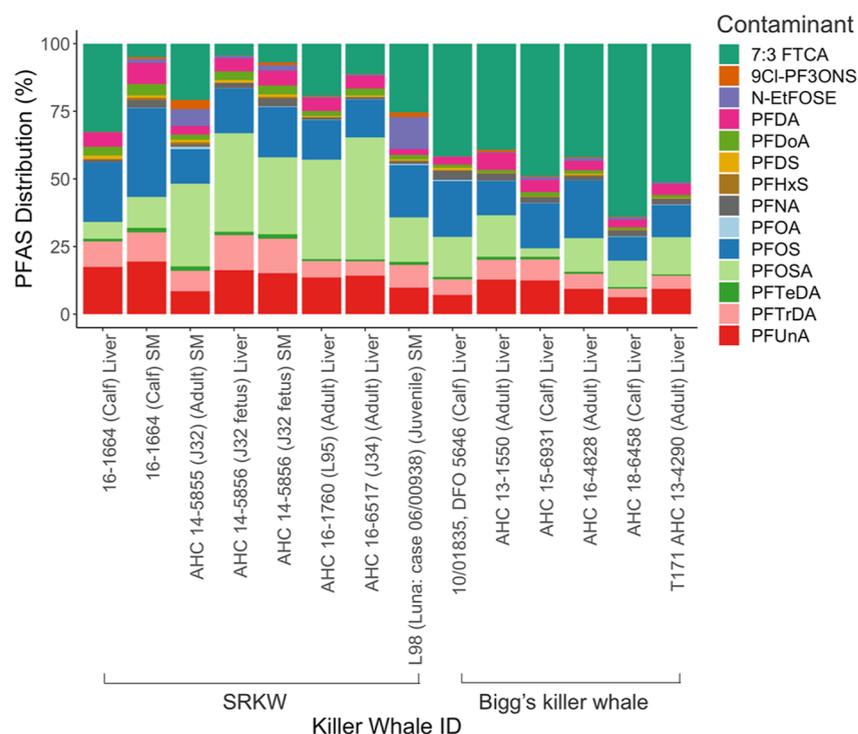


Figure 3. Per- and polyfluoroalkyl substances (PFAS) contaminant concentration (wet weight) distribution summary (%) for each killer whale (*O. orca*; SRKW and Bigg's killer whale) sample ($n = 14$, SM and liver). The composition pattern shows that 7:3 FTCA accounted for a high proportion of total PFAS concentrations, mainly in killer whale liver samples.

presented HBCDD concentrations similar to those found in the present study; for instance, HBCDD concentrations in bacon samples of Baird's beaked whale (*Berardius bairdii*) and bottlenose dolphin (*Tursiops truncatus*) from Japan ranged from 25 to 256 ng/g lw, respectively.⁶⁰ Concentrations of this contaminant were also reported in harbor porpoises and the common dolphin (*Delphinus delphis*) stranded in European seas,^{8,61} where the coasts of Ireland and Scotland presented high values (median of 2900 ng/g lw, maximum 9600 ng/g lw) with lower concentrations detected along the south coast of Ireland (median 1200 ng/g lw), the Netherlands (median 1100 ng/g lw), and both Belgium and the North Sea (770 ng/g lw). These concentrations were higher than those detected in the present study.

Differences in HBCDD levels in various geographic regions have been attributed to regional HBCDD application and use. For example, in the early 2000s, there was a greater demand and, therefore, use of these compounds in Europe than in America.⁶² Although regulated under the Stockholm convention, this contaminant can still be found as flame retardant additives in clothing, building insulation, furniture textiles, and electrical equipment, and can easily be released to the environment through leaching and weathering.^{59,63} Only some governing bodies have taken measures to further restrict, manufacture, use, sale, and import HBCDD (e.g., CEPA and ECHA have banned the manufacture and import of this contaminant). Results from this study indicate alpha-HBCDD is ubiquitous and bioaccumulative in apex marine mammals, such as the endangered SRKW and Bigg's killer whales.

Triclosan and Methyl Triclosan. Triclosan was detected in all SRKW and Bigg's killer whale samples, whereas methyl triclosan was identified in all but three samples (Table S1). Triclosan concentrations ranged from 0.003 to 0.43 ng/g ww (median 0.053 ng/g ww) and methyl triclosan ranged from

0.0005 (MDL 0.0006) to 0.085 ng/g ww (median 0.004 ng/g ww; Table S2). Triclosan accounted for 0.016% of the total contaminant concentration across all samples, while methyl triclosan accounted for 0.0017% (Figure 2A,B).

These results were consistent with prior studies analyzing triclosan in cetaceans. Plasma samples from free-ranging bottlenose dolphins (*T. truncatus*) off the coast of South Carolina and Texas, USA, showed a detectable presence of triclosan, with mean concentrations ranging from 0.18 to 0.072 ng/g ww depending on the geographic location.⁶⁴ This contaminant has also been measured in blood samples of a captive Bigg's killer whale, in which triclosan was ranked to be the third highest contaminant concentration (9.0 ng/g ww), following polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) compounds.⁶⁵ In this whale, triclosan accumulation was attributed primarily to its herring-based diet harvested from the NEP. This observation suggests that even with a disproportionately small percentage of herring in the diet of free ranging killer whales, this prey species may be a contributor to triclosan exposure and accumulation in these mammals.

Triclosan, a pharmaceutical and personal care product (PCP), is prevalent in society as an antibacterial agent that can be found in consumer products such as toothpaste, soaps, detergents, toys, and cleaning products, and may enter the marine environment through residential wastewater and sewage effluent.^{66,67} Regulatory actions of triclosan have been taken by CEPA, ECHA, and the US Food and Drug Administration. Certain countries including Japan have maximum allowable limits of triclosan in consumer products.⁶⁸ To the best of our knowledge, this is the first study to report methyl triclosan in free-ranging cetaceans along the BC coast and the first to report the presence of triclosan in SRKWs and Bigg's killer whales.

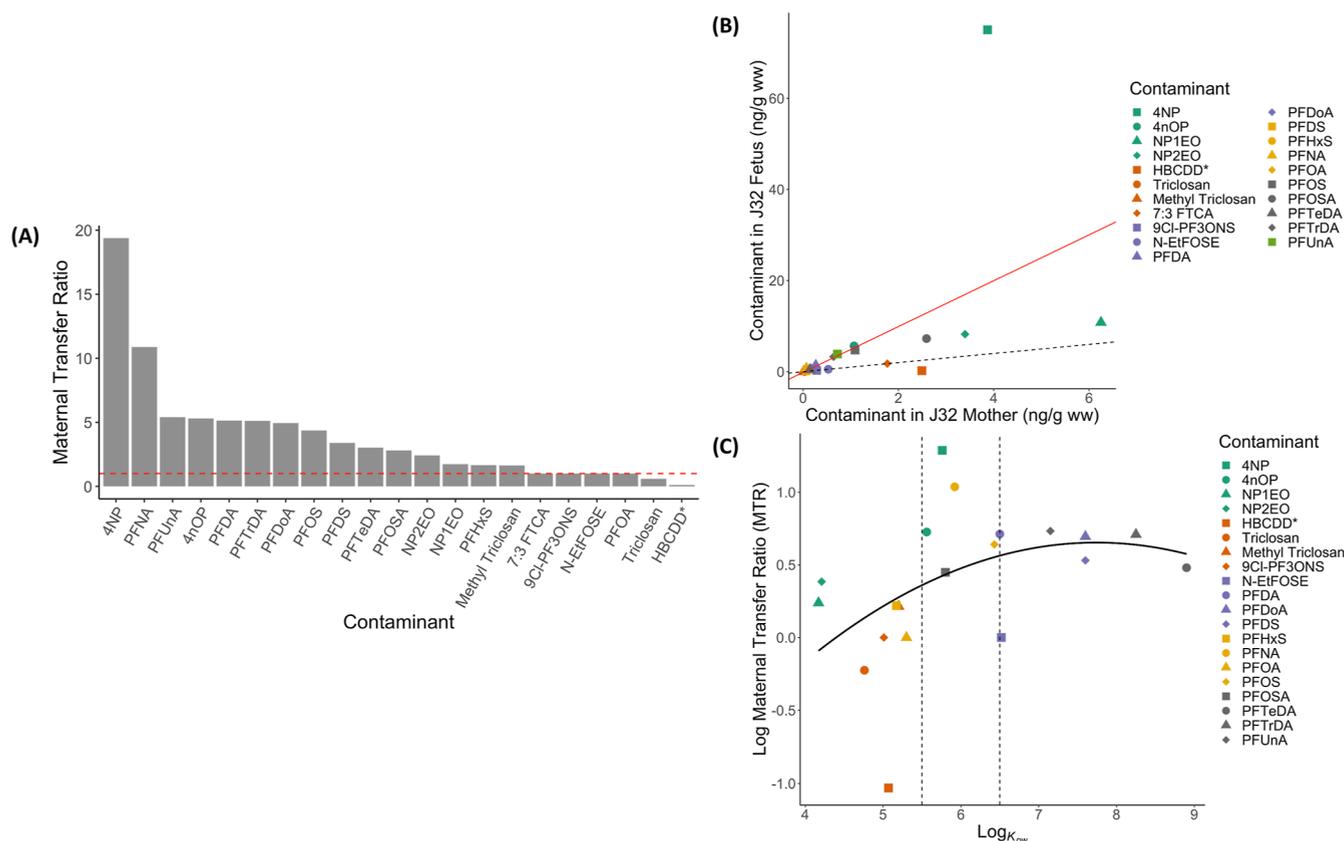


Figure 4. Analysis of contaminants (wet weight) in SM samples of the SRKW mother-fetus pair (J32 Mother) and J32 Fetus). (A) MTR calculated based on SRKW (*O. orca*) J32 Mother and her calf, J32 Fetus, contaminant concentrations. Contaminants with MTR >1 indicate the given contaminant was efficiently and preferentially exposed to the fetus, while contaminants with MTR <1 suggest scarce of lack of maternal transfer. The dashed red line indicates MTR = 1, representing equal partitioning of contaminant concentrations between fetus and mother. (B) Relationship (red line; $p = 1.55 \times 10^{-6}$, $r = 0.83$, slope = 5.03) between respective contaminant concentrations in J32 Mother and J32 Fetus. The dashed line indicates a 1:1 concentration ratio between these individual samples. Contaminants above the 1:1 line indicate a higher contaminant concentration in J32 Fetus compared to J32 Mother. (C) Relationship between the octanol–water partition coefficient ($\log K_{ow}$) and MTR of each contaminant derived from J32 Mother and J32 Fetus samples. Shown is the best-fit quadratic curve and the dashed lines highlight the $\log K_{ow}$ range in which higher MTR values are found. Note for (C): The data points are presented in log scale and a $\log K_{ow}$ value was not found for 7:3 FTCA. *HBCDD is presented in wet weight.

Per- and Polyfluoroalkyl Substances. Analysis of killer whale samples identified 14 of 40 PFAS contaminants as 26 PFAS congeners were below RL (<RL) in more than 50% of the samples. The sum of PFAS concentrations in each sample ranged from 8.48 to 938.69 ng/g ww (median 266.35 ng/g ww). Total contaminant concentration across all tissue samples comprised 51.63% PFAS (Figure 2A).

Interestingly, the PFAS accounting for the majority of the overall concentrations was 7:3-fluorotelomer carboxylic acid (7:3 FTCA), contributing to 41.32% of total PFAS contamination across all samples, followed by PFOS (15.86%) and PFOSA (15.43%), as shown in Figures 2B and 3. In contrast to prior studies of PFAS in marine mammals, PFOS was not the dominant PFAS in our samples (Figure 3; Table S2).^{41,69–74}

Prior studies have quantified the presence of PFAS in a variety of marine mammal species. Mean PFAS concentrations of each sample in our study (315.05 ± 133.39 ng/g ww) were higher than those recorded in killer whales off the coast of Greenland (269 ± 90 ng/g ww).²⁰ For example, mean PFOS concentrations measured here (49.97 ± 11.29 ww) were lower than those observed in Greenland killer whales (122 ± 42 ng/g ww).²⁰ PFAS concentrations have been widely studied in

toothed cetaceans, with mean concentrations ranging from 927 ng/g ww in plasma of bottlenose dolphins (*T. truncatus*) from Sarasota, Florida (US) to 1738 ng/g ww in bottlenose dolphin plasma of the Gulf of Mexico and the Atlantic Ocean.^{41,70} Stockin et al.²¹ reported a maximum of 6975 ng/g ww in coastal-estuarine Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) liver samples of Australia based on preliminary data reported in the gray literature by Stephens et al.⁷⁵ The levels in our killer whale samples were lower to those averages previously stated. As PFAS has previously been reported to be underestimated in marine mammals from the Northern Hemisphere, our results further contribute to the prevalence of this contaminant in threatened killer whales in the NEP.⁷²

Spaan et al.⁷² reported 7:3 FTCA in cetaceans for the first time, with the highest reported concentration found in killer whales from East Greenland (614 ± 49 ng/g ww). This is a considerably higher burden than 7:3 FTCA concentrations measured in other vertebrate species such as birds, fish, and human blood, but not as high as concentrations found in polar bears (~ 1000 ng/g ww).⁷² In the present study, 7:3 FTCA concentrations ranged from 1.77 to 481 ng/g ww (mean 130.18 ± 41.07 ng/g ww). Bigg's killer whale T171 (AHC 13–4290 liver sample) exhibited the highest concentration and was

comparable to those found in killer whales from East Greenland. In contrast to our findings, however, Spaan et al.⁷² found higher PFOS concentrations compared to 7:3 FTCA.

PFAS contaminants, also referred to as 'forever chemicals' due to their strong and very long persistence in the environment, are widely used in industrial settings; they have hydrophilic and lipophilic properties which allows for their frequent application in food packaging materials, stain and water-repellent fabrics, cooking ware, and fire extinguishers.⁵ The international mandate of the Stockholm Convention on POPs prompted many countries, such as Canada, the US, and European Nations, to recognize the need for PFAS regulation, specifically PFOS, PFOA, and PFHxS. There are no known restrictions for 7:3 FTCA; however, one of its potential precursors, 8:2 fluorotelomer alcohol (8:2 FTOH), is classified as a "PFAS of Interest" under the EPA and is part of a list of chemicals proposed as new POPs to the Stockholm Convention by ECHA. Other potential precursors to 7:3 FTCA include 8:2 fluorotelomer sulfonates, C8-based perfluoroalkyl phosphonic acids (PFPA), polyfluoroalkyl phosphates (PAPs), and polyfluoroalkyl phosphate diesters (di-PAPs). The present study provides further evidence of the prevalence of PFAS contaminants in top marine predators, specifically killer whales found in the NEP.

Maternal Transfer. Our results indicated *in utero* maternal transfer of pollutants during fetal development of offspring. To assess the degree of contaminant maternofetal transfer, SM tissue samples from a mother-fetus pair (J32 Mother and J32 Fetus) were analyzed. Tissue analysis disclosed *in utero* maternal transfer of all contaminants, with efficient and preferential (long-term exposure; MTR >1) transfer of 15 contaminants from the dam to the developing fetus (Figure 4A). The top three highest ratios were 4NP (MTR = 19.39), PFNA (MTR = 10.89), and PFUnA (MTR = 5.4; Table S3). The highest *in utero* MTR was 4NP, almost two times higher than PFNA, with 4NP concentration reported at 75.04 ng/g ww in J32 Fetus and 3.87 ng/g ww in J32 Mother. HBCDD (ww and lw basis) and triclosan had MTR values below one, indicating scarcity or lack of maternal transfer of these chemical contaminants via placenta from J32 Mother to J32 Fetus. Maternal transfer rates of 4NP were the highest at 95.1% followed by PFNA (91.59%) and PFUnA (84.38%). These results suggests that maternal transfer is an exposure pathway of alkylphenols and PFAS contaminants to killer whale fetuses and can result in greater fetal assimilation compared to levels in the mother (Figures 4A,B).

To better understand the transfer of contaminants from J32 Mother to J32 Fetus, the relationship between MTRs and octanol–water partition ratios was explored. An octanol–water partition coefficient (K_{ow}) is a common bioaccumulation metric expressing the lipophilicity of a chemical contaminant. Because the contaminant must travel through hydrophilic and hydrophobic environments, a compound's lipophilic nature can affect absorption and distribution throughout an organism.⁷⁶ A compound's lipophilic nature and trophic magnification factor tends to increase with K_{ow} .¹³ Results from this study showed a significant positive correlation between $\log K_{ow}$ and MTR ($p = 0.015$, $r = 0.54$) and suggest that contaminants with a higher $\log K_{ow}$ ($\log K_{ow} > 5.5$) were more readily transferred across placental membranes compared to contaminants with a lower $\log K_{ow}$ (i.e., $\log K_{ow} < 5.5$, as shown in Figure 4C). In particular, contaminants such as 4NP

and PFNA with $5.5 < \log K_{ow} < 6.5$ showed the highest MTRs (Figure 4C and Table S3).

Conversely, relatively lower MTR values are also observed with $\log K_{ow} \geq 6.5$. As a comparison, for instance, lower transplacental transfer ratios for PCBs were reported above a $\log K_{ow}$ of 7.5 in Steller sea lions (*Eumetopias jubatus*), beluga whales (*Delphinapterus leucas*), and California sea lions (*Zalophus californianus*).^{77–79} The degree of chlorination or fluorination (i.e., lower or less chlorinated or fluorinated vs more persistent chlorinated or fluorinated compounds) in tandem with the contaminants' molecular weight (i.e., low molecular weight vs high molecular weight) are factors that may influence the transplacental transfer across *in utero* membranes. PFAS contaminants are known to partition with protein-rich compartments (e.g., blood and liver), with longer fluorinated compounds having higher bioaccumulation potential; it has been demonstrated that such PFAS contaminants are readily transported to human follicle fluid.^{80,81} This is supported in the present study as long fluorinated carbon-chained PFAS contaminants such as PFDA, PFUnA, and PFDoA showed higher MTRs from J32 Mother to J32 Fetus (Figure 4C). Additionally, difference in molecular weight between 4NP and PFTeDA (493.76 kg/mol) may have contributed to 4NP being over six times more transferable than PFTeDA (Figure 4A,C).

This study is the first to document *in utero* maternal transfer of selected CECs and new POPs in killer whales inhabiting the NEP. Maternal transfer of PFAS has been studied in marine mammals in which contaminant loads in fetuses and neonates have been compared to dams in cetaceans and pinnipeds.^{70,82–85} Few killer whale MTR analyses have been completed; however, Andvik et al.¹⁹ reported maternal transfer of PFAS in a mother-fetus killer whale pair from Norway and claimed lipid rich milk and transplacental transfer were responsible for contaminant exposure. A study on a mother-fetus killer whale pair from Greenland also documents maternal transfer; however, contrary to the present study, all contaminant burdens were higher in the mother compared to the fetus.²⁰ Although there is limited ability to compare results with Gebbink et al.²⁰ as contaminant burdens were not calculated in the present study (see Methods), aspects impacting differences in MTR results between studies are discussed below.

Maternofetal transfer of contaminants is important to understand as calves are sensitive to toxicity in development and are at increased risk of pollutant exposure before birth. Additional mother-fetus pairs are needed to further assess maternal transfer of contaminants in killer whale species as discrepancies were found between the results of the present study and other studies analyzing maternal transfer in humans and cetaceans. For example in humans, Midasch et al.⁸⁶ reported consistently higher concentrations of PFOS in maternal-sampled blood compared to umbilical cord plasma samples, while PFOA showed only minor differences in concentration between the two sample types. This indicates the ability of PFOA to cross the placental barrier unhindered and contradicts the findings of our study as PFOS was lower in J32 Mother compared to J32 Fetus and PFOA had equal concentrations in the mother-fetus pair. Conversely, similar to that in the present study, Grønnestad et al.⁸⁵ found that sulfonated PFAS contaminants (e.g., PFOS) are more readily transferred across placental barriers compared to carboxylated PFAS contaminants (e.g., PFOA) in hooded seals (*Cystophora*

crystata), and differences were attributed to protein-binding efficiencies and compound-specific persistence and retention. Also in agreement with the present study were higher concentrations of PFHxS, PFOS, PFD_oA, and PFTrDA found in hood seal pups compared to mothers which implies these compounds may be more readily eliminated from the mother (e.g., through metabolism, excretion, and placental transfer), while the fetus is unable to biotransform and eliminate such contaminants. This presents differences in detoxification mechanisms in J32 Mother and J32 fetus as a potential source of MTR variation in this pair.

Research regarding *in utero* transfer of 4NP has been performed in human samples, supporting the transfer of this contaminant from the mother to the fetus.^{87–89} Li et al.⁸⁹ detected approximately 20% lower non-POP (e.g., 4NP) contaminant concentrations in the umbilical cord than maternal blood samples compared to polybrominated diphenyl ether (PBDE) compounds which showed significantly higher concentrations in umbilical-cord blood. It was concluded that the placental barrier provided only a slight decrease in non-POP contaminant exposure to fetuses. Although PBDE compounds were not studied here, 4NP was much higher than all other contaminants analyzed (Figure 4A) which suggests little protection from 4NP by the placental barrier. Other factors such as foraging in contaminant hotspots in critical habitats may have contributed to the high 4NP levels found in J32 Fetus.⁸⁴ General aspects potentially impacting differences in MTR between individuals and species include placental thickness, diffuse placentation, blood pH, and lipid solubility.⁹⁰

The high MTR of 4NP in the present study may be considered an outlier in the data; however, given few studies have analyzed the presence of 4NP in cetaceans and MTR findings here are based on a sample size of one, it is difficult to confidently make such conclusions. Inconsistencies in results between studies and sparse research regarding *in utero* maternal transfer of CEC (such as 4NP) and new POP contaminants in different ecotype of killer whales with diverse feeding and dietary preferences (e.g., fish-eating vs marine mammal-eating) further emphasizes the need for additional studies on this topic to make more robust interpretations of the results found in this study.

Killer Whale Calf Population Contaminant Comparisons and Killer Whale Exposure Sources. Concentrations of CECs and new POPs in calf samples of SRKW ($n = 3$) and Bigg's killer whales ($n = 4$) were compared to explore for differences in contaminant concentrations. The calf cohort of SRKWs and Bigg's killer whales were grouped to include neonates and fetuses. The concentration of 4NP was significantly higher ($p = 0.02$) in SRKW calves, whereas HBCDD ($p = 0.026$, lipid weight basis), 7:3 FTCA ($p = 0.04$), PFHxS ($p = 0.025$), PFNA ($p = 0.019$), and PFOA ($p = 0.044$) concentrations were significantly higher in Bigg's killer whale calves (Figure S4). These findings may well indicate that SRKW calves were most exposed to a putative 4NP source nearby to their critical habitat relative to the more mobile Bigg's killer whale calves, which were most exposed to HBCDD and PFAS contaminants.

Significant differences in contaminant concentrations between SRKWs and Bigg's killer whale calves may be due to habitat.⁹¹ Bigg's killer whales range throughout the west coast of North America, from Southeast Alaska to California, and transit both the outer coast and protected inshore areas,

while SRKWs remain seasonally inshore or nearby coastal waters, with the Georgia and Johnstone Strait, BC, considered as their critical habitats.^{16,18} A significantly higher concentration of 4NP in SRKWs may well be attributed to their primary habitats surrounding industrial and residential hubs as well as their association to a more estuarine trophic chain. As toilet paper is a major source of 4NP, sewage effluent may have exposed this species to elevated concentrations of this contaminant. Estuaries are considered heavily impacted by anthropogenic 4NP pollution; therefore, this CEC may have been more readily absorbed in SRKWs throughout the associated food web.⁵⁴ In contrast to SRKWs, Bigg's killer whales may be more exposed to HBCDD and PFOS in more remote regions as these contaminants have a strong propensity for long-range environmental and atmospheric transport and may be less exposed by local sources in the ocean.⁴ These compounds are commonly found in flame retardant additives and PFOS specifically can be present in hydraulic fluids, electric parts, and textiles.

The unique presence of 7:3 FTCA in these killer whales warrants further investigation of potential sources and exposure pathways, including point and nonpoint pollution sources from urban, agricultural, or industrial areas at the regional level, as well as presumptive wet deposition via long-range atmospheric transport. 7:3 FTCA is a stable metabolite but can also be an intermediate product; for example, it can result from the metabolism of 8:2 FTOH.^{92,93} Fluorinated telomer alcohols (FTOHs) are used as surfactants and in the production of PFAS contaminants and are prone to undergo atmospheric oxidation which can produce fluorotelomer carboxylic acids (FTCAs).⁹⁴ It is unclear whether FTOHs may serve as the precursor of 7:3 FTCA in the ocean atmosphere and marine environment of SRKW and Bigg's killer whales.

Contaminants such as legacy and emerging POPs and some CECs have the capacity to bioaccumulate in killer whales via biomagnification at each trophic level across their food webs;^{31,33,91,95} thus, differences in contaminant concentrations in SRKW and Bigg's killer whale calf populations may also be attributed in part to foraging behavior and dietary preferences.^{22,31,46,48,91,96} Fish-eating SRKWs had lower contaminant concentrations compared to marine mammal-eaters such as Bigg's killer whales in prior studies and the present study. This was exemplified in Norway where killer whales preying upon seals reported four times the concentration of PCBs than those feeding on fish.²⁴ In the NEP, SRKWs have shown significantly lower PCB and PBDE concentration levels compared to Bigg's killer whales, which has been attributed to the difference in trophic levels between these sympatric populations.^{26,46,97} Chinook salmon, the primary prey of SRKWs, have shown PCB concentrations ranging from 516 to 3099 ng/g lw (estuary) and 521 to 760 ng/g lw (hatchery) while harbor seals, top constituents of Bigg's killer whale prey, had PCB levels ranging from 1,143 to 18,135 ng/g lw.^{33,48,98,99} Based on extrapolation from pinniped studies, recent research has stated that PCB concentrations in these killer whale populations have surpassed the toxic effect concentration thresholds for PCBs (i.e., immunotoxicity and endocrine disruption).^{24,46,91,95,100,101}

As both geographical location and food web composition and structure influence contamination in killer whales, this study highlights the need for further analyses of CECs and new

POPs in the habitat and prey of these apex predators of the NEP.

Killer Whale Pathology and Association to CEC and New POP Exposure. Based on prior studies of harbor porpoises in the United Kingdom and bottlenose dolphins (*T. truncatus*) near Charleston, North Carolina (US), killer whales' chemical contamination may have surpassed thresholds and impacted homeostasis.^{25,102} In free-ranging bottlenose dolphins, for instance, PFAS concentrations ranging from 500–9000 $\mu\text{g/L}$ ww were associated with immunological and hematological alterations.¹⁰² In our study, the top three highest concentrations of PFAS were detected in the liver of Bigg's killer whales, including two calves [i.e., 10/01835, DFO 5646 had 546.1 ng/g or part per billion (ppb) ww, and AHC 18–6458 had 580.8 ng/g ww] and one adult female (i.e., T171 AHC 13–4290 had \sim 940 ng/g ww). This represents 21% of the total killer whales sampled, and exceeds the minimum range of 500 $\mu\text{g/L}$ (ppb) associated with alterations on the haematology and circulating immune cell populations of bottlenose dolphins.

Additionally, in the bottlenose dolphins, elevated PFOS levels were associated with changes in phagocytic function and immune modulation,¹⁰² which may have indirectly contributed to pathology, suboptimal health, and BCI in the sampled cohort of the present study (Table 1). Causes of killer whale mortalities in the present study ranged from infections, emaciation, blunt force trauma, dystocia, and intraspecific interactions.³⁸ In speculation, suboptimal body condition, coupled with elevated contaminant loads may have predisposed or exacerbated AHC 16–1760 (L95) to mucormycosis, a rare but serious fungal infection caused by *Rhizomucor pusillus*, *Lichtheimia corymbifera*, and *Cunninghamella bertholletiae*, recently observed in marine mammals stranded in the NEP.¹⁰³ In AHC 15–6931, it is difficult to infer a specific cause and effect between presumptive metabolic derangements, hypoglycemia, and dystocia^{38,103} and elevated PFOSA and 7:3 FTCA, which were most dominant in this mammal (Table 1). At present, however, there is insufficient information regarding the impacts of CECs and new POPs to infer a specific cause and effect.

Many of these contaminants may have been associated or attributed to disruption of homeostasis. In humans, PFAS contaminants have been reported to alter immune and hepatic functions, disrupt glucose metabolism, and cause reproductive risks.^{6,104} Studies have reported dystocia to be related to hormonal imbalances and endocrine disorders.^{105,106} Moreover, alkylphenols have shown to alter the endocrine system which may create difficulties in the birthing process.⁵¹

As previously mentioned, the studied cohort is too small to assess variation in contaminant concentrations with ante-mortem, morbidity, reproductive failure, or the loss of these animals. Additionally, toxic thresholds for the analyzed CECs and most new POPs in marine mammals are not yet fluent in the literature. Further studies linking contaminant loads to pathological findings in a variety of marine mammal species are warranted.

Tissue analysis of necropsied SRKW and Bigg's killer whales in the NEP demonstrated that CECs (AP, triclosan, methyl triclosan, and selected PFAS compounds) and new POPs (PFOS, PFOA, PFHxS, and HBCDD) are prevalent along the marine-coastal ecosystems of British Columbia, Canada. AP and PFAS pollutants were most common across killer whale samples. The AP group predominantly consisted of

4NP, a novel contaminant that has been studied in few marine mammal species; this is the first study to report 4NP levels in killer whales. Overall, 7:3 FTCA was the primary PFAS contaminant and was observed here for the first time in SRKWs and Bigg's killer whales. This contaminant was first detected in cetaceans in 2020;⁷² therefore, little is known about the kinetics and metabolism of this contaminant in marine mammals. Interestingly, PFOS was not the dominant PFAS contaminant, as usually detected in marine mammals. Triclosan, methyl triclosan, and HBCDD accounted for a very small fraction of contamination across all samples. In addition to studying the prevalence of CECs in conjunction with new POPs regulated under the Stockholm Convention, we evaluated *in utero* maternal transfer of pollutants in the J32 Mother-J32 Fetus SM-sample pair. Efficient and preferential exposure of APs, particularly 4NP, and PFAS contaminants were detected in J32 Fetus (MTR >1). This raises concerns regarding the persistence of these emerging chemicals and potential impacts on fetal development and post-partum survival.

As previously discussed, it is difficult at this point to confidently assess both the prevalence and impacts of the studied contaminants in this species; the novel findings of this research should be considered as a preliminary baseline for future studies which can provide more robust and statistically convincing results through larger samples size analyses. Although access to skeletal muscle and liver samples of stranded and necropsied killer whales is uncommon and opportunistic, it is essential that contaminant distribution and prevalence continues to be monitored in endangered SRKWs and threatened Bigg's killer whales to expand our understanding of their ecotoxicological consequences. Additional toxicological risk assessments should also be done on these chemicals to support risk management and global regulation efforts of these substances. Nonetheless, the present study helps to establish baseline knowledge on CECs and new POPs in these charismatic and sentinel species, and our findings provide scientifically-derived evidence to inform policy to support and enhance regulations to mitigate pollutant exposure in marine ecosystems. These measures may also contribute to management strategies and conservation efforts of SRKWs and their critical habitat, as well as the habitat of other marine mammals living within BC's coastal ecosystem.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.2c04126>.

Raw data for each sample (XLSX)

Wet and lipid-normalized weight data for each sample (XLSX)

Additional background information on the analyzed "contaminants of emerging concern" and new POPs; further procedure details regarding sample collection and contaminant analytical methods; killer whale stranding locations; panel of figures presenting significant correlations between BCI and contaminant concentration; lipid weight distribution of HBCDD for each sample; contaminant concentration comparisons in calves of different ecotypes; and MTRs for each contaminant and their associated octanol–water partition coefficient ($\log K_{ow}$) (PDF)

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Notes

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