Maternal Transfer of Flame Retardants in Sharks from the Western North Atlantic Ocean

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S *Supporting Information*

ABSTRACT: The present work represents a comprehensive study of *in utero* maternal transfer of legacy and emerging flame retardants (FRs) in marine predators. We analyzed liver tissues from pregnant sharks of five viviparous species, including blacknose shark (*Carcharhinus acronotus*; n = 12), blacktip shark (*Carcharhinus limbatus*; n = 2), bonnethead (*Sphyrna tiburo*; n = 2), Atlantic sharpnose shark (*Rhizoprionodon terraenovae*; n = 2), and spinner shark (*Carcharhinus brevipinna*; n = 2), as well as their embryos (n = 84 in total from five species), collected from the western North Atlantic Ocean. Concentrations of frequently detected emerging FRs in adult female blacknose sharks were determined to be 6.1−83.3 ng/g lipid weight (lw) for dechlorane analogues, 2.5−29.8 ng/g lw for tetrabromo-*o*-chlorotoluene, and nondetection −32.6 ng/g lw for hexabromobenzene. These concentrations were 1−2 orders of magnitude lower than those of legacy polybrominated diphenyl ether flame retardants (85.7−398 ng/g lw). Similar contamination profiles were also found in the other four species, although FR concentrations varied in different species. A total of 21 FRs were commonly found in developing embryos of female sharks from five species, demonstrating maternal transfer *in utero*. The maternal transfer ratio (i.e., ratio of the mean litter concentration to their mother's concentration) determined in blacknose shark mother/embryo groups for each FR chemical was negatively associated with its octanol−water partition coefficient. Our work lays a solid foundation for future investigation of the underlying mechanisms of *in utero* transfer and additional physical or chemical factors that affect maternal transfer.
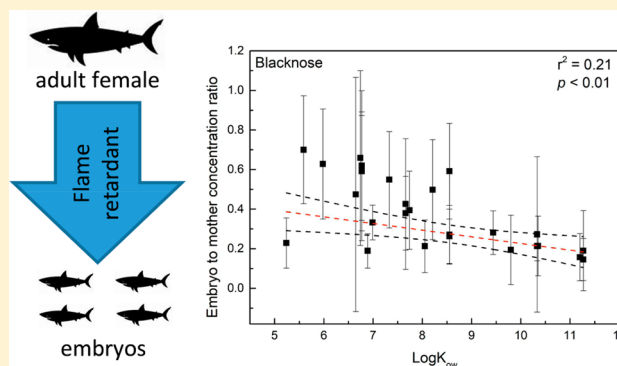
INTRODUCTION

For decades, polybrominated diphenyl ethers (PBDEs) have been the primary flame retardants (FRs) for industrial and commercial applications worldwide. Many studies have assessed the exposure of PBDEs in wildlife and humans and identified this group of halogenated substances as persistent organic pollutants (POPs).[1−5] PentaBDE, OctaBDE, and DecaBDE mixtures have all been added to the POPs list of the Stockholm Convention (chm.pops.int). The phase-out of major PBDE products in North America and Europe has led to the increased use of alternative flame-retardant chemicals to meet flammability standards.[6] To date, more than 75 brominated and chlorinated compounds other than PBDEs have been commercially manufactured.[6] Typical chlorinated alternative FRs include *syn*- and *anti*-dechlorane plus (DP) and

its analogues, such as dechlorane (Dec-) 602, 603, and 604.[7] Typical brominated alternatives include 2-ethylhexyltetrabromobenzoate (EH-TBB), bis(2-ethylhexyl)-3,4,5,6-tetrabromobenzoate (BEH-TEBP), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), and decabromodiphenylethane (DBDPE).[6] Many alternative FRs possess physicochemical properties (e.g., logarithmic octanol−water partition coefficient or log K_{ow} greater than 5) favoring environmental persistence and bioaccumulation.[6] Indeed, some of these alternatives have already been detected in house dust, air, wastewater, sediment,

fish, and wildlife.[6−8] However, the current understanding of alternative flame retardants in biota of the Atlantic Ocean is limited, although there have been a variety of studies on PBDEs and wildlife, including elasmobranchs, in this region.[5,9,10]

Compared to postnatal exposure, prenatal exposure to emerging FRs via gestational maternal transfer is much less understood in many taxa, including mammals and fishes. The maternal transfer of organochlorine pesticides, polychlorinated biphenyls (PCBs), and PBDEs has been reported in marine mammals and elasmobranchs employing viviparous (live-bearing) reproductive strategies.[10−13] Organochlorines, including PCBs, dichlorodiphenyltrichloroethanes (DDTs), and other legacy pesticides, were detected in maternal tissues, embryonic tissues, and yolky stomach contents of the embryos of the common thresher shark (*Alopias vulpinus*), clearly demonstrating maternal transfer *in utero*.[12] Significant maternal transfer of organochlorines was also observed in scalloped hammerhead shark (*Sphyrna lewini*) females and their embryos, with females offloading approximately 0.03−2.3% of their hepatic contaminant load to their offspring.[13] A comparison of maternal offloading of organohalogenated chemicals between species with aplacental (e.g., Atlantic stingray, *Hypanus sabinus*) and placental viviparity (e.g., blacktip shark, *Carcharhinus limbatus*; bonnethead, *Sphyrna tiburo*) revealed greater rates of transfer for organhohalogens in the placental species.[10] Maternal offloading of contaminants has also been shown to be a significant pathway for contaminant accumulation other than dietary exposure in the offspring of multiple high trophic level marine predators.[11,14,15]

Compared with organochlorines, maternal offloading of FRs, particularly alternative FRs, has received comparatively little attention in marine mammals and fishes, including elasmo-branchs employing viviparous reproduction. Exposure to FRs during the embryonic stage of development can lead to adverse effects on offspring, including immune disruption, endocrine disrupting effects, and developmental neurotoxicity.[16,17] There-fore, understanding which FRs are subject to maternal transfer and to what degree are critical components for evaluating and managing wildlife with regards to long-term FR exposure. The use of embryos and associated adult females from viviparous species allows for effective assessments of both prenatal exposure in embryos and maternal offloading of contaminants.[13] As a chemical's K_{ow} typically represents its lipophilicity and is closely related to its uptake and bioaccumulation potency,[18] we hypothesized that K_{ow} plays an important role in the maternal transfer of FR chemicals. Therefore, the specific objectives of this study were to investigate (1) if maternal transfer of both legacy and emerging FRs occurred in selected viviparous shark species from the Atlantic Ocean and (2) if the K_{ow} significantly influences maternal offloading of FRs.

■ MATERIALS AND METHODS

Samples. Pregnant sharks from five viviparous species, including blacknose shark (*Carcharhinus acronotus*, n = 12), blacktip shark (n = 2), bonnethead (n = 2), Atlantic sharpnose shark (*Rhizoprionodon terraenovae*, n = 2), and spinner shark (*Carcharhinus brevipinna*, n = 2), were collected from offshore waters from South Carolina to Florida and adjacent waters (Figure S1). Adult female sharks were sampled using research longlines or rod and reel gear types. Liver tissues were collected from female sharks and their embryos (n = 84 in total from five species) and stored in clean, sterile polyethylene

containers (Fisher Scientific, Hanover Park, IL) at −20 °C. In this study the number of embryos from individual adult female sharks is summarized as 2−6 (median: 4) for blacknose sharks, 6−9 for spinner sharks, 4 for blacktip sharks, 4 for bonnetheads, and 5 for Atlantic sharpnose sharks. All samples were collected from legally landed sharks within regional fisheries or from ongoing fish research operations by federal or state fisheries agencies within their respective coastal waters. None of the species are prohibited from capture during legal fishing operations. Thus, no specific state or federal permit or ethical committee approval was required for this study. The conservation status, diet, and other biological information on each species are summarized in Table S1.

Chemicals and Reagents. A total of 35 non-PBDE flame retardants and 20 PBDE congeners were analyzed in this study (Table S2). Reference standards of PBDE congeners and most other FRs were purchased from AccuStandard (New Haven, CT) or Wellington Laboratories (Guelph, ON, Canada), except for Br-Dec604, Br$_2$-Dec604, Br$_2$Cl$_2$-Dec604, and Cl$_4$-Dec604 which were obtained from Toronto Research Chemicals (Toronto, Canada). The surrogate standards of 4′-fluoro-2,3′,4,6-tetrabromodiphenyl ether (F-BDE69), 4′-fluoro-2,3,3′,4,5,6-hexabromodiphenyl ether (F-BDE160), 2,2′,3,3′,4,5,5′,6,6′-nonabromo-4′-chlorodiphenyl ether (4PC-BDE208), and d$_{18}$-labeled α-hexabromocyclododecane (HBCDD), as well as the internal standard, 3′-fluoro-2,2′,4,4′,5,6′-hexabromodiphenyl ether (F-BDE154), were obtained from AccuStandard or Wellington Laboratories. Other supplies, including diatomaceous earth, sodium sulfate (10−60 mesh), copper (50 mesh, granular reagent grade), and high-performance liquid chromatography (HPLC) grade solvents, were bought from Fisher Scientific. Silica sorbent (Isolute, average pore size: 60) was purchased from Biotage Inc. (Charlotte, NC) and baked at 130 °C overnight prior to use.

Chemical Residue Analysis. Chemical residue analysis followed the same procedures as reported in a previous publication with minor modifications.[19] In brief, 0.2−1.5 g per sample was ground with diatomaceous earth, spiked with surrogate standards, and then subjected to accelerated solvent extraction (ASE350; Thermo Scientific, Sunnyvale CA, USA) with dichloromethane (DCM) at 100 °C and 1500 psi. After moisture removal through sodium sulfate, 10% of the extract was measured for lipid content by gravimetric determination. The remainder of the extract was run through a Shimadzu Prominence Semi-Prep HPLC (Shimadzu America Inc., Columbia, MD) outfitted with a Phenogel gel permeation chromatography (GPC) column (300 × 21.2 mm, 10 μ, 100 Å) for bulk lipid removal. The resulting extract was further cleaned through a solid phase extraction (SPE) cartridge containing 2 g of Isolute silica sorbent. The first fraction was eluted with 3 mL of hexane and was discarded. The second fraction, eluted with 11 mL of a 60:40 hexane:DCM mixture, contained all analytes of interest. The latter fraction was spiked with internal standard (FBDE-154) before instrumental analysis.

All FRs (except HBCDD diastereomers) were analyzed on an Agilent 7890B gas chromatograph (GC) coupled to a 5966 A mass spectrometer (MS; Agilent Technologies, Palo Alto, CA). The GC column was a 15-m DB-5HT column (0.18 mm i.d., 0.1 mm film thickness, J&W Scientific, Folsom, CA). Initial column temperature was set at 50 °C for 4 min, then ramped up to 300 °C at 10 °C/min, and held for 15 min.

Table 1. Ranges and Median Values (in Parentheses, for Blacknose Shark Only) of Flame Retardant Concentrations (ng/g lipid weight) in Adult Female Sharks

	blacknose shark *Carcharhinus acronotus*	blacktip shark *Carcharhinus limbatus*	bonnethead *Sphyrna tiburo*	Atlantic sharpnose shark *Rhizoprionodon terraenovae*	spinner shark *Carcharhinus brevipinna*
N	12	2	2	2	2
lipid%	27.4−61 (34.2)	26.6−30.8	56.1−77.4	11.8−46.5	46−66.7
ATE	nd[a]-2.1 (0.7)	nd-0.6	nd-2.5	nd	nd
BB-101	<LOQ[b]-38.8 (7.8)	15.6−30.8	3.8−9.5	1.9−16.2	71.7−179
BTBPE	nd-3.5 (<LOQ)	nd	nd-7.3	<LOQ-2.5	1.4−15.6
HBBZ	nd-32.6 (5)	5.9−14.2	2.3−15.2	3.7−13.7	10.8−17.1
TBCT	2.5−29.8 (5.9)	4.3−94	1−19	9.7−160	74.7−270
β-TBECH	nd-138 (<LOQ)	nd-2.4	5.5−70	nd	0.5−4.6
TBX	nd-2.4 (<LOQ)	nd-1.2	1.3−13.4	nd	nd-2.1
Cplus	3.1−21.4 (5.9)	2.9−37.2	<LOQ-9.9	13.8−18.2	18.2−72.8
Cl11-DP	nd-1.5 (0.3)	0.3−0.5	<LOQ-0.9	nd-0.6	0.6−5
Dec-602	1.8−34.1 (8.3)	3.8−112	0.6−9	7.5−58.7	58.7−372
Dec-603	1.2−19.1 (5.1)	21.3−2.9	<LOQ-3.8	10.9−11.9	10.9−88.1
Dec-604	nd-1.5 (<LOQ)	<LOQ-2.3	nd	0.9−1.3	0.8−5.8
syn-DP	nd-3.0 (0.8)	0.3−0.7	nd-1.7	0.6−2	2−13.1
anti-DP	nd-8.2 (1.6)	0.6−1.8	<LOQ-4	0.6−3.2	3.2−30
∑Dechloranes	6.1−83.3 (20.7)	11−175	0.6−29	35.6−94.4	94.3−587
α-HBCDD	2.2−30.1 (10.1)	22.2−290	12−14	13.4−105	68−805
β-HBCDD	nd-2.1 (<LOQ)	0.9−5	0.5−0.5	0.9−2.1	1.2−15
γ-HBCDD	nd-4.7 (1.2)	1.7−21.1	1.0−1.5	1.5−8.4	5.2−61.2
∑HBCDDs	2.2−36.9 (11.0)	24.8−316	13.5−16	15.8−116	75−881
∑PBDEs	85.7−398 (128)	258−2640	148−173	493−4470	2950−16190

[a]nd = not detected. [b]<LOQ = less than limit of quantification.

Identification and quantification of FRs were done with selected ion monitoring (SIM) of characteristic ions in electron capture negative ionization mode (ECNI) (Table S2). A 30-m HP-5MS column (0.25 mm i.d., 0.25 mm film thickness, J&W Scientific) was used to confirm and quantify Dec-604 concentrations because Dec-604 may coelute with BDE-183 or other heptaBDE congeners.[20] The limit of detection (LOD) or limit of quantification (LOQ) of a FR chemical, defined as three or ten times the standard deviation of the noise from the GC-MS determination, ranged from 0.1 to 1.2 ng/g lipid weight (lw) or 0.4−3.5 ng/g lw, respectively.

Determination of native HBCDD diastereomers (including α-, β-, and, γ-) and d_{18}-labeled α-HBCDD was achieved with an Agilent 1260 HPLC with a Waters Xterra phenyl column (2.1 mm × 100 mm, 3.5 μm particle size), which was interfaced with a 3200 Q Trap triple quadrupole MS (Applied Biosystems, MDS SCIEX; Toronto, Canada). The MS utilized a TurbolonSpray electrospray ionization (ESI) probe in multiple reaction monitoring mode for quantitative assessment of HBCDDs (see the Supporting Information for details).

Quality Assurance and Control. Multiple measures, including analyzing spiked matrix samples, Standard References Materials (SRMs), the inclusion of procedural blanks in each batch of samples, and surrogate standard recoveries in authentic samples, were utilized. A bag of Tilapia fillets was purchased from a local retail source, and approximately 100 g of fillets was combined into a composite for spiking experiments. The composite was tested prior to spiking experiments, and the only analyte detected was BDE-47 with a mean concentration of 1.5 ng/g lw. Approximately 3 g of composite fish was spiked with a mixture of FR analytes (50−100 ng each) and processed using the same methodology for authentic samples to assess analytical recoveries and efficiencies. The mean (±standard deviation) recoveries of spiked FR analytes ranged from 72.4 ± 12% to 98 ± 8.4% in five replicates (Table S3). The analyses of the National Institute of Standards Technology (NIST) SRM 1947 Lake Michigan Fish ($n = 5$) revealed recovery efficiencies of PBDE congeners ranging from 82.1 ± 6.0% to 95.3 ± 7.8% of the certified concentrations. One procedural blank was included with every five to ten samples to check for laboratory contamination; the only analyte detected was BDE-47 which was detected in 65% of the blanks with all concentrations below the LOQ. Polyethylene containers used for field sample collection were also tested for contamination. Precleaned sodium sulfate was kept in the container for 48 h and then processed with the aforementioned method. The results revealed no contamination of any target chemicals of interest. Recoveries of surrogate standards FBDE-69, FBDE-160, 4PC-BDE208, and d_{18}-α-HBCDD in authentic samples ranged from 68.5 to 91.6%, 77.5−106.2%, 51.6−82.3%, and 79.8−90.2%, respectively.

Data Analysis. All flame retardant concentrations were expressed as ng/g lw. Residual levels of FRs were adjusted based on the recoveries of FBDE-69 (for analytes with GC retention times earlier than BDE-85), 4PC-BDE208 (for BDE-209 and DBDPE), or FBDE-160 (for all other analytes). The selection of different surrogate chemicals was based on spiking tests to determine which surrogate can best represent the recovery of a target analyte during sample pretreatment.[19,20] For measurements below LOD (i.e., nondetectable) or LOQ, a half LOD or half LOQ was assigned for statistical analysis, respectively. Logarithmic transformation was used on non-normally distributed data to approximate a normal distribution. ∑PBDEs and ∑Dechloranes represent the total concentration of all detected PBDE congeners and dechlorane analogues, respectively. Concentration of ∑HBCDDs included α-, β- and γ- diastereomers. The maternal transfer ratio for a FR chemical

was determined as a ratio of the mean litter concentration to the mother's concentration on a lipid weight basis. Linear regression used to assess the relationship between log K_{ow} and maternal transfer ratio and the *Kruskal−Wallis* analysis of variance (ANOVA) were conducted with PASW Statistics 18.0 (IBM Inc.). The level of significance was set at $\alpha = 0.05$. Differences in composition patterns between mothers and embryos were determined with Principal Component Analysis (PCA) (SAS 9.1; SAS Institute, Inc.). A range of concentrations or maternal transfer ratios was reported for all species. Median values were reported only for blacknose sharks and not for the other species as only two adult females per species were assessed.

■ RESULTS AND DISCUSSION

FR Concentrations and Profiles in Adult Females. PBDEs were the most abundant type of FRs determined in adult female sharks. Concentrations of $\sum$PBDEs ranged from 85.7−398 ng/g lw (median: 128 ng/g lw) and accounted for a percentage of 55.4%−78.3% (mean: 66.1%) of the total FR load in 12 adult female blacknose sharks (Table 1). In other studied species concentrations of $\sum$PBDEs were determined to be 258−2640 ng/g lw in female blacktip sharks, 148−173 ng/g lw in female bonnetheads, 493−4470 ng/g lw in female Atlantic sharpnose sharks, and 2950−16190 ng/g lw in female spinner sharks (Table 1). Previous studies have reported the wide occurrence of PBDEs in North American marine mammals and sharks, with mean concentrations ranging from 3.4 to 7500 ng/g lw in different species.[5,21−23]

Several dechlorane analogues, including *syn*-DP, *anti*-DP, Cplus, Dec-602, and Dec-603, were detected in adult female sharks investigated in the present study at a frequency of over 86%, whereas DPMA, Cl10-DP, Dec-601, Dec-604CB, Br-Dec604, Br_2-Dec604, Br_2Cl_2-Dec604, and Cl_4-Dec604 were generally not detected and Dec-604 was quantifiable in 43% of the samples. Concentrations of $\sum$Dechloranes ranged from 6.1−83.3 ng/g lw (median: 15 ng/g lw) in adult female blacknose sharks, 11−175 ng/g lw in female blacktip sharks, 0.6−29 in female bonnetheads, 35.6−94.4 ng/g lw in female Atlantic sharpnose sharks, and 94.3−587 ng/g lw in female spinner sharks (Table 1). These levels were generally 1 order of magnitude lower than those of $\sum$PBDEs in most adult female sharks. The dechlorane composition profile in adult female blacknose sharks was dominated by Dec-602, followed by Cplus and Dec-603 (Figure 1). Similar profiles were observed in other species. Dechlorane analogues other than *syn*- and *anti*-DP have not been frequently described for global fish and wildlife. Our findings indicate broad exposure to and subsequent bioaccumulation of this group of chlorinated FRs in western North Atlantic sharks.

The various non-PBDE brominated FRs, including BB-101, HBCDD, TBCT, and HBBZ, exhibited a high detection frequency (i.e., 90−100%). Median concentrations of $\sum$HBCDDs, TBCT, HBBZ, and BB-101 were 11, 5.9, 5.0, and 7.8 ng/g lw in adult female blacknose sharks, respectively. Concentrations of these four flame retardants were also determined to be 13.5−881, 1.0−270, 2.3−17.1, and 1.9−179 ng/g lw in the eight individual adult females from other four studied species, respectively (Table 1). BTBPE was also detected in 43% and TBX in 38% of samples. BB-101 is one of the polybrominated biphenyls that were used as flame retardants until the early 1980s.[24] Other alternative FRs have not been subject to any specific regulations in North America,
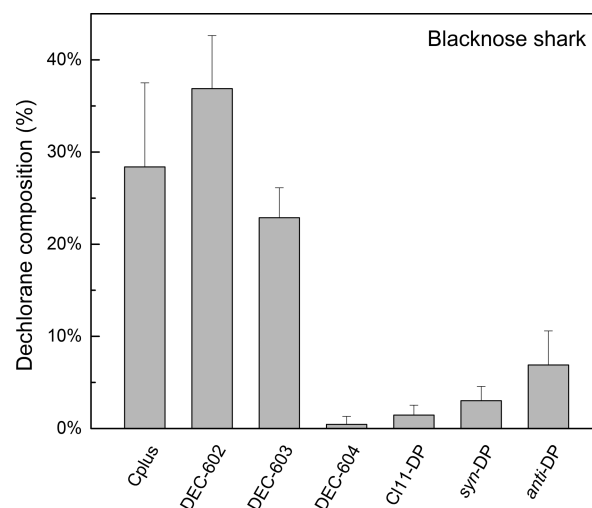
Figure 1. Compositional profile of dechlorane analogues in adult female blacknose sharks.

except for HBCDD which was added to the Stockholm Convention on Persistent Organic Pollutants in 2013.[25] Some of these alternative FRs are considered replacements for PBDEs. For example, BTBPE and EH-TBB are considered replacements for OctaBDE and PentaBDE, respectively.[6,26] EH-TBB is listed as a high production volume (HPV) chemical and (along with BEH-TEBP) an important component of several commercial FR mixtures including Firemaster 550 and Firemaster BZ-54.[27] Some alternative FRs (i.e., BTBPE, EH-TBB, BEH-TEBP, and TBX) have recently been reported in marine mammals,[21,24,28] but available field studies have generally agreed with laboratory *in vitro* and *in vivo* biotransformation studies in indicating their limited bioavailability.[29−31]

The analysis of between-species variation in FR accumulation was not the main objective of our study and was limited by small sample sizes for most studied species. Nevertheless, some of the biological factors, such as reproductive strategy, body size, growth rate, and diet, differ between the studied species (Table S1) and may contribute to between-species variation in FR contamination. For example, PBDE concentrations in adult female spinner sharks were 1 order of magnitude greater than those in female blacknose sharks (Table 1). These two species have similar diets (i.e., primarily teleost fish) and occupy the same trophic level.[32] However, they differ in reproductive strategy, body size, and age at maturity. In the Atlantic Ocean, females of both spinner shark and blacknose shark produce offspring approximately every two years, although spinner sharks typically have longer gestation periods (11−15 months) than blacknose sharks (9−11 months).[33−36] Female spinner sharks are relatively slow to mature, not reaching maturity until 7−8 years, and have a typical total length or TL of 150−155 cm when reaching maturity, whereas female blacknose sharks mature at approximately 4.5 years and have a typical TL of 100−105 cm when reaching maturity.[33−36] Age- or size-dependent accumulation of PBDEs has been reported in sharks and marine mammals.[21,23,37] These different biological and reproductive characteristics may contribute to different bioaccumulation patterns and FR concentration levels between female spinner sharks and blacknose sharks. Geographical factors were not investigated in this study as all the species of

Table 2. Range of Embryo to Mother Concentration Ratios of Flame Retardants in Five Different Shark Species[c]

	blacknose shark *Carcharhinus acronotus* $(n = 12)^a$	blacktip shark *Carcharhinus limbatus* $(n = 2)$	bonnethead *Sphyrna tiburo* $(n = 2)$	Atlantic sharpnose shark *Rhizoprionodon terraenovae* $(n = 2)$	spinner shark *Carcharhinus brevipinna* $(n = 2)$
ATE	0.15−1.05 (0.70)	na[b]-0.24	na-0.23	na	na
BB-101	0.26−0.78 (0.50)	0.45−0.47	0.26- 0.51	0.07−0.77	0.07−0.11
HBBZ	0.25−0.78 (0.55)	0.44−0.67	na-0.07	0.25−0.40	0.24−0.25
TBCT	0.17−1.55 (0.66)	0.18−1.06	0.04−0.52	0.13−0.23	0.13−0.37
β-TBECH	0.09−0.34 (0.23)	na-0.43	na-0.02	na	na-0.72
TBX	0.12−1.16 (0.47)	na-0.81	na-0.05	na	na-0.70
α-HBCDD	0.12−0.69 (0.39)	0.06−0.48	0.25−0.34	0.13−0.25	0.13−0.13
Cplus	0.03−0.69 (0.19)	0.02−0.09	na-0.18	0.01−0.08	0.01−0.01
Cl11-DP	0.08−0.42 (0.21)	na-0.17	na-0.12	na-0.01	na
Dec-602	0.03−0.57 (0.21)	0.01−0.19	0.21−0.52	0.01−0.06	0.01−0.01
Dec-603	0.01−0.50 (0.16)	0.01−0.13	0.19−0.26	0.01−0.08	0.01−0.02
syn-DP	0.05−0.78 (0.19)	0.02−0.13	na-0.16	0.01−0.12	na
anti-DP	0.03−0.42 (0.15)	0.02−0.23	na-0.14	0.01−0.10	0.01−0.02
$\sum$*Dechloranes*	0.03−0.55 (0.18)	0.02−0.16	0.12−0.18	0.01−0.08	0.01−0.01
BDE-28	0.17−0.99 (0.63)	0.28−0.35	0.35−0.58	0.24−0.51	0.24−0.92
BDE-47	0.13−1.49 (0.62)	0.21−0.73	0.21−0.41	0.11−0.23	0.11−0.52
BDE-49	0.20−1.02 (0.61)	0.10−0.30	0.16−0.39	0.10−0.47	0.10−0.15
BDE-66	0.13−1.05 (0.59)	0.15−0.53	na-0.08	0.10−0.34	0.10−0.20
BDE-99	0.06−1.26 (0.43)	0.05−0.27	0.17−0.26	0.05−0.09	0.05−0.09
BDE-100	0.08−0.71 (0.38)	0.06−0.54	0.23−0.38	0.04−0.12	0.04−0.10
BDE-153	0.03−0.53 (0.26)	0.04−0.24	0.01−0.26	0.02−0.11	0.01−0.02
BDE-154	0.01−0.56 (0.27)	0.03−0.24	0.19−0.42	0.01−0.10	0.01−0.02
BDE-183	0.13−0.31 (0.28)	na-0.21	na-0.19	na	na
$\sum$*PBDEs*	0.16−0.80 (0.49)	0.11−0.54	0.26−0.38	0.07−0.20	0.08−0.19

[a]n represents the number of mother/embryo groups per species. [b]na = data not available in one or both of the mother/embryo groups as the flame retardant chemical was not detectable or quantifiable in the group. [c]Mean values (in parentheses) are only provided for blacknose shark.

interest are highly migratory and cover significant spatial areas during the course of a year.[38,39] Investigation of the variety of biological and ecological factors will facilitate the understanding of other between-species differences in flame retardant exposure and bioaccumulation. Due to the difficulties in collecting pregnant females with specific term litters for the majority of species examined in this study, our species-specific interpretations will focus on species with the most robust sample sizes.

Maternal Transfer. The shark species investigated in our study all employ placental viviparous reproduction,[10,33,35,40,41] in which embryos initially derive nutrients from the yolk sac during the first stage of gestation, while in the later stages of gestation the depleted yolk sac becomes highly vascularized providing nutrients to the embryos directly from the maternal bloodstream by functioning as a pseudoplacenta.[10,41,42] Thus, maternal transfer of contaminants likely occurs when lipophilic contaminants are mobilized along with lipids from maternal fat stores (e.g., liver tissue) and diet through yolk sac during early stages of gestation and via bloodstream when the pseudoplacenta forms later in gestation.[42−44] While many factors contribute to the contaminant burden in young fish and wildlife, maternal transfer is known to be a primary route of POPs exposure for embryos, neonates, and young-of-the-year (YOY) of various species.[11−13,42,43] Many flame retardants are known to be lipophilic and bioaccumulative, which suggests that they may also be subject to maternal offloading; however, direct evidence of this process has not been extensively studied in sharks.

The maternal transfer ratios were determined to range from 0.07 to 0.80 for $\sum$PBDEs, 0.06−0.69 for α-HBCDD, 0.04−1.55 for TBCT, and 0.01−0.55 for $\sum$Dechloranes in a total of

20 mother/embryo groups from five species (Table 2). Variation in maternal transfer ratios was observed both between and within species. For example, the maternal transfer ratios for $\sum$PBDEs and $\sum$Dechloranes ranged from 0.16 to 0.80 and 0.03−0.55 within blacknose shark mother/embryo groups and 0.08−0.19 and 0.01−0.01 within spinner shark mother/embryo groups, respectively (Table 2). Previous studies also reported large variations in maternal transfer ratios of PBDEs (i.e., 0.03−1.57), PCBs (0.04−2.85), and organochlorine pesticides (0.04−2.43) in different marine mammals, sharks, and Atlantic stingrays (summarized in Table S4).

Some of the observed between-species variation in maternal transfer ratios may be linked to a variety of ecological and reproductive factors, including but not limited to trophic position of females, age-at-maturity, gestational period, and reproductive strategy, as well as female feeding ecology and habitat use.[11] For example, litter size may affect maternal transfer ratios. As aforementioned, spinner sharks tended to exhibit relatively lower maternal transfer ratios than blacknose sharks, although the small sample size of spinner sharks restricted valid statistical comparison. Female spinner sharks give birth to litters of three to 20 pups (usually 7−11), whereas female blacknose sharks typically give birth to one to six pups.[33,35] Although more embryos may tend to enable a female to offload an overall greater amount of contaminants,[13] larger litter size may result in a relatively lower partition of contaminants between the female and each individual embryo. Similarly, Lyons and Adams reported that female hammerhead sharks offloaded greater amounts of PCBs and DDTs to their embryos compared with a thresher shark female.[13] However, in the same study greater offloading ratios between embryos and

their mother were observed for the latter species which typically has a smaller litter size than hammerhead sharks.[13] However, expanded analyses of additional mother/embryo groups are required for more comprehensive comparisons of maternal offloading between species.

Some within species variation may be attributed to different embryonic stages or the number of past reproduction events experienced by females examined.[10] However, the analysis of blacknose shark data revealed no differences in maternal transfer ratios among three embryonic stages (i.e., early gestation, midearly gestation, and full term) for PBDEs, dechloranes, TBCT, or HBBZ (Table S5). Borrell et al. suggested that long-finned pilot whale (*Globicephala melas*) females tend to offload more organochlorine contaminants (i.e., DDT and PCBs) to offspring during the first reproduction event and less to subsequent litters.[44] However, the information on the number of past reproduction events was not available in this study. There was also some variation in FR concentrations among littermates from the same mother. For example, for $\sum$PBDEs the relative standard deviations (% RSD) in littermates were less than 15% for nine blacknose shark litters, but additional three blacknose litters exhibited relatively greater %RSDs (i.e., 19−38%). Additionally, the % RSDs for $\sum$Dechloranes (14−66%) in a total of 12 blacknose shark litters were significantly greater than those for $\sum$PBDEs (9−38%) and α-HBCDD (3−28%) ($p < 0.05$). The causal factors of these differences require additional investigation.

Maternal offloading may be expected to contribute a dominant portion of FRs in the YOY stage of some marine species. Previous studies revealed that concentrations of PCBs and organochlorine pesticides decreased with the size of the YOY sharks of scalloped hammerhead shark, common thresher shark, and shortfin mako (*Isurus oxyrinchus*) after parturition, suggesting that their own dietary acquisition of contaminants during their entire first year of life was relatively lower compared to direct maternal transfer *in utero*.[11,13] A similar pattern may be expected for FRs such as PBDEs, given their similarity with PCBs in bioaccumulation and persistence. Thus, initial FR burdens in neonate and early YOY sharks result from what they received *in utero* and represent a maternal signature. Flame retardant signatures are likely modified when exogenous feeding begins after parturition. Therefore, maternal offloading may play an important role in potential adverse effects induced by bioaccumulative halogenated contaminants not only *in utero* but also during the critical postnatal developmental stages in neonate and YOY sharks. Future studies on the potential adverse effects of these contaminants on neonatal and YOY sharks are warranted.

Maternal Transfer and Log K_{ow}. The maternal transfer ratio of FRs was significantly influenced by their log K_{ow} values. A significant, negative correlation was observed between log K_{ow} of all major FRs (ranging from 5 to 12) and their maternal transfer ratios in the mother/embryo groups of blacknose sharks ($p < 0.01$, $r^2 = 0.21$) (Figure 2). The correlation was also significant when combining all species ($p < 0.001$, $r^2 = 0.34$). However, between-species variation in reproductive strategy, trophic status, and other factors may confound the correlative analysis when combining all species. Nevertheless, this pattern suggests that although lipophilic contaminants are prone to be maternally transferred, extremely high lipophilicity may decrease a chemical's mobility, uptake, and bioaccumulation *in utero*, thus reducing its maternal transfer ratio.[13,18] Chemicals with a high log K_{ow} value (i.e., > 7.5) generally
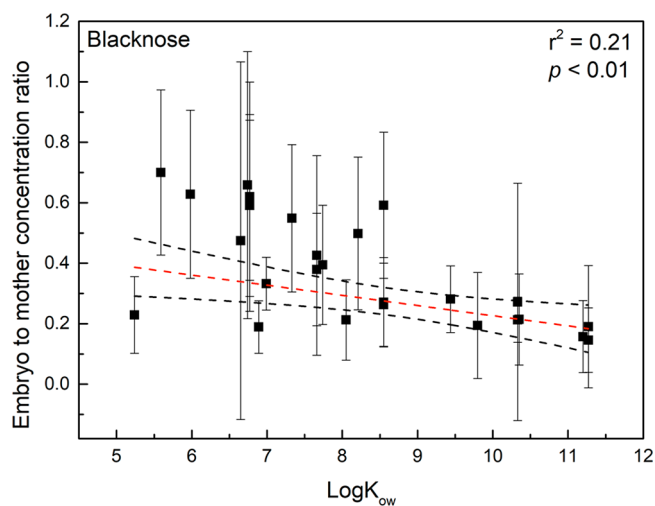
Figure 2. Correlation between the embryo to mother concentration ratios of flame retardants in blacknose sharks and the chemicals' octanol−water partition coefficients (K_{ow}). The log K_{ow} was estimated based on the U.S. Environmental Protection Agency (EPA) Estimation Program Interface (EPI) Suite Version 4.11. Red and black dashed lines represent the linear regression line and ±95% confidence bars, respectively.

possess a great molecular weight and/or size, making them less readily cross cell membranes *in utero*.[18] Therefore, bioaccumulative FRs with lower molecular weights are subject to greater maternal offloading than higher molecular weight FRs. By contrast, the accumulation of chemicals with a relatively higher log K_{ow} in marine organisms after the neonate and YOY stages is more likely influenced by dietary intake than remaining signature from earlier maternal offloading.
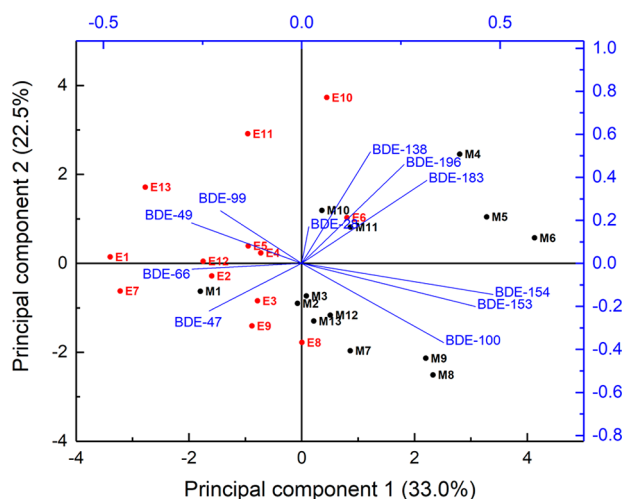
Similar to our findings, declining transfer efficiency along with the increasing lipophilicity of PBDE congeners was also reported in mother/embryo groups of Japanese melon-headed whales and Arctic beluga whales.[45,46] A negative relationship between mother-to-embryo, mother-to-pup, or mother-to-calf transfer ratio and the log K_{ow} (or the level of chlorination) of PCB congeners has also been reported in scalloped hammerhead sharks from the southeastern U.S. Atlantic coast,[13] thresher sharks from southern California,[12] blacktip sharks from the Atlantic coast of Florida,[10] melon-headed whales and killer whales (*Orcinus orca*) from Japan,[15,45] and Canadian Arctic beluga whales.[46] These data collectively demonstrate the important role of a chemical's lipophilicity in its maternal transfer potency in sharks and marine mammals.

In addition to log K_{ow}, metabolism may also affect a chemical's maternal transfer ratio. Chemicals with a relatively low log K_{ow} may be subjected to elevated metabolism and thus are less likely transferred *in utero*. This may explain a slight shift of β-TBECH (log K_{ow} = 5.24) of the correlation between the maternal transfer ratio and log K_{ow} (Figure 2). Metabolic transformation of TBECH has been reported *in vitro* by rat or human liver microsomes.[47,48] Thus, the correlation model may not be able to predict the maternal transfer potency of chemicals with a relatively low log K_{ow} (i.e., less than 5).

Other factors may also affect maternal offloading. For example, Lyons et al. observed a positive relationship between YOY lamniform sharks and maternal trophic position, indicating that trophic ecology plays an important role in maternal offloading of legacy organic contaminants.[11] As aforementioned, the sequence of reproductive activity may also

affect maternal offloading in long-finned pilot whales, with the first reproduction event offloading more organochlorines than subsequent reproduction.[44] However, these factors are not addressed for our studied sharks but merit future investigation.

To further understand how chemical-specific maternal transfer affected FR compositions in embryos, principal component analyses (PCA) were performed on the composition profiles of PBDEs and dechloranes in blacknose shark mother/embryo groups. Congener/analogue profiles were expressed as concentration percentages of individual congeners/analogues to ∑PBDEs or ∑Dechloranes. Most of the embryos cluster together on the biplot of PBDE PCA, primarily due to the relatively higher contribution of lower brominated congeners (i.e., BDE-47, -49, -66, and −99) and lower contributions of more heavily brominated congeners in embryos when compared to the mothers (Figure 3). The PCA

Figure 4. Biplot from the principal component analysis (Component 1, 49.3% of total variance; Component 2, 23.4% of total variance) of dechlorane analogue compositions in adult female blacknose sharks and their embryos. M = mother; E = embryo; the number following M or E represents mother/embryo group ID.

maternal offloading of chemicals, such as trophic position, reproductive strategy, and female feeding ecology, also need further investigation with expanded analyses. The health implications of FR exposure for female sharks or their embryos are currently unknown due to a lack of toxic threshold data regarding any shark species. Ongoing studies will attempt to address some of these limitations by increasing sample sizes and investigating more biological and ecological aspects of individuals of multiple shark species.

Maternal transfer of bioaccumulative FRs *in utero* represents a potential risk to embryonic development and may represent the largest source of FR input to offspring during the first few years of life.[13] Chemical-specific maternal transfer suggests the important influence by physiochemical properties of contaminants, in addition to ecological and reproductive factors, on the maternal offloading of individual contaminants. However, maternal transfer studies are still limited with respect to chemical types and species. Knowledge regarding many aspects of maternal transfer remains scarce, including (1) the underlying mechanisms driving maternal transfer in placental species; (2) factors other than K_{ow} that may affect maternal transfer, including chemical, biological, and ecological factors; (3) real-scenario risks to embryos, neonates, and YOYs due to *in utero* maternal transfer at individual and population levels; and (4) the influence of *in utero* transfer on the sex-dependent bioaccumulation and food-web transfer of FRs or other emerging contaminants in aquatic and terrestrial wildlife. Similar knowledge gaps remain not just for marine wildlife but also for humans, meriting further investigations.
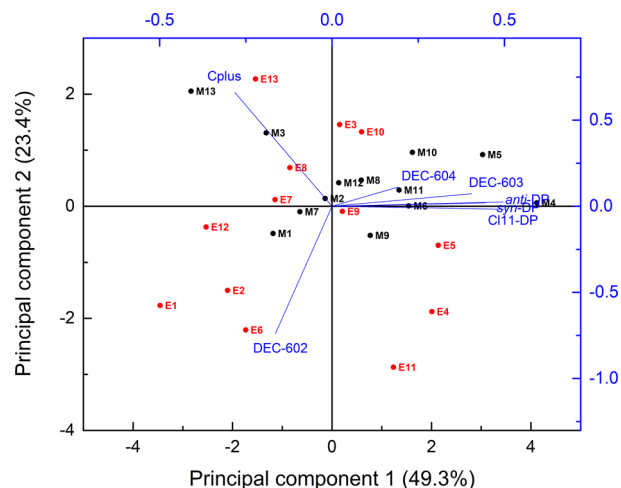

Figure 3. Biplot from the principal component analysis (Component 1, 33.0% of total variance; Component 2, 22.5% of total variance) of PBDE congener compositions in adult female blacknose shark and embryos. M = mother; E = embryo; the number following M or E represents mother/embryo group ID.

biplot reveals an elevated contribution by Dec-602 in most embryos compared with their mothers, whereas in mothers other analogues (i.e., Dec-603, *syn*-DP, *anti*-DP, and C₁₁-DP) with relatively greater log K_{ow} than Dec-602 showed elevated compositions (Figure 4). However, large variations between individual mother/embryo groups were also observed from the dechlorane PCA analysis. Further investigation is needed to determine if individual level factors that determine preferential transfer of certain analogues exist.

Result Implications. Our study provides a comprehensive investigation of maternal offloading of flame retardants in multiple shark species and is the first to examine emerging FRs in any shark species. Our findings broaden the knowledge on the exposure of Western North Atlantic sharks to a large variety of emerging FRs and the potential risks to their embryos through maternal offloading. These results further explored the role of a chemical's physicochemical properties in its maternal transfer potency toward a better understanding of the factors influencing maternal offloading.

Expanded sampling and analysis of additional mother/embryo groups will provide more in-depth interpretations of inter- or within species variations in FRs' maternal transfer ratios. Factors other than lipophilicity that may influence

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b01613.

Tables S1−S5 and Figure S1, as well as a detailed description of the HBCDD analytical method (PDF)

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Notes

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