Temporal Trends and Suspect Screening of Halogenated Flame Retardants and Their Metabolites in Blubbers of Cetaceans Stranded in Hong Kong Waters during 2013–2020

Qi Wang, Yuefei Ruan,* Linjie Jin, Brian C. W. Kot, Kenneth Mei Yee Leung, and Paul K. S. Lam*

ABSTRACT: Halogenated flame retardants (HFRs) are a large class of chemical additives intended to meet flammability safety requirements, and at present, they are ubiquitous in the environment. Herein, we conducted the target analysis and suspect screening of legacy and novel HFRs and their metabolites in the blubber of finless porpoises (Neophocaena phocaenoides; n = 70) and Indo-Pacific humpback dolphins (Sousa chinensis; n = 35) stranded in Hong Kong, a coastal city in the South China Sea, between 2013 and 2020. The average concentrations of total target HFRs (ΣHFRs) were $6.48 \times 10^3 \pm 1.01 \times 10^4$ and $1.40 \times 10^4 \pm 1.51 \times 10^4$ ng/g lipid weight in porpoises and dolphins, respectively. Significant decreasing temporal trends were observed in the concentrations of tetra-/penta-/hexa-bromodiphenyl ethers (tetra-/penta-/hexa-BDEs) in adult porpoises stranded from 2013–2015 to 2016–2020 ($p < 0.05$), probably because of their phasing out in China. No significant difference was found for the concentrations of decabromodiphenyl ether and the ban in China until 2025 and 2021, respectively. Eight brominated compounds were additionally identified via suspect screening. A positive correlation was found between the concentrations of tetra-BDE and methyl-methoxy-tetra-BDE (Me-MeO-tetra-BDE) ($p < 0.05$), indicating that the metabolism of tetra-BDE may be a potential source of Me-MeO-tetra-BDE in marine mammals.

KEYWORDS: polybrominated diphenyl ethers, Me-MeO-tetra-BDE, high-resolution mass spectrometry, south china sea, marine mammal, metabolites, 2,3-dibromopropyl-2,4,6-tribromophenyl ether, biomonitor

INTRODUCTION

Halogenated flame retardants (HFRs) are a group of chemical substances that are extensively incorporated into plastics, textiles, and appliances to prevent the combustion of materials or hinder the propagation and progression of fire. ¹,² Legacy HFRs mainly include polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls, hexabromocyclododecane (HBCD), and tetrabromobisphenol A (TBBPA). These chemicals have exhibited persistence and bioaccumulation potential and toxic effects on environmental and human health. ²,³ Consequently, in 2009, commercial octabromodiphenyl ether (octa-BDE), hexabromobiphenyl, and commercial pentabromodiphenyl ether (penta-BDE) were listed as persistent organic pollutants (POPs) in Annex A of the Stockholm Convention for global elimination.⁴ Subsequently, commercial decabromodiphenyl ether (deca-BDE) and an HBCD technical mixture were also included as POPs in the Stockholm Convention in 2013 and 2015, respectively.⁵,⁶ With the phasing out of these legacy HFRs, many novel HFRs have emerged as replacements and have been increasingly used. For example, 2-ethylhexyl-2,3,4,5-tetramethyloctyl ether (EHHTBB) and bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BHTEBP) have been used to replace penta-BDE, whereas 1,2-bis-(pentabromodiphenyl)ethane (DBDPE) and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) have been used as deca- and octa-BDE alternatives, respectively. ³ Moreover, some HFRs have already been used for decades, but they were usually neglected and omitted from environmental investigations until recent years, for example, 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE).⁷ For the purpose of clarity, HFRs investigated other than PBDEs and HBCD were defined as novel HFRs in this work. The global production of novel HFRs is reported to range from 100 to 180 kt annually.⁸ The ubiquitous occurrence and adverse effects of novel HFRs

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in the environment have raised global concerns in recent years.\textsuperscript{2,3}

Some novel HFRs have chemical structures similar to those of legacy HFRs, indicating the importance of the investigations on the occurrence and distribution of novel HFRs and their inherent correlations with legacy HFRs. Target analysis of HFRs usually focuses on a limited number of novel HFRs as the reference standards for novel HFRs are not often available.\textsuperscript{2} Moreover, many HFRs have biotransformation potential, for example, PBDEs could undergo dihydroxylation and methoxylation in avian liver microsomes and fish,\textsuperscript{10,11} while TBBPA was reported to form sulfation/glucuronidation conjugates in rodents.\textsuperscript{12} However, HFR metabolites are less studied compared with their parent compounds, which could partly be attributed to the absence of relevant reference standards.\textsuperscript{13} High-resolution mass spectrometry (HRMS), which can provide accurate masses, isotopic distributions, and MS/MS spectra, has become an indispensable technique for suspect screening of emerging pollutants, including novel HFRs.\textsuperscript{9,14} HRMS is also an effective tool for tentative identification of the countless reported (or inferred) HFR metabolites in the absence of reference standards.\textsuperscript{15,16} The combination of target analysis and suspect screening can help identify a large variety of HFRs and their metabolites, which will help better understand the pollution status and transformation processes of HFRs, and subsequently, contribute to the risk evaluation of HFRs.

The Pearl River Delta (PRD) region, adjacent to the northern South China Sea (SCS), is one of the most developed, industrialized, and urbanized regions in China. Rapid economic and industrial development in this region has elevated the burden of environmental pollution.\textsuperscript{17--19} Among various pollutants found in the PRD, HFRs are of considerable significance because of the vigorous growth of electronic, plastic, household appliance, and textile manufacturing activities in this region.\textsuperscript{17} The Pearl River flows through the PRD and ultimately ends in the SCS. As top predators, marine mammals can bioaccumulate high amounts of POPs and are particularly susceptible to HFR exposure.\textsuperscript{2,20,21} This makes them an ideal biomonitor of the source and temporal trend of HFRs in the marine environment. Indo-Pacific humpback dolphins (Sousa chinensis) and finless porpoises (Neophocaena phocaenoides) are two resident marine mammals in the SCS. Our previous study reported the widespread presence of novel HFRs in seawater and sediment from the coasts of SCS with concentrations comparable to those of PBDEs.\textsuperscript{17} As the use of novel HFRs increases, it is imperative to investigate the pollution status and temporal trend of HFRs in the marine mammals in this region.

Thus, we conducted comprehensive target analysis and suspect screening of HFRs in blubber samples from 105 marine cetaceans stranded in Hong Kong waters between 2013 and 2020. We aimed to (1) investigate the pollution status of legacy and novel HFRs in the SCS using the cetacean blubbers as the biomonitor, (2) examine the temporal trends of concentrations and composition profiles of the legacy and novel HFRs in the marine mammals, and (3) use HRMS to screen the novel HFRs and potential HFR metabolites. The results unravel the recent contamination status of HFRs and provide evidence for evaluating the effectiveness of various control measures (e.g., ban of use of certain groups of HFRs).

### MATERIALS AND METHODS

**Reagents and Standards.** Details of solvents and reagents used are provided in the Supporting Information (SI). Basic information on 26 target legacy and novel HFRs analyzed in this study are provided in Table S1.

**Sample Collection.** Blubber samples of finless porpoises (\( n = 70 \)) and Indo-Pacific humpback dolphins (\( n = 35 \)) were obtained from stranded cetaceans collected by the Agriculture, Fisheries, and Conservation Department in Hong Kong, China, between 2013 and 2020 (Table S2). Once in the laboratory, the samples were freeze-dried and stored at \( -50 \) °C until further processing.

**Extraction and Cleanup.** Analyses of blubber samples were performed using a previously established method.\textsuperscript{21} Briefly, \( \sim 1 \) g of the blubber sample was ground with anhydrous sodium sulfate and spiked with mass-labeled surrogates of 10 ng each. Then, the sample was extracted via pressurized fluid extraction. Each extract was purified before lipid removal via gel permeation chromatography and then further purified by successive elution through anhydrous sodium sulfate, activated aluminum oxide, and activated silica gel. The final reduced extract was spiked with 10 ng of the respective internal standard, concentrated to dryness under a gentle nitrogen stream, and reconstituted to 100 \( \mu L \) with methanol for instrumental analysis.

**Target Analysis.** Target analysis of HFRs was performed through ultrahigh-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) using an Agilent 1290 Infinity liquid chromatograph (Palo Alto, CA, USA) coupled to SCIEX QTRAP 5000 (Woodlands, Singapore) with an atmospheric pressure chemical ionization (APCI) interface operated in the negative multiple-reaction monitoring mode. Details of the instrumental parameters are provided in the SI and Table S3.

**Suspect Screening.** UPLC–quadrupole time-of-flight (QToF) HRMS was used for the suspect screening of HFRs. The UPLC method for QToF-HRMS was the same as that for target analysis. HRMS screening was performed using a Sciex X500R mass spectrometer (Foster City, CA, USA) in the negative APCI mode. The information-dependent acquisition (IDA) experiment was conducted with a mass-to-charge ratio (\( m/z \)) ranging from 200 to 1200, and dependent MS/MS was operated with \( m/z \) ranging from 30 to 1200 in the high-resolution mode. Dynamic background subtraction was applied in the IDA criteria for dynamic exclusion.

The peak list was extracted from the raw QToF data in SCIEX OS. The following parameters were set for initial filtering: (1) signal-to-noise ratio (S/N) > 3, (2) LC peak width < 30 s, and (3) intensity > five times the intensity in the procedural blank. Suspect screening was conducted using a suspect list that included three parts: (1) novel HFRs reported in recent years, (2) reported HFR metabolites, and (3) inferred HFR metabolites.\textsuperscript{13,14,22--25} Eight common metabolic pathways were considered, including methylation, debrumination, hydroxylation, dihydroxylation, methoxylation, demethylation, sulfation, and glucuronidation.\textsuperscript{13} The monoisotopic mass of the highest abundance was used for screening, while the monoisotopic mass of the second and third highest abundance was used for verification. The ions for HFRs were considered to be generated in \([ M - Br + O ] ^ { - } , [ M - H ] ^ { - } , \) and \([ M + O _ { 2 } ] ^ { - } \) in the negative APCI mode, as previously reported.\textsuperscript{17,22,26} All screening processes were performed using

Quality Assurance and Quality Control. For target analysis, the limit of quantification was defined as S/N ≥ 10:1 (Table S4). For each batch of five samples, a procedural blank was included for quality assurance. Recoveries of spiked surrogates in the cetacean blubber were all in the range of 71%–96%.

In HRMS analysis, a procedural blank sample was used to monitor blank contamination during data analysis. The instrument was automatically calibrated after every six sample injections to check the mass accuracy of the instrument. The mass error was set as <10 ppm, and the isotope ratio difference was <10%. For each candidate, at least three peaks (two peaks for formula containing only one Br) that fit the isotopic distribution were kept. The screened candidates were then manually cross-checked with their MS/MS spectra using Sciex OS. Only the candidates with the Br− fragment were used.

Statistical Analysis. Temporal trend analysis was carried out using log-linear regression following our previous study.21,27 The nonparametric Mann–Whitney test was used to examine the significant difference in the concentration of the same chemical contaminant in porpoise bubblers collected between two time periods (2013–2015 versus 2016–2020). Spearman’s test was used for correlation analysis. Statistical significance was accepted at p < 0.05. Temporal trend analysis was performed using simple log-linear regression following the method in our previous studies.21,27 Yearly median concentrations in the bubblers of dolphins and porpoises were used to perform the regression of the whole monitoring dataset, respectively. Statistical analysis was performed using IBM SPSS software (version 22.0, IBM Corp., NY, USA) and the R program.

RESULTS AND DISCUSSION

Overview of Legacy and Novel HFRs. Nineteen legacy and novel HFRs were detected in the investigated marine cetacean blubber samples among 26 target analytes analyzed in the present study. Concentrations of nine legacy HFRs were quantified in more than 21 cetacean individuals (i.e., quantification frequency (QF) > 20%), including 2,4,4′-tribromodiphenyl ether (BDE-28), 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153), 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154), 2,2′,3,4,4′,5,6-heptabromodiphenyl ether (BDE-183), decabromodiphenyl ether (BDE-209), and HBCD. Five novel HFRs, i.e., DPTE, 2,4,6-tribromophenol (TBP), pentabromo-toluene (PBT), hexabromobenzene (HBBz), and BEHTBP, exhibited quantification frequencies of >20%, while other novel HFRs were rarely detected. The comparisons of the HFR concentrations and compositions between the two cetacean species are shown in Figure 1.

The concentrations of total target HFRs (ΣHFRs) in the blubber samples ranged from 207 to 5.28 × 10⁴ ng/g lipid weight (lw) (mean = 0.65 × 10⁴ ± 1.01 × 10⁴ ng/g lw) for finless porpoises (referred to as porpoises if unspecified) and from 101 to 5.72 × 10⁴ ng/g lw (mean = 1.40 × 10⁴ ± 1.51 × 10⁴ ng/g lw) for Indo-Pacific humpback dolphins (referred to as dolphins if unspecified) (Figure 1A).

Figure 1. Concentrations and compositions of (A, B) legacy and (C, D) novel HFRs in the blubber of dolphins (n = 35) and finless porpoises (n = 70).
truncatus) collected from the Florida coast (151 ng/g lw), ringed seals (Pusa hispida botanica) collected from the Baltic Sea (86 ng/g lw), and finless porpoises (Neophocaena asioorientalis) collected from Korean coastal waters (290 ng/g lw). High industrialization and urbanization with a dense population in the PRD might contribute to the emission of HFRs in the coastal SCS, leading to the high bioaccumulation of some HFRs in the resident marine cetaceans. ΣHFR concentrations were significantly higher in dolphins than porpoises \((p < 0.05)\). The main reason is the habitat for the dolphins is closer to the coastal regions of the Pearl River Estuary, where the aquatic environment is more contaminated by HFRs, while porpoises generally inhabit the southern and eastern Hong Kong waters (adjacent to open seas) where HFRs are at lower levels as compared to the Pearl River Estuary.\(^{17,21,31,32}\) Nevertheless, differences in the bioaccumulation and metabolism of HFRs in these two marine mammal species should not be neglected, which deserves further investigation.

HFR compositions in the studied blubber samples were similar for porpoises and dolphins. BDE-47 was the predominant HFR, accounting for 60% and 68% of ΣHFR levels in porpoises and dolphins, respectively (Figure 1B). In the seawater samples collected in 2018 in the coastal SCS where the studied marine mammals inhabit, BDE-47 was found to be the only detected PBDE from tri- to octa-BDE congeners.\(^{37}\) Globally, BDE-47 was also found to be the predominant HFR in marine animals, including invertebrates, fishes, and mammals in most reported studies, mainly due to its broad application and extensive use as well as its high bioaccumulation and persistence potential.\(^{30,33–35}\) Apart from BDE-47, other six BDE congeners, including BDE-28, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183, were found in most of the investigated cetaceans in this study \((QF > 95\%)\), with individual levels contributing to 2%–11% of the ΣHFR concentrations. Significant positive correlations were found among these BDE congeners \((p < 0.05, \text{Tables SS and S6})\), indicating that they likely had a similar contamination source in the studied cetaceans. It is worth noting that these six PBDE congeners were not found in seawater samples collected in 2018 in the coastal SCS,\(^{17}\) partly because of the strong hydrophobicity of these congeners. BDE-209, the highest brominated PBDE, was detected in less than half of the studied cetaceans \((QF = 38\%)\). BDE-209 was found to be the predominant HFR in the seawater and sediment from the coastal SCS.\(^{17}\) However, BDE-209 only contributed to <1% of ΣHFR levels in the investigated marine mammals inhabiting the same region, probably because of the relatively lower bioavailability of BDE-209 than other PBDEs that are lower-brominated.\(^{34}\) In addition, no significant correlation was found between the concentrations of BDE-209 and other BDE congeners \((p > 0.05)\), which implies that the source of BDE-209 in the stranded cetaceans may be different from that of other BDE analogs. Among all the studied HFRs, PBDEs were still the predominant compounds \((95\% \text{ to } ΣHFR)\) in the cetaceans in this study, which implies that PBDE contamination is an ongoing matter of concern. α-HBCD was detected in almost all of the blubber samples \(104 \text{ out of } 105\) and contributed to 4% of ΣHFR levels, while two other diastereomers \(\text{i.e., } β- \text{ and } γ-HBCD\) were not found in any samples, probably because of the much lower biomagnification potential of these two isomers in the marine food web.\(^{29}\)

Unlike the widespread occurrence and high proportion of legacy HFRs (PBDEs and α-HBCD), concentrations of novel HFRs in the blubber samples were much lower, and novel HFRs made a small contribution to ΣHFR levels \((<2\%)\) (Figure 1A,C). This is probably because the production and use of novel HFRs are much lower than those of legacy HFRs in China.\(^{25,36}\) Another reason could be that novel HFRs are not stable in the marine environment owing to their relatively short solar-irradiation-related half-lives \((1.5−165 \text{ days})\) and their potential metabolization in mammal species.\(^{13,37}\) While there are many reports on legacy HFRs in marine mammals, there are fewer studies on novel HFRs in them, and levels of novel HFRs are usually several orders of magnitude lower than those of PBDEs. Global studies on the concentrations of detected novel HFRs in the marine mammal blubber are summarized in Table S7. Similar results were also observed in the current study where the average concentrations of novel HFRs \(\text{porpoises: } 71.2 ± 159.2 \text{ ng/g lw; dolphins: } 284 ± 395 \text{ ng/g lw}\) were much lower than those of legacy HFRs \(\text{porpoises: } 6.41 ± 10.04 \times 10^2 \text{ ng/g lw; dolphins: } 1.37 ± 1.48 \times 10^2 \text{ ng/g lw}\). DPTE was the predominant novel HFR with a QF of 58% in the investigated samples; the average concentrations of DPTE were \(61 ± 151 \text{ ng/g lw and } 277 ± 393 \text{ ng/g lw in porpoises and dolphins, which contributed to }97.4\%\) and 86.2% of the total novel HFR levels (Figure 1C,D), respectively. Reports on DPTE in marine mammals are very limited. To the best of our knowledge, one study has reported that the concentrations of DPTE in the blubber of hooded seal \(\text{(Cystophora cristata)}\) collected from the Barents Sea in 1991 ranged from 322 to 470 ng/g lw,\(^{38}\) and this study is the only report on the presence of DPTE in marine mammals in the last 30 years (Table S7). DPTE was first produced by Chemische Fabrik Kalk in Germany under the trademark Bromkal 73-5 PE.\(^{37}\) The recorded commercial production of DPTE has been gradually phased out since 1993, and information about its current production and use is unclear.\(^{39}\) However, DPTE has been frequently detected in wildlife globally and is usually the predominant novel HFR, indicating its wide application, which deserves further investigation.\(^{39–41}\) In this study, the QFs of other novel HFRs in the cetacean blubbers, including TBP, HBBr, PBT, and BEHTBP, ranged from 20% to 34%, and in total, they contributed to <1% of ΣHFR levels (Figure 1D). The limited production and use of these novel HFRs in China is probably the main reason for their relatively infrequent occurrences, which is consistent with their fewer detections globally (Table S7).\(^{42}\) A significant positive correlation was found between the concentrations of DPTE, TBP, HBBr, and PBT and legacy HFRs \(\text{(i.e., } \text{PBDEs and } \alpha-\text{HBCD)}\) (Table S6). With the gradual phasing out of legacy HFRs, novel HFRs were used as replacements to meet flammability standards for materials and products.\(^{32,33}\) Although commercial penta- and octa-BDE mixtures, HBCD, and commercial deca-BDE mixtures were listed in Annex A under the Stockholm Convention for global elimination in 2009, 2013, and 2017, respectively, the legislative action on a total ban did not come into effect in China until 2013, 2021, and 2023,\(^{42}\) respectively. This could result in a continuous accumulation (and a possible subsequent “delayed decline”) of legacy HFR concentrations in the studied cetacean blubber during the investigated period (2013–2020) in the present study. Thus, the significant positive correlation between these novel HFRs and legacy HFRs in the investigated cetaceans indicated that they may have a similar contamination source.
Figure 2. Concentrations of selected HFRs in the blubber of finless porpoises stranded during 2013–2015 and 2016–2020. (*) represents $p < 0.05$ and ** represents $p < 0.01$ based on Mann–Whitney tests.

Table 1. Exact Masses, Fragment Formula, and Relative Abundances of Novel HFRs and Potential HFR Metabolites Identified via Suspect Screening

<table>
<thead>
<tr>
<th>compound</th>
<th>fragment formula</th>
<th>retention time (min)</th>
<th>theoretical $m/z$</th>
<th>observed $m/z$</th>
<th>error (ppm)</th>
<th>relative abundance (%)</th>
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<td>ATE</td>
<td>$\text{C}_n\text{H}_2\text{Br}_2\text{O}^-$</td>
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<td>450.8019</td>
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<tr>
<td>MeO-penta-BDE</td>
<td>$\text{C}_n\text{H}<em>2\text{Br}</em>{23}\text{Br}_14\text{BrO}^-$</td>
<td>8.23/10.47</td>
<td>528.7113</td>
<td>528.7127</td>
<td>2.6</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>$\text{C}_n\text{H}<em>2\text{Br}</em>{24}\text{Br}_15\text{BrO}^-$</td>
<td></td>
<td>530.7093</td>
<td>530.7103</td>
<td>1.8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>$\text{C}_n\text{H}<em>2\text{Br}</em>{25}\text{Br}_16\text{BrO}^-$</td>
<td></td>
<td>532.7072</td>
<td>532.7075</td>
<td>0.5</td>
<td>65</td>
</tr>
<tr>
<td>Me-MeO-tetra-BDE</td>
<td>$\text{C}_n\text{H}<em>2\text{Br}</em>{26}\text{Br}_17\text{BrO}^-$</td>
<td>10.30</td>
<td>462.8185</td>
<td>462.8195</td>
<td>2.1</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>$\text{C}_n\text{H}<em>2\text{Br}</em>{27}\text{Br}_18\text{BrO}^-$</td>
<td></td>
<td>464.8165</td>
<td>464.8185</td>
<td>−1.6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>$\text{C}_n\text{H}<em>2\text{Br}</em>{28}\text{Br}_19\text{BrO}^-$</td>
<td></td>
<td>466.8144</td>
<td>466.8142</td>
<td>−0.5</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>$\text{C}_n\text{H}<em>2\text{Br}</em>{29}\text{Br}_20\text{BrO}^-$</td>
<td></td>
<td>468.8124</td>
<td>468.8131</td>
<td>1.4</td>
<td>32</td>
</tr>
</tbody>
</table>

Temporal Trends of HFR Levels. Only adult samples (body lengths of dolphins and porpoises greater than 200 and 120 cm, respectively) were used for statistical analysis to minimize the possible age-related differences, and 53 porpoise and 17 dolphin samples were included in the analysis. Considering the relatively small sample size of dolphin samples, only porpoise samples were used for the temporal trend discussion.

No significant temporal trend was found for any target HFR in the investigated marine cetaceans. The adult porpoise blubber samples ($n = 53$) were divided into two groups, 2013–2015 ($n = 26$) and 2016–2020 ($n = 27$), according to the stranded years to reflect temporal changes in recent years, with the consideration of the long elimination half-lives of PBDEs in marine mammals as well as a balance in sample size between the two periods. Significantly higher levels of tetra-/penta-/hexa-BDEs (i.e., BDE-28, −47, −99, −100, −153, and −154)
were found in porpoises stranded between 2013 and 2015 than those between 2016 and 2020 (p < 0.05) (Figure 2), indicating a decrease in consumption of the lower brominated BDE congeners in recent years. This observed reduction could be attributed to the legislative action on commercial penta- and octa-BDE mixtures in China since 2014, as these mixtures were banned by the Chinese government during this period. However, an increasing trend of DPTE concentrations in porpoises from the Bohai Sea in China was observed between 2013 and 2015 (p < 0.05) versus 2016 (Table 1).

The levels of novel HFRs have increased in various environmental matrices in most regions around the world because of the phase-out of PBDEs and HBCD and their replacements. The relatively low QFs of novel HFRs in our results limit our discussion on their temporal trend. DPTE was the only novel HFR detected in more than half of the adult porpoise samples in this study. Significantly higher levels of DPTE were found in the porpoises stranded between 2013 and 2015 than those between 2016 and 2020 (p < 0.01; Figure 2), indicating a decreasing trend of DPTE in recent years. This result is consistent with that of Dreyer et al. (2019), who reported that DPTE concentrations have generally decreased since 2000 in environmental abiotic matrices and wildlife in the German and polar regions. However, an increasing trend of DPTE concentrations in mollusks from the Bohai Sea in China was observed between 2011 and 2018. Currently, information on the scale and status of DPTE production/use is still ambiguous. Further investigations on the emission pathways and ecotoxicity of DPTE are needed because of its widespread global occurrence.

Identification of HFR Metabolites and Novel HFRs in Cetacean Blubber. Eight HFR metabolites and novel HFRs were identified using our suspect screening strategy with a mass error of <10 ppm and an isotope ratio difference of <0.05. Table 1 summarizes the HFR metabolites identified via suspect screening along with their formulas and monoisotopic theoretical/observed m/z. Semi-quantification was conducted for each identified HFR and HFR metabolite by comparing their intensity with that of a similar analyte whose reference standard was available and correct with an internal standard (13C12-TBP).

2,4,6-Tribromophenyl Allyl Ether. 2,4,6-Tribromophenyl allyl ether (ATE) in porpoise and dolphin samples was screened using its [M − Br + O]− format with diagnostic ions of 304.8846 (C12H7BrO3; mass error = 9.00 ppm), 306.8816 (C12H7Br3O3; mass error = 6.00 ppm), and 306.8767 (C12H7Br2O3; mass error = −3.4 ppm) as well as an isotopic intensity ratio of 51:100:49. ATE (trade name: PHE-65) is commonly added to expandable or foamed polystyrene, which is currently produced by Chemtura Corp. ATE can exhibit significant trophic magnification potential in the aquatic food web, which could lead to its considerable bioaccumulation in the studied marine mammals from the coastal SCS. Apart from the direct source, ATE is also a degradation product of DPTE, which usually co-occurs with 2-bromoallyl-2,4,6-tribromophenyl ether (BATE). However, no BATE was found in any of the investigated blubber samples in the current study. Besides, no statistically significant correlation was found between the concentrations of ATE and DPTE in either dolphin or porpoise blubber samples based on our semi-quantification results. Therefore, ATE in the studied marine mammals from the coastal SCS probably originated from the direct source rather than the biotransformation of DPTE (i.e., indirect source).

Bromobenzenes. Three bromobenzenes, pentabromobenzene (PBBz), tribromobenzene (TrBBz), and tribromobenzene (TBBz), were detected via suspect screening of the blubber samples. All three bromobenzenes were screened using their [M − Br + O]− fragment with diagnostic ions of 408.6735 (C12H7Br2O3; mass error = 2.3 ppm), 328.7636 (C12H7BrO3; mass error = −1.4 ppm), and 250.8534 (C12H7BrO3; mass error = −0.6 ppm). Combined with HBBz quantified via target analysis, in total, four bromobenzenes were found in the cetacean blubber samples in this study. PBBz, TeBBz, and TrBBz were reported to be the step-by-step debromination products of HBBz under abiotic/biotic degradation/metabolism conditions. No significant correlation was found between the concentrations of PBBz/TeBBz/TrBBz and HBBz in either porpoise or dolphin blubber samples, suggesting that the source of TrBBz/TeBBz/PBBz and HBBz is different in the investigated samples, and the debromination of HBBz may not be the main source of PBBz, TeBBz, and TrBBz in the investigated marine mammals. PBBz is widely used in polymeric materials, electronic equipment, and household furniture and has become ubiquitous in the aquatic environment and biota owing to relatively high bioaccumulation potential, while reports on the direct production and use of TrBBz and TeBBz are lacking.
and TeBBz is not available, the indirect source, i.e., the
debromination of PBBz, might be an important source of
TrBBz and TeBBz in the marine mammals stranded in the
coastal SCS between 2013 and 2020.

Hydroxylated and Methoxylated BDEs (OH- and MeO-
BDEs). OH-tetra-BDE in porpoise and dolphin samples was
screened using its \([M - Br + O]^-\) fragment with diagnostic
ions of 434.7871 (C_{12}H_{67}Br_{3}O_{3}^-; mass error = -0.3 ppm),
436.7863 (C_{12}H_{68}Br_{2}81BrO_{3}^-; mass error = 2.4 ppm),
438.7846 (C_{12}H_{69}Br_{3}81BrO_{3}^-; mass error = 3.3 ppm), and
440.7821 (C_{12}H_{70}Br_{3}O_{3}^-; mass error = 2.2 ppm) with isotope
intensity ratios of 34:100:97:32. Two MeO-tetra-BDE isomers
in porpoise and dolphin samples with retention times (RTs) of
7.63 and 9.49 min were screened using their \([M - Br + O]^-\) fragment with diagnostic ions of 448.8046 (C_{13}H_{71}Br_{3}O_{3}^-; mass error = 3.7 ppm),
450.8019 (C_{13}H_{72}Br_{2}81BrO_{3}^-; mass error = 2.3 ppm),
452.7999 (C_{13}H_{73}Br_{3}81BrO_{3}^-; mass error = 2.3 ppm), and
454.7983 (C_{13}H_{74}Br_{3}O_{3}^-; mass error = 3.4 ppm) with isotope intensity ratios of 34:100:97:32. Two MeO-
penta-BDE isomers in porpoise and dolphin samples with RTs of
8.23 and 10.47 min were screened using their \([M - Br + O]^-\) fragment with diagnostic ions of 528.7127 (C_{13}H_{79}Br_{3}81BrO_{3}^-; mass error = 2.6 ppm), 530.7103 (C_{13}H_{80}Br_{3}81BrO_{3}^-; mass error = 1.8 ppm), and 532.7075 (C_{13}H_{81}Br_{3}81BrO_{3}^-; mass error = 0.5 ppm) with isotope intensity ratios of 69:100:65. MeO- and OH-BDEs are not
manufactured; however, they can be naturally formed in
marine organisms.\(^{55,54}\)

It was reported that OH-BDEs did not
biomagnify in the marine food web, and OH-BDEs in marine
mammals mainly originate from (1) metabolism of PBDEs
and/or MeO-BDEs and (2) the direct uptake from the natural
source.\(^{55-57}\) A significant positive correlation was found
between concentrations of OH-tetra-BDE and BDE-47/
MeO-tetra-BDE in both porpoises and dolphins (\(p < 0.05;\)
Table S6), suggesting that the indirect source (i.e., metabolism
of PBDEs and/or MeO-BDEs) could be the main origin for
OH-tetra-BDE in the investigated cetaceans. MeO-BDEs were
reported to have biomagnification potential, and MeO-BDEs
present in marine mammals were generally considered to come
from natural sources.\(^{55-57}\) Nevertheless, a significant positive
correlation was observed between concentrations of MeO-
BDEs and native PBDEs in both porpoises and dolphins (\(p < 0.05;\)
Table S6), indicating the possible contribution of the
biotransformation of PBDEs to MeO-BDEs in the investigated
marine mammals. The origin of MeO-BDEs in marine
cetaceans is likely to be attributed to both natural source
and PBDE metabolism, which deserves future investigation.
This is the first report on the presence of OH-BDEs and MeO-
BDEs in top marine predators from the SCS. To better understand the source of OH- and MeO-BDEs in the studied marine mammals, more investigation is needed on the presence of OH-/MeO-BDEs in different trophic levels of marine organisms in the coastal SCS. In addition, our semi-quantification based on the intensity of OH-/MeO-BDEs and their native PBDEs could make the results inaccurate, and their congener information is insufficient. Thus, target analysis of the identified compounds should be conducted in the future.

Methylated MeO-BDE (Me-MeO-BDE). Me-MeO-tetra-BDE in porpoise and dolphin samples was identified using its [M − Br + O − Br]− fragment with diagnostic ions of 462.8195 \((C_{14}H_{10}Br_2O_3)^−\); mass error = 2.1 ppm), 464.8158 \((C_{14}H_{10}Br_2O_5)^−\); mass error = −1.6 ppm), 466.8142 \((C_{14}H_{10}Br_3O_3)^−\); mass error = −0.5 ppm), and 468.8131 \((C_{14}H_{10}Br_3O_5)^−\); mass error = 1.4 ppm) with isotope intensity ratios of 34:100:7:32 (Figure 3A). The empirical structure of \(C_{14}H_{10}Br_2O_3\) could be a tetra-brominated di-MeO-tetra-PBBz or MeO-tetra-BDE with an additional methyl group (i.e., Me-MeO-tetra-BDE).\(^{78,39}\) In the MS/MS spectra, fragments including \(C_{14}H_{10}Br_2O_3\) \([M − Br + O − CH_3]−\); mass error = −2.5 ppm) and \(C_{14}H_{10}Br_3O_3\) \([M − Br + O − CH_3 − OCH_3]−\); mass error = 4.0 ppm) were observed while no \([M − Br + O − OCH_3 − OCH_3]−\) fragment was found (Figure 3B). This result shows that di-MeO-tetra-PBBz may not be present, as the \([M − Br + O − CH_3 − OCH_3]−\) fragment should be present in the MS/MS spectrum of di-MeO-tetra-PBBz.

However, the substituent position of the methyl group, methoxy atoms, and bromine atoms on the benzene ring of the identified Me-MeO-BDE cannot be determined based on the information on mass spectra obtained in the present work alone (Figure 3). There are very few reports on the occurrence of Me-MeO-BDE in the environment, and the source of Me-MeO-BDE is still unclear. To the best of our knowledge, only two studies from the same group have reported the presence of Me-MeO-BDE in the blubber of northern bottlenose whales through gas chromatography–HRMS and nuclear magnetic resonance spectroscopy where only one individual was involved in these two studies.\(^{39,60}\) In the present work, a significant positive correlation was found between concentrations of Me-MeO-tetra-BDE (based on semi-quantification) and tetra-BDE (i.e., BDE-47), the predominant HFR in porpoise and dolphin blubber samples (Table S6). This indicates that Me-MeO-tetra-BDE may have a source similar to that of tetra-BDE, and Me-MeO-tetra-BDE could be a possible tetra-BDE metabolite in the marine environment. It should be noted that Me-MeO-tetra-BDE was detected in 61 out of the 105 investigated marine mammal blubber samples. In addition, when BDE-47 was used as a reference standard, Me-MeO-tetra-BDE had the highest semi-quantification concentration (up to 6000 ng/g lw) identified via our suspect screening approach. The similar structure between Me-MeO-tetra-BDE and BDE-47 indicates that they may pose similar adverse effect on marine organisms but it is not revealed yet. The widespread detection and high concentration of Me-MeO-tetra-BDE in the top marine predators call for urgent investigations on the source and ecotoxicity of Me-MeO-BDE, an emerging PBDE-like organobromine and a potential BDE metabolite.

STUDY PERSPECTIVES AND IMPLICATIONS

Marine cetaceans, as top predators at high trophic levels with long life spans, are ideal biomonitors for tracing the long-term pollution status and trends of bioaccumulative pollutants (e.g., PBDEs, HBCD, and several novel HFRs) in the marine environment. Herein, target analysis and suspect screening of legacy and novel HFRs and HFR metabolites were conducted for 105 marine cetacean individuals from the SCS stranded between 2013 and 2020. Target HFRs were found at \(\mu g/g\) lw levels in the blubber samples, revealing their high pollution burden. PBDEs and α-HBCD accounted for more than 95% of ΣHFRs, indicating the ongoing problem of legacy-HFR pollution in recent years.

Using cetacean blubber as a biomonitor, significant decreasing temporal trends were observed in the concentrations of tetra-/penta-/hexa-BDEs in adult porpoises stranded in 2013–2015 than those stranded in 2016–2020. Such observed declines coincided with the phasing out of these PBDEs in China, reflecting the effectiveness of this environmental policy. Nevertheless, tetra-/penta-/hexa-BDEs were still at high levels in the most recent samples obtained in the present work, indicating the necessity for continuous monitoring of these banned PBDEs. No decreasing trend was observed in the levels of deca-BDE or HBCD, probably due to their exemption from the ban in China until 2025 and 2021, respectively, and continuous monitoring of deca-BDE and HBCD is required. The current results provide a baseline for verifying if the ban of the exemption will be effective in lowering deca-BDE and HBCD in the future.

A significant positive correlation between these legacy and novel HFRs in the investigated cetaceans indicates their similar contamination source. With the ban on PBDEs and HBCD, novel HFRs have been increasingly used as replacements; nevertheless, the environmental occurrence and ecotoxicology of novel HFRs are much less reported as compared with the legacy ones, which calls for more research efforts. DPTE, a novel HFR, was found at high levels in the investigated marine cetaceans and accounted for more than 85% of the total novel HFRs. More monitoring data on DPTE are needed, especially for top predators considering the relatively high bioaccumulation potential of DPTE.

Eight novel HFR and potential HFR metabolites were additionally identified via suspect screening. Semi-quantification results indicated the existence of Me-MeO-tetra-BDE at the \(\mu g/g\)-level in the cetacean blubber samples. A significant positive correlation was found between concentrations of tetra-BDE and Me-MeO-tetra-BDE, indicating that the metabolism of tetra-BDE may be a potential source of Me-MeO-tetra-BDE in marine mammals. Though the adverse effects of PBDE have been revealed thoroughly by research studies in recent years, reports on the environmental occurrence, toxicokinetic, and toxicodynamic characteristics of Me-MeO-tetra-BDE are very limited. More research efforts in these aspects are warranted.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c00684.

Details on materials and sample treatment; instrumental methods; information on target HFRs; sample list; matrix LOQs; correlation analysis result for concentrations of HFR and their metabolites in porpoise and dolphin samples, and comparison of novel HFR concentrations (PDF)
Corresponding Authors
Yuefei Ruan – State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong 999077, China; Southern Marine Science and Engineering, Guangdong Laboratory (Guangzhou), Guangzhou 511458, China; Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen 518057, China; orcid.org/0000-0003-4696-5708; Phone: +852 3442-7833; Email: yruan8@cityu.edu.hk, ryfeser@gmail.com; Fax: +852 3442-0524
Paul K. S. Lam – State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong 999077, China; Southern Marine Science and Engineering, Guangdong Laboratory (Guangzhou), Guangzhou 511458, China; Department of Science, School of Science and Technology, Hong Kong Metropolitan University, Hong Kong 999077, China; orcid.org/0000-0002-2134-3710; Phone: +852 2768-8089; Email: paullam@hkmu.edu.hk, bhpksl@cityu.edu.hk; Fax: +852 3442-0524

Authors
Qi Wang – State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong 999077, China; Southern Marine Science and Engineering, Guangdong Laboratory (Guangzhou), Guangzhou 511458, China; Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen 518057, China
Linjie Jin – State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong 999077, China; Southern Marine Science and Engineering, Guangdong Laboratory (Guangzhou), Guangzhou 511458, China; Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen 518057, China
Brian C. W. Kot – State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong 999077, China; Southern Marine Science and Engineering, Guangdong Laboratory (Guangzhou), Guangzhou 511458, China
Kenneth Mei Yee Leung – State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong 999077, China; Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen 518057, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.3c00684

Notes
The authors declare no competing financial interest.

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