Legacy and Emerging Per- and Polyfluoroalkyl Substances in a Subtropical Marine Food Web: Suspect Screening, Isomer Profile, and Identification of Analytical Interference

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ABSTRACT: The ban/elimination of legacy per- and polyfluoroalkyl substances (PFASs) has led to a dramatic increase in the production and use of various emerging PFASs over the past decade. However, trophodynamics of many emerging PFASs in aquatic food webs remain poorly understood. In this study, samples of seawaters and marine organisms including 15 fish species, 21 crustacean species, and two cetacean species were collected from the northern South China Sea (SCS) to investigate the trophic biomagnification potential of legacy and emerging PFASs. Bis(trifluoromethylsulfonyl)imide was found in seawater via suspect screening (concentration up to 1.50 ng/L) but not in the biota, indicating its negligible bioaccumulation potential. A chlorinated perfluorocarboxylate sulfonate (PFOS) analytical interfering compound was identified with a predicted formula of C_{16}H_{35}O_{5}S_{2}Cl_{4}− (most abundant at m/z = 514.9373). Significant trophic magnification was observed for 22 PFASs, and the trophic magnification factors of cis- and trans-perfluoroethylcyclohexane sulfonate isomers (1.92 and 2.25, respectively) were reported for the first time. Perfluorohexanoic acid was trophic-magnified, possibly attributed to the PFAS precursor degradation. The hazard index of PFOS was close to 1, implying a potential human health risk via dietary exposure to PFASs in seafood on the premise of continuous PFAS discharge to the SCS.

KEYWORDS: NTf2, Cl-PFESA, H-PFESA, PFECHS, Cl-PFOS, trophic magnification factor, relative potency factor

1. INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a class of synthetic organofluorine substances with excellent biochemical stability and high surface activity.1 PFASs have been manufactured and widely used in industrial and daily products in recent decades.2 Since the early 2000s, one major group of PFASs, perfluoroalkyl acids (PFAAs), have attracted substantial public attention because of their highly persistent, bioaccumulative, and toxic properties as well as their long-range transport potential.2−4 Consequently, certain PFAAs, including perfluorooctane sulfonate (PFOS), perfluoroctanoate (PFOA), and perfluorohexane sulfonate (PFHxS), have been identified as persistent organic pollutants (POPs) and listed according to the Stockholm Convention for global restriction/elimination since 2009, 2019, and 2022, respectively.5−7 Furthermore, in 2022, it was proposed that long-chain perfluorocarboxylic acids (PFCAs) with perfluorinated carbon chain lengths from 8 to 20 should be listed as POPs.8 With the phasing out of these legacy PFASs, many PFASs with structures similar to PFAAs (emerging PFASs) have been manufactured for complementary applications, e.g., 2,3,3,3-tetrafluoro-2-propanoate and hexafluoropropylene oxide dimer acid.9 Despite being used for decades, the environmental occurrence of certain PFASs, e.g., 6:2 chlorinated polyfluorinated ether sulfonate (Cl-PFESA) and perfluoroethylcyclohexane sulfonate (PFECHS), has only recently been reported owing to the continuous improvement in analytical techniques and the increasing focus on PFAS pollution.10−12 These PFASs are usually regarded as emerging PFASs because they were previously neglected. Nevertheless, with similar physicochemical and biochemical properties, emerging PFASs usually share similar environmental fate and toxicity with legacy PFASs, although the former has not been studied as much as the latter.13,14 Historical production of PFAAs using electrochemical fluorination (ECF) yields 70−95% of the desired...
linear PFAAs, whereas production via telomerization yields isometrically pure product (typically linear PFASs).\textsuperscript{15} Linear and branched PFAAs exhibit different behaviors in the environment and in organisms.\textsuperscript{16} Therefore, the investigation of the PFAA isomer profile could help to track the source of PFASs and deepen the understanding of their ecological risks.

Due to relatively high solubility and polarity, PFASs, especially PFAAs, can be readily transported in aquatic systems, and oceans are believed to be one of their final global sinks. Many studies have reported the bioaccumulation (and biomagnification) of long-chain PFCAs and PFOS in the marine food web, in which linear PFAs were found to be more bioaccumulative than branched ones.\textsuperscript{17−20} The biomagnification of some emerging PFASs has also been reported in recent years. For example, perfluoroalkyl ether carboxylic acids with perfluorinated carbon chain lengths ≥6 and 6:2 Cl-PFESA were reported to be biomagnified along the food chain in estuarine and marine food webs.\textsuperscript{21−23} PFECHEs was found to have biomagnification potential but moderately bioaccumulative.\textsuperscript{24} However, compared with legacy PFASs, studies on the bioaccumulation potential of emerging PFASs are still relatively limited, for which isomer-specific studies on PFASs other than PFOS and PFOA are rare. Considering the increasing use and detection of emerging PFASs, as well as the potential discrepancy in the environmental fate of different PFAS isomers, further relevant research needs to be conducted.

Globally, China has been the largest producer of PFOS and PFOA since 2004, as estimated in 2014 and 2015, respectively.\textsuperscript{25,26} The Greater Bay Area (GBA), located within the Pearl River Estuary (PRE), is one of the most developed regions in China, and the rapid industrialization and urbanization in this region have resulted in the substantial increase of PFASs in the environment and in organisms.\textsuperscript{27} The northern South China Sea (SCS) adjacent to the PRE is highly influenced by the PFASs discharged from the GBA, which pose a possible risk to local marine organisms.\textsuperscript{28} Our previous studies reported that concentrations of PFOS and 6:2 Cl-PFESA in the liver of resident marine cetaceans (μg/g dry weight level) in SCS were the highest among those in marine mammal studies published worldwide since 2006.\textsuperscript{29} Moreover, 44 PFASs were found using high-resolution mass spectrometry (HRMS) in the absence of reference standards.\textsuperscript{30,31} These results indicate the high and complex PFAS contamination status in the PRE and SCS. Therefore, it is imperative to investigate the biomagnification characteristics of PFASs, particularly emerging PFASs, in the marine food web of this region, and the information will be useful to water quality management and marine conservation. In this work, seawater (n = 26) and marine organisms (n = 62), including 21 crustacean species, 15 fish species, and 2 marine mammal species, were collected in the northern SCS in 2020. Target analysis and nontarget screening of isomer-specific PFASs were performed on these samples. The objectives of this study were (1) to investigate the occurrence of PFASs in the investigated marine ecosystem from the northern SCS using both target quantification and suspect screening identification approaches and (2) to reveal the trophodynamics of legacy and emerging PFASs in the studied subtropical marine food web with special focus on isomer-specific patterns.

2. MATERIALS AND METHODS

2.1. Chemicals. High-purity (>97%) analytical standards for target PFASs were purchased from Wellington Laboratories Inc. (Guelph, Canada) except for 6:2 and 8:2 hydrogen-substituted PFESAs (H-PFESAs, purities of >95%), which were donated by the Chinese Academy of Sciences (Beijing, China). Detailed information on the studied PFASs is listed in Table S1 of the Supporting Information. The details of the reagents and solvents used are provided in the Supporting Information.

2.2. Sample Collection. Seawater and marine organisms were collected from the northern SCS in 2020. Surface and bottom seawater samples were collected (Table S2), while biota samples comprising 21 crustacean species (9 shrimp species and 12 crab species) and 15 fish species were collected via bottom trawling in the same region (Table S3). For crustaceans, 5−10 individuals of the same species were grouped as one sample for analysis, whereas for fishes, 3−5 individuals were grouped as one sample. The whole-body samples (including skin) of crustaceans and fishes were homogenized and used for PFAS analysis and stable isotope determination. Liver samples of two marine mammal species inhabiting the same region, namely, the finless porpoises (Neophocaena phocaenoides, n = 9) and the Indo-Pacific humpback dolphins (Sousa chinensis, n = 4), which were stranded along the coast of Hong Kong, China were collected between 2020 and 2021. These marine species constitute a subtropical marine food web comprising low-trophic-level lives to top predators (Table S4).

2.3. Sample Treatment and Instrumental Analysis. Sample extraction and cleanup methods were performed following the procedures used in our previous studies.\textsuperscript{25,32} Briefly, seawater samples were extracted using Oasis WAX cartridges, whereas homogenized freeze-dried biota samples were extracted by sonication in alkaline acetonitrile and purified using ENV1-Carb cartridges and Oasis WAX cartridges. All eluates were concentrated to 0.5 mL under a gentle stream of high-purity nitrogen prior to instrumental analysis.

Instrumental analysis of PFASs was performed following the procedures used in previous studies.\textsuperscript{31−33} Target analysis was performed using an Agilent 1290 Infinity ultra-performance liquid chromatograph (UPLC) (Palo Alto, CA, USA) interfaced with a Sciex 5500 QTRAP tandem MS (MS/MS) (Foster City, CA, USA) under multiple reaction monitoring modes with negative electrospray ionization (ESI). HRMS screening was performed using an Agilent 1290 Infinity UPLC (Palo Alto, CA, USA) interfaced with a Sciex X500R quadrupole time-of-flight (QTOF) MS (Foster City, CA, USA). The suspect list matched that developed in our previous study.\textsuperscript{21,31} An Acquity UPLC system coupled with a G2-XS QTOF (Waters Corporation, Milford, USA) was applied as an aid for the further identification of PFASs in some samples. MS confidence levels were assigned following the procedure of Schymanski et al. (2014).\textsuperscript{34} Detailed information on the sample treatment and instrumental analysis is provided in the Supporting Information and Table S5.

2.4. Nomenclature and Quantification of PFAS Isomers. The nomenclature for specific PFAA isomers followed that of a previous study.\textsuperscript{35} Linear and perfluoro-isopropyl branches are abbreviated as n- and iso-PFAAs, respectively. The m refers to the perfluoromethyl branch, and
the preceding number indicates the carbon position of the branching point.

The levels of perfluoro-7-methyloctanesulfonate (iso-PFNS), perfluoro-6-methylheptanoic acid (iso-PFOA), perfluoro-5-methylheptanoic acid (5m-PFOA), and perfluoro-7-methyl-octanoic acid (iso-PFNA) isomers were determined using their exact native standards. The PFHxS, PFOS, and PFECHS isomer levels were quantified using standard mixtures with known isomeric ratios (Figure S1). All PFAS isomer standards were purchased from Wellington Laboratories (Guelph, ON, Canada).

2.5. Quality Assurance and Quality Control. The matrix-spike recoveries (n = 3) of individual native PFASs ranged from 55 to 101% (Table S6). A procedural blank and a reference standard were included for each batch of 10 samples. The method quantification limit (MQL) was defined as an instrumental signal-to-noise ratio of 10:1 if the analyte was not found in the blanks. Otherwise, the MQL was defined as the average concentration of the procedural blank plus three times their standard deviation. Surrogate standards were added before the sample treatment, and the individual PFAS concentrations were corrected using the corresponding surrogates.

For HRMS analysis, a procedural blank sample was used to monitor blank contamination, and the intensity in the samples was more than 10 times of that in the procedural blank. QTOF was automatically calibrated after every six sample injections to check the mass accuracy of the instrument, and the accurate mass error was less than 5 ppm and the isotope ratio difference less than 10%.

2.6. Calculations on the Trophic Level and Hazard Index. The details of the stable isotope analysis are given in the Supporting Information. The trophic level (TL) of individual organisms was determined based on the assumption that zooplankton occupies a TL of 2.0 using the following equation

\[ TL_{\text{consumer}} = 2.0 + \left( \delta^{15}N_{\text{primary consumer}} - \delta^{15}N_{\text{primary consumer}} \right) / 3.8 \]

where \( \delta^{15}N_{\text{primary consumer}} \) is the stable nitrogen isotope value of zooplankton (9.7) and 3.8 is the isotopic trophic enrichment factor.\(^{36,37}\) The trophic magnification factor (TMF) was used to interpret the biomagnification potential, noting that whole-body samples of crustaceans and fishes were used for the TMF calculation. The calculation is based on the correlation between the concentration of an individual PFAS in a species (\( C_{\text{biota}} \)) and the TL of this species using the following equation

\[ \ln C_{\text{biota}} = a + b \times TL \]

where \( a \) and \( b \) represent the constant and the slope of the linear regression, respectively. Slope \( b \) was used to calculate the TMF using the following equation

\[ \text{TMF} = e^b \]

The estimated daily intake (EDI) for human exposure to PFASs was calculated using the following equation
EDI = C_{biota} \times Q/BW \quad (4)

where Q is the average daily seafood consumption (g/day) and BW is the average body weight (kg) of adults. The hazard index (HI) was then calculated as

HI = EDI/R_{fD} \quad (5)

where R_{fD} is the reference dose for PFASs. HI > 1 indicates a potential exposure risk of PFASs for human health; otherwise, the risk is relatively low.

2.7. Statistical Analysis. A nonparametric Mann–Whitney (MW) test was used to examine the significant difference in the total concentration of PFASs (\(\sum\) PFASs) between medians of surface and bottom water samples. Spearman’s test was used for the correlation analysis using a two-tailed test. Statistical analysis was performed using IBM SPSS software (version 22.0, IBM Corp., NY, USA) and the R program (R v3.6.2, R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was accepted at \(p < 0.05\).

3. RESULTS AND DISCUSSION

3.1. PFASs in Seawater. In seawater samples, 33 PFASs were detected, including 12 PFCAs (including one PFOA branch isomer), nine perfluorooctyl sulfoic acids (PFOSas, including four PFOS branch isomers), five emerging PFASs, and seven PFAS precursors/intermediates (Table S7). PFOA was the predominant PFAS in the seawater followed by perfluorobutanoic acid and perfluorobutane sulfoic acid, which accounted for 33, 17, and 11%, respectively, of the \(\sum\) PFASs. Short-chain PFCAs (having perfluorinated carbon chain lengths < 7) accounted for a higher proportion (40%) than long-chain PFCAs (36%) and PFASs (19%), whereas PFAS precursors/intermediates, PFESAs, and emerging PFASs excluding PFESAs only accounted for 2, 2, and 1% of the \(\sum\) PFASs, respectively (Figure 1). Significantly higher \(\sum\) PFAS concentrations were found in the surface (18.3 ± 11.1 ng/L) than that in the bottom water (9.09 ± 7.51 ng/L) (MW test; \(p < 0.05\)). The average concentrations in the surface and bottom water were used for further calculations.

PFOS isomers were detected in water samples from 9 of the 13 sampling sites. Five isomers were detected, including linear PFOS (n-PFOS) (57%), iso-PFOS (14%), 5m-PFOS (11%), 4m-PFOS (10%), and 3m-PFOS (9%). The high proportion of branched PFOS (br-PFOS) isomers (43% of the total PFOS) indicated that ECF-based PFOS was an important source of PFOS in the northern SCS. The proportion of br-PFOS in the local seawater (43%) was even higher than that from the ECF manufacturing source (30%), which could be attributed to n-PFOS having a higher n-octanol–water partition coefficient \(K_{ow}\) and bioaccumulation potential than the branched isomers. Thus, n-PFOS was more likely to adsorb onto suspended particulate matter than to settle as sediment and be uptaken by marine organisms, resulting in a relatively lower n-PFOS proportion in the seawater. Two PFOA isomers, n-PFOA (97%) and iso-PFOA (3%), were detected at two sampling sites, and thus, telomerization-based PFOA production, which only produces n-PFOA, is expected to be the main PFOA source in the northern SCS. To the best of our knowledge, this is the first study to report on the isomeric profile of PFASs in the SCS, which partly reveals the potential source of PFASs in this region.

Three PFASs were additionally found in the seawater via suspect screening (Table S8), which were also reported in a recent paper on suspect screening of PFASs in the Pearl River. Sodium p-perfluorooctanesulfonate (OBS, \(C_{10}H_{22}F_7SO_4\), \(m/z = 602.9564\)), which is mainly used as a surfactant in oil production, was identified in 2 of the 13 sites with a confidence level of 2b. Additionally, 5:2 Cl-PFESA (\(C_{5}F_{11}SO_2H\), \(m/z = 480.8988\)), a homologue of 6:2 Cl-PFESA, was identified in 5 of the 13 sites with a confidence level of 2b. Homologues of unsaturated PFECs (uPFECs) with perfluorinated carbon chain lengths of C6–C9 were found in the seawater samples. uPFCA homologues with perfluorinated carbon chain lengths of C3–C12 and C14 were also reported to be widely detected in the Pearl River. Nevertheless, the uPFECs identified in the present work might be attributed to ESI in-source fragmentation of PFECs since the proposed uPFCA homologues could also have been present in our high-purity PFCA standards (10 ng/mL) and could not be differentiated by retention time under the current LC conditions. This phenomenon was also observed in a previous study. An ultra-short-chain PFAS, bis-(trifluoromethylsulfonyl)imide (NTf2, \(C_{2}F_{2}NOSO_2\), \(m/z = 279.9168\)), was found in 3 out of the 13 sites and identified with a confidence level of 3 (Figure S2). Using an external calibration curve for determination according to the HRMS result, the concentrations of NTf2 were estimated to be 0.396, 1.16, and 1.50 ng/L in these three seawater samples. The retention time, isotope distribution, and MS/MS spectrum of NTf2 found in the seawater matched those of the high-purity NTf2 lithium salt standard, providing a confidence level of 1. NTf2 is a fluorinated anion predominantly used in ionic liquids; the environmental occurrence of NTf2 is rarely reported. This study is the first to document the presence of NTf2 in the marine environment, and further field data on the environmental behavior of NTf2 is urgently needed.

3.2. PFASs in Marine Organisms. 3.2.1. Target Analysis. The PFAS compositions of the biota samples are shown in Figure 1 (Tables S9 and S10). Unlike the high proportion of short-chain PFCAs in seawater (40%), short-chain PFCAs only contributed 3, 2, and 0.4% to the \(\sum\) PFASs in crustaceans, fishes, and cetaceans, respectively. This could result from the insignificant bioaccumulation potential of short-chain PFCAs, as previously reported. PFOS was the predominant PFAS in all of the investigated biota, accounting for 40, 57, and 37% of the \(\sum\) PFASs in crustaceans, fishes, and cetaceans, respectively. The n-PFOS proportions to all PFOS isomers were > 85% in all taxonomic groups, which were much higher than that in the seawater (57%), indicating that n-PFOS had a higher bioaccumulation potential than br-PFOS (Figure 1B). br-PFOS was only detected in the cetacean liver samples, accounting for < 1% of the total PFOA (although accounting for 3% in the seawater). The reason for the low levels of br-PFOS in the biota samples might be attributed to their lower \(K_{ow}\) and thus a lower potential for bioaccumulation in marine organisms at high TLs, especially top predators such as cetaceans. The ratios between cis- and trans-PFECHS in marine organisms were reported for the first time in this study and were 43:57, 41:59, and 32:68 in crustaceans, fishes, and cetaceans, respectively (Figure 1C).

3.2.2. Suspect Screening. Apart from the target analysis, six PFASs were additionally identified via suspect screening in the cetacean liver samples, including 5:2, 7:2, and 10:2 Cl-PFESAs, 5:2 and 10:2 H-PFESAs, and OBS with confidence levels of 3.
Additionally, perfluorohexane sulfonamide, perfluorooctane sulfonamide, and 8:3 and 9:3 fluorotelomer carboxylic acids were identified via suspect screening (with confidence levels of 4) due to the lack of their MS/MS information in the investigated samples. The additionally identified PFASs were also found in our previous study on nontarget and suspect screening of PFASs in marine mammals stranded between 2012 and 2018. However, most of the identified PFASs in the former study were not found in the seawater and other marine biotas in the present work. This could be attributed to the relatively high biomagnification potential of many of these newly identified PFASs, making them only detectable in the top predator marine mammals. Furthermore, the air-breathing behavior of marine mammals could lead to PFAS exposure from the atmosphere, which is inapplicable to other investigated marine organisms. 5:2 Cl-PFESA and OBS were found in crustaceans and fishes, which was expected because they were also found in the seawater, indicating a greater extent of contamination. However, another PFAS identified in the seawater, NTf2, was not found in any biosample, suggesting that this ultra-short-chain PFAS (with two perfluorinated carbons) is less bioaccumulative. Another possible reason is that NTf2 could undergo rapid metabolism in the investigated marine organisms. Because toxicokinetic and toxicodynamic studies on NTf2 are limited, more data are necessary with respect to its emerging occurrence in the marine environment.42

3.2.3. Identification of Cl-PFOS Analytical Interference. In the target analysis under multiple reaction monitoring modes by UPLC-MS/MS, a “suspected Cl-PFOS” compound (a Cl-PFOS-interfering substance) was found in crustaceans via two LC methods [Agilent Zorbax Eclipse Plus C18 LC column and Ascentis Express F5 LC column with pentfluoroarylpropyl (PFP) as the stationary phase] (Figures S3A,B). For the analysis of Cl-PFOS, 514.9 → 79.9 (nominal C7F16ClSO3− → SO3−) and 514.9 → 98.9 (nominal C7F16ClSO3− → FSO3−) are the most commonly used transition pair because they have been found to have higher signal than other transitions (Figure S4).31,45–47 The “suspected Cl-PFOS” compound was detected in both two transitions, and this compound has a close retention time to that of the Cl-PFOS identity (the retention time intervals between these two compounds were 0.2 min for the C18 column and 0.07 min for the PFP column). This “suspected Cl-PFOS” compound was then re-analyzed via HRMS for identification. As shown in Figure 2A, the isotope distribution suggests the presence of a chlorine atom, as confirmed by the MS/MS data (Figure 2B) (the neutral loss of HCl and the presence of Cl−). The presence of the SO3 group in this compound was confirmed by the HSO3− (96.9603) fragment. The formula of this Cl-PFOS-interfering substance was then searched in the ChemSpider and PubChem libraries (e.g., containing six chlorine atoms and at least one sulfur atom with mass error <10 ppm); however, no compound met the criteria. With the following allowable elements and limits: C, 5–30; H, 0–100; N, 0–10; S, 1–3; F, 0–20; O, 3–10; Cl, 0–6; and P, 0–5 (mass error ≤ 5 ppm), an empirical formula was predicted for the compound. The only plausible prediction was C14H7O3S3Cl6 (mass error = −0.8 ppm). More details on the identification of this interfering substance can be found in the Supporting Information.

Cl-PFOS was found to have comparable bioaccumulation potential and similar tissue-distribution characteristics with PFOS in fish43 and exhibited a strong persistence in human blood (estimated half-life: 5.0 years).45 Nevertheless, the source of Cl-PFOS is still unclear, and the occurrence of Cl-PFOS in wildlife was not reported until 2020.45,46 Hence, more investigation on Cl-PFOS in biota is necessary, and the influence of the analytical interfering substance should attract broader attention. Moreover, it is possible that Cl-PFOS exists as several different isomers,48 any one of which can be misidentified as Cl-PFOS identity, especially for isomer-specific studies. Our results indicate that using low-resolution LC–MS/MS to analyze Cl-PFOS in the biosamples (or other complex matrices) could influence the result due to the interference of Cl4H29O3SCl−. Therefore, to avoid false detection, LC–HRMS is recommended in future studies for the identification of Cl-PFOS in biosamples. Moreover, considering the limited MS2 information and the potential fragment rearrangement in the collision cell, the proposed structure of the interfering substance is still tentative and needs to be confirmed via other approaches (e.g., nuclear magnetic resonance) in future work.

3.3. Trophic Transfer of PFASs in the Marine Food Web. A correlation analysis between the PFAS concentrations and TLs was conducted for 21 crustacean species and 15 fish species. A significant positive correlation was found for 22 PFASs (Spearmann’s r ≥ 0.321; p < 0.05), indicating their biomagnification potential. The calculated TMFs of these PFASs are listed in Table 1. The TMFs of most detected long-chain PFASs were higher than 1, which has also been reported for other aquatic food webs.17–20 The TMF of one short-chain PFAS, perfluorooctanoic acid (PFHxA), was also higher than 1, indicating its considerable biomagnification potential. However, the bioaccumulation potential of PFHxA in fish has been reported to be negligible in some laboratory experiments.42,45 In another field study conducted by our research group, PFHxA exhibited considerable biocentr-
tion potential in wild oysters collected in the PRE, which could be attributed to the contribution of indirect PFHxA sources, for example, the in vivo transformation of PFHxA precursors [e.g., 6:2 fluorotelomer sulfonate (6:2 FTSA) and 6:2 fluorotelomer sulfonamide alkylbetaine]. Among the investigated PFAs, 8:2 CI-PFESA had the highest TMF (6.44), indicating the substantially high biomagnification potential of this long-chain emerging PFAS. This TMF was higher than that of 6:2 CI-PFESA (5.65), although these two CI-PFESAs were reported to have close bioconcentration factors in laboratory-exposed marine medaka (Oryzias melastigma; 8:2 CI-PFESA: 3980 ± 410 L/kg; and 6:2 CI-PFESA: 3650 ± 670 L/kg). This result indicates that bioconcentration may not be the main exposure route for CI-PFESAs in the investigated marine organisms, and some other pathways (e.g., dietary intake) can significantly affect the occurrence of CI-PFESAs in marine organisms. Furthermore, the TMFs of these two emerging PFASs were higher than that of PFOS (4.88), which is a legacy PFAS. In the investigated marine food web, the TMFs of 6:2 H-PFESA (3.27) and 8:2 H-PFESA (4.08) were lower than the TMFs of 6:2 and 8:2 CI-PFESAs, indicating their lower biomagnification potential than the corresponding CI-PFESAs. Note that H-PFESAs are impurities in F-53B, for which 6:2 Cl-PFESA is the main component. Nevertheless, other PFAA isomers were rarely detected in our marine organism samples; thus, the evaluation of their TMFs was unavailable. A significant positive correlation was found between both the cis- and trans-PFECHS

<table>
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<th>analyte</th>
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<td>PFHxA</td>
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<tr>
<td>8:2 CI-PFESA</td>
<td>6.44</td>
<td>In C = 1.86 × TL−6.50</td>
<td>0.819</td>
<td>&lt;0.001</td>
<td>54</td>
</tr>
<tr>
<td>6:2 H-PFESA</td>
<td>3.37</td>
<td>In C = 1.19 × TL−4.82</td>
<td>0.420</td>
<td>0.032</td>
<td>59</td>
</tr>
<tr>
<td>8:2 H-PFESA</td>
<td>4.08</td>
<td>In C = 1.41 × TL−5.87</td>
<td>0.424</td>
<td>0.031</td>
<td>25</td>
</tr>
<tr>
<td>cis-PFECHS</td>
<td>1.92</td>
<td>In C = 0.653 × TL−6.30</td>
<td>0.354</td>
<td>0.005</td>
<td>26</td>
</tr>
<tr>
<td>trans-PFECHS</td>
<td>2.25</td>
<td>In C = 0.813 × TL−6.42</td>
<td>0.321</td>
<td>0.011</td>
<td>26</td>
</tr>
<tr>
<td>8:2 FTSA</td>
<td>2.95</td>
<td>In C = 1.08 × TL−6.86</td>
<td>0.660</td>
<td>&lt;0.001</td>
<td>38</td>
</tr>
<tr>
<td>10:2 FTSA</td>
<td>4.15</td>
<td>In C = 1.42 × TL−7.29</td>
<td>0.502</td>
<td>0.001</td>
<td>32</td>
</tr>
<tr>
<td>FHpPA</td>
<td>5.82</td>
<td>In C = 1.76 × TL−6.59</td>
<td>0.672</td>
<td>&lt;0.001</td>
<td>61</td>
</tr>
<tr>
<td>N-EtFOSAA</td>
<td>2.93</td>
<td>In C = 1.07 × TL−5.38</td>
<td>0.466</td>
<td>&lt;0.001</td>
<td>34</td>
</tr>
</tbody>
</table>

C represents individual PFAS concentration in biota. PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpS = perfluorohexanesulfonic acid; FHpPA = 7:3 fluorotelomer carboxylic acid; and N-EtFOSAA = N-ethyl perfluorooctane sulfonamido acetic acid.

Figure 3. Relationship between the ln concentrations of cis- (A) and trans- (B) PFECHS and their TLs in crustaceans and fishes.
concentrations and their TLs in the studied marine food web (Spearman’s $r = 0.365$ and 0.321, respectively; $p < 0.05$), indicating the biomagnification of both PFECHS isomers in the marine food web (Figure 3). Herein, the biomagnification potential of different PFECHS isomers was reported for the first time. The TMF of trans-PFECHS (2.25) was generally slightly higher than that of cis-PFECHS (1.92); no significant correlation was found between the two PFECHS isomer ratios and their TLs in the studied marine food web ($p > 0.05$).

The TLs of two investigated marine mammals, finless porpoises, and Indo-Pacific humpback dolphins, were calculated according to the minimum risk levels recommended by the United States Registry of Poisons and Diseases and the local food consumption data from the Hong Kong government. The estimated potential of different PFECHS isomers was reported for the first time. The RPF was adopted from Bil et al. (2021). The threshold values were calculated according to the minimum risk levels recommended by the United States Registry of Poisons and Diseases and the local food consumption data from the Hong Kong government.

3.4. Risk Assessment of PFAS Exposure via Seafood Consumption. Data on the daily intake of shrimps, crabs, and fishes by local residents of Hong Kong and the average BWs of adults were retrieved from a recent report from the Food and Environmental Hygiene Department of Hong Kong (Table S11). The RDIs were retrieved from the intermediate-duration temporary minimum risk levels recommended by the United States Registry of Poisons and Diseases, which were 2, 3, and 3 ng/kg/day for PFOS, PFOA, and PFNA, respectively.

The HI was calculated to evaluate the potential hazards from exposure to PFASs in humans via seafood consumption. The HIs of PFOA and PFNA are relatively low (<0.06), indicating a low health risk of exposure to these two PFASs (Table 2), which is consistent with a previous report on seafood from the coastal region of the SCS. Nevertheless, it should be noted that the median HIs of PFOS (0.872 and 0.981 for males and females, respectively) were close to 1.0, which implies that, on the premise of continuous PFAS discharge from the GBA to the PRE and SCS and with the increasing PFOS levels in resident marine organisms, there could be potential human health risk for the local population via dietary exposure to PFASs in seafood. In addition, to estimate the health risk of dietary exposure to PFAS mixtures, a more refined RPF approach (RPF) was applied to achieve a PFOA-equivalent HI. Fifteen PFAs were included in the RPF calculation, and their corresponding RPF factors are listed in Table S12. Concentrations of individual PFASs were multiplied by their corresponding RPF factors and then summed to obtain a PFOA-equivalent concentration for subsequent health risk assessment. Using the RPF approach, the PFOA-equivalent intakes via seafood consumption were 536 and 494 ng/d for males and females, respectively, which were higher than the guideline values (209 and 171 ng/d for males and females, respectively). Among the 15 PFAs, PFOS contributed the most (46%) to the total PFOA-equivalent intake, followed by PFDA (27%), PFUnDA (12%), PFNA (8%), PFDoDA (4%), and PFOA (2%), while other PFASs in total contributed less than 1% to the total PFOA-equivalent intake (Table S12). This result reveals that the combined human health risk via dietary exposure to PFASs in seafood will be high when all detected PFAs are included for risk assessment, with PFOA and PFDA being the determinants driving the PFAS exposure risk for local residents. It is noteworthy that the lack of toxicity data on emerging PFASs (e.g., 6:2 Cl-PFESA and PFECHS) and PFAS precursors (e.g., 6:2 FTSA) precludes the derivation of their threshold values and RPFs. Clearly, more detailed toxicodynamic and toxicokinetic data on these chemicals are indispensable. Moreover, due to the uncertainty regarding the interaction mode of PFOA-like effects in vivo (e.g., synergistic, additive, or antagonistic) with respect to exposure to PFAS mixtures, a more refined RPF risk assessment approach for PFASs is urgently needed.

3.5. Environmental Implications. Target analysis and suspect screening of PFASs were conducted in a subtropical marine food web. Thirty-eight species were involved, including 21 crustaceans, 15 fishes, and 2 cetaceans. Significant biomagnification was found for 22 legacy and emerging PFASs, including a short-chain PFCA named PFHxA, indicating the contribution of PFAS precursor degradation. More investigation on the screening of PFAS precursors (e.g., cationic PFAS screening and TOP assay) in the investigated marine food web (Figure 3). Herein, the biomagnification potential of different PFECHS isomers was reported for the first time. The TMF of trans-PFECHS (2.25) was generally slightly higher than that of cis-PFECHS (1.92); no significant correlation was found between the two PFECHS isomer ratios and their TLs in the studied marine food web (Figure 3).

**Table 2. Daily Median PFAS Intake (ng/d)** via Seafood Consumption

<table>
<thead>
<tr>
<th>PFAS intake (ng/day)</th>
<th>PFOS</th>
<th>PFOA</th>
<th>PFNA</th>
<th>PFOA-equivalent *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Fish</td>
<td>114</td>
<td>107</td>
<td>7.88</td>
<td>7.38</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>6.86</td>
<td>4.80</td>
<td>2.59</td>
<td>1.81</td>
</tr>
<tr>
<td>Mollusks</td>
<td>0.490</td>
<td>0.480</td>
<td>0.326</td>
<td>0.320</td>
</tr>
<tr>
<td>Total seafood</td>
<td>121</td>
<td>112</td>
<td>10.8</td>
<td>9.51</td>
</tr>
<tr>
<td>Threshold</td>
<td>139</td>
<td>114</td>
<td>209</td>
<td>171</td>
</tr>
</tbody>
</table>

*The RPF was adopted from Bil et al. (2021). The threshold values were calculated according to the minimum risk levels recommended by the United States Registry of Poisons and Diseases and the local food consumption data from the Hong Kong government.
region shall be conducted. The proportion of \( n \)-PFOS in biota was higher than that in seawater, and the TMF of \( n \)-PFOS was higher than those of \( br \)-PFOS isomers. These results are consistent with those reported in previous investigations where \( n \)-PFAs showed higher bioaccumulation and biomagnification potentials than \( br \)-PFAs. PFECHS was not detected in seawater and hardly observed in crustacean samples; however, it was widely found in fish and cetacean samples, suggesting its biomagnification potential. This study, for the first time, reported the difference in biomagnification potential between two PFECHS isomers, where \( trans \)-PFECHS had a slightly higher TMF than \( cis \)-PFECHS, but no significant difference was observed in the studied marine food web (\( p > 0.05 \)). Currently, studies regarding the isomer-specific occurrence of emerging PFASs in the environment are still limited. Considering the increasing use of emerging PFASs in the modern society, more investigations should be conducted on the environmental fate of emerging PFASs, including their isomers, so as to further our understanding of the ecological risks of these emerging contaminants.

TMFs of 6:2 and 8:2 Cl-PFESAs were higher than that of PFOS, indicating the higher biomagnification potential of these two emerging PFASs compared to the legacy C8 PFAS. One Cl-PFOS interfering substance with the proposed formula of \( C_{14}H_{25}O_7S_{16} \) was identified in the studied marine organisms using HRMS. Considering the similar structures between Cl-PFOS and PFOS, they may share similar bioaccumulative and toxic characteristics. Thus, more investigation on the environmental behavior of Cl-PFOS is needed, and attention to the Cl-PFOS interference should be paid during the analysis of Cl-PFOS in complex matrices so as to avoid any false detection. The occurrence of NTf2, a fluorinated ionic liquid anion, in the seawater was revealed. Considering that NTf2 was not detected in any of the studied marine organisms, it could be inferred that this ultra-short-chain PFAS might have a low bioaccumulation potential. Environmental studies on NTf2 are scant, which calls for more studies on NTf2 regarding its sources and potential homologues, environmental fate, and toxic characteristics.

The daily intake of PFOS via seafood consumption for the local population was very close to the threshold, and the exposure risk of PFOA-equivalent daily intake via seafood consumption for local residents was estimated to be high. These results suggest a requisite for further efforts on reducing the production and use of PFOS in the GBA so as to lessen the subsequent discharge and eventual deposit of these contaminants in marine ecosystems. More detailed toxicity data on emerging PFASs and PFAS precursors are needed, and a more refined RPF risk assessment approach is highly recommended for the health risk evaluation of exposure to PFAS mixtures.

### ASSOCIATED CONTENT

- **Supporting Information**
  The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c00374.
  Details on standards and reagents, sample treatment, and instrumental analysis; sampling information; matrix-spiked MQLs and recoveries; PFAS concentrations in seawater and biota samples; identification of Cl-PFOS interfering compounds; PFASs found through suspect screening; chromatograms of PFHxS, PFOS, and PFECHS isomers; and MS2 spectrum of Cl-PFOS (PDF)

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