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Structure-Related Thyroid Disrupting Effect of Perfluorooctanesulfonate-like Substances in Zebrafish Larvae

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ABSTRACT: Chlorinated polyfluorooctane ether sulfonate (6:2 Cl-PFESA), hydrogenated polyfluorooctane ether sulfonate (6:2 H-PFESA), and chlorinated polyfluorooctanesulfonate (Cl-PFOS) share structural similarities with the regulated perfluorooctanesulfonate (PFOS), but their toxic potential is rarely known. Here, the thyroid disrupting potential of these four compounds in zebrafish larvae has been comparably investigated. PFOS, Cl-PFOS, and 6:2 Cl-PFESA were accumulated in the larvae at similar levels, approximately 1.3−1.6 times higher than 6:2 H-PFESA. Additionally, PFOS, Cl-PFOS, and 6:2 Cl-PFESA exhibited stronger disruption than 6:2 H-PFESA on genetic regulation, particularly concerning thyroid hormone (TH) activation and action and on TH homeostasis in both free and total forms of thyroxine (T4) and 3,5,3′-triiodothyronine (T3). These results indicate that chlorination or oxygen insertion does not substantially alter the thyrotoxicity of PFOS, but hydrogenation mitigates it. Molecular docking analysis and the luciferase reporter gene assay provided mechanistic perspectives that the PFOS-like substances could competitively replace THs to bind with TH plasma and membrane transporters, thereby disrupting TH transport and action, respectively. Moreover, they are also potent to disrupt TH synthesis and activation through Na^+/K^+-dependent transport of I^- or competitive binding to the sites of deiodinases.

KEYWORDS: *chlorinated polyfluoroalkyl substance, hydrogenated polyfluoroalkyl substance, zebrafish, hyperthyroidism, structure-dependent response, competitive binding*

1. INTRODUCTION

As alternatives to perfluorooctanesulfonate (PFOS), chlorinated polyfluoroalkyl ether sulfonates (Cl-PFESAs, trade name F-53B) have been extensively employed as mist suppressants in the metal plating industry since 1970s.[1,2] The 6:2 chlorinated polyfluorooctane ether sulfonate (6:2 Cl-PFESA) constitutes over 90% of the formulation of F-53B (>90%), which has been demonstrated to be widely distributed and frequently detected in surface water (mean: 10−50 ng/L),[2] atmospheric particulate matter (mean: 722 pg/m^3 in 2014),[3] wildlife (mean: 41.9 ng/g in fish blood),[4] and humans (mean: 0.019−5040 ng/mL in human serum)[5] at comparable levels to PFOS. The 6:2 hydrogenated polyfluorooctane ether sulfonate (6:2 H-PFESA) as a minor component of F-53B (<0.1%), though, can originate from 6:2 Cl-PFESA via reductive dechlorination observed in fish,[6,7] rats,[8] and anaerobic microbial inoculum,[9] and thus was also detectable in environments (e.g., 560 pg/L in river water).[10] Although the detected concentrations of 6:2 H-PFESA were relatively lower than that of 6:2 Cl-PFESA in groundwater, the concentration ratio of 6:2 H-PFESA to 6:2 Cl-PFESA under this anaerobic condition (mean: 6−27%) was dramatically higher than that in the commercial product F-53B (mean: 1%).[11] An additional PFOS-like substance, chlorinated polyfluorooctanesulfonate (Cl-PFOS), has been recently identified in the wastewater discharges from a fluorochemical manufacturing park near the Yangtze River (nontarget analysis),[12] as well as in bald eagle eggs collected from the North American Great Lakes (0.53 ng/g ww),[13] and among the aqueous film-forming foam (AFFF)-exposed firefighters recruited across Australia (nontarget s analysis),[14] sparking growing concerns about its potential toxicity. Cl-PFOS could potentially originate from its use as a precursor in the electrochemical fluorination process to produce PFOS,[14,15] even though its production history remains unconfirmed. The PFOS-like substances were designed to be less toxic in organisms, though our knowledge of their biosafety remains limited. So far, the vast majority of the research on the toxic effects of PFOS-like substances pertains to Cl-PFESAs, particularly 6:2 Cl-PFESA, which consistently suggests that 6:2 Cl-PFESA is potent to cause strong detrimental effects on endocrine functions (e.g., thyroidal and lipid steatosis) in organisms as PFOS, or even stronger.[16−19] The only study to look into the toxicity of Cl-PFOS reported its potential to elicit comparable hepatotoxicity to PFOS in zebrafish larvae.[16] As the recently identified reductive transformation product of 6:2

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Cl-PFESA, information about 6:2 H-PFESA is heavily sparse. Its toxicity has not been defined in any species; however, the available data suggested that 6:2 H-PFESA was less bioaccumulative than its predecessor 6:2 Cl-PFESA in rat,[8] which is thus inferred to be less toxic.

Across all vertebrates, the thyroid endocrine system assumes the crucial role of sensing and translating central and peripheral signals to regulate an array of physiological processes encompassing bone maintenance, lipid metabolism, cardiovascular function, and fetal neurodevelopment.[20−22] Thyroid hormone (TH) homeostasis is based on a proper thyroidal set-point involving a multiloop feedback mechanism mediated by the hypothalamus−pituitary−thyroid (HPT) axis, which is comprised of TH secretion stimulation, synthesis, distribution/transport, activation, and action.[20,23] A comprehensive review by Ghassabian et al.[24] highlights the potential for PFAS to disrupt any step in the TH metabolism process, among which PFOS has been extensively studied.[25] For example, PFOS could hinder TH biosynthesis via inhibiting the iodide uptake mediated by sodium/iodide symporter (NIS) in FRTL-5 cells[26] and reducing thyroperoxidase (TPO) activity in tumor thyroid cells.[27] Additionally, PFOS can competitively bind with THs to the plasma transport protein, transthyretin (TTR), resulting in a higher fraction of unbound THs in circulation.[28,29] Furthermore, PFOS might also directly interact with TH receptors, which consequently disrupt TH-mediated downstream gene expression, i.e., TH action.[30−32]

Here, we employed zebrafish larvae as a model organism to investigate the thyroid disrupting effects of three novel PFOS-like substances, including Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA, in direct comparison with PFOS itself. We sought to establish the connections between chemical exposure and thyrotoxic consequences by evaluating an integrated network of biomarkers across three molecular tiers: chemical bioaccumulation, hormonal alterations, and transcriptional profiles of the hypothalamus−pituitary−thyroid (HPT) axis within zebrafish.

Our investigation included comprehensive cross-comparisons designed to unveil distinct structural influences arising from chlorination (PFOS vs Cl-PFOS), oxygen insertion (Cl-PFOS vs 6:2 Cl-PFESA), and hydrogenation (6:2 Cl-PFESA vs 6:2 H-PFESA). To deepen our understanding, we employed both in silico molecular docking models and in vitro luciferase reporter gene assays, enabling to explore the intricate interactions between these four chemicals and pivotal proteins associated with TH transport and action.

2. MATERIALS AND METHODS

2.1. Chemicals. PFOS, Cl-PFOS, 6:2 Cl-PFESA, and isotope-labeled internal standard ($^{13}C_4$−PFOS) were purchased from Wellington Laboratories Inc. (ON, Canada) with purity >98%. 6:2 H-PFESA (with purity >95%) was provided by Jiang's laboratory (Chinese Academy of Sciences).[33] A visual representation of the chemical structures employed in this study can be found in Figure S1. Individual stock solutions were initially prepared in dimethyl sulfoxide (DMSO) and then diluted with embryonic rearing water (40 mg/L instant ocean salt in distilled water) to attain concentrations of 0.01, 0.1, and 1 μmol/L, respectively, with the final DMSO concentration in the test solution not exceeding 0.1% v/v. The selection of the concentration regimen is 2-fold. On the one hand, it is based on their environmental concentrations. For example, PFOS concentrations as high as 2.0 μmol/L have been reported in river water,[34] and 6:2 Cl-PFESA levels up to 0.2 μmol/L have been observed in surface water in the vicinity of a municipal wastewater treatment plant.[2] On the other hand, it is stemmed from the toxicological tests examining thyroidal toxic end points (both toxic and nontoxic) of PFAS-exposed zebrafish for establishing the dosage.[25] RNA stabilization reagents, RNeasy mini kit, first strand cDNA synthesis kits, and SYBR Green qPCR mix kits were purchased from Sparkjade Biotechnology Co., Ltd. (Shandong, China). Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Haling Biotechnology Co., Ltd. (Shanghai, China). BCA protein assay kit was purchased from Solarbio Biotechnology Co., Ltd. (Beijing, China). Luciferase reporter assay system kit was purchased from Promega Biotechnology Co., Ltd. (Shanghai, China).

2.2. Animal Maintenance and Exposure Experiment. Adult zebrafish (approximately three months old, AB strain) were purchased from a local aquarium fish market (Tianjin, China) and acclimated in the laboratory for 2 weeks. A semiautomatic water system with temperature at 26 ± 1 °C and a 14/10 h light/dark photoperiod were employed. Adult, embryonic, and larval zebrafish maintenance adhered to our previously established method,[16] in alignment with OECD FET. Fertilized embryos were rinsed with embryonic rearing water and examined for viability under a stereomicroscope (Olympus SZX7, Japan). For hormone measurement and mRNA expression analyses, 120 normally shaped fertilized embryos that reached the blastula stage (i.e., 4 h post fertilization, hpf) were selected and randomly assigned to a 96-well plate (two embryos in each well), after which each well was filled with 200 μL of test or vehicle control (0.1% DMSO, v/v) solution. To analyze the chemical concentrations accumulated within the larvae, 30 zebrafish larvae were pooled and exacted, after the same exposure regimen as above. All of the exposures for zebrafish embryos and larvae were conducted in a static mode. Each test in this study was conducted in triplicate. The experiment was terminated on day 6 post fertilization (dpf), a duration chosen to ensure that the zebrafish had developed into free-swimming larvae, allowing the detection of gene expressions along the HPT axis.[35−37] Prior to harvest, morphological alterations in the embryos/larvae were examined, recording lethal, hatching, and malformation under a stereomicroscope at 6, 12, 24, 48, 54, and 58 h post fertilization (hpf) and 3, 4, 5, 6 dpf. Spinal curvature, pericardial edema, and yolk edema were recorded as malformation symptoms. Ultimately, all toxic end points, including the lethal rate, malformation rate, and hatching rate, were expressed as accumulated numbers recorded at 6 dpf.

All animal-related procedures conducted within this study were approved by the Committee for Animal Experimentation at the College of Life Sciences, Nankai University, and were executed in strict adherence to the established guidelines set forth by the Animal Care Center.

2.3. Sample Preparation and LC-MS/MS Analysis. Quantification of the target chemicals in test and control solutions as well as in total body zebrafish larvae was conducted before and after exposure, performed on a Waters UPLC system coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA) (LC-MS/MS). The detailed protocols for sample extraction, instrumental analysis, and quality assurance and quality control (QA/QC) are provided in Text S1−S3. Overall, the measured concentrations of all of the four test chemicals in the exposure solutions were

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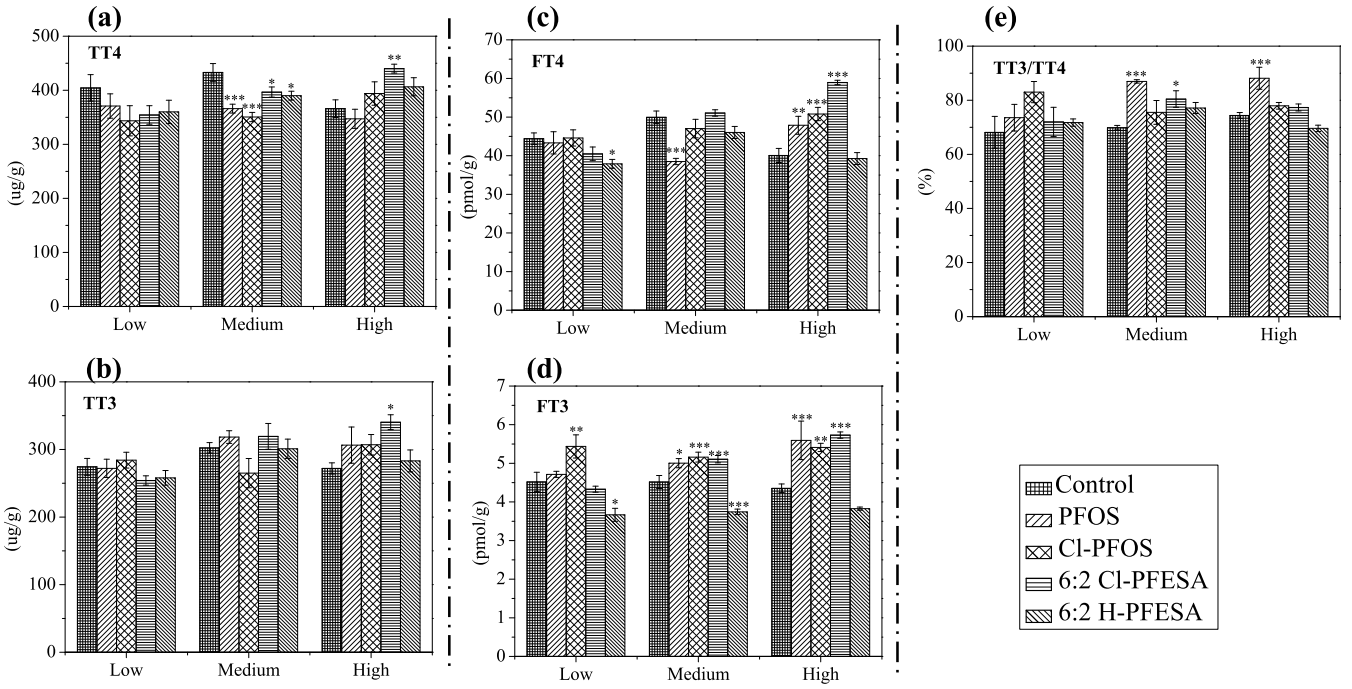

Figure 1. Thyroid hormones in zebrafish larvae upon 6-d exposure to control vehicle (DMSO) or PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA (corresponding to the columns from left to right) with low- (0.01 μmol/L), medium- (0.1 μmol/L), and high-dose (1 μmol/L) treatments. (a, b) Total thyroid hormones, TT4 and TT3; (c, d) free thyroid hormones, FT4 and FT3; and (e) ratio of active metabolite T3 to precursor T4, TT3/TT4. Results are expressed as the mean ± SD of three replicate samples. Significant difference between exposure and control groups is indicated at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

close to the nominate concentrations with <15% variation throughout the exposure duration (Table S3), which was in compliance with OECD Guidelines for testing of chemicals 203 (1992).

2.4. Thyroid Hormone Assay. Thyroid hormones (THs) including both total and free forms of thyroxine (TT4 and FT4, respectively) and 3,5,3′-triiodothyronine (TT3 and FT3, respectively) were all measured by ELISA[38] for the zebrafish larvae subsequent to 6-d exposure. Approximately 120 zebrafish larvae collected from each group were pooled, rinsed with 3 mL of 0.01 M ice-cold phosphate buffered solution (PBS, pH = 7.2 to 7.4), and homogenized by intermittent sonication using a Scientz-IID Ultrasonic cell grinder (Ningbo Scientz Biotechnology Co., LTD, China) for 10 min on ice. The homogenate was centrifuged for 10 min at 13000 g and 4 °C, and the supernatant was collected for TT4, FT4, TT3, and FT3 analyses using ELISA kits (Haling Biotechnology Co., Ltd. China). Each group contained three replicates, and each replicate was assayed in quadruplicate. The interassay (n = 3) and intra-assay (n = 4) variabilities were below 10% or 5% of coefficient of variation. TH contents in the extracts were further corrected by the total protein quantities, following the manufacturer's protocol provided by the BCA protein assay kit (Solarbio Biotechnology, China). The limits of detection of TT4, TT3, FT4, and FT3 were 5−160 μg/L, 3.75−120 μg/L, 0.6−16 pmol/L, and 0.075−2.4 pmol/L, respectively.

2.5. mRNA Analysis. Isolation of total mRNA was performed by using the RNeasy mini kit (Sparkjade Biotechnology Co., Ltd. China), and quantification and verification were performed in accordance with a previously reported protocol.[39] The detailed procedures are provided in Text S4.

2.6. Molecular Docking. The binding interactions of PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA with TTR and TRβ (in complex with either agonist or antagonist) were evaluated by a molecular docking model performed on AutoDock Vina (Scripps Research Institute, CA), and the detailed procedures and parameters are provided in Text S5.

2.7. TRβ-Mediated Luciferase Reporter Gene Assay. Quantitative assessment of agonistic and antagonistic activity of PFOS-like substances toward TRβ at the cellular level was carried out through a luciferase reporter gene assay, building upon the established methodology[40,41] with some modifications. The detailed procedures are provided in Text S6.

2.8. Data Analysis. Whole body burden of each chemical was calculated as proposed previously[4] to denote the accumulative amount of the chemical in each zebrafish larvae over the 6-day exposure:

$$\text{whole body burden} = M_{\text{fish}} \times C_{\text{whole body}} \quad (1)$$

where M_{fish} is the average fish weight obtained at 6 dpf (g, ww) and $C_{\text{whole body}}$ is the concentration of the target chemical in zebrafish larvae (μmol/g).

2.9. Statistical Analysis. All statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL). After testing for normality and homogeneity of variances by the Kolmogorov−Smirnov test and Levene's test, respectively, one-way analysis of variance (ANOVA) with the Tukey test was used to evaluate the differences between the control and test groups. Pearson correlation with a two-tailed test was used to examine the correlation coefficient (referred to as r value) between different variables. Statistical significance was indicated at three different levels (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). All data were expressed as mean ± standard deviation (SD).

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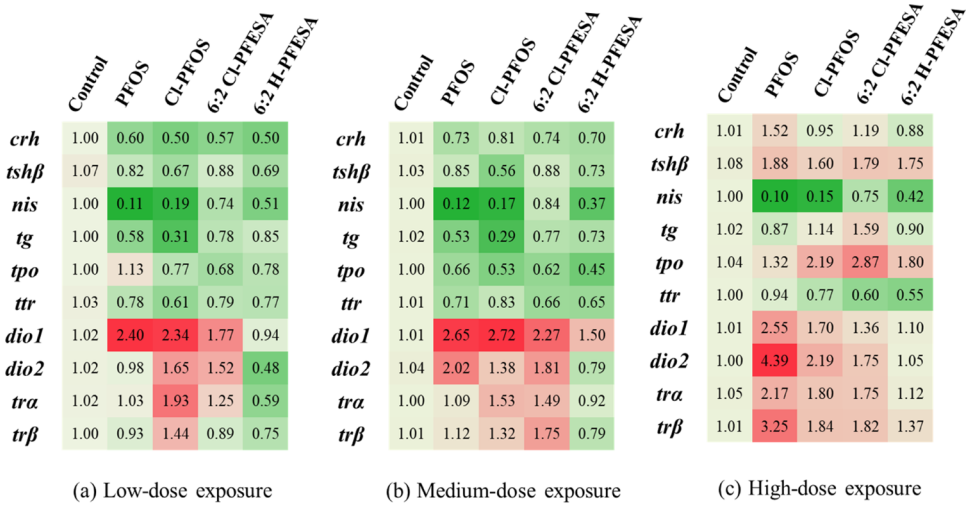

Figure 2. Heatmap of the HPT axis-related gene expression in zebrafish larvae after treatment with the control vehicle (DMSO) or PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA at (a) low (0.01 μmol/L), (b) medium (0.1 μmol/L), and (c) high (1 μmol/L) doses. Each chemical is listed in columns, and each gene in lines. Green indicates downregulation, whereas red indicates upregulation. Color intensity represents the extent of the fold changes. Results are expressed as means of three replicate samples.

3. RESULTS

3.1. Accumulation and Biotransformation of the Target Chemicals. PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA accumulated in zebrafish larvae as a result of exposure for 6 days, and the accumulated amounts were positively proportional to the dosed levels in the solutions (Tables S3 and S4). Following the 6-d exposure, PFOS, Cl-PFOS, and 6:2 Cl-PFESA exhibited comparable enrichments in the larvae, showing magnitudes significantly greater than those of 6:2 H-PFESA across all levels of exposure ($p < 0.05$). For example, the cumulative whole-body accumulation of 6:2 Cl-PFESA in the zebrafish subject to low-, medium- and high-dose exposure amounted to 0.11 ± 0.01, 2.1 ± 0.3, and 22 ± 2.4 pmol per fish, respectively, higher than the respective concentrations of 6:2 H-PFESA of 0.085 ± 0.009, 1.4 ± 0.1, and 16 ± 1.9 pmol per fish. The hypothesized biotransformation of 6:2 Cl-PFESA to 6:2 H-PFESA, as previously outlined in our investigations,[6,8] was not observed in the exposed zebrafish based on pseudotargeted analysis.

3.2. Morphologic Changes in Zebrafish Larvae. The exposure concentrations were selected according to previous studies to avoid any acute toxicity or mortality of zebrafish.[2,42] Our observations revealed that exposure to PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA across all concentrations did not elicit obvious toxic effects in zebrafish larvae with respect to hatching, malformation, and survival rates accumulated upon 6 dpf ($p > 0.05$) (Table S5). Notably, the most sensitive indicators of potential thyrotoxic symptoms were the co-occurrence of spinal curvature and pericardial edema, particularly notable in the high-dose group. Our findings highlighted that exposure to these four chemicals resulted in an increase in malformation rates between 4 and 6 dpf, although the difference between the treatment and control groups was not statistically significant (Table S5).

3.3. Thyroid Hormone Assessment. The alterations of total THs, including TT4 and TT3, in the zebrafish larvae are illustrated in Figure 1a and 1b, respectively. The incorporation of multiple TH indicators is grounded in the interrelationship of these indicators. In the low-dose exposure group, the TT4 levels exhibited reduction in all treatment groups but not significantly different from the control group ($p > 0.05$). However, a significant decrease in TT4 levels was observed in the medium-dose exposure groups, with particular significance for PFOS and Cl-PFOS treatments ($p < 0.005$). Conversely, an increase in TT4 levels was noted in the high-dose exposure groups, particularly notable for 6:2 Cl-PFESA treatment ($p < 0.005$). Likewise, the TT3 levels were not significantly affected in the low- and medium-dose exposure groups but were significantly promoted in the high-dose exposure groups. This increase was most pronounced in response to 6:2 Cl-PFESA treatment ($p < 0.05$). Correspondingly, the FT4 and FT3 levels concomitantly changed with TT4 and TT3 levels, and the changing degrees of FT3 were more remarkable than those of FT4 (Figure 1c,d). Among all of the THs, FT3 was the most sensitive biomarker upon exposure to PFOS-like substances, exhibiting the most significant changes. Except for 6:2 H-PFESA treatment, substantial increase in FT3 levels was recorded in response to PFOS, Cl-PFOS, and 6:2 Cl-PFESA treatments. Furthermore, it should be noted that the abundance ratio of FT4 to TT4 (molecular weight of T4 = 777 Da) stood at approximately 0.01%,[43] which aligned closely with the defined level of 0.03%. In contrast, the abundance ratio of FT3 to TT3 (molecular weight of T3 = 651 Da) registered at around 0.001%, which was 300 times lower than the defined level of 0.3%.[43] The relatively lower FT3/TT3 ratio was attributed to the abnormally high TT3 level observed. Under normal conditions, the TT3/TT4 ratio hovers around 2%.[44] In our study, however, the TT3 level was comparable to the TT4 level (Figure 1a,b), indicating a 50 times higher TT3 concentration than the norm and thus a decreased FT3/TT3 ratio.

To quantitatively assess the dynamic flux of THs between their active and inactive forms, we examined the *de novo* transformation ratio from T4 to active T3, denoted as the TT3/TT4 ratio (Figure 1e). At all exposure levels, the conversion from T4 to T3 was minimally disturbed by the 6:2 H-PFESA treatment, in contrast with the pronounced enhancement triggered by PFOS, Cl-PFOS, and 6:2 Cl-PFESA treatments. Notably, the most significant elevation was

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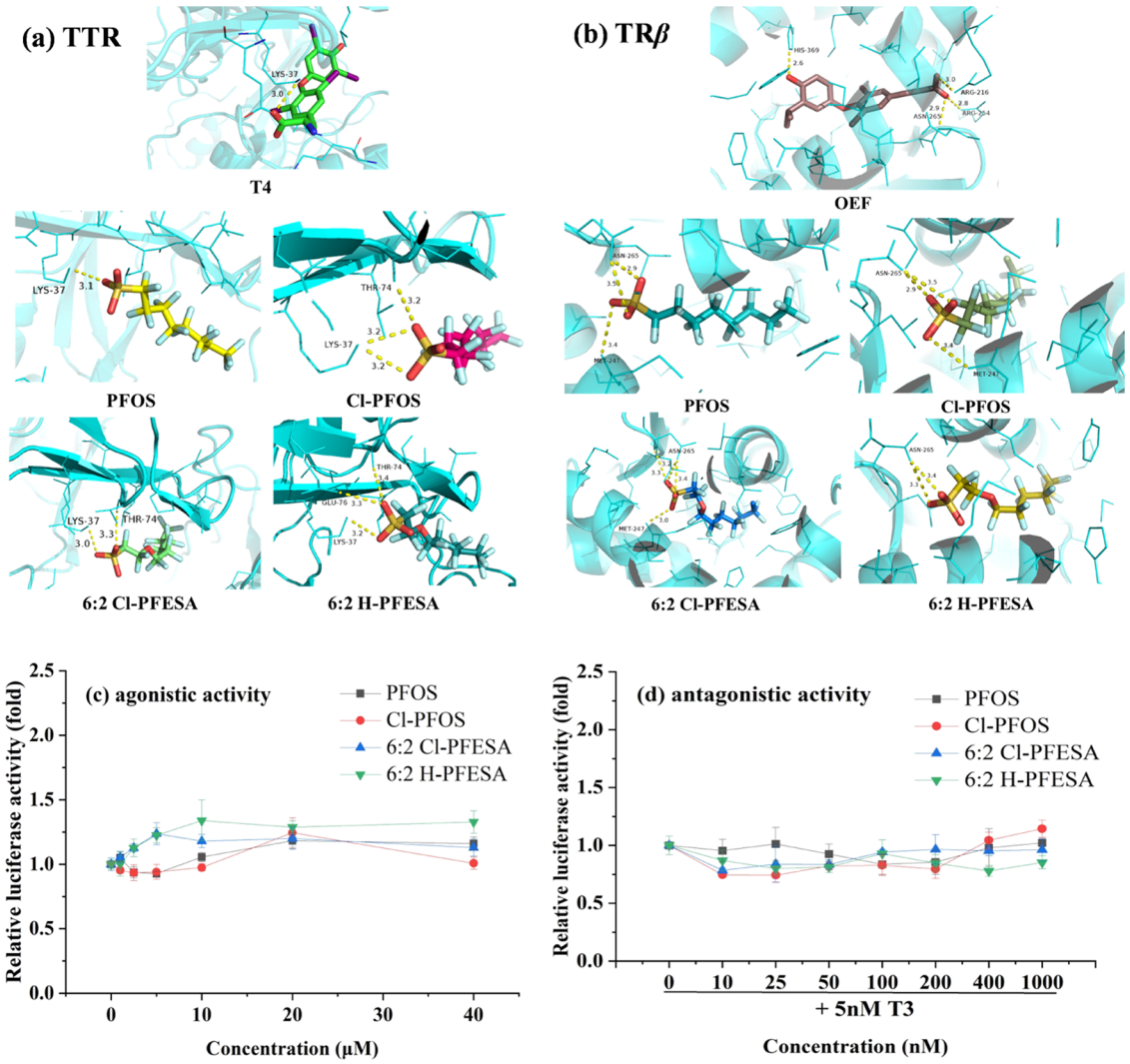

Figure 3. Molecular docking results of (a) T4 and four PFOS-like substances at the active site of TTR; and (b) 3,5-dibromo-4-(3-isopropylphenoxy) benzoic acid (OEF) and four PFOS-like substances at the active site of TRβ. Hydrogen bonds between the ligand and protein are represented by dashed yellow lines. Luciferase reporter gene transcription activity induced by four PFOS-like substances toward (c) TRβ agonism, and (d) TRβ antagonism. Results are expressed as means ± SD of three replicate samples.

recorded in the case of PFOS treatment, especially at medium and high doses.

3.4. Gene Transcription of the HPT Axis. The transcript levels of genes related to the HPT axis, which encode TH secretion stimulation, synthesis, distribution/transport, activation, and action processes, were measured in zebrafish larvae at 6 dpf (Figure 2).

Of the TH secretion stimulation genes, corticotropin-releasing hormone (*crh*) and thyroid-stimulating hormone β (*tsh*β) expression levels were concomitantly reduced upon low- and medium-dose exposure to PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA. In contrast, a noticeable elevation in their transcriptional levels, particularly *tsh*β, was observed upon exposure to all four compounds at a high dose. Remarkably, the most profound promotion on *crh* (1.5-fold, $p < 0.005$) and *tsh*β (1.9-fold, $p < 0.005$) transcripts was induced by PFOS treatment. A strongly positive correlation was established between the *crh* and *tsh*β mRNA levels ($r = 0.73$, $p < 0.005$).

Among the TH synthesis genes, sodium/iodide symporter (*nis*), thyroglobulin (*tg*), and thyroid peroxidase (*tpo*) were downregulated after exposure to PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA at low and medium doses. PFOS and Cl-PFOS treatments exhibited the most significant repression on these three genes, of which the *nis* expression was mostly reduced by 5-fold and the *tpo* and *tg* expressions by 2−3-fold. In contrast, the *tg* and *tpo* transcripts were upregulated after high-dose exposure to PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA, and the maximum upregulation was 1.6-fold for *tg* ($p < 0.005$) and 2.9-fold for *tpo* ($p < 0.005$). However, the *nis* expression remained consistently repressed, even after high-dose exposure.

For the TH distribution and transport gene, transthyretin (*ttr*) was finely downregulated in all treatment groups after low-, medium-, and high-dose exposure. There was no significant difference in *ttr* expression between the treatment groups except for a relatively minor attenuation induced by PFOS exposure at high dose. Of the two subtypes of deiodinase (*dio*) genes, namely *dio1* and *dio2*, which are in charge of TH activation by converting T4 into the biologically more active T3, their transcripts were significantly promoted after treatments with PFOS, Cl-PFOS, and 6:2 Cl-PFESA. This enhancement exhibited an increasing trend in tandem with

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increasing exposure dosage. A nearly 2-fold increase in *dio1* and *dio2* transcripts was observed after low- and medium-dose treatments, which further increased by 4-fold after high-dose treatment. However, the 6:2 H-PFESA treatment did not manifest a substantial capacity to stimulate *dio1* and *dio2* expressions. Instead, we observed a 52% ($p < 0.005$) and 21% ($p < 0.05$) decrease in *dio2* expression, respectively, after low- and medium-dose treatment with 6:2 H-PFESA. Of the nuclear thyroid hormone receptor (*tr*) genes responsible for TH action, the *trα* and *trβ* mRNA levels exhibited a dose-dependent enhancement following treatments with PFOS, Cl-PFOS, and 6:2 Cl-PFESA, but such enhancement was absent in the treatment with 6:2 H-PFESA at all exposure levels. The most significant promotion in *trα* and *trβ* expressions was recorded for Cl-PFOS by 1.4−1.9-fold ($p < 0.005$) in the low-dose treatment, for 6:2 Cl-PFESA by 1.5−1.8-fold ($p < 0.005$) in the medium-dose treatment, and for PFOS by 2.2−3.3-fold ($p < 0.005$) in the high-dose treatment.

Taken together, the gene-responsive patterns were closely related to both the chemical structure and the exposure levels. The modulation patterns of gene expression observed at low dose exhibited a remarkable resemblance to those at medium dose yet were significantly different from those at high dose. The transactivating activities of genes encoding TH secretion (*crh* and *tshβ*) and synthesis (*nis*, *tg*, *tpo*) were repressed after low- and medium-dose treatments but were stimulated upon high-dose exposure. Besides, similar modulations of the HPT axis, manifesting in a consistent manner and to a comparable degree, were noted in response to exposures to PFOS, Cl-PFOS, and 6:2 Cl-PFESA. This pattern notably differed from the observed trends in 6:2 H-PFESA treatment. The most significant difference was linked to TH activation (*dio1* and *dio2*) and action (*trα* and *trβ*) activities, which were strongly promoted by PFOS, Cl-PFOS, and 6:2 Cl-PFESA treatments, whereas they were scarcely affected by 6:2 H-PFESA treatment, implying a weaker disruption effect of the hydrogenated analogue on TH functioning.

3.5. Molecular Docking. Molecular docking was carried out to discern potential binding interactions of the four chemicals with TTR and TR*β*. Natural ligands of TTR and TR*β* were also included in the docking analysis for comparison. Inclusion of natural ligands of TTR and TR*β* in the docking analysis facilitated a comparative assessment. The configurations of the ligand−protein complexes are illustrated in Figure 3, while information regarding the binding affinity and the active amino acid residues in forming hydrogen bonds with the ligand is provided in Tables S6 and S7.

The PFOS-like substances all fit into the interior of the ligand binding pocket in TTR with an orientation that is very similar to each other and also resemble the one with T4 (Figure 3a). The sulfonic acid moiety in PFAS interacted with LYS37 and THR74 to form hydrogen bonds (Table S6). In addition, no significant difference in the binding potency was observed for the four PFAS in complex with TTR, ranging from −3.9 to −4.1 kcal/mol (Table S6).

When testing either agonistic or antagonistic activity of the PFOS-like substances toward TR*β*, we observed that none of these substances could be successfully docked into the ligand binding pocket in TR*β* in an agonistic conformation, but they were able to bind to its antagonistic conformation with appreciable affinities within the range of −7.9 to −8.2 kcal/mol (Table S7). This is evidenced by the arrangement depicted in Figure 3b, wherein interactive residues such as ASN265 and MET247 contribute to the activation of TR*β* antagonism. Inferred from the docked structures, the PFOS-like substances were stabilized in the binding pocket via hydrogen-bond interactions facilitated by the sulfonic acid group in PFAS (Table S7). This property renders them plausible candidates for TR*β* antagonistic ligands.

3.6. Activities of PFOS-like Substances toward TR*β*. The more quantitatively agonistic and antagonistic activity of the test chemicals toward TR*β* was further corroborated via TR*β*-mediated luciferase transcriptional activity (Figure 3c,d). For agonistic assay, the tested compounds, PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA, all could not effectively enhance the TR*β*-mediated luciferase transcriptional activity compared to the negative control (0.90−1.24-fold, $p > 0.05$, Figure 3c). For antagonist assay, none of the four chemicals significantly suppressed the TR*β*-mediated luciferase transcriptional activity, even at 200 times higher exposure level than T3 (0.92−1.21-fold, $p > 0.05$, Figure 3d). No significant difference was observed among the chemical treatment groups in both assays. These results suggest that the PFOS-like substances have neither agonistic nor antagonistic activity toward TR*β* under test concentrations.

4. DISCUSSION

Dechlorination of 6:2 Cl-PFESA was not observed in this study, possibly due to the limited enzymatic activity of zebrafish, especially in their early life stage. Consistently, it was observed that the percentage of 6:2 Cl-PFESA undergoing reductive dechlorination in rainbow trout liver was less than 0.02%.[6] Cl-PFOS and 6:2 Cl-PFESA exhibited comparable accumulative potency to PFOS in zebrafish larvae, whereas the dechlorinated molecule 6:2 H-PFESA showed lower enrichment. This was in alignment with our earlier rat-based findings, indicating that 6:2 H-PFESA was preferentially excreted through urine in comparison to 6:2 Cl-PFESA.[8] These results suggest that the low degree of chlorination and introduction of a single ether bond into the carbon backbone of PFOS might not considerably affect the bioaccumulation potential, at least during short-term exposure. However, hydrogenation of one Cl- at the end tail of 6:2 Cl-PFESA distinctly diminished the bioaccumulation potential, leading to reduced body burden. A close relationship between PFAS ability to accumulate in fish and their hydrophobicity, often represented by K_{ow} values, is extensively presented in previous studies.[45] However, experimental or modeled K_{ow} values for the novel PFAS, namely, 6:2 H-PFESA and Cl-PFOS, are currently unavailable at this stage. Thus, we employed three models from VEGA software, including the LogP model (Meylan−Kowwin) 1.1.5, LogP model (MlogP) 1.0.1, and LogP model (AlogP) 1.0.1, in an attempt to predict the K_{ow} values for PFOS, Cl-PFOS, 6:2 H-PFESA, and 6:2 Cl-PFESA. However, all four PFAS fell outside the applicability domains of these models. This limitation stems from a scarcity of substances structurally similar to PFAS in the training sets of these K_{ow} prediction models, rendering them inadequate for predicting K_{ow} values for PFAS. Additionally, we explored the COMPTOX database to gather experimental and modeled K_{ow} values for PFOS, but it revealed substantial disparities among different experimental measurements and model predictions. Consequently, K_{ow} values for the four PFAS are not provided in this study. Nevertheless, based on their retention times in liquid chromatography, the hydrophobicity of the four PFAS can be tentatively assessed in the order of 6:2 H-PFESA (4.0 min) < PFOS (4.4 min) <

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Cl-PFOS (4.5 min) < 6:2 Cl-PFESA (4.6 min). Consistently, our previous research demonstrated that despite the structural similarity between 6:2 H-PFESA and 6:2 Cl-PFESA, with the only difference being a H atom, the chloro-analogue accumulates 1.1-fold more in rats[8] and exhibits a 5.5-fold higher solid−water partitioning coefficient in anaerobic sludge collected from a wastewater treatment plant,[9] compared to the hydro-analogue. This indicates that subtle structural differences could significantly impact their bioaccumulation potential, which is in essence related to the molecular size and hydrophobicity, accounting for varying binding affinities of PFAS to relevant proteins.[46] For the same reason, the binding affinities of structure-varying PFAS to TH-related proteins should be a key factor affecting TH secretion stimulation, synthesis, distribution/transport, activation, and action processes.

It has been summarized that several potential end points in developing embryos/larvae can be used to assess the functions of the thyroid gland, including specific expressions in differentiated thyroid follicular cells (e.g., TG), activation of the HPT axis (TSHβ) and levels of THs.[47] In our study, we specifically focused on the effects of PFAS on gene expression along the HPT axis and TH homeostasis. Our results demonstrated that the four chemicals exerted varying degrees of disruption of TH homeostasis, leading to an imbalance between T4 and T3, in both total and free forms. Concurrently, these chemicals dysregulated the expression of the responsive genes along the HPT axis responsible for TH secretion stimulation, synthesis, distribution, transport, activation, and action.

In fish, the hypothalamus secretes CRH to stimulate the release of TSHβ from the pituitary, which exerts a stimulatory effect on the synthesis and release of T4 by thyroid follicles.[48,49] Cell lines and animal studies proposed that the expressions of *crh* and *tsh*β were subject to the feedback modulation by THs, targeting either the hypothalamic[50] or pituitary,[51,52] or both to negatively regulate *crh* and *tsh*β expressions. However, in this study, the *crh* and *tsh*β transcripts were negatively regulated by THs at the low- and medium-dose exposure groups, but they were not negatively correlated with either T4 or T3 at the high exposure level. Similarly, a previous epidemiological study reported that neither CRH nor TSH was negatively affected by the elevated FT4 level in the individuals, but the CRH concentration was positively correlated with the serum level of PFAS.[53] The mechanistic explanation for the absence of feedback regulation of THs on *crh* and *tsh*β expressions upon high dose of PFAS is discussed in detail in the following text.

When TSH is transferred into thyroid, THs (mainly T4) are produced with the assistance of NIS, TG, and TPO.[54,55] NIS, serving as a transmembrane glycoprotein, is stimulated by TSH to transport sodium and iodide across the basolateral plasma membrane into thyroid follicle cells, followed by a series of steps involving TPO to catalyze the coupling of iodine and tyrosine residues within the TG molecule, leading to the TH synthesis.[54,55] The causal relationship between pituitary control and thyroid gland for TH production/release was identified in this study by the strong associations between *tsh*β and *tg* ($r = 0.67$, $p < 0.005$) as well as *tpo* ($r = 0.80$, $p < 0.005$) transcripts. Besides, the transcriptional levels of *tg* and *tpo* were significantly correlated to the TT4 levels ($r = 0.64$, $p < 0.005$; $r = 0.50$, $p < 0.005$), signifying the critical roles of *tg* and *tpo* in T4 synthesis. Strikingly, *nis* was heavily downregulated by all four chemicals at all exposure levels, especially notable for PFOS and Cl-PFOS. It is well established that the *nis* expression is modulated via two main pathways: direct modulation dominated by the *tsh* gene, and feedback regulation mediated by cytoplasmic I^- concentration.[56] NIS couples the inward transport of two Na^+ ions and one I^- ion into the cytoplasm of the thyroid follicular cells.[57] Depending on the fact that NIS enzymatic activity is Na^+-dependent, the soluble Na^+ in PFOS and Cl-PFOS (their sodium salts were used in this study) would promote the NIS activity accounting for higher influx of I^- into cytoplasm, which in turn represses the *nis* expression via a feedback mechanism. This is in agreement with the Wolff−Chaikoff effect, which involves a sharp downregulation of the *nis* expression in various organisms induced by the high intracellular I^- level.[58−60] In comparison, the effect of K^+ in 6:2 Cl-PFESA and 6:2 H-PFESA (their potassium salts were used) on the NIS enzymatic activity was not straightforward. However, another concerning fact is that the Na^+ gradient which provides the driving force for I^- uptake is maintained by the Na^+/K^+ ATPase,[54] and it was evidenced that K^+ can activate and stabilize the enzymatic activity of Na^+/K^+ ATPase.[61] Therefore, the less-significant yet still noticeable repression of the *nis* expression by 6:2 Cl-PFESA and 6:2 H-PFESA is possibly due to the accumulated K^+ promoting an overload of cytoplasmic I^-. Taken together, *nis*, *tg*, and *tpo* are responsible for the T4 synthesis and release, whose transactivating activities are subject to upstream regulation by *tsh*β, as well as the Na^+ and K^+ concentrations in the exposure solution. Thus, PFAS are proposed as potent disruptors of the *nis* expression due to their Na^+ and K^+ moieties showing binding affinity to Na^+/K^+-dependent transporters. In line with our hypothesis, a previous study demonstrated Na^+-dependent transport for PFAS (including PFOS) in human and rat cells, which relied on the Na^+/taurocholate cotransporting polypeptide (NTCP) and sodium-dependent bile salt transporter (ASBT).[62] Further studies to delineate the thyrotoxicity of a large suit of Na^+- and K^+-containing PFAS are necessary to understand the saline effect on the thyroid synthesis process.

After synthesis, THs are released from the thyroid gland into the bloodstream. More than 99% of the synthesized THs in blood are bound to extracellular TH distributor proteins, and only a small fraction (<1%) of THs that dissociate from plasma proteins, known as FT4 and FT3, are biologically active and available for cellular uptake.[63] The protein-bound fraction serves as a buffered pool to ensure constant delivery of THs into cells and prevent avid nonspecific partitioning of THs into membranes.[63,64] In fish, TTR serves as the primary plasma protein for THs.[63] Accordingly, our hypothesis posits that the *ttr* expression is directly related to the maintenance of TH levels in bloodstream. Within expectations, the decreased *ttr* expression in larvae corresponded to the reduction of the total amount of THs, particularly TT4. Worse, the availability of TTR for TH binding could be further reduced due to the competitive binding of PFAS to TTR. Previous studies reported that PFAS could turf THs from the TTR-ligand binding sites, leading to the release of THs from TTR.[28,65,66] The space filling model revealed that PFOS, with a medium chain length and a sulfonate acid group which nearly fill the TTR-ligand binding pocket, was optimal for TTR binding.[66] Although PFOS showed a 5-fold lower affinity for TTR binding compared to T4,[66] the accumulated PFAS amounts in exposed larvae at high dose were 10−20-fold higher than the

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total TH concentrations. Thus, the competitive binding of PFAS to TTR should not be neglected. In support of our hypothesis, molecular docking results revealed that PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA exhibited comparable potency to competitively replace T4 from the binding site in TTR. Along the same line is the finding that PFOS and 6:2 Cl-PFESA could displace TH from TTR in plasma by competitive binding.[32] This may consequently lead to increased unbound TH levels in plasma circulation.[28,29]

Although thyroid mainly resembles and releases T4, Dios enzymes are in charge of converting it to a more biologically active T3 in central and peripheral tissues (e.g., brain, gut, liver).[67] It is estimated that approximately 60% (in rats) to 80% (in humans) of T3 is derived from the deiodination of T4 by Dio1 and Dio2.[68,69] Consistently, we observed a positive correlation between the TT3/TT4 ratio and *dios* expression ($r = 0.62$, $p < 0.005$ for *dio1*; $r = 0.61$, $p < 0.005$ for *dio2*). Among them, PFOS, Cl-PFOS, and 6:2 Cl-PFESA exhibited strong activation of the *dios* expression, leading to an enhanced production of T3. 6:2 H-PFESA exerted little effect on *dios* activation. These results magnified the fact that the potent stimulatory effect of PFOS, Cl-PFOS, and 6:2 Cl-PFESA on T3 production was due to the activation of the *dio1* and *dio2* genes, while the weak interference of 6:2 H-PFESA on these two genes contributed to its marginal influence on T3 production. Similar results were reported with waterborne exposure to PFOS causing a significant increase of T3 level and *dios* expression in zebrafish larvae,[70] and consecutive oral administration of PFOS concomitantly inhibiting the T3 level and the *dios* expression in rats.[71]

However, it is worth noting that the promoted *dios* expression by these chemicals could not guarantee that the Dios enzymatic activity is consistently facilitated. It was evidenced that the deiodination mechanism of T4 was linked to the halogen bonding (XB, in which X = I) to the selenocysteine (Sec) residue at the dio active site,[72] while halogenated organic compounds could inhibit TH deiodination by competitively binding to the active site Sec via XB interactions (in which X = I, Br, or Cl).[73] As a result, debromination or dechlorination of the ligands would occur upon the formation of XB. Polybrominated diphenyl ethers (PBDEs), for example, were debrominated by Dios in animals.[74−76] Although dehalogenation of the target chemicals did not extensively occur in zebrafish larvae, it could happen to a great degree in mammals.[8] These results collectively suggest that the chlorinated analogues like Cl-PFOS and 6:2 Cl-PFESA, containing larger halogen atoms conducive to forming XB interactions,[77] are potent in preventing T4 deiodination by forming chlorine bonds within Dios. Meanwhile, the results also highlight the likelihood of Dios responsible for the dechlorination of 6:2 Cl-PFESA observed in previous studies.[6,8]

Once T3 is formed in the cytoplasm, it will be transferred across the nuclear membrane for functioning. TRs are nuclear receptors expressed primarily in the brain that TRα regulates the cardiac rate and contractility, while TRβ is important to maintain cholesterol homeostasis.[78] Studies using transgenic animals demonstrated the dominant role of TRs in T3-mediated downstream gene expression, signifying a TR-dependent mechanism of T3 action on the HPT axis, such as the negative feedback loop among *crh*, *tshβ*, and T3.[79,80] Particularly, with clinical observations[81] and findings from mouse studies,[82−84] TRβ was identified as the predominant receptor that was preferentially bound to T3 for action. Xenobiotic chemicals can disrupt TRβ functioning via either replacing T3 for binding to TRβ or interfering with coactivator/corepressor recruitment for the TRβ complex.[78] Previous studies reached controversial conclusions about PFAS as potential TRβ agonists or antagonists. For example, Xin et al.[40] demonstrated that PFOS and 6:2 Cl-PFESA acted as agonists in activating TRβ transcription in human HEK 293 cell line. Conversely, Du et al.[41] found that PFOS depressed TRβ expression as a potent antagonist in African green monkey kidney CV-1 cell line. Moreover, Chen et al.[85] reported that polyfluoroalkyl phosphate diesters (diPAPs) could bind to TRβ at the site for antagonists using molecular docking. In this study, the molecular docking model supported the potent antagonism of PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-PFESA toward TRβ. However, according to the luciferase reporter gene results, these four PFAS neither effectively countered the agonistic activity of T3 toward TRβ nor competitively activated TRβ similar to T3. Collectively, it is hypothesized that these PFAS are potent antagonists for TRβ based on structures, but they barely outcompete agonistic T3 even at significantly higher concentrations (200 times). Our results were consistent with the US EPA ToxCast library of luciferase reporter gene assays, in which PFAS, including PFOS, are inactive in inducing TR agonism (Tox21 TR LUC_GH3_Agonist) or antagonism (Tox21 TR_LUC_GH3_Antagonist). In line with the same experimental design, a total of 150 PFAS samples were further elaborately explained by Houck et al., in which all PFAS exhibited inertness to agonize or antagonize TRs.[86] Hence, the reason T3 failed to negatively regulate the *crh* and *tshβ* expressions was not attributed to the PFAS replacing T3 for TR binding. Instead, it could arise from the replacement of T3 from membrane transporters, such as the mostly recognized monocarboxylate transporters (MCTs) and organic anion transporting polypeptides (OATPs),[87] to enter target cells (e.g., hypothalamic cells) for subsequent TR binding and action. Previous investigations have demonstrated that PFAS could serve as the substrates of OATPs.[88] Although the role of competitive binding of PFAS to MCTs and OATPs in affecting T3 functioning falls out of the scope of the current research, it holds significant relevance for future investigations. Consequently, the diminished bioaccessibility of T3 renders target cells hungry for T3, and in response, the body increases TSH to stimulate additional T4 production and consequently swift T3 recovery. It may explain the concomitant increase in the *tshβ* expression and the TT3 level, rather than the negative relationship, in high-dose exposure groups.

Ultimately, a comprehensive assessment of TSH, together with T3 and T4, serves to delineate between a primary and a secondary cause of thyroid dysfunction.[89] A TSH change that concurs with the opposite direction of T3 and T4 indicates a primary problem in the thyroid gland itself; in contrast, a change in TSH that parallels T3 and T4 changes toward the same direction suggests a secondary problem originating in the anterior pituitary. According to the observations in the present study, it suggests that PFAS, at a high exposure level, are capable of causing secondary hyperthyroidism via disrupting the T3−TR−TSH−T4−T3 loop. In secondary hyperthyroidism, the anterior pituitary lost sensitivity to elevated THs and produced additional TSH instead, which, in turn, intrigued the thyroid follicular cells to secrete THs in excessive amount.

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5. ENVIRONMENTAL IMPLICATIONS

The present study compared the genetic and hormonal consequences of thyrotoxicity of PFOS and emerging PFOS-like substances (such as Cl-PFOS for the first time) in relation to exposure concentrations and chemical structures. From the perspective of their toxic mode of action, it is highly probable that PFOS-like substances primarily impact TH synthesis, distribution/transport, and activation processes through direct interactions with relevant enzymes and plasma transport proteins. Furthermore, these substances likely disrupt TH action by binding to membrane proteins rather than directly interacting with TH receptors, leading to a reduced cellular uptake of THs. These constitute the molecular initiating events of the PFOS-like substance-induced thyrotoxicity. Mechanically, all of these toxic effects were achieved via competitive binding of PFOS-like substances to relevant proteins owing to either structural resemblance with THs or Na^+/K^+-driven activity. Although further targeted research is imperative for robust validation, our results here provide the first line of evidence for elucidating PFAS-induced thyrotoxicity relating to the Na^+ or K^+ moieties, which so far has been overlooked in previous studies.

From the perspective of their toxic potential, chemical structures affected thyrotoxicity remarkably. PFOS, Cl-PFOS, and 6:2 Cl-PFESA exhibited comparable thyrotoxicity as PFOS both at hormonal and transcriptional levels, which were notably more pronounced than 6:2 H-PFESA, particularly for TH activation, action, and hormonal homeostasis. This aligns with the enrichment degrees of these chemicals in larvae. Alternatively, the differences in hydrophobicity of these four PFAS are crucial in determining the binding affinities of PFAS to TH-related carrier or receptor proteins, so the weakened hydrophobicity of 6:2 H-PFESA (compared to 6:2 Cl-PFESA) may lead to its reduced binding affinity with relevant proteins and correspondingly fewer adverse effects. The comparable toxic potential of 6:2 Cl-PFESA to PFOS may have brought concerns over its safety as a desirable substitute to PFOS though the inertness of 6:2 H-PFESA is reassuring. Although 6:2 H-PFESA is not directly produced as an industrial product, our earlier studies implied the occurrence of 6:2 H-PFESA in anaerobic environments (~99% in wastewater treatment plants, landfills as well as groundwater)[9] and mammals (13.6% in rat liver)[8] as a dechlorinating product of 6:2 Cl-PFESA is favorable. Further investigation into the extensive hydrogenation of PFOS to determine its toxicity will provide valuable insights into designing biologically safer PFOS alternatives in the future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c07003.

Text, figures, and tables addressing sample extraction; instrumental analysis by UPLC-MS/MS; quality assurance and quality control (QA/QC); mRNA analysis; molecular docking analysis assessing the binding of PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-FPESA with TTR and TRβ; luciferase reporter gene assay determining activities of PFOS, Cl-PFOS, 6:2 Cl-PFESA, and 6:2 H-FPESA toward TRβ; limits of quantification (LOQs) and matrix spike recoveries of the target chemicals in exposure solutions and zebrafish larvae; primer sequences for quantitative real-time polymerase chain reaction (qRT-PCR); quantification of the target chemicals in exposure solutions before and after exposure; quantification of the target chemicals accumulated in zebrafish larvae after exposure; morphological changes (hatching, malformation, and lethal rates); results of hydrogen-bond interactions and binding energy of T4 and four PFAS with TTR by molecular docking analysis; results of hydrogen-bond interactions and binding energy of OEF and four PFAS with TRβ by molecular docking analysis; structures of chemicals used in this study (PDF).

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Notes

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■ REFERENCES

(1) Wang, Z.; Cousins, I. T.; Scheringer, M.; Buck, R. C.; Hungerbühler, K. Global emission inventories for C4−C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources. *Environ. Int.* **2014**, *70*, 62−75.

(2) Wang, S.; Huang, J.; Yang, Y.; Hui, Y.; Ge, Y.; Larssen, T.; Yu, G.; Deng, S.; Wang, B.; Harman, C. First report of a Chinese PFOS alternative overlooked for 30 years: its toxicity, persistence, and presence in the environment. *Environ. Sci. Technol.* **2013**, *47* (18), 10163−10170.

(3) Liu, W.; Qin, H.; Li, J.; Zhang, Q.; Zhang, H.; Wang, Z.; He, X. Atmospheric chlorinated polyfluorinated ether sulfonate and ionic perfluoroalkyl acids in 2006 to 2014 in Dalian, China. *Environ. Toxicol. Chem.* **2017**, *36* (10), 2581−2586.

(4) Shi, Y.; Vestergren, R.; Zhou, Z.; Song, X.; Xu, L.; Liang, Y.; Cai, Y. Tissue distribution and whole body burden of the chlorinated polyfluoroalkyl ether sulfonic acid F-53B in crucian carp (*Carassius carassius*): Evidence for a highly bioaccumulative contaminant of emerging concern. *Environ. Sci. Technol.* **2015**, *49* (24), 14156−14165.

(5) Shi, Y.; Vestergren, R.; Xu, L.; Zhou, Z.; Li, C.; Liang, Y.; Cai, Y. Human exposure and elimination kinetics of chlorinated polyfluoroalkyl ether sulfonic acids (Cl-PFESAs). *Environ. Sci. Technol.* **2016**, *50* (5), 2396−2404.

(6) Yi, S.; Zhu, L.; Mabury, S. A. First report on in vivo pharmacokinetics and biotransformation of chlorinated polyfluoroalkyl ether sulfonates in rainbow trout. *Environ. Sci. Technol.* **2020**, *54* (1), 345−354.

(7) Wang, Q.; Ruan, Y.; Jin, L.; Lin, H.; Yan, M.; Gu, J.; Yuen, C. N. T.; Leung, K. M. Y.; Lam, P. K. S. Tissue-specific uptake, depuration kinetics, and suspected metabolites of three emerging per- and polyfluoroalkyl substances (PFASs) in marine medaka. *Environ. Sci. Technol.* **2022**, *56* (10), 6182−6191.

(8) Yi, S.; Yang, D.; Zhu, L.; Mabury, S. A. Significant reductive transformation of 6:2 chlorinated polyfluorooctane ether sulfonate to form hydrogen-substituted polyfluorooctane ether sulfonate and their toxicokinetics in male Sprague−Dawley rats. *Environ. Sci. Technol.* **2022**, *56* (10), 6123−6132.

(9) Yi, S.; Morson, N.; Edwards, E. A.; Yang, D.; Liu, R.; Zhu, L.; Mabury, S. A. Anaerobic microbial dechlorination of 6:2 chlorinated polyfluorooctane ether sulfonate and the underlying mechanisms. *Environ. Sci. Technol.* **2022**, *56* (2), 907−916.

(10) Feng, X.; Ye, M.; Li, Y.; Zhou, J.; Sun, B.; Zhu, Y.; Zhu, L. Potential sources and sediment-pore water partitioning behaviors of emerging per/polyfluoroalkyl substances in the South Yellow Sea. *J. Hazard. Mater.* **2020**, *389*, No. 122124.

(11) Feng, X.; Yi, S.; Shan, G.; Chen, X.; Yang, Y.; Yang, L.; Jia, Y.; Zhu, Y.; Zhu, L. Occurrence of perfluoroalkyl substances in the environment compartments near a mega fluorochemical industry: Implication of specific behaviors and emission estimation. *J. Hazard. Mater.* **2023**, *445*, No. 130473.

(12) Wang, Y.; Yu, N.; Zhu, X.; Guo, H.; Jiang, J.; Wang, X.; Shi, W.; Wu, J.; Yu, H.; Wei, S. Suspect and nontarget screening of per-and polyfluoroalkyl substances in wastewater from a fluorochemical manufacturing park. *Environ. Sci. Technol.* **2018**, *52* (19), 11007−11016.

(13) Wu, Y.; Simon, K. L.; Best, D. A.; Bowerman, W.; Venier, M. Novel and legacy per- and polyfluoroalkyl substances in bald eagle eggs from the Great Lakes region. *Environ. Pollut.* **2020**, *260*, No. 113811.

(14) Rotander, A.; Kärrman, A.; Toms, L. M.; Kay, M.; Mueller, J. F.; Gómez Ramos, M. J. Novel fluorinated surfactants tentatively identified in firefighters using liquid chromatography quadrupole time-of-flight tandem mass spectrometry and a case-control approach. *Environ. Sci. Technol.* **2015**, *49* (4), 2434−2442.

(15) Pabon, M.; Corpart, J. M. Fluorinated surfactants: synthesis, properties, effluent treatment. *J. Fluorine Chem.* **2002**, *114* (2), 149−156.

(16) Yi, S.; Chen, P.; Yang, L.; Zhu, L. Probing the hepatotoxicity mechanisms of novel chlorinated polyfluoroalkyl sulfonates to zebrafish larvae: Implication of structural specificity. *Environ. Int.* **2019**, *133*, No. 105262.

(17) Zhang, H.; Zhou, X.; Sheng, N.; Cui, R.; Cui, Q.; Guo, H.; Guo, Y.; Sun, Y.; Dai, J. Subchronic hepatotoxicity effects of 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA), a novel perfluorooctanesulfonate (PFOS) alternative, on adult male mice. *Environ. Sci. Technol.* **2018**, *52* (21), 12809−12818.

(18) Wang, Z.; Yao, J.; Guo, H.; Sheng, N.; Guo, Y.; Dai, J. Comparative hepatotoxicity of a novel perfluoroalkyl ether sulfonic acid, nafion byproduct 2 (H-PFMO2OSA), and legacy perfluorooctane sulfonate (PFOS) in adult male mice. *Environ. Sci. Technol.* **2022**, *56* (14), 10183−10192.

(19) Li, Q.-Q.; Liu, J.-J.; Su, F.; Zhang, Y.-T.; Wu, L.-Y.; Chu, C.; Zhou, Y.; Shen, X.; Xiong, S.; Geiger, S. D.; Qian, Z. M.; McMillin, S. E.; Dong, G.-H.; Zeng, X.-W. Chlorinated polyfluorinated ether sulfonates and thyroid hormone levels in adults: Isomers of C8 health project in China. *Environ. Sci. Technol.* **2022**, *56* (10), 6152−6161.

(20) Deal, C. K.; Volkoff, H. The role of the thyroid axis in fish. *Front. Endocrinol.* **2020**, *11*, No. 596585.

(21) Taylor, P. N.; Razvi, S.; Pearce, S. H.; Dayan, C. M. Clinical review: A review of the clinical consequences of variation in thyroid function within the reference range. *J. Clin. Endocrinol. Metab.* **2013**, *98* (9), 3562−3571.

(22) Moog, N. K.; Entringer, S.; Heim, C.; Wadhwa, P. D.; Kathmann, N.; Buss, C. Influence of maternal thyroid hormones during gestation on fetal brain development. *Neuroscience* **2017**, *342*, 68−100.

(23) Murk, A. J.; Rijntjes, E.; Blaauboer, B. J.; Clewell, R.; Crofton, K. M.; Dingemans, M. M. L.; Furlow, J. D.; Kavlock, R.; Köhrle, J.; Opitz, R.; Traas, T.; Visser, T. J.; Xia, M.; Gutleb, A. C. Mechanism-based testing strategy using in vitro approaches for identification of thyroid hormone disrupting chemicals. *Toxicol. In Vitro* **2013**, *27* (4), 1320−1346.

(24) Ghassabian, A.; Trasande, L. Disruption in thyroid signaling pathway: A mechanism for the effect of endocrine-disrupting chemicals on child neurodevelopment. *Front. Endocrinol.* **2018**, *9*, No. 204.

(25) Coperchini, F.; Croce, L.; Ricci, G.; Magri, F.; Rotondi, M.; Imbriani, M.; Chiovato, L. Thyroid disrupting effects of old and new generation PFAS. *Front. Endocrinol.* **2021**, *11* (1077), No. 612320.

(26) Conti, A.; Strazzeri, C.; Rhoden, K. J. Perfluorooctane sulfonic acid, a persistent organic pollutant, inhibits iodide accumulation by thyroid follicular cells in vitro. *Mol. Cell. Endocrinol.* **2020**, *515*, No. 110922.

(27) Song, M.; Kim, Y.-J.; Park, Y.-K.; Ryu, J.-C. Changes in thyroid peroxidase activity in response to various chemicals. *J. Environ. Monit.* **2012**, *14* (8), 2121−2126.

(28) Kar, S.; Sepúlveda, M. S.; Roy, K.; Leszczynski, J. Endocrine-disrupting activity of per-and polyfluoroalkyl substances: Exploring combined approaches of ligand and structure based modeling. *Chemosphere* **2017**, *184*, 514−523.

(29) Chang, S.-C.; Thibodeaux, J. R.; Eastvold, M. L.; Ehresman, D. J.; Bjork, J. A.; Froehlich, J. W.; Lau, C.; Singh, R. J.; Wallace, K. B.; Butenhoff, J. L. Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). *Toxicology* **2008**, *243* (3), 330−339.

https://doi.org/10.1021/acs.est.3c07003
Environ. Sci. Technol. 2024, 58, 182−193

(30) Kim, S.; Ji, K.; Lee, S.; Lee, J.; Kim, J.; Kim, S.; Kho, Y.; Choi, K. Perfluorooctane sulfonic acid exposure increases cadmium toxicity in early life stage of zebrafish, *Danio rerio*. *Environ. Toxicol. Chem.* **2011**, *30* (4), 870−877.
(31) Chang, S. C.; Thibodeaux, J. R.; Eastvold, M. L.; Ehresman, D. J.; Bjork, J. A.; Froehlich, J. W.; Lau, C.; Singh, R. J.; Wallace, K. B.; Butenhoff, J. L. Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). *Toxicology* **2008**, *243* (3), 330−339.
(32) Xin, Y.; Ren, X. M.; Ruan, T.; et al. Chlorinated polyfluoroalkyl ether sulfonates exhibit similar binding potency and activity to thyroid hormone transport proteins and nuclear receptors as perfluorooctanesulfonate. *Environ. Sci. Technol.* **2018**, *52* (16), 9412−9418.
(33) Lin, Y.; Ruan, T.; Liu, A.; Jiang, G. Identification of novel hydrogen-substituted polyfluoroalkyl ether sulfonates in environmental matrices near metal-plating facilities. *Environ. Sci. Technol.* **2017**, *51* (20), 11588−11596.
(34) Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. A. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. *Environ. Sci. Technol.* **2002**, *36* (4), 545−551.
(35) Alt, B.; Reibe, S.; Feitosa, N. M.; Elsalini, O. A.; Wendl, T.; Rohr, K. B. Analysis of origin and growth of the thyroid gland in zebrafish. *Dev. Dyn.* **2006**, *235* (7), 1872−1883.
(36) Elsalini, O. A.; von Gartzen, J.; Cramer, M.; Rohr, K. B. Zebrafish *hhex*, *nk2.1a*, and *pax2.1* regulate thyroid growth and differentiation downstream of Nodal-dependent transcription factors. *Dev. Biol.* **2003**, *263* (1), 67−80.
(37) Luo, L.; Chen, A.; Hu, C.; Lu, W. Dynamic expression pattern of corticotropin-releasing hormone, urotensin I and II genes under acute salinity and temperature challenge during early development of zebrafish. *Fish Physiol. Biochem.* **2014**, *40* (6), 1877−1886.
(38) Agharanya, J. C. Clinical usefulness of ELISA technique in the assessment of thyroid function. *West Afr. J. Med.* **1990**, *9* (4), 258−263.
(39) Liu, C.; Yan, W.; Zhou, B.; Guo, Y.; Liu, H.; Yu, H.; Giesy, J. P.; Wang, J.; Li, G.; Zhang, X. Characterization of a bystander effect induced by the endocrine-disrupting chemical 6-propyl-2-thiouracil in zebrafish embryos. *Aquat. Toxicol.* **2012**, *118-119*, 108−115.
(40) Xin, Y.; Ren, X.-M.; Ruan, T.; Li, C.-H.; Guo, L.-H.; Jiang, G. Chlorinated polyfluoroalkyl ether sulfonates exhibit similar binding potency and activity to thyroid hormone transport proteins and nuclear receptors as perfluorooctanesulfonate. *Environ. Sci. Technol.* **2018**, *52* (16), 9412−9418.
(41) Du, G.; Hu, J.; Huang, H.; Qin, Y.; Han, X.; Wu, D.; Song, L.; Xia, Y.; Wang, X. Perfluorooctane sulfonate (PFOS) affects hormone receptor activity, steroidogenesis, and expression of endocrine-related genes in vitro and in vivo. *Environ. Toxicol. Chem.* **2013**, *32* (2), 353−360.
(42) Shi, G.; Cui, Q.; Pan, Y.; Sheng, N.; Sun, S.; Guo, Y.; Dai, J. 6:2 Chlorinated polyfluorinated ether sulfonate, a PFOS alternative, induces embryotoxicity and disrupts cardiac development in zebrafish embryos. *Aquat. Toxicol.* **2017**, *185*, 67−75.
(43) Walker, H. K.; Hall, W. D.; Hurst, J. W. *Clinical Methods: The History, Physical, and Laboratory Examinations.*; Butterworths: Boston, USA, 1990.
(44) Narkar, R. R.; Mishra, I.; Baliarsinha, A. K.; Choudhury, A. K. Rapid differential diagnosis of thyrotoxicosis using T3/T4 ratio, FT3/FT4 ratio and color doppler of thyroid gland. *Indian J. Endocrinol. Metab.* **2021**, *25* (3), 193−197.
(45) Semerád, J.; Horká, P.; Filipová, A.; Kukla, J.; Holubová, K.; Musilová, Z.; Jandová, K.; Frouz, J.; Cajthaml, T. The driving factors of per- and polyfluorinated alkyl substance (PFAS) accumulation in selected fish species: The influence of position in river continuum, fish feed composition, and pollutant properties. *Sci. Total Environ.* **2022**, *816*, No. 151662.
(46) Zhao, L.; Teng, M.; Zhao, X.; Li, Y.; Sun, J.; Zhao, W.; Ruan, Y.; Leung, K. M. Y.; Wu, F. Insight into the binding model of per- and polyfluoroalkyl substances to proteins and membranes. *Environ. Int.* **2023**, *175*, No. 107951.
(47) Zhu, B.; Wang, Q.; Wang, X.; Zhou, B. Impact of co-exposure with lead and decabromodiphenyl ether (BDE-209) on thyroid function in zebrafish larvae. *Aquat. Toxicol.* **2014**, *157*, 186−195.
(48) Larsen, D. A.; Swanson, P.; Dickey, J. T.; Rivier, J.; Dickhoff, W. W. In vitro thyrotropin-releasing activity of corticotropin-releasing hormone-family peptides in coho salmon, Oncorhynchus kisutch. *Gen. Comp. Endrocrinol.* **1998**, *109* (2), 276−285.
(49) De Groef, B.; Van der Geyten, S.; Darras, V. M.; Kühn, E. R. Role of corticotropin-releasing hormone as a thyrotropin-releasing factor in non-mammalian vertebrates. *Gen. Comp. Endrocrinol.* **2006**, *146* (1), 62−68.
(50) Segerson, T. P.; Kauer, J.; Wolfe, H.; Mobtaker, H.; Wu, P.; Jackson, I.; Lechan, R. Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science* **1987**, *238* (4823), 78−80.
(51) MacKenzie, D. S.; Jones, R. A.; Miller, T. C. Thyrotropin in teleost fish. *Gen. Comp. Endrocrinol.* **2009**, *161* (1), 83−89.
(52) Shupnik, M. A.; Chin, W. W.; Habener, J. F.; Ridgway, E. C. Transcriptional regulation of the thyrotropin subunit genes by thyroid hormone. *J. Biol. Chem.* **1985**, *260* (5), 2900−2903.
(53) Eick, S. M.; Goin, D. E.; Cushing, L.; DeMicco, E.; Smith, S.; Park, J.-S.; Padula, A. M.; Woodruff, T. J.; Morello-Frosch, R. Joint effects of prenatal exposure to per- and poly-fluoroalkyl substances and psychosocial stressors on corticotropin-releasing hormone during pregnancy. *J. Exposure Sci. Environ. Epidemiol.* **2022**, *32* (2), 27−36.
(54) Dohán, O.; Carrasco, N. Advances in Na^+/I^- symporter (NIS) research in the thyroid and beyond. *Mol. Cell. Endocrinol.* **2003**, *213* (1), 59−70.
(55) Soundarrajan, M.; Kopp, P. A. Thyroid Hormone Biosynthesis and Physiology. In *Thyroid Disease and Reproduction: A Clinical Guide to Diagnosis and Management*; Springer International Publishing: Cham, 2019; pp 1−17.
(56) Dohán, O.; De la Vieja, A.; Paroder, V.; Riedel, C.; Artani, M.; Reed, M.; Ginter, C. S.; Carrasco, N. The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. *Endocr. Rev.* **2003**, *24* (1), 48−77.
(57) Eskandari, S.; Loo, D. D.; Dai, G.; Levy, O.; Wright, E. M.; Carrasco, N. Thyroid Na^+/I^- symporter. Mechanism, stoichiometry, and specificity. *J. Biol. Chem.* **1997**, *272* (43), 27230−27238.
(58) Lebsir, D.; Manens, L.; Grison, S.; Lestaevel, P.; Ebrahimian, T.; Suhard, D.; Phan, G.; Dublineau, I.; Tack, K.; Benderitter, M.; Pech, A.; Jourdain, J.-R.; Souidi, M. Effects of repeated potassium iodide administration on genes involved in synthesis and secretion of thyroid hormone in adult male rat. *Mol. Cell. Endocrinol.* **2018**, *474*, 119−126.
(59) Wolff, J.; Chaikoff, I. L. Plasma inorganic iodide as a homeostatic regulator of thyroid function. *J. Biol. Chem.* **1948**, *174* (2), 555−564.
(60) Dayem, M.; Navarro, V.; Marsault, R.; Darcourt, J.; Lindenthal, S.; Pourcher, T. From the molecular characterization of iodide transporters to the prevention of radioactive iodide exposure. *Biochimie* **2006**, *88* (11), 1793−1806.
(61) Kaufman, S. B.; González-Flecha, F. L.; González-Lebrero, R. M. Opposing effects of Na+ and K+ on the thermal stability of Na+,K+-ATPase. *J. Phys. Chem. B* **2012**, *116* (10), 3421−3429.
(62) Zhao, W.; Zitzow, J. D.; Ehresman, D. J.; Chang, S. C.; Butenhoff, J. L.; Forster, J.; Hagenbuch, B. Na+/Taurocholate cotransporting polypeptide and apical sodium-dependent bile acid transporter are involved in the disposition of perfluoroalkyl sulfonates in humans and rats. *Toxicol. Sci.* **2015**, *146* (2), 363−373.
(63) Richardson, S. J. Cell and molecular biology of transthyretin and thyroid hormones. *Int. Rev. Cytol.* **2007**, *258*, 137−193.
(64) Alshehri, B.; D'Souza, D. G.; Lee, J. Y.; Petratos, S.; Richardson, S. J. The diversity of mechanisms influenced by transthyretin in neurobiology: development, disease and endocrine disruption. *J. Neuroendocrinol.* **2015**, *27* (5), 303−323.

https://doi.org/10.1021/acs.est.3c07003
Environ. Sci. Technol. 2024, 58, 182−193

(65) Weiss, J. M.; Andersson, P. L.; Lamoree, M. H.; Leonards, P. E.; van Leeuwen, S. P.; Hamers, T. Competitive binding of poly-and perfluorinated compounds to the thyroid hormone transport protein transthyretin. *Toxicol. Sci.* **2009**, *109* (2), 206−216.
(66) Ren, X.-M.; Qin, W.-P.; Cao, L.-Y.; Zhang, J.; Yang, Y.; Wan, B.; Guo, L.-H. Binding interactions of perfluoroalkyl substances with thyroid hormone transport proteins and potential toxicological implications. *Toxicology* **2016**, *366*−*367*, 32−42.
(67) Orozco, A.; Valverde-R, C. Thyroid hormone deiodination in fish. *Thyroid* **2005**, *15* (8), 799−813.
(68) Bianco, A. C.; Salvatore, D.; Gereben, B.; Berry, M. J.; Larsen, P. R. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev.* **2002**, *23* (1), 38−89.
(69) Bianco, A. C.; Kim, B. W. Deiodinases: implications of the local control of thyroid hormone action. *J. Clin. Invest.* **2006**, *116* (10), 2571−2579.
(70) Shi, X.; Liu, C.; Wu, G.; Zhou, B. Waterborne exposure to PFOS causes disruption of the hypothalamus−pituitary−thyroid axis in zebrafish larvae. *Chemosphere* **2009**, *77* (7), 1010−1018.
(71) Martin, M. T.; Brennan, R. J.; Hu, W.; Ayanoglu, E.; Lau, C.; Ren, H.; Wood, C. R.; Corton, J. C.; Kavlock, R. J.; Dix, D. J. Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. *Toxicol. Sci.* **2007**, *97* (2), 595−613.
(72) Bayse, C. A.; Rafferty, E. R. Is halogen bonding the basis for iodothyronine deiodinase activity? *Inorg. Chem.* **2010**, *49* (12), 5365−5367.
(73) Marsan, E. S.; Bayse, C. A. Halogen-bonding interactions of polybrominated diphenyl ethers and thyroid hormone derivatives: A potential mechanism for the inhibition of iodothyronine deiodinase. *Chem. - Eur. J.* **2017**, *23* (27), 6625−6633.
(74) Roberts, S. C.; Bianco, A. C.; Stapleton, H. M. Disruption of type 2 iodothyronine deiodinase activity in cultured human glial cells by polybrominated diphenyl ethers. *Chem. Res. Toxicol.* **2015**, *28* (6), 1265−1274.
(75) François, A.; Verreault, J. Interaction between deca-BDE and hepatic deiodinase in a highly PBDE-exposed bird. *Environ. Res.* **2018**, *163*, 108−114.
(76) Van den Steen, E.; Covaci, A.; Jaspers, V. L.; Dauwe, T.; Voorspoels, S.; Eens, M.; Pinxten, R. Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (Sturnus vulgaris). *Environ. Pollut.* **2007**, *148* (2), 648−653.
(77) Clark, T.; Hennemann, M.; Murray, J. S.; Politzer, P. Halogen bonding: the σ-hole. *J. Mol. Model.* **2007**, *13* (2), 291−296.
(78) Sadana, P.; Hwang, J. Y.; Attia, R. R.; Arnold, L. A.; Neale, G.; Guy, R. K. Similarities and differences between two modes of antagonism of the thyroid hormone receptor. *ACS Chem. Biol.* **2011**, *6* (10), 1096−1106.
(79) Chiamolera, M. I.; Wondisford, F. E. Minireview: Thyrotropin-releasing hormone and the thyroid hormone feedback mechanism. *Endocrinology* **2009**, *150* (3), 1091−1096.
(80) Marchand, O.; Safi, R.; Escriva, H.; Van Rompaey, E.; Prunet, P.; Laudet, V. Molecular cloning and characterization of thyroid hormone receptors in teleost fish. *J. Mol. Endocrinol.* **2001**, *26* (1), 51−65.
(81) Bochukova, E.; Schoenmakers, N.; Agostini, M.; Schoenmakers, E.; Rajanayagam, O.; Keogh, J. M.; Henning, E.; Reinemund, J.; Gevers, E.; Sarri, M.; Downes, K.; Offiah, A.; Albanese, A.; Halsall, D.; Schwabe, J. W.; Bain, M.; Lindley, K.; Muntoni, F.; Vargha-Khadem, F.; Dattani, M.; Farooqi, I. S.; Gurnell, M.; Chatterjee, K. A mutation in the thyroid hormone receptor alpha gene. *N. Engl. J. Med.* **2012**, *366* (3), 243−249.
(82) Gauthier, K.; Chassande, O.; Plateroti, M.; Roux, J.-P.; Legrand, C.; Pain, B.; Rousset, B.; Weiss, R.; Trouillas, J.; Samarut, J. Different functions for the thyroid hormone receptors TRα and TRβ in the control of thyroid hormone production and post-natal development. *EMBO J.* **1999**, *18* (3), 623−631.
(83) Abel, E. D.; Ahima, R. S.; Boers, M. E.; Elmquist, J. K.; Wondisford, F. E. Critical role for thyroid hormone receptor beta2 in the regulation of paraventricular thyrotropin-releasing hormone neurons. *J. Clin. Invest.* **2001**, *107* (8), 1017−1023.
(84) Gauthier, K.; Plateroti, M.; Harvey, C. B.; Williams, G. R.; Weiss, R. E.; Refetoff, S.; Willott, J. F.; Sundin, V.; Roux, J. P.; Malaval, L.; Hara, M.; Samarut, J.; Chassande, O. Genetic analysis reveals different functions for the products of the thyroid hormone receptor alpha locus. *Mol. Cell. Biol.* **2001**, *21* (14), 4748−4760.
(85) Chen, P.; Yang, J.; Chen, G.; Yi, S.; Liu, M.; Zhu, L. Thyroid-disrupting effects of 6:2 and 8:2 polyfluoroalkyl phosphate diester (diPAPs) at environmentally relevant concentrations from integrated *in silico* and *in vivo* studies. *Environ. Sci. Technol. Lett.* **2020**, *7* (5), 330−336.
(86) Houck, K. A.; Patlewicz, G.; Richard, A. M.; Williams, A. J.; Shobair, M. A.; Smeltz, M.; Clifton, M. S.; Wetmore, B.; Medvedev, A.; Makarov, S. Bioactivity profiling of per- and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure. *Toxicology* **2021**, *457*, No. 152789.
(87) Mendoza, A.; Hollenberg, A. N. New insights into thyroid hormone action. *Pharmacol. Ther.* **2017**, *173*, 135−145.
(88) Zhao, W.; Zitzow, J. D.; Weaver, Y.; Ehresman, D. J.; Chang, S.-C.; Butenhoff, J. L.; Hagenbuch, B. Organic anion transporting polypeptides contribute to the disposition of perfluoroalkyl acids in humans and rats. *Toxicol. Sci.* **2016**, *156* (1), 84−95.
(89) Pirahanchi, Y.; Toro, F.; Jialal, I. *Physiology, Thyroid Stimulating Hormone*; StatPearls, 2023.

https://doi.org/10.1021/acs.est.3c07003
Environ. Sci. Technol. 2024, 58, 182−193