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Analysis on the Toxicology of Per-/polyfluoro Alkyl Substances (PFAS)

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Abstract. The high persistence, bioaccumulation, and possible health risks of perfluoroalkyl substances (PFAS) have made them emerging environmental contaminants of global concern. Early studies focused on representative compounds such as PFOA and PFOS, but in recent years have expanded to tens of thousands of PFAS with different structures. According to studies, PFAS can bind to a range of human proteins and are found in large quantities in the environment. This can potentially disrupt regular physiological processes. In particular, its binding to human serum albumin (HSA) and cytochrome P450 enzymes (such as CYP3A7) suggests that it may affect the transport, metabolism and detoxification of substances in the body. Numerous conditions affecting the immunological, hepatic, metabolic, and endocrine systems have been linked to PFAS exposure. However, the precise molecular toxicity mechanisms of the many PFAS types remain unclear. This article systematically reviews the toxicological properties and mechanisms of action of PFAS, focusing on its molecular interactions with HSA and CYP3A7, and the possible interference effects in multiple systems. This study contributes to a better knowledge of the health concerns associated with PFAS and serves as a guide for next evaluations of systemic toxicity. Subsequent research should focus on comparative analysis of different PFAS subclasses and their long-term effect mechanisms.

Keywords: Perfluoroalkyl substances, human serum albumin, toxicology.

1. Introduction

Because of their distinct physicochemical characteristics, per- and polyfluoroalkyl substances (PFAS) are a family of synthetic organofluorine chemicals that are extensively used in a variety of industrial applications and consumer goods [1]. Per- and polyfluoroalkyl substances (PFAS) have, nevertheless, attracted more attention worldwide since the early 2000s because of their detrimental effects on human health, remarkable environmental durability, and high potential for bioaccumulation. The main issues involved perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), two thoroughly studied compounds acknowledged for their toxicity and prevalence. There is growing attention on various other PFAS compounds, many of which remain insufficiently characterized.

Differing definitions and categorization approaches have led to inconsistencies in PFAS identification and regulatory coverage. For instance, Robert C. Buck et al. used fundamental criteria

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based on composition and structure for the identification and categorization PFAS. They subsequently identified categories of PFAS substances suitable for risk assessment, resulting in the formulation of effective regulatory risk assessments for economically significant chemicals. Emily Hammel et al. screened 360 organofluorine drugs approved and used worldwide between 1954 and 2021, standardizing their identification using nine definitions prepared by different stakeholders [2]. For PFAS that are easily identified by their chemical structure, G. Patlewicz et al. created a chemical categorization system based on chain length and chemical fingerprints [3,4]. Recent efforts have helped to clarify the PFAS landscape and promote a more harmonized terminology.

There are four major issues with the previous definition of the PFAS domain: First, homologous compounds with fully fluorinated aliphatic cyclic structures, with or without fully fluorinated alkyl side chains, are not consistently classified; second, compounds with functional groups at both ends of the perfluorinated carbon chain (such as perfluoroalkyldicarboxylic acids) are not included; third, compounds with aromatic rings inside their nonfluorinated functional groups are not included, which could be cleaved under biological or environmental conditions; and fourth, the vague term "highly fluorinated," which lacks specific structural criteria, is used. In order to address these issues, the U.S. Environmental Protection Agency created a thorough definition of PFASs: Per- and polyfluorinated chemicals, or PFASs, are identified by the structural unit R–(CF2)–C(F)(R1)R2. The CF2 and CF moieties are both saturated carbon entities, and none of the R groups (R, R1, or R2) may be hydrogen. Stakeholders may classify their compounds consistently and rationally according to the Organization for Economic Co-operation and Development's (OECD) established framework for the systematic categorization of various PFASs based on their molecular structural properties [5].

Furthermore, PFAS are becoming more widely acknowledged as contaminants of growing concern as a result of their prevalence in food, drinking water, and packaging materials. They mostly impair women's reproductive health and are linked to a number of harmful health consequences, including as hepatotoxicity and endocrine disruption. In order to provide a thorough grasp of the toxicological behaviors and biological impacts of PFAS, this study attempts to compile the toxicity features, mechanisms, and epidemiological research of these chemicals.

2. Mechanisms of toxic effects of PFAS

Human serum albumin (HSA), the most abundant soluble protein in human plasma, is synthesized in the liver and typically occurs at a physiological concentration of approximately 0.64 mM [6].. In addition to maintaining colloidal osmotic pressure, albumin is crucial for the movement, distribution, and metabolism of numerous endogenous and exogenous substances, including medications and fatty acids (FA). There are several binding sites for HSA. For instance, medium and long chain fatty acids are strongly favored by Sudlow site II. The plasma concentration of free fatty acids will increase if this site is occupied, leading to accumulation. This can lead to multiple metabolic disturbances, including impaired insulin-mediated glycogen synthesis and decreased lactate production. Mingguo Peng et al. selected six substances for the analysis of the interaction between PFAS and HSAS: PFOA, Perfluoro-2,5-dimethyl-3,6-dioxanonanoic Acid (HFPO-TA), Perfluorononanoic Acid (PFNA), Perfluoro-3,6,9-trioxadecanoic Acid (PFO3DA), Perfluoroheptanoic Acid (PFHpA), and Dodecafluorosuberic Acid (DFSA) [7].

HOMO and LUMO are critical parameters in the analysis of Frontier Molecular Orbital (FMO) [7]. The Highest Occupied Molecular Orbital (HOMO) functions as an electron donor, whereas the Lowest Unoccupied Molecular Orbital (LUMO) accepts electron in chemical reactions. The stability and reactivity of a molecule are signified by its energy gap, denoted as ΔEHOMO-LUMO. The

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computational results indicate that the six PFAS exhibited ΔEHOMO-LUMO values between 0.3802 eV and 0.3970 eV, suggesting that these chemicals facilitate chemical processes.

As with the other five PFAS, Table 1 demonstrates that the predominant orbital electron density of PFNA is distinctly concentrated on the carbonyl oxygen within the carboxyl group (-COOH) [7]. This result indicates that free radical processes may transpire within the carboxyl group. In particular, since DFSA has two carboxyl groups, its orbital distribution differs significantly from that of the other five PFAS. The carboxyl group (-COOH), the oxygen atom connected to the carboxyl group, and the carbon-fluorine (CF) bond—all of which are possible reaction sites—are where the HOMO orbital's charge is mostly found.

Table 1. Calculation results of electron density at the free radical front of six PFNA [7]

Matter	Label	Atom	HOMO Electron Density (×100%)	LUMO Electron Density (×100%)	Radical Frontier Electron Densities(fr Values×100%)
PFNA	1	F	0.620	0.110	0.730
	2	F	0.620	0.110	0.730
	3	F	0.348	0.046	0.394

PFAS interacts with several amino acid residues on HSA by hydrogen bonds, van der Waals forces, and halogen bonds, as indicated by molecular docking data [7]. The stability of the ligand-protein complex is contingent upon the polar terminus of the PFNA carboxyl group establishing a hydrogen bond with the protein's SER-192 residue. This corroborates the FMO findings, indicating that the principal binding mechanism between PFNA and HSA is hydrogen bonding. Moreover, it was discovered that the positively charged regions of the GLN-196 and ARG-257 residues established halogen bonds with the Fa atom in PFNA. Similar to hydrogen bonding, halogen bonding constitutes a non-covalent connection. The affinity and specificity of PFOA for HSA are enhanced when the halogen atom of PFOA establishes a halogen bond with the nucleophilic site of HSA. PFO3DA and PFNA exhibited comparable docking results. Hydrogen bonding, halogen bonding, and hydrophobic interactions are mechanisms via which the four PFAS (HFPO-TA/PFOA/PFHpA/DFSA) interact with HSA. These results demonstrate the binding mechanism of PFAS and HSA, revealing another potential risk of PFAS. The study of binding sites also provides targeted ideas for subsequent drug design.

3. PFAS and cytochrome P450 CYP3A7

CYP3A7, a human hepatic CYP isoform, makes up around half of the total cytochrome P450 (CYP) enzyme content in the fetal and neonatal liver. CYP3A7 is essential for the metabolism of endogenous substrates as well as medicinal substances. Its hydroxylation of dehydroepiandrosterone 3-sulfate (DHEA-S) to create 16α-hydroxy-DHEA-S, a crucial step in the production of estriol during pregnancy, is one prominent example.Estriol production is critical for maintaining full-term pregnancy and is associated with enhanced uterine vasodilation. In addition, CYP3A7 contributes to the developmental regulation of retinoid signaling by metabolizing all-trans retinoic acid (atRA). Impaired hepatic clearance of atRA may lead to its accumulation, resulting in hepatotoxicity and teratogenic effects, including spontaneous miscarriage and congenital malformations such as craniofacial anomalies. The broad substrate specificity of CYP3A isoforms enables them to interact with a wide range of structurally similar compounds (Table 2) [8]. In this context, Michaela Hvizdak et al. investigated the interactions between CYP3A7 and selected per- and polyfluoroalkyl

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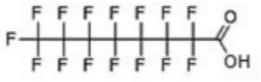
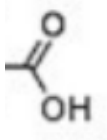
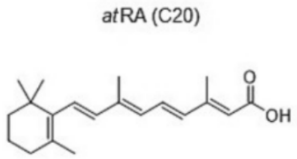
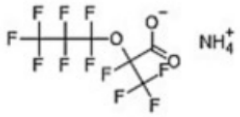
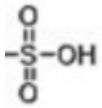
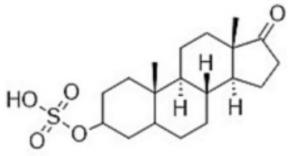
substances (PFAS), as some of these compounds exhibit structural and functional similarity to known CYP3A7 ligands. For instance, the sulfonic acid head groups of PFOS, PFNS, and PFHxS closely resemble that of DHEA-S, while the carboxylic acid termini of PFOA, GenX, and PFNA exhibit notable similarity to that of atRA. Owing to these shared chemical features, the active site of CYP3A7 is hypothesized to accommodate these PFAS, potentially facilitating their binding and subsequent metabolic transformation.

Table 2. Comparison of the chemical structures of the six major PFAS compounds and two endogenous ligands of CYP3A7 [8]

Abbreviation	Structure	Similar functional group	Endogenous ligands of CYP3A7
PFOA			
PFNA			
GenX			
PFOS			
PFNS			
PFHxS			

The heme iron located within the binding pocket of cytochrome P450 (CYP) enzymes serves as the primary interaction site for ligand binding. Conventional CYP absorbance spectra are typically classified into three types: Type I, Type II, and Reverse Type I. In both Type I and Reverse Type I binding modes, ligand binding displaces the axial water molecule coordinated to the heme iron. However, Reverse Type I spectra arise when additional interactions occur between the ligand's heteroatoms (e.g., oxygen or sulfur) and the heme iron center. In contrast, Type II binding involves coordination of a nitrogen atom from the ligand directly to the heme iron, resulting in the formation of a coordinate covalent bond and the emergence of characteristic Type II spectral bands.

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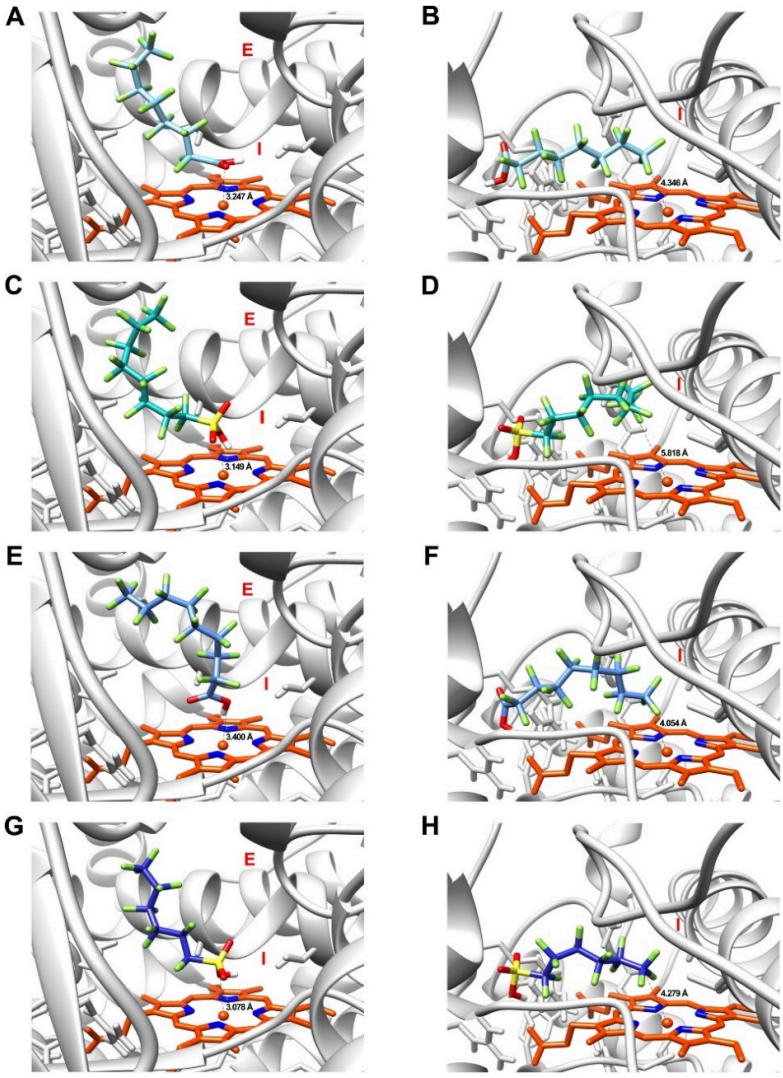

Figure 1. The in-silico docking of PFAS with the CYP3A7 active site and cutaways of the PFNA, PFOS, PFOA, and PFHxS docked structures [8]

Cross-sectional views of docked configurations for PFOA, PFOS, PFNA, and PFHxS around the heme group (shown in orange) are shown in Figure 1, which displays molecular docking simulations of several PFAS molecules with the CYP3A7 active site [8].The ligand binding regions are localized near the CYP3A7 I and E helices, and are characterized as either Reverse Type I (Panels A, C, E, and G) or Type I (Panels B, D, F, and H) interactions. Binding distances between each ligand and the heme iron are indicated by dashed black lines and reported in Ångströms.

Reverse Type I binding interactions were observed for PFOA (3.427 Å), PFOS (3.149 Å), PFNA (3.400 Å), and PFHxS (3.078 Å), corresponding to the distance between the heme iron and the nearest heteroatom. In contrast, Type I interactions for the same compounds displayed longer distances between the heme and the ω-1 carbon: PFOA (4.346 Å), PFOS (5.818 Å), PFNA (4.054 Å), and PFHxS (4.279 Å). In Type I binding conformations, PFAS molecules are generally oriented parallel to the heme plane, with the ω-1 carbon positioned above the heme iron and the hydrophilic head group directed away from the active site. Conversely, in Reverse Type I conformations, the PFAS molecules adopt a perpendicular orientation, with the hydrophilic head group directed toward

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the heme center and the alkyl tail extending outward. These configurations represent energetically favorable docking poses, consistent with experimentally observed interaction trends.

Michaela Hvizdak et al. characterized the initial interaction between PFAS and the cytochrome P450 (CYP) active site as a Reverse Type I binding mode [8]. In this configuration, the ligand either formed an indirect interaction with the heme iron via a bridging water molecule, or the negatively charged oxygen atoms of the PFAS head group displaced the axial water ligand, leading to the formation of a coordinate covalent bond with the heme iron within the CYP binding pocket. However, as PFAS concentration increased during titration, the binding behavior shifted toward a more energetically favorable Type I interaction. Following saturation of the original Reverse Type I complex, the PFAS molecules reoriented to adopt a parallel alignment with respect to the heme plane, consistent with Type I binding. This transition may serve to minimize electrostatic repulsion between negatively charged heteroatoms in the PFAS head group. The disruption of the initial coordination ultimately resulted in the emergence of a Type I absorbance spectrum. These research results reveal that the binding mechanism between PFAS and CYP3A7 varies according to concentration, locks in some important binding sites, and provides information and ideas for related drug design.

4. Degradation of PFAS in the human body

The carbon-fluorine (C-F) bond possesses the highest bond dissociation energy of any natural product, reaching up to 120 kcal·mol−1, making it the strongest single bond associated with a carbon atom [9]. But there are different types of defluorinase enzymes including hydrolases, oxygenases, reductases, and lyases. The current view on the environmental persistence of PFAS is that thes C-F bond is chemically stable.

However, according to Lawrence P. Wackett, the poisonous fluoride anion result of the universal defluorinase process may represent an evolutionary constraint on biological defluorination since enzymes that can cleave C-F bonds are not uncommon [9]. In other words, fluoride is toxic to all cells and exerts negative selection pressure, hindering the evolution of new metabolisms that cannot biodegrade PFAS (Table 3).

Table 3. Enzymes catalyzing defluorination of PFAS moieties [9]

PFAS moiety	Enzyme	Defluorination mechanism
-CF_2-	Fluoroacetate dehalogenase Dehalogenase for haloalkanes Dioxygenase of toluene	Hydrolytic, 2 F^- displaced Hydrolytic displacement of 2 F Oxygenolytic, displacement of 2 F
-CF_3	Arene dioxygenase Diol dehydratase for propane	Difluoro-quinone methide via way of Through a drastic change
-OCF_3	Reductase of caffeine-CoA	CF_3OH formation and decay
-CF=CF-	Caffeoyl-CoA reductase	Formation and degradation of CF_3OH
Aryl-F	Dioxygenase of 2-fluorobenzoate CoA reductase benzoyl	Elimination and dioxygenation Ring removal and reduction

5. Conclusion

This paper reviews the interaction between PFAS and human proteins, focusing on the analysis of their binding mechanisms with HSA and liver cytochrome (CYP3A7). Studies have shown that a

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variety of PFAS bind to HSA through hydrogen bonds, halogen bonds and hydrophobic interactions, affecting its transport function; at the same time, some PFAS coordinate with the active site of CYP3A7, interfering with key metabolic pathways. Combined with FMO analysis, molecular docking simulation and other methods, this paper reveals the toxic behavior of PFAS at the molecular level, providing a basis for a deeper understanding of its biological toxicity mechanism.

These results not only expand the understanding of the toxicological mechanism of PFAS, but also further respond to the problem of "structural diversity leading to unclear mechanisms" mentioned in the introduction. Through an in-depth analysis of the binding characteristics of PFAS molecular structure and functional proteins, this paper provides an important reference for subsequent toxicity evaluation, drug antagonism design and environmental remediation research.

However, the analysis in this paper still has certain limitations. First, although a variety of computational simulation and theoretical analysis methods were used, there is a lack of systematic experimental verification, and further confirmation is still needed through in vivo and in vitro experiments. Secondly, there are many types of PFAS. This study only selected some representative structures, which cannot fully reflect the toxicity differences of all PFAS. Future research can be expanded in the following aspects: first, conduct structural toxicity association studies on more PFAS subclasses to establish a unified evaluation framework; second, combine high-throughput screening with in vivo experiments to achieve a systematic evaluation of long-term low-dose exposure effects; third, explore the metabolic degradation pathways of PFAS to provide mechanism support for the persistent governance of PFAS in the environment.

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