Toxic Effects of Poly-Fluoroalkyl Substances in Organisms

Subjects: Toxicology | Public, Environmental & Occupational Health

Contributor: Consolato Schiavone , Chiara Portesi

More than 7000 synthetic compounds known as per- and poly-fluoroalkyl substances (PFAS) are applied to food packaging and other materials to provide fat, fire, and/or water resistance properties. These compounds have exceptional environmental stability and persistence due to the strong C-F chemical bond, earning them the moniker “forever chemicals”. Emission of PFAS from industrial waste leads to water, air, and soil contamination. Due to this ubiquitous nature, combined with the fact that PFAS in humans are known to have carcinogenic and reprotoxic effects and to cause vaccine resistance and depression of the immunity system, PFAS may constitute a major threat to human health. For this reason, the attention of the scientific community and of control bodies is increasing and as a consequence legislation and the scientific literature on PFAS are constantly evolving.

PFAS Toxic effects Environment Food safety

1. Introduction

The broad category of hundreds of synthetic chemicals known as per- and polyfluoroalkyl substances (PFASs) is widely utilized for a wide range of applications, from Gore-Tex materials and anti-adherent pans to firefighting foams. They are, however, more frequently identified as environmental contaminants, and some of them have been connected to harmful effects on human health [1]. The PFAS backbone is constituted by single-bonded carbon atoms with fluorine atoms covalently bonded to the alkyl chain, one of the strongest in chemistry, and therefore PFAS do not break down easily in the environment [2]. In addition, PFASs can be classified in different families [3]: perfluoroalkyl substances and polyfluoroalkyl substances for the non-polymers group; and fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers for the polymers group. The chemical structures of the different classes are depicted in **Figure 1**.

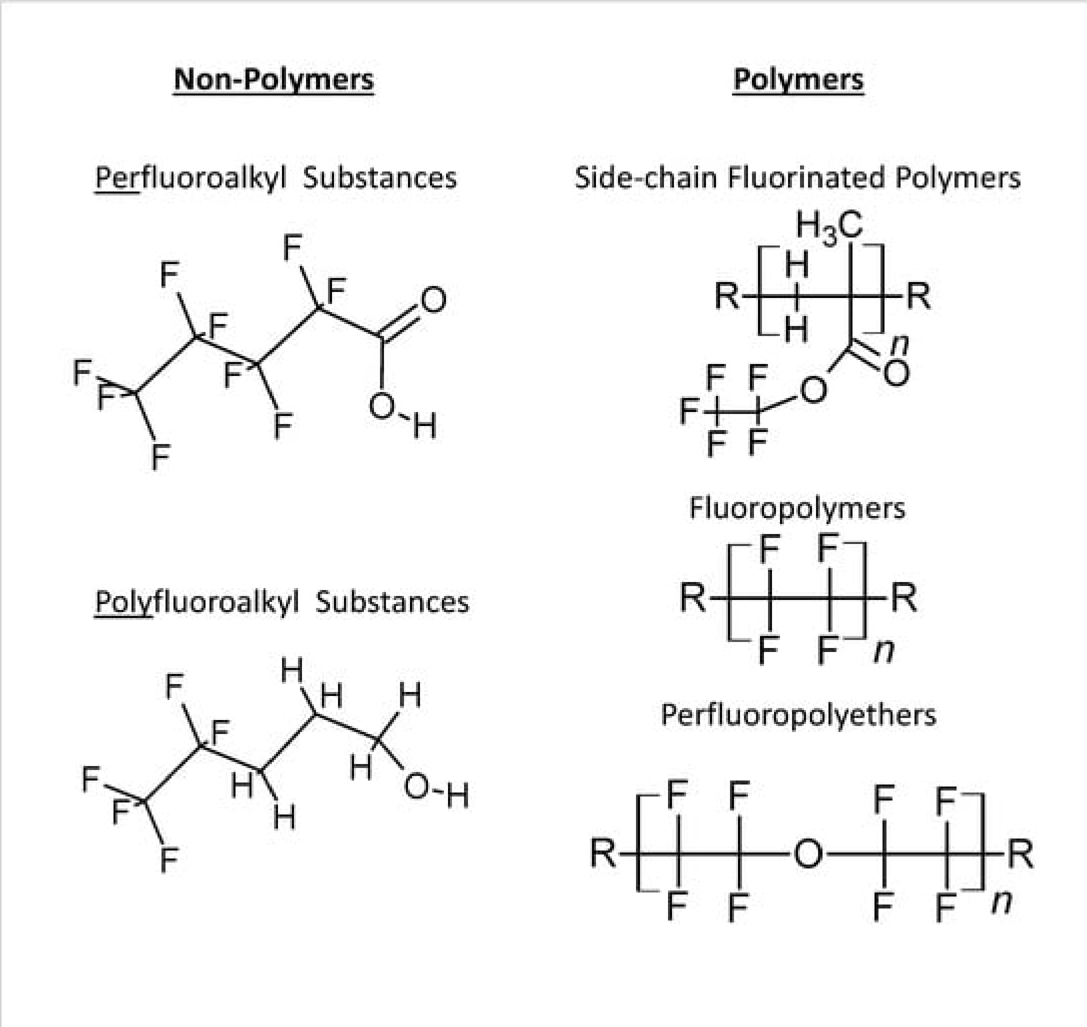

Figure 1. Classification of PFASs major groups.

PFAS are known to be ubiquitous forever chemicals that can come in contact with humans through contaminated environmental and food matrices such as drinking waters and eggs [4][5][6] and therefore the attention is constantly increasing at the scientific level and also outside the scientific community. For instance, companies are starting to use the "PFAS free" label on their products to demonstrate safety to consumers. The results of a search on Google Trends, **Figure 2** [7], show the increasing interest around PFAS over time on the net, where searches in the last two decades (from January 2004 to the current year) have exponentially grown.

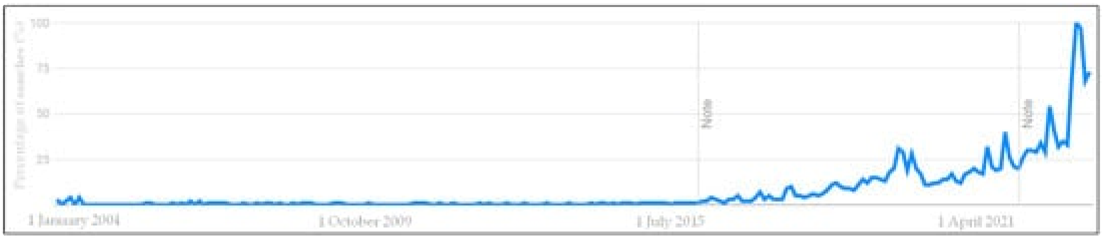

Figure 2. Interest over time of the word "PFAS" in Google searches since 2004. Data source: Google Trends (https://trends.google.com/trends/explore?date=all&q=PFAS (accessed on 12 May 2023)) [7].

As depicted in **Figure 3**, the attention of the scientific community to this topic has also largely increased in the last 15 years. By exploring the Web of Science^{TM} platform [8], by searching the scientific papers published per year that include the terms "PFAS" and "per" to avoid false responses based on PFAS acronyms that are not unique, a clear visualization of the increasing number of scientific papers published on the PFAS topic in the last decade is obtained, and from 2017 to 2022 a tenfold increase has occurred.

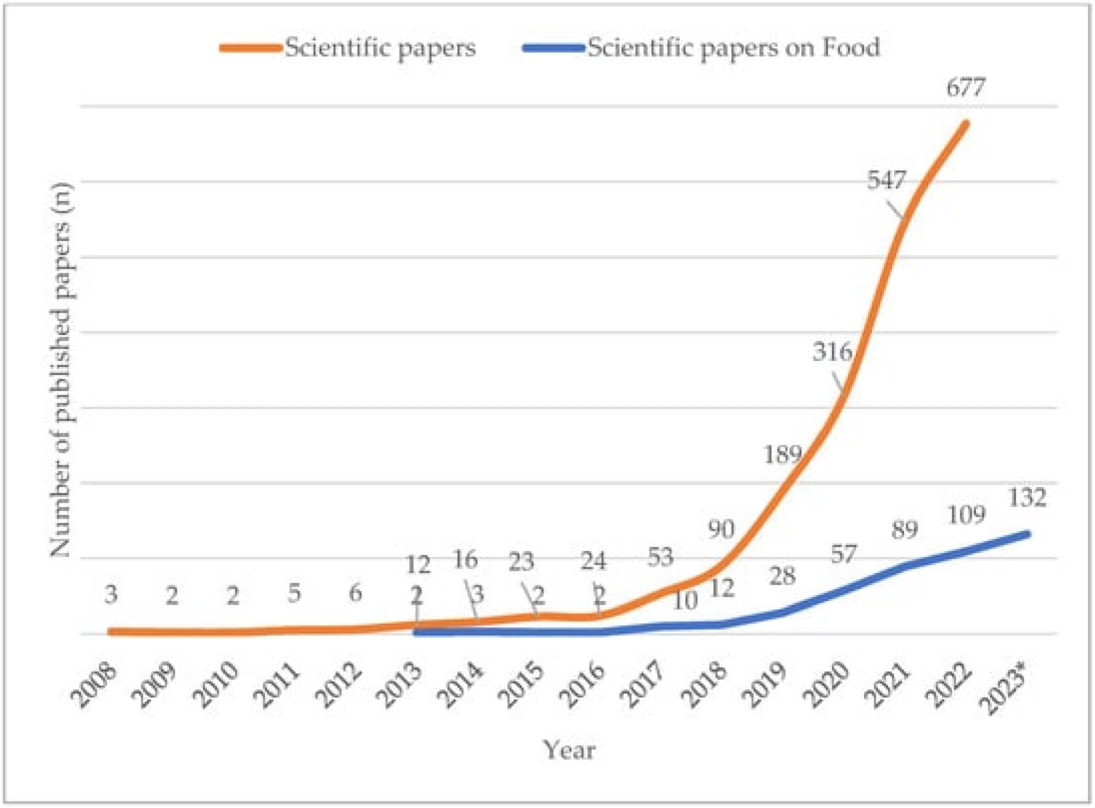

Figure 3. Chart for scientific papers and scientific papers on food published with the words "PFAS", "per" and "food" collecting data from Web of Science^{TM} site since 2008 (2023* data were estimated).

Like other chemical, PFAS are potentially capable to have an adverse effect on human health [9] and, in parallel to the discovery of the accumulation of different type of PFAS in the environment and in food, authorities have begun looking into the toxicity of PFAS and in estimating the human uptake. Regarding toxicity, Velez et al. [10] found that perfluorooctanoic acid (PFOA) and perfluorohexane sulfonic acid (PFHxS), even at lower levels than previously reported [11][12][13][14], may diminish fecundability.

Grandjean et al. [15] underlined the possible need for a specific protection for infants and children, in particular in relation to their immune system. Abraham et al. [16] demonstrated that the transdermal absorption of PFOA (and consequently other PFASs) from cosmetic products can significantly contribute to the total exposure to PFAS for costumers. Blomberg et al. [17] demonstrated changes in PFAS levels in human milk during breastfeeding depending on the level of exposure (from almost zero to elevated levels). In particular, PFOS levels increased over time, from colostrum to milk matured, while PFOA ones were observed to decrease. The level of exposure for children depends on both the specific mix of PFAS and the source of exposure.

In a final determination published in March 2021 “Regulatory Determinations for Contaminants on the Fourth Contaminant Candidate List”, the Environmental Protection Agency (EPA) states that two of the so-called “legacy PFAS”, PFOA and perfluorooctane sulfonic acid (PFOS), need to be regulated in drinking water due to their already known contamination and toxicity. On 14 March 2023, the EPA proposed a national drinking water regulation for PFOA and PFOS. Along with taking this step, the EPA is also addressing additional PFAS and specific measures for defined PFAS groups. By the end of 2023, the EPA expects to have the regulation in its final form [18]. In addition, according to the FDA’s (Food and Drug Administration of US) testing of foods grown or produced in regions with documented environmental PFAS contamination in the soil, water, or air can be absorbed by plants and animals, resulting in contaminated foods [19]. In addition, the European Food Safety Authority (EFSA) published a scientific opinion on their journal on 2020 stating that fish meat, fruit and fruit products, and eggs and egg products were the top contributing categories for the combined exposure to PFOA, perfluorononanoic acid (PFNA), PFHxS, and PFOS for all demographic groups. For the majority of people, diet is the main way they are exposed to PFAS, although other factors such as breathing in indoor air and ingesting dust may also play a big part. This focus on food requires EFSA researchers to establish a Tolerable Weekly Intake (TWI) of 4.4 ng/kg(body weight)/week based on the intake of the sum of PFOA, PFNA, PFHxS, and PFOS [20]. In 2022, the European Union Reference Laboratory for Persistent Organic Pollutants (EURL-POPs) published the “Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed,” which established a path for analytical laboratories around the world to follow in developing and validating robust and reliable methods for the discovery and quantification of PFAS, as well as implementing a lower limit of quantification in food and feed to assess the level of contamination [21].

2. Toxic Effects of PFAS in Organisms

In recent decades, major studies on the toxicological effects of perfluoroalkyl substances have focused on two of the first legacy compounds discovered, PFOS and PFOA, that are the most representative molecules for perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs), respectively [22][23][24][25][26]. Laine et al. [27] addressed, beside PFOA, PFHxA as a potential ecotoxicological compound for the freshwater microbial community. Statistical analysis showed that both PFOA and PFHxA at elevated doses considerably modified the composition of the microbial community. Moreover, statistical analysis demonstrated the more intense toxicity of PFOA, although unexpected outcomes were also found when PFHxA treatments were contrasted. PFOA reduced the biovolume of the bacteria, but it was unable to show that they reduced activity by monitoring cell respiration.

An important factor to consider is the trophic transfer and bioaccumulation of PFAS. The study made by Cara et al. [28] examined many locations in the Belgian portion of the Western Scheldt Estuary and the North Sea, sampling various fish and crustacean species. All matrices included only long-chain PFAS; fish muscle and liver tissue had seven chemicals, whereas crustaceans contained five. Regardless of the kind of fish or the location, liver tissue exhibited considerably higher PFAS levels than muscle tissue overall. The fact that the current study’s PFOS concentrations in fish (*P. platessa*) and crustaceans (*C. crangon* and *crab* sp.) are lower than those of previous studies is a noteworthy finding. The most likely cause is the PFOS and PFOA phase-out performed by 3M in 2000 [29]. Several studies demonstrate a steady decline in PFOS levels throughout the biota.

Li et al. [30] combined targeted and untargeted approaches to predict toxicity using LC-HRMS, LC-MS, and GC-MS. The study results in a strong positive relationship between body weight and liver damage for 13 PFASs. Eick et al. [31] investigated dietary predictors of prenatal PFAS exposure and discovered seven of them in approximately 65% of subjects. The ingestion of dairy products at least once a week was modestly related to higher levels of PFNA and perfluorodecanoic acid (PFDeA). For dairy milk and cheese, the relationships were shown with PFDeA and PFNA, respectively. The level of PFOA, PFDeA, PFNA, and PFOS were found to correlate with the consumption of fish, chicken, and red meat at least once a week. The work of Liu et al. [32] contributes to a better understanding of children's exposure to PFAS. In particular, 107 raw milk samples and 70 cow feed samples from nine Chinese provinces were analyzed in order to investigate the correlation between the level of PFAS of milk and the contamination of PFASs in milk and feed. The findings showed that children are more vulnerable to the risk of PFAS intake from milk than adults.

The findings of the scientific community aim to assist institutions in defining limits and maximum levels of contamination and/or exposure that can be tolerated. The PFOS-induced transcriptional dose-response data were first collected by Chen et al. [33] in both in vitro and in vivo human and animal studies. After the application of statistical models that were demonstrated to be feasible and reliable to perform a human health risk assessment, it was shown that the reference doses for the most sensitive diseases and pathways were below the EPA reference dose, but similar to the tolerable doses settled by EFSA. Cytotoxic effects in humans were also addressed by recent studies. Amstutz et al. [34] investigated the correlation between structure-activity of linear PFASs and their toxicity, with a focus on their impact on hepatotoxicity in HepG2 cells. The data clearly demonstrate a relationship between the chemical structure of PFAS and their Reactive Oxygen Species (ROS) production and cytotoxic activities in HepG2 cells.

The analysis of the impact takes into account both the exposure period and the chemical structure. Solan et al. [35] showed that appropriate data on the impact on health of short-chain PFAS may be obtained using the same strategies used for in vitro human cellular models. Human cell lines from six distinct tissues were exposed to five short-chain PFASs and two legacy PFASs: colon (CaCo-2), brain (HMC-3), kidney (HEK293), lung (MRC-5), liver (HepaRG), and muscle (RMS-13). RMS-13 showed the highest decline in viability after being exposed to PFBS for 48 h, whereas HepaRG appeared to be the most susceptible to hexafluoropropylene oxide-dimer acid (HFPO-DA) exposure. For the long-chain PFAS cytotoxicity, Caco-2 and MRC-5 were less vulnerable to PFOA exposure, whereas PFOA had the largest impact on viability in HepaRG, HMC-3, and RMS-13 comparable to 6:2 FTOH for the short-chain group. An independent t-test was used to compare these data, but no differences were found. Batzella et al. [36] studied the relationship between high PFASs exposure and cardiovascular diseases and cardiometabolic outcomes. Blood samples of 232 male ex-workers in Europe's most important PFAS manufacturing factory between 1968 and 2018 were tested, and four out of twelve PFASs were found in at least half of the samples. The combination of four PFASs combined were positively correlated with TC, LDL-C, and SBP (markers of cardiovascular diseases). Lin et al. [37] showed for the first time a link between PFAS concentrations in serum and thrombograms of a panel of young and middle-aged persons in Asia, particularly in Taiwan. Moreover, for PFOS and PFOA, concentrations were higher than the 50th percentile, while the average number of platelets was at its lowest.

The Toxicology studies on PFAS are incredibly important, as these compounds have been linked with serious health effects such as cancer, liver damage, and reproductive issues. Further studies in this area are needed to i. provide valuable additional information about the potential effects of exposure to different levels of PFAS; ii. support authorities to take informed decisions on how best to protect public health; and iii. develop informed policies aimed at regulating the levels of PFAS in the environment and consumer products, in an effort to protect people from unwanted exposure.

References

1. Available online: https://echa.europa.eu/it/hot-topics/perfluoroalkyl-chemicals-pfas (accessed on 12 May 2023).
2. Available online: https://www.niehs.nih.gov/health/topics/agents/pfc/index.cfm (accessed on 12 May 2023).
3. Buck, R.C.; Franklin, J.; Berger, U.; Conder, J.M.; Cousins, I.T.; De Voogt, P.; Jensen, A.A.; Kannan, K.; Mabury, S.A.; van Leeuwen, S.P.J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. Integr. Environ. Assess. Manag. 2011, 7, 513–541.
4. Daly, E.R.; Chan, B.P.; Talbot, E.A.; Nassif, J.; Bean, C.; Cavallo, S.J.; Metcalf, E.; Simone, K.; Woolf, A.D. Per- and polyfluoroalkyl substance (PFAS) exposure assessment in a community exposed to contaminated drinking water, New Hampshire, 2015. Int. J. Hyg. Environ. Health 2018, 221, 569–577.
5. Jurikova, M.; Dvorakova, D.; Pulkrabova, J. The occurrence of perfluoroalkyl substances (PFAS) in drinking water in the Czech Republic: A pilot study. Environ. Sci. Pollut. Res. 2022, 29, 60341–60353.
6. Gazzotti, T.; Sirri, F.; Ghelli, E.; Zironi, E.; Zampiga, M.; Pagliuca, G. Perfluoroalkyl contaminants in eggs from backyard chickens reared in Italy. Food Chem. 2021, 362, 130178.
7. Available online: https://trends.google.com/trends/explore?date=all&q=PFAS (accessed on 12 May 2023).
8. Available online: https://www.webofscience.com/wos/woscc/basic-search (accessed on 12 May 2023).

9. Fenton, S.E.; Ducatman, A.; Boobis, A.; DeWitt, J.C.; Lau, C.; Ng, C.; Smith, J.S.; Roberts, S.M. Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. Environ. Toxicol. Chem. 2021, 40, 606–630.
10. Vélez, M.P.; Arbuckle, T.E.; Fraser, W.D. Maternal exposure to perfluorinated chemicals and reduced fecundity: The MIREC study. Hum. Reprod. 2015, 30, 701–709.
11. Fei, C.; McLaughlin, J.K.; Lipworth, L.; Olsen, J. Maternal levels of perfluorinated chemicals and subfecundity. Hum. Reprod. 2009, 24, 1200–1205.
12. Whitworth, K.W.; Haug, L.S.; Baird, D.D.; Becher, G.; Hoppin, J.A.; Skjaerven, R.; Thomsen, C.; Eggesbo, M.; Travlos, G.; Wilson, R.; et al. Perfluorinated compounds and subfecundity in pregnant women. Epidemiology 2012, 23, 257–263.
13. Vestergaard, S.; Nielsen, F.; Andersson, A.M.; Hjøllund, N.H.; Grandjean, P.; Andersen, H.R.; Jensen, T.K. Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive. Hum. Reprod. 2012, 27, 873–880.
14. Buck Louis, G.M.; Sundaram, R.; Schisterman, E.F.; Sweeney, A.M.; Lynch, C.D.; Gore-Langton, R.E.; Maisog, J.; Kim, S.; Chen, Z.; Barr, D.B. Persistent environmental pollutants and couple fecundity: The LIFE study. Environ. Health Perspect. 2013, 121, 231–236.
15. Grandjean, P.; Heilmann, C.; Weihe, P.; Nielsen, F.; Mogensen, U.B.; Timmermann, A.; Budtz-Jørgensen, E. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. J. Immunotoxicol. 2017, 14, 188–195.
16. Abraham, K.; Monien, B.H. Transdermal absorption of 13C4-perfluorooctanoic acid (13C4-PFOA) from a sunscreen in a male volunteer—What could be the contribution of cosmetics to the internal exposure of perfluoroalkyl substances (PFAS)? Environ. Int. 2022, 169, 107549.
17. Blomberg, A.J.; Haug, L.S.; Lindh, C.; Sabaredzovic, A.; Pineda, D.; Jakobsson, K.; Nielsen, C. Changes in perfluoroalkyl substances (PFAS) concentrations in human milk over the course of lactation: A study in Ronneby mother-child cohort. Environ. Res. 2023, 219, 115096.
18. Available online: https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas#:~:text=EPA%20is%20developing%20a%20proposed,by%20the%20end%20of%202023. (accessed on 12 May 2023).
19. Available online: https://www.fda.gov/food/environmental-contaminants-food/and-polyfluoroalkyl-substances-pfas (accessed on 12 May 2023).
20. Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.; Leblanc, J.C.; Nebbia, C.S.; et al. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J. 2020, 18, e06223.

21. Jahnke, A.; Marchand, P. Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed; EURL POPs: Freiburg, Germany, 2022; pp. 1–26.

22. Briels, N.; Ciesielski, T.M.; Herzke, D.; Jaspers, V.L.B. Developmental Toxicity of Perfluorooctanesulfonate (PFOS) and Its Chlorinated Polyfluoroalkyl Ether Sulfonate Alternative F-53B in the Domestic Chicken. Environ. Sci. Technol. 2018, 52, 12859–12867.

23. Bursian, S.J.; Link, J.E.; McCarty, M.; Simcik, M.F. The Subacute Toxicity of Perfluorooctane Sulfonate and/or Perfluorooctanoic Acid and Legacy Aqueous Film-Forming Foams to Japanese Quail (Coturnix japonica) Chicks. Environ. Toxicol. Chem. 2021, 40, 695–710.

24. Bartlett, A.J.; De Silva, A.O.; Schissler, D.M.; Hedges, A.M.; Brown, L.R.; Shires, K.; Miller, J.; Sullivan, C.; Spencer, C.; Parrott, J.L. Lethal and sublethal toxicity of perfluorooctanoic acid (PFOA) in chronic tests with Hyalella azteca (amphipod) and early-life stage tests with Pimephales promelas (Fathead minnow). Ecotoxicol. Environ. Saf. 2021, 207, 111250.

25. Zhang, L.; Niu, J.; Li, Y.; Wang, Y.; Sun, D. Evaluating the sub-lethal toxicity of PFOS and PFOA using rotifer Brachionus calyciflorus. Environ. Pollut. 2013, 180, 34–40.

26. Shi, X.; Du, Y.; Lam, P.K.S.; Wu, R.S.S.; Zhou, B. Developmental toxicity and alteration of gene expression in zebrafish embryos exposed to PFOS. Toxicol. Appl. Pharmacol. 2008, 230, 23–32.

27. Laine, M.B.; Vesamäki, J.S.; Puupponen, V.M.; Tiirola, M.; Taipale, S.J. Comparing the Ecotoxicological Effects of Perfluorooctanoic Acid (PFOA) and Perfluorohexanoic Acid (PFHxA) on Freshwater Microbial Community. Front. Environ. Sci. 2022, 10, 516.

28. Cara, B.; Lies, T.; Thimo, G.; Robin, L.; Lieven, B. Bioaccumulation and trophic transfer of perfluorinated alkyl substances (PFAS) in marine biota from the Belgian North Sea: Distribution and human health risk implications. Environ. Pollut. 2022, 311, 119907.

29. United Nations Industrial Development Organization (UNIDO). Preparing for HCFC Phase-Out: Fundamentals of Uses, Alternatives, Implications and Funding for Article 5 Countries; UNIDO: Vienna, Austria, 2009.

30. Li, L.; Yu, N.; Wang, X.; Shi, W.; Liu, H.; Zhang, X.; Yang, L.; Pan, B.; Yu, H.; Wei, S. Comprehensive Exposure Studies of Per- and Polyfluoroalkyl Substances in the General Population: Target, Nontarget Screening, and Toxicity Prediction. Environ. Sci. Technol. 2022, 56, 14617–14626.

31. Eick, S.M.; Goin, D.E.; Trowbridge, J.; Cushing, L.; Smith, S.C.; Park, J.S.; DeMicco, E.; Padula, A.M.; Woodruff, T.J.; Morello-Frosch, R. Dietary predictors of prenatal per- and poly-fluoroalkyl substances exposure. J. Expo. Sci. Environ. Epidemiol. 2023, 33, 32–39.

32. Liu, Y.; Zhang, Q.; Li, Y.; Hao, Y.; Li, J.; Zhang, L.; Wang, P.; Yin, Y.; Zhang, S.; Li, T.; et al. Occurrence of per- and polyfluoroalkyl substances (PFASs) in raw milk and feed from nine

Chinese provinces and human exposure risk assessment. Chemosphere 2022, 300, 134521.

33. Chen, Q.; Chou, W.C.; Lin, Z. Integration of Toxicogenomics and Physiologically Based Pharmacokinetic Modeling in Human Health Risk Assessment of Perfluorooctane Sulfonate. Environ. Sci. Technol. 2022, 56, 3623–3633.
34. Amstutz, V.H.; Cengo, A.; Gehres, F.; Sijm, D.T.H.M.; Vrolijk, M.F. Investigating the cytotoxicity of per- and polyfluoroalkyl substances in HepG2 cells: A structure-activity relationship approach. Toxicology 2022, 480, 153312.
35. Solan, M.E.; Senthilkumar, S.; Aquino, G.V.; Bruce, E.D.; Lavado, R. Comparative cytotoxicity of seven per- and polyfluoroalkyl substances (PFAS) in six human cell lines. Toxicology 2022, 477, 153281.
36. Batzella, E.; Girardi, P.; Russo, F.; Pitter, G.; Da Re, F.; Fletcher, T.; Canova, C. Perfluoroalkyl substance mixtures and cardio-metabolic outcomes in highly exposed male workers in the Veneto Region: A mixture-based approach. Environ. Res. 2022, 212, 113225.
37. Lin, C.Y.; Wang, C.; Sung, F.C.; Su, T.C. Association between serum per- and polyfluoroalkyl substances and thrombograms in young and middle-aged Taiwanese populations. Ecotoxicol. Environ. Saf. 2022, 236, 113457.