Invited Perspective: PFAS Bioconcentration and Biotransformation in Early Life Stage Zebrafish and Its Implications for Human Health Protection

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Per- and polyfluoroalkyl substances (PFAS) widely occur in the environment and are routinely detected in sera obtained from human adults, adolescents, children, infants, and fetuses (Chohan et al. 2020; European Food Safety Authority 2020). Part of PFAS lore is their pop-culture categorization as so-called “forever chemicals” (Perkins 2021), meaning they persist, without alteration, for extensive periods of time in humans and the environment. This assumption is generally based on well-studied PFAS, such as perfluorooctanesulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS), and perfluorooctanoic acid (PFOA), which contain a single, indestructible chain of fluorinated carbons attached to sulfonic or carboxylic acid terminal groups. Collectively, these PFAS exhibit extended mean-estimated half-lives in humans ranging from 1.7 to 7.3 y (Olsen et al. 2007; Li et al. 2018; Xu et al. 2020). Human epidemiological studies have associated PFOS, PFHxS, and PFOA serum concentrations with a variety of adverse health effects, including altered immune and thyroid function, liver disease, and lipid dysregulation (Chohan et al. 2020; European Food Safety Authority 2020). Parties to the Stockholm Convention have therefore pledged to restrict the production and use of PFOS and PFOA (United Nations Environment Programme 2019). Despite increasing understanding of the exposure and effects of PFOS, PFHxS, and PFOA, there are more than 9,200 structurally diverse PFAS (U.S. EPA 2021) that widely lack corresponding bioconcentration factors (i.e., internal dose) and toxicity data. This lack of corresponding bioconcentration and toxicity data poses a significant challenge to regulatory agencies tasked with environmental health protection.

In this issue of *Environmental Health Perspectives*, Han et al. (2021) determined the bioconcentration factors and biotransformation potentials of 36 PFAS in the alternative early life stage zebrafish model. Bioconcentration factors (BCFs) are ratios between internal PFAS concentrations in whole body of 5-d zebrafish and PFAS exposure concentrations and therefore serve as an aggregate measure for key toxicokinetic processes: absorption, distribution, metabolism (i.e., biotransformation), and elimination, collectively referred to as ADME. These same toxicokinetic principals orchestrate PFAS concentrations detected in specific biological matrices such as serum, blood plasma, and urine and therefore universally dictate PFAS accumulation across all organisms, including humans. In addition to measuring parent PFAS in 5-d zebrafish, Han et al. (2021) performed rapid toxicokinetic profiling via non-target analysis to identify biotransformation products. The study demonstrates that many PFAS accumulate in zebrafish and undergo biotransformation into a wide range of structurally diverse products, including those that persist for extensive periods of time in humans and the environment.

**PFAS Bioconcentration**

Of the 36 evaluated PFAS, 28 accumulate in zebralife larvae (Han et al. 2021). Biotransformation, saturation, and column retention time, but not hydrophobicity (i.e., log Kow), influence variation in PFAS accumulation (Han et al. 2021). Interestingly, experimentally determined retention times, used as a proxy measurement for hydrophobicity, correlate well with PFAS accumulation and substantially outperformed widely used structure-based hydrophobicity predictions (Han et al. 2021). To place BCF data from Han et al. (2021) in the context of previously published work, we compared PFAS accumulation data between seven early-life zebrafish and adult fish studies for a handful of well-studied PFAS, including PFOS, PFHxS, and PFOA (Figure 1 and Table 1). We used weighted least squares regression analysis to evaluate the relationship between log-transformed PFAS accumulation in fish (i.e., BCFs) and log-transformed exposure concentrations (Cw). Our analysis confirms previous findings (Vogs et al. 2019; Gaballah et al. 2020) that early life stage zebrafish BCFs are inversely related to exposure concentrations for PFOS, PFHxS, and PFOA (Figure 1). Han et al. (2021) extends this phenomenon to 24 out of 28 PFAS that accumulate in early life stage zebrafish. Notably, the inverse relationship seems to occur across fish life stage (Figure 1), although further studies are needed to confirm this observation.

We and others speculate that active transport-mediated ADME mechanisms might limit PFAS accumulation in zebralife larvae at higher exposure concentrations due to saturation (Ng and Hungerbühler 2013; Ng and Hungerbühler 2015; Vogs et al. 2019; Gaballah et al. 2020). In support of this, active transport of perfluorooalkyl sulfonic acids (e.g., PFOS) and perfluorooalkyl carboxylic acids [e.g., PFOA, PFNA (perfluorononanoic acid)] PFAS have been described in human cells for a range of transporters involved in enterohepatic circulation, which leads to preferential PFAS uptake in the liver (Zhao et al. 2017). However, it remains unknown whether lower concentrations of PFAS detected in drinking water and food sources are also associated with higher PFAS serum accumulation in humans. This key data gap needs to be explored to determine whether the inverse PFAS accumulation phenomenon is relevant to human exposure scenarios. In addition to integrating exposure and human biomonitoring data, this can be achieved via the elucidation of mechanisms that govern active PFAS ADME in zebrafish and the determination of whether such mechanisms are conserved in humans.

Surprisingly, our BCF comparison across seven studies reveals that similar levels of PFAS accumulation in adult fish resulted from...
∼2–4 orders of magnitude lower exposure concentrations, relative to early life stage zebrafish (Figure 1). Explanation of the observed variation in PFAS accumulation across life stage requires new toxicokinetic studies that enable the comparison of estimated ADME parameters (Figure 1). Ultimately, reverse toxicokinetic models are critically needed to extrapolate dose in alternative animal models to human serum concentrations. However, despite the lack of appropriate models for reverse toxicokinetics, the early life stage zebrafish model enables rapid ranking and prioritization of compounds that exhibit a high risk for PFAS accumulation. The strategy used in Han et al. (2021) is particularly relevant for two reasons: a) The vast number of PFAS compounds in commerce prevent this type of comparative assessment using guideline animal studies, and b) In vitro models lack the necessary complexity to detect organismal ADME processes.

**PFAS Biotransformation**

Han et al. (2021) provides a tantalizing example of how PFAS biotransformation processes can be rapidly elucidated. The study identified 25 parent PFAS that undergo in vivo biotransformation in 5-d zebrafish. Broadly, this work shows that a single parent compound can give rise to a wide range of stable and persistent biotransformation products. Exposure to the perfluorooctanesulfonamido ammonium (PFOSAmS), for example, resulted in the detection of 74 biotransformation products (Han et al. 2021). This number is in line with the 92 parent and biotransformation products detected in mice exposed to PFAS-containing aqueous film-forming foam (AFFF) (McDonough et al. 2020). Accordingly, human PFAS risk assessment strategies likely need to consider internal PFAS mixtures from direct accumulation and, as an additional source, biotransformation.

Hydrolysis, determined by Han et al. (2021) to be a central PFAS biotransformation reaction, was enzymatically validated in vitro using a human enzyme. Multiple large polyfluoroalkyl sulfonamides were shown to undergo hydrolysis to form PFOS and perfluorooctanesulfonamide (PFOSa) (Han et al. 2021). PFOS is also a predominant biotransformation product generated from the same class of PFAS precursors in adult fish (Chen et al. 2015), human

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**Figure 1.** Comparison of PFAS exposure concentrations in water (C_w) and corresponding bioconcentration factors (BCFs) across life stage reported in seven studies. To place Han et al. (2021) in context with previously published work, a comparison of published bioaccumulation data for well-studied PFAS in early life stage zebrafish and adult fish studies was performed (Table 1). We performed a weighted least squares regression analysis and calculated the coefficient of determination (R^2) to evaluate the relationship between log-transformed PFAS BCFs and log-transformed exposure concentrations [GraphPad Prism (version 9.1.0; GraphPad Inc.)]. Comparison of BCFs (L/kg) for PFOS, PFOA, and PFHxS in early life stage zebrafish (Vogs et al. 2019; Gaballah et al. 2020; Menger et al. 2020; Han et al. 2021) and adult fish (Martin et al. 2003; Inoue et al. 2011; Chen et al. 2016) reveals two key points: a) For accumulative PFAS, there is an inverse correlation between water concentrations and BCFs, supporting the concept that there are saturable transport-mediated uptake mechanisms that govern the bioconcentration of some PFAS and b) Life stage–specific bioconcentration of PFAS were observed, where adult fish accumulate PFAS at ∼2–4 orders of magnitude lower water concentrations, relative to zebrafish larvae. See Table 1 for underlying data. Gray circles (adult fish – liver); yellow squares (adult fish – whole body), purple up triangles (adult fish – blood/plasma), pink down triangles (zebrafish larvae – previous publications), orange diamonds [data from Han et al. (2021)], solid regression line reflects adult fish data, and dashed regression line reflects data collected in zebrafish larvae. Note: BCF, bioconcentration factor; PFHxS, perfluorohexanesulfonic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.
Looking Forward

Han et al. (2021) advances the use of early life stage zebrafish to rapidly identify parent PFAS that are biotransformed into toxic and indistinguishable products known to adversely affect human health. Human studies have generally neglected the relevance and consequences of PFAS biotransformation as an additional exposure source. The incorporation of physiologically based pharmacokinetic models in epidemiological studies might enable estimates of the contribution of both direct accumulation and the generation of the same PFAS via biotransformation. The overall ambition should be to better regulate all chemical exposure that leads to toxic PFAS. Such a straightforward concept is quite challenging, however, because gross imbalances in commercially used PFAS, relative to those measured in humans and the environment, still exist.

This research lays the groundwork for the integration of multiple phenotype-based toxicity end points with the internal doses of parent PFAS, and their biotransformation products, in a metabolically competent, alternative testing system. Data integration is necessary, particularly from an environmentally relevant perspective where organisms, including humans, are exposed to diverse mixtures of PFAS (Gebbink et al. 2017; McCord and Strynar 2019). To ultimately use early life stage zebrafish toxicokinetic data for human risk assessment, qualitative and quantitative validation studies are essential to determine whether PFAS accumulation and biotransformation processes are comparable between early life stage zebrafish and humans. To address this major knowledge gap, reverse toxicokinetic models are urgently needed. Despite this gap in knowledge, Han et al. (2021) provides a blueprint for the rapid identification of relevant transformation products that rapidly accumulate in zebrafish and potentially cause toxicity.

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