Determination of Bioconcentration Factors for Per- and Polyfluorinated Alkyl Substances in *Hyalella azteca*

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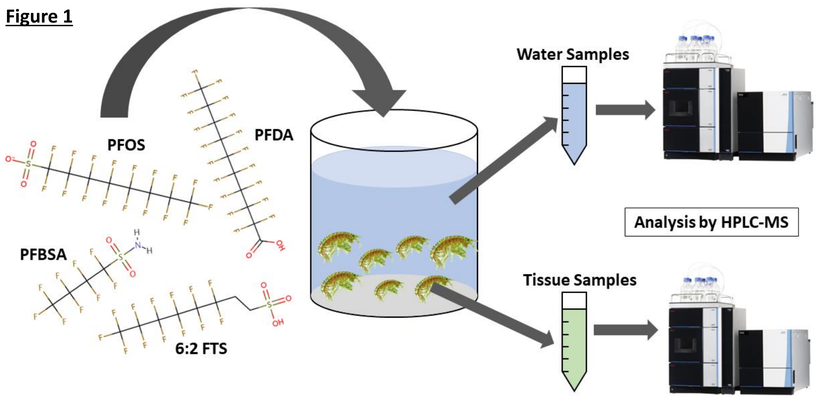
Introduction

Per- and polyfluorinated alkyl substances (PFAS) have become a focal point of toxicity research in recent years, owing to their near ubiquitous environmental distribution and recalcitrance to degradation. Yet high quality data on bioaccumulation of these chemicals in lower trophic level organisms remains sparse, making it difficult to model movement and accumulation of PFAS within food webs. Uptake by low trophic level organisms is also of interest as a component in research at EPA on water column toxicity of PFAS with several small aquatic invertebrates. Within this work, measured bioconcentration factors (BCFs) can be used to relate water column effect concentrations to internal dose. However, the lack of relevant measured BCFs currently leaves this line of analysis unavailable. To meet these dual needs, this research aims to measure PFAS BCFs in *Hyalella azteca*, a small aquatic invertebrate used for sublethal toxicity testing. The PFAS tested thus far consist of those within the homologous series of perfluorinated carboxylic acids, sulfonates, sulfonamides, and fluorotelomer sulfonates.

Figure 1



Methods

Water Samples were collected by stabilizing an aliquot in acetonitrile (ACN) or methanol to a maximum 50% aqueous composition. Samples were then centrifuged (10,000 RPM, 5 mins) and diluted as needed into autosampler vials for analysis.

Tissue Samples were generated with organisms pooled as needed to produce a sufficient tissue mass. Organisms were processed by rinsing gently with DI water and blotting dry under vacuum before freezing (0° C) in 2 mL PP microcentrifuge tubes. Sample processing was carried out by homogenization in 1-1.5 mL ACN on a beadmill (30 Hz, 2 mins), followed by freezing to -80° C, thawing, centrifugation (10,000 RPM, 10 mins) and dilution of an aliquot for analysis.

Sample Analysis was carried out via high-performance liquid chromatography coupled to a single quadrupole mass spectrometer by electrospray ionization. Data was processed using Chromeleon version 7.2.

Curve Fitting for calculating rate constants and BCF values was carried out in R and excel using the method of least squares. Fitting was carried out using equations (1), (2), and (3) as the data demanded.

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Note: The views expressed herein are those of the author and do not represent those of the U.S. EPA.

Fit Equations

(1) $C_{biota} = \frac{k_u}{k_e} \cdot C_w \cdot (1 - e^{-k_e t})$ **Uptake-only fit**

(2) $\ln(C_{biota}) = \ln(C_{t_0}) + k_e(t - t_0)$ **Depuration-only fit**

(3) $C_{biota} = U \cdot \frac{k_u}{k_e} \cdot C_w \cdot \left(1 - e^{-k_e t}\right) + E \cdot C_{t_0} \cdot (e^{-k_e(t-t_0)})$ **Combined fit of uptake and depuration data simultaneously**

C_{biota} - Tissue concentration
k_u - Uptake rate constant
k_e - Elimination rate constant
C_w - Water concentration
t - Time
t_0 - Time at start of depuration
C_{t_0} - Tissue concentration at start of depuration
U - Binary constant (1 if in uptake, 0 if in depuration)
E - Binary constant (0 if in uptake, 1 if in depuration)

Results

Figure 2

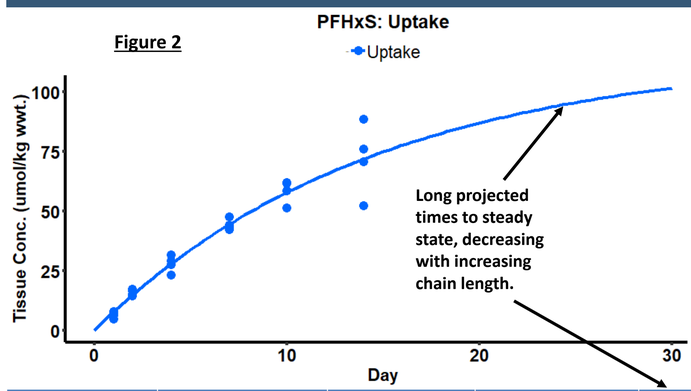


Figure 2: An uptake curve from a 14-day uptake experiment using a mixture of PFHxS, PFOS, PFHpA, and PFNA. **Table 1:** Rate constants and kinetic BCFs obtained from the 14-day uptake experiment. Calculated times to 95% steady state are also shown.

Table 1

Compound	k_u† (L/kg·day)	k_e† (1/day)	BCF‡ (L/kg wwt.)	Estimated Time to 95% S.S. (days)
PFHxS	2.6 ± 0.3	0.068 ± 0.02	38 ± 20	44
PFOS	31 ± 5	0.26 ± 0.06	120 ± 70	12
PFHpA	0.91 ± 0.1	0.064 ± 0.02	14 ± 10	47
PFNA	11 ± 2	0.084 ± 0.03	140 ± 100	36

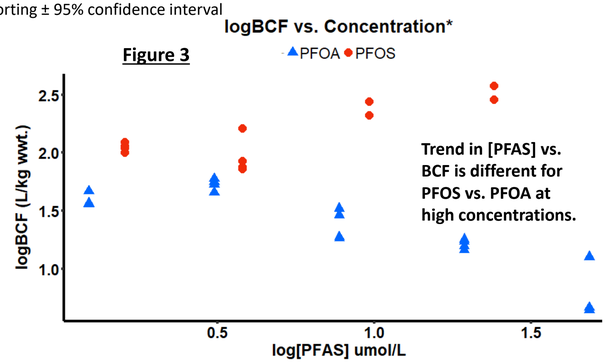
†Reporting ± standard error ‡Reporting ± 95% confidence interval

Table 2

Compound	BCF‡ (L/kg wwt.)
PFOS	110 ± 10
PFOA	42 ± 6

Table 2 and Figure 3: BCF values obtained from tissues generated in a 42-day chronic study.
* Data is preliminary as water measurements have not yet been tabulated.

Figure 3



Results Continued

Figure 4

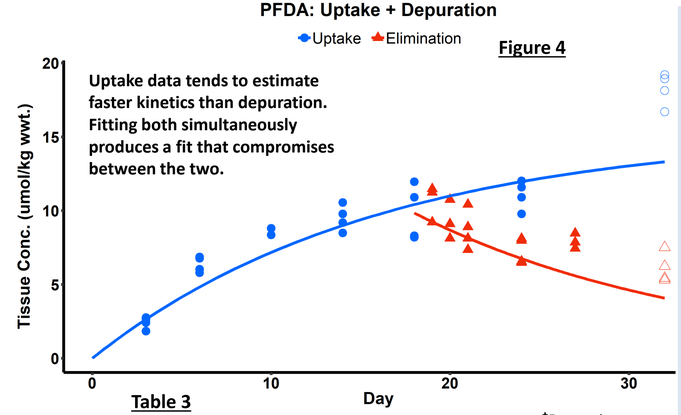


Figure 4: An uptake and depuration curve from a 32-day uptake and 14-day depuration study with a mixture of PFDA, 6:2 FTS, PFOS, and PFBSA. Note that Day 32 data is censored due to suspect weights. **Table 3:** Rate constants and kinetic BCFs measured in the 32-day uptake and 14-day depuration study. Curve fitting was done by fitting depuration and elimination data simultaneously (Equation 3).

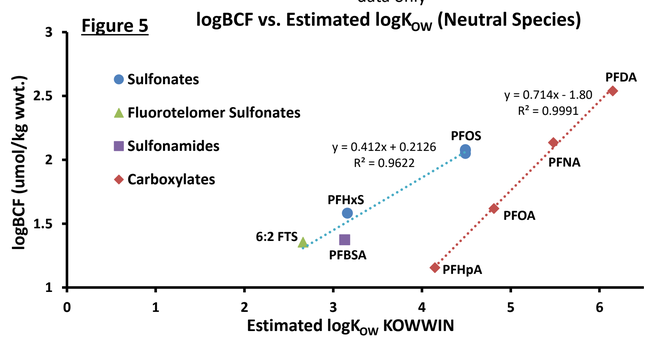
Table 3

Compound	k_u† (L/kg·day)	k_e† (1/day)	BCF‡ (L/kg wwt.)
PFBSA*	9.3 ± 2	0.39 ± 0.1	24 ± 20
6:2 FTS	1.3 ± 0.1	0.059 ± 0.01	23 ± 9
PFOS	16 ± 2	0.13 ± 0.02	120 ± 40
PFDA	22 ± 2	0.063 ± 0.01	350 ± 100

†Reporting ± standard error
‡Reporting ± 95% confidence interval
*Fit to uptake data only

Figure 5: A plot summarizing the trends in measured logBCF vs. estimated $\log K_{OW}$ of the neutral species. Note the strong linear trend for the four carboxylates (PFHpA, PFOA, PFNA, and PFDA) and the offset compared with the other chemicals.

Figure 5



Conclusions and Future Work

- Times to steady state were much longer than anticipated, increasing experimental demands.
- BCF measurements for PFOS are highly reproducible across different experiments (3 in total).
- Uptake curves predict faster kinetics than depuration curves (i.e. larger k_u and k_e values).
- Carboxylates show a strong linear trend for logBCF vs. estimated $\log K_{OW}$ for the neutral species.
- Future work will expand uptake measurements to *C. dilutus* and fathead minnow (*P. promelas*) and add further compounds to the slate of *H. azteca* measurements (e.g. PFHxSA, PFOSA)

Citations

(1) OECD (2012), *Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure*, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris.

(2) US EPA. (2022). Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.