Summary of Concawe Research Project Entitled "Developing Ex-Vivo Approaches to Support PFAS Water Permitting and Effluent Monitoring for Industrial Site Application"

Background

In 2024, a research team consisting of NewFields, Oregon State University, The University of Pittsburg and the US Army Corps of Engineers (NewFields et al.) completed a research project entitled "Developing Ex-Vivo Approaches to Support PFAS Water Permitting and Effluent Monitoring for Industrial Site Application." This two-year project was funded by Concawe Biological Effects/Measures Special Taskforce (STF-32), and Soil & Groundwater Special Taskforce (STF-33).

In a 2022 proposal the European Commission proposed PFAS regulation in surface and groundwater by means of environmental quality standards (EQSs) for the sum of 24 per- and poly-fluoroalkyl substances (PFAS).[1] The EQSs in the proposed regulation are developed using the relative potency factor (RPF) approach described in Bil et a., 2021.[2] There are several challenges to using this approach, including but not limited to lack of bioavailability data for all 24 PFAS and the use of "PFOA equivalents" to regulate PFAS as a group. It is outside the scope of this memo to describe the proposed regulation in detail, though a 2022 memo by the European Commission Scientific Committee on Health, Environment and Emerging Risks (SCHEER) describes some of these challenges.[3]

The aim of the Concawe-contracted research project was to develop biologically relevant partition coefficients for PFAS which would allow scientists and regulators to better understand and model bioavailability.

Partition coefficients are parameters that describe the affinity of a chemical for a material. For example, the octanol-water partition coefficient (K_{OW}) describes how much of a chemical will partition into octanol and how much will partition into water. It is a proxy for the affinity of a chemical for storage lipids in organisms (i.e., the liver and fats within an organism that serve as long-term energy reserves). K_{OW} is often expressed on a logarithmic scale: the higher the $logK_{OW}$, the more a chemical will partition into storage lipids.

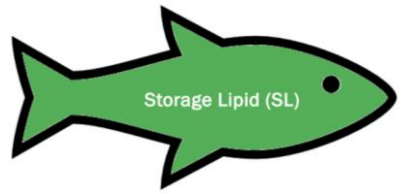
[1] European Commission, Proposal for a Directive of the European Parliament and of the Council amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration and Directive 2008/105/EC on environmental quality standards in the field of water policy, Brussels, 26.10.2022, 2022/0344 (COD) https://environment.ec.europa.eu/document/download/6e618dec-c528-4ba8-8900-1e020eefe393_en?filename=Proposal%20for%20a%20Directive%20amending%20the%20Water%20Framework%20Directive%2C%20the%20Groundwater%20Directive%20and%20the%20Environmental%20Quality%20Standards%20Directive.pdf

[2] W. Bil, M. Zeilmaker, S. Fragki, J. Lijzen, E. Verbruggen, B. Bokkers Risk Assessment of Per- and Polyfluoroalkyl Substance Mixtures: A Relative Potency Factor Approach. Environmental Toxicology and Chemistry. 2021 Mar;40(3):859-870. doi: 10.1002/etc.4835.

[3] SCHEER (Scientific Committee on Health, Environmental and Emerging Risks), Final Opinion on Draft Environmental Quality Standards for Priority Substances under the Water Framework Directive - PFAS, 18 August 2022 https://health.ec.europa.eu/publications/scheer-scientific-opinion-draft-environmental-quality-standards-priority-substances-under-water_en#files

The BCFs of legacy contaminants, such as polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and polycyclic aromatic hydrocarbons (PAHs), can modeled from values of K_{OW}. In other words, the bioavailability of legacy contaminants is largely or entirely described by their affinity for storage lipids. Figure 1 illustrates how BCF is modeled for a chemical that solely partitions into storage lipids.

***Figure 1*: Calculation of BCF for a chemical that solely partitions into storage lipids.**



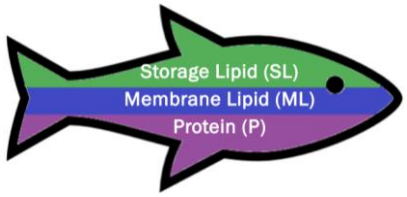
$$BCF_{organism} \cong f_{SL} \times K_{OW}$$

The bioconcentration factor (BCF) of a chemical can be modeled from the fraction of storage lipid (f_{SL}) times the octanol-water partition coefficient (K_{OW}) of the chemical.

The BCFs of PFAS cannot be calculated in this fashion, because their bioavailability is more complicated. Like legacy contaminants, PFAS have an affinity for storage lipids, so K_{OW} is needed to model BCF. However, unlike legacy contaminants, PFAS also have an affinity for other parts of organisms, such as membrane lipids (i.e., the compounds that form part of cell walls) and proteins. Therefore, one would need to know the partition coefficients of PFAS for these three biological compartments to model BCF: K_{OW} to understand partitioning onto storage lipids; the membrane lipid-water partition coefficient (K_{ML}) to evaluate the partitioning onto membrane lipids; and the protein-water partition coefficient (K_P) to understand the partitioning onto proteins. Figure 2 illustrates how BCF could be modeled for a chemical that partitions into storage lipids, membrane lipids and proteins.

***Figure 2*: Calculation of BCF for a chemical that partitions into storage lipids, membrane lipids and proteins. Note: the equation is color-coded to illustrate which portions of the equation pertain to partitioning into different parts of the organism.**

$$BCF \cong (f_{SL} \times K_{OW}) + (f_{ML} \times K_{ML}) + (f_P \times K_P)$$



The bioconcentration factor (BCF) of a chemical can be modeled from the fraction of storage lipid (f_{SL}) times the octanol-water partition coefficient (K_{OW}) of the chemical, plus the fraction of membrane lipid (f_{ML}) times the membrane lipid-water partition coefficient (K_{ML}), plus the fraction of storage lipid (f_P) times the protein-water partition coefficient (K_P).

The barrier to using this approach to model BCFs for different PFAS, is that values of K_{OW} for some PFAS and values K_{ML} and K_P for most PFAS are not known.

The Concawe-funded research project utilized three different approaches to determine biologically relevant partition coefficients, including 1) traditional *in vitro* experiments to empirically measure partition coefficients, 2) a novel approach using biomimetic chromatography to measure partition coefficients, and 3) a computational approach using molecular dynamics and quantitative structure-activity relationship (QSAR) to model partition coefficients. Each of these approaches is discussed in the following paragraphs.

Traditional *In Vitro* Experiments

Traditional *in vitro* experiments were conducted at the University of Pittsburgh using solid-supported lipid membranes (SSLM) or equilibrium dialysis. SSLM mimic the structure and function of natural cell membranes and were used to determine K_{ML}. Equilibrium dialysis is a technique that measures the extent to which a chemical binds to plasma proteins (i.e., the proteins in blood) and was used to determine K_P. Using these *in vitro* techniques, K_{ML} and K_P were determined for 60 individual PFAS, including 20 of the 24 PFAS covered by the European Commission EQS. Table 1 provides these partition coefficients, represented on a logarithmic scale. Comprehensive details of this part of the project can be found in the corresponding article in the peer-reviewed scientific journal Environmental Science & Technology of January 2025[4].

***Table 1*: Results of the *in vitro* experiments conducted at the University of Pittsburgh. PFAS in bold represent the 20 PFAS covered by the European Commission EQS. Note: K_{MW} stands for membrane-water partition coefficient and is equivalent to K_{ML}; and K_A stands for albumin-water partition coefficient and is equivalent to K_P, specifically for albumin, the protein found in the liver and blood.**

PFAS	Group	$logK_{MW}$ (at pH 7.0)	$logK_A$ (at pH 7.0)
PFBA	PFCA	**1.63 ± 0.193**	**84.9 ± 15.95**
PFPeA		**2.02 ± 0.149**	**16.27 ± 3.58**
PFHxA		**2.42 ± 0.156**	**13.98 ± 2.79**
PFHpA		**2.85 ± 0.115**	**6.2 ± 1.13**
PFOA		**3.28 ± 0.135**	**2.57 ± 0.45**
PFNA		**3.75 ± 0.163**	**3.75 ± 0.77**
PFDA		**4.18 ± 0.213**	**3.64 ± 0.85**
PFUnA		**4.5 ± 0.215**	**4.73 ± 1.08**
PFDoA		**4.82 ± 0.1**	**15.76 ± 3.34**
PFTrDA		**5.11 ± 0.187**	**31.44 ± 5.09**
PFTeDA		**5.36 ± 0.241**	**13.09 ± 3.01**
PFHxDA		**5.73 ± 0.25**	**10.45 ± 1.71**
PFBS	PFSA	**2.72 ± 0.308**	**15.6 ± 2.68**
PFPeS		**3.16 ± 0.127**	**17.15 ± 2.58**
PFHxS		**3.65 ± 0.115**	**1.31 ± 0.25**
PFHpS		**4.09 ± 0.174**	**1.68 ± 0.36**
PFOS		**4.5 ± 0.145**	**0.94 ± 0.23**
PFNS		4.82 ± 0.179	6.56 ± 1.36
PFDS		**5.15 ± 0.224**	**1.88 ± 0.4**
PFDoS		5.66 ± 0.121	1.29 ± 0.21
4:2 FTS	FTS	2.21 ± 0.132	18.96 ± 3.59
6:2 FTS		3.12 ± 0.135	2.42 ± 0.6
8:2 FTS		4.07 ± 0.264	4.49 ± 0.84
10:2 FTS		4.57 ± 0.071	2.31 ± 0.51
PFOSA	FASA	3.91 ± 0.162	11.18 ± 1.92
NMeFOSA		4.17 ± 0.172	12.22 ± 1.94
NEtFOSA		4.41 ± 0.23	12.98 ± 2.06

[4] R. Chen, D. Muensterman, J. Field, and C. Ng. Deriving Membrane–Water and Protein–Water Partition Coefficients from In Vitro Experiments for Per- and Polyfluoroalkyl Substances (PFAS), Environmental Science & Technology 2025 59 (1), 82-91, DOI: 10.1021/acs.est.4c06734

PFAS	Group	$logK_{MW}$ (at pH 7.0)	$logK_A$ (at pH 7.0)
Table 1 continued			
NMeFOSAA	FASAA	4.21 ± 0.179	6.63 ± 1.49
NEtFOSAA		4.4 ± 0.153	5.44 ± 1.28
FOSAA		4.28 ± 0.257	7.4 ± 1.69
NMeFOSE	FASE	3.96 ± 0.12	5.88 ± 0.97
NEtFOSE		4.21 ± 0.089	6.09 ± 1.39
HFPO-DA	PFECA	**2.07 ± 0.129**	**6.57 ± 1.49**
ADONA		**2.07**	**5.68 ± 0.89**
PFMPA		2 ± 0.223	47.55 ± 7.91
PFMBA		2.04 ± 0.144	0.22 ± 0.04
NFDHA		2	17.34 ± 3.01
9Cl-PF3ONS	Cl-PFESA	4.38 ± 0.191	1.58 ± 0.39
11Cl-PF3OUdS		4.75 ± 0.263	3.52 ± 0.77
PFEESA	PFESA	2.49 ± 0.246	5.15 ± 1.22
3:3 FTCA	FTCA	2.14 ± 0.135	70.57 ± 14.94
5:3 FTCA		2.47 ± 0.1	10.75 ± 2
7:3 FTCA	FTCA	3.4 ± 0.208	1.82 ± 0.3
6:2 FTCA		2.34 ± 0.103	3.64 ± 0.74
8:2 FTCA		3.23 ± 0.203	1.99 ± 0.37
10:2 FTCA		4.1 ± 0.083	9.06 ± 1.61
8Cl-PFOS	Other Sulfonates	4.73 ± 0.198	0.87 ± 0.17
PFEtCHxS		3.81 ± 0.156	1.62 ± 0.34
FBSA	FASA	2.07 ± 0.243	5.58 ± 0.92
FHxSA		3 ± 0.203	3.07 ± 0.57
6:2 FTUCA	FTUCA	2.16 ± 0.023	4.23 ± 0.89
8:2 FTUCA		3.13 ± 0.196	2.26 ± 0.37
6:2 diPAP	X:2 diPAP	4.62 ± 0.18	2.71 ± 0.41
8:2 diPAP		5.17 ± 0.15	14.4 ± 3.11
diSAmPAP		5.06 ± 0.072	5.94 ± 1.1
PFHxSaAm	Zwitterionic	3.52	6.19 ± 1.01
6:2 FtSaB		2.98 ± 0.2	16.97 ± 3.52
N-TAmP-FHxSA		3.14	33.68 ± 6
5:3 FTB		1.63 ± 0.1	30.44 ± 5.78
5:1:2 FTB		2.01 ± 0.1	49.32 ± 10.1

Biomimetic Chromatography

Biomimetic chromatography experiments were conducted at Oregon State University. Biomimetic chromatography is a tool that has been used in the pharmaceutical industry, and this project represents, to our knowledge, the first such application in environmental science. Effectively, biomimetic chromatography relates the retention time (RT) of chemicals on specialized high-performance liquid chromatography (HPLC) columns to partition coefficients. A detailed description of biomimetic chromatography is available elsewhere.[5] The research team determined the partition coefficients for 81 PFAS, including 23 of the 24 PFAS covered by the European Commission EQS using four different specialized HPLC columns (Table 2).

[5] K.L. Valko. Application of biomimetic HPLC to estimate in vivo behavior of early drug discovery compounds, Future Drug Discovery, 1:1, 2019.

Table 2: **Specialize HPLC columns and the partition coefficients using biomimetic chromatography. Note: $CHI\ logD$ stands for the logarithmic chromatographic hydrophobicity index distribution coefficient K_{IAM} stands for immobilized artificial membrane partition coefficient; K_{HSA} stands for human serum albumin partition coefficient; and K_{AGP} stands for α1-Glycoprotein partition coefficient.**

Column	Partition Coefficient	Comment
"Standard" C18 Column	$CHI\ logD$	Similar to $logK_{OW}$
Immobilized Artificial Membrane (IAM)	K_{IAM}	Akin to K_{ML}
Human Serum Albumin (HSA)	K_{HSA}	Akin to K_P specifically for human serum albumin (HSA), the protein found in human serum.
α1-Glycoprotein (AGP)	K_{AGP}	Akin to K_P specifically for α1-Glycoprotein (AGP), a protein found in blood.

There were many characteristics of PFAS physiochemical properties and behavior learned from the biomimetic chromatography experiments, and it is outside the scope of this summary to review them all. For a comprehensive discussion of this information, an article has been submitted to the peer-reviewed scientific journal Environmental Science & Technology[6]. To summarize:

- Most of the PFAS were characterized as strong acids, meaning the $CHI\ logD$ values were greater at low pH (2.6) compared to neutral (7.4) or high (10.5) pH.
- K_{IAM} varied between PFAS. Different headgroups had different effects on the K_{IAM}, but within any class of PFAS, an increase in the length of the perfluorinated chain resulted in an increase in K_{IAM}.
- Biomimetic chromatography may produce more reliable values for K_{IAM} compared to the traditional SSLM experiments, particularly for PFAS with very short and very long perfluorinated chains.
- PFAS have a much stronger affinity for HSA than they do AGP. For a given PFAS, values of $logK_{HSA}$ values were often two or more orders of magnitude greater than values of $logK_{AGP}$.
- Values of $logK_{HSA}$ increased within each class of PFAS and often reached a "maxima" chain length. In other words, the highest values of $logK_{HSA}$ for PFAS in the same class were often observed for intermediate chain lengths. PFAS with shorter and longer perfluorinated chain lengths had lower values of $logK_{HSA}$.

[6] D. J. Muensterman, K. Valko, R. Chen, C. Ng, M. Benotti and J. Field. Biomimetic Chromatography: A Novel Approach for Measuring Phospholipid Membrane-Water and Protein-Water Partition Coefficients for Target and Suspect PFAS, *submitted* to Environmental Science & Technology in August 2024.

Molecular Dynamics and Quantitative Structure-Activity Relationship (QSAR)

Molecular Dynamics (MD) and Quantitative Structure-Activity Relationship (QSAR) experiments were conducted by the US Army Corps of Engineers (USACE). MD is a computational method that simulates the physical movement of atoms and molecules over time. In this context, MD was used to evaluate mechanisms that drive PFAS transport, partitioning and bioaccumulation and to identify "descriptors" (i.e., important chemical or physical features or characteristics) for input into the QSAR model. QSAR is a computational method that uses mathematical models to predict the biological availability of chemical compounds based on their structure. MD and QSAR were primarily used to evaluate the transport of PFAS across membranes. Results show that PFAS require the lowest energy to position themselves at the membrane interface and the highest energy to move through the middle of the membrane. In other words, while they have an affinity for membranes, the hydrophobic core within the membrane serves as a barrier to PFAS transport across the membrane, as they prefer to remain partitioned at the interface. Additionally, there was good agreement for partition coefficients determined from MD and QSAR approaches and those obtained from traditional in vitro or biomimetic chromatography approaches. Detailed results and discussion for this part of the project have been submitted as an article to the peer-reviewed scientific journal Physical Chemistry Chemical Physics[7].

Relevance and Future Work

This project generated some of the first partition coefficients, in particular K_{ML} and K_P, for 60-80 PFAS. Given that PFAS bioavailability is due, in part, to partitioning to membrane lipids and proteins, these data will serve as a basis for scientists and regulators to model BCFs. These BCFs, in turn, will provide an alternative, and perhaps more robust approach for helping scientists determine appropriate regulations, where applicable.

Beyond the data generated in this project, biomimetic chromatography is a particularly promising tool for assessing PFAS bioavailability. While there is some up-front work to develop a method and model partition coefficient from retention times, once established, it can generate data quickly. A typical analysis takes minutes and whereas a laboratory experiment to determine partition coefficients takes days to weeks. Additionally, given that the model relies on a retention time, and does not necessarily rely on the identity of the analyte, this approach could provide a valuable way to prioritize chemicals for regulation. In other words, it is possible that this tool could develop to a point where one is able to evaluate wastewater from a municipal and industrial site and identify whether there are chemicals present that pose a risk from a bioavailability standpoint. If there are not, then it may be possible to quickly demonstrate risk compliance. If there are, then researchers can prioritize identification of those chemicals (if their identity is not known) and determine concentration.

To that end, a project team led by Dr. Jennifer Field, a professor at Oregon State University and a co-PI on this project, was awarded a Strategic Environmental Research and Development Program (SERDP) award to further develop biomimetic chromatography. This project is entitled "Biomimetic Chromatography for Rapid Assessment of Bioaccumulation (BioCRAB) in PFAS-impacted Aquatic Food Webs," and the project team includes collaborators from Oregon State University, the University of Pittsburgh, The Environmental

[7] M.L. Mayo, R.M. Warner, T.C. Schutt, B.D. Etz, and P. Rana. Molecular Dynamics Informed Prediction of Per- and Polyfluoroalkyl Substances (PFAS) Partitioning into Phospholipid Bilayer Environments *submitted* to Physical Chemistry Chemical Physics in January 2025.

Forensics Group (formerly NewFields) and Geosyntec.[8] The overall goal of this SERDP project is to evaluate the ability of biomimetic chromatography and tissue fractionation measurements to provide a mechanistic understanding PFAS bioaccumulation. In turn, these values could be applied to PFAS risk assessment, especially for the dozens to hundreds of PFAS for which empirical PFAS bioaccumulation metrics are currently unavailable. If successful, these research techniques have the capability to save money and time conducting research; reduce uncertainty with current site investigations and new firefighting foams; and advance a new paradigm for understanding the biological behavior of PFAS.

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The Environmental Forensics Group, LLC
21 July 2025

[8] https://serdp-estcp.mil/projects/details/3f1411a3-a789-4fbd-b070-ce388ef9a236/biomimetic-chromatography-for-rapid-assessment-of-bioaccumulation-biocrab-in-pfas-impacted-aquatic-food-webs