Can Hemoglobin (Hb) Binding Models be Used to Predict Toxicity of Mixtures of Forever Chemicals?

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Introduction

- Per- and poly-fluoroalkyl substances (PFAS): ubiquitous and persistent environmental contaminants and pressing hazard to human/environmental health.
- PFAS often bioaccumulate and bind to proteins, including Hb.
- PFAS mixture effects are not well understood, despite that mixture exposures are the reality for humans and wildlife.
- Pressing need for methods to predict the effects of PFAS singly and as mixtures: testing 14,00 PFAS/all possible mixtures impossible.
- Midge *Chironomus dilutus* is ideal model to test how PFAS/protein interactions might enable prediction of toxicity:
  - Midges are extremely sensitive to PFAS, possibly due to effects on oxygen transport
  - 95% of hemolymph is Hb protein

Fig. 2. Photo of 4th instar (L4) midge, *Chironomus dilutus, an ideal model given its sensitivity to PFAS exposure and extremely high Hb content.*

Objectives

1. Determine if PFAS potency can be ranked based on their binding affinity to Hb and whether this interaction will be driven by chain length and functional group (i.e., structural characteristics).
2. Determine if PFAS mixtures act additively using *in vitro* assays.
3. Validate models optimized by *in vitro* tests using *in vivo* toxicity tests.

Methods

In Silico QSAR Modeling:

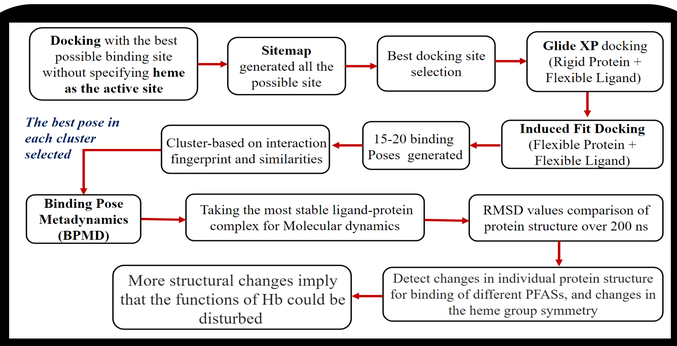

Fig. 3. Summary computational pipeline used to determine predicted toxicities of PFAS and their mixtures based on structure-activity relationships.

In Vitro Modeling via Two Complimentary Methods:

Fluorescence Spectrophotometry:

- Shifts in fluorescence will be used to determine patterns in conformational change of oxygen-carrying heme due to Hb/PFAS binding.

Equilibrium Dialysis:

- Equilibrium dialysis quantifies binding affinities of PFAS to Hb, expressed as disassociation constant (K_d).

In Vivo Toxicity Testing:

10-day Dose Response Assays with *C. dilutus*:

- Subset of PFAS/mixtures predicted to be most toxic via models selected for midge exposure studies.
- 6 doses per PFAS exposure: control, 3 doses below LC_{50}, 1 dose at LC_{50}, 1 dose above LC_{50} (Fig. 1, Panel 3, for example with PFOS).
  - Measure growth, survival, and Hb expression via quantitative polymerase chain reaction (qPCR) for two *C. dilutus* Hb isoforms.

Central Hypothesis: **Binding of PFAS to Hb is a tractable and sensitive physiological signal that can be used to predict the toxicity of PFAS mixtures.**

Approach:

1. **Computational modeling uses 3D Hb & PFAS structures to simulate binding. Resulting changes in protein conformation/oxygen carrying capacity ranks predicted toxicities of PFAS and mixtures.**
2. ***In vitro* assays test model predictions. Used iteratively to revise computer models if predictions not accurate. If model predictions validated, proceed to *in vivo* testing.**
3. **Use *in vivo* dose response experiments with the midge, *Chironomus dilutus*, to provide final validation of model predictions in whole organisms.**

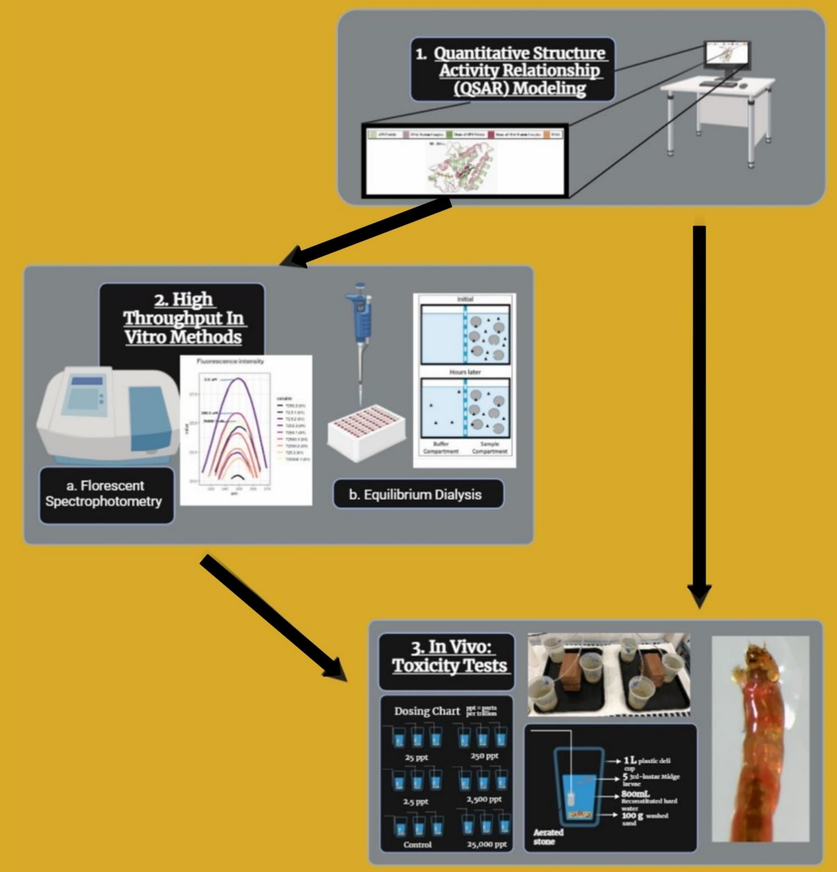

Fig. 1. Conceptual diagram showing how the interplay among QSAR models, high-throughput *in vitro* assays, and *in vivo* toxicity tests will be leveraged to iteratively refine models to ultimately predict toxic effects of PFAS and mixtures for which experimental data are lacking.

Preliminary Results

Toxicity Ranking	PFAS	Chain Length
1	Perfluorononanoic acid (PFNA)	C9
2	Heptafluoropropoxy propanoic acid (Gen X)	C6
3	Perfluorooctanesulfonic acid (PFOS)	C8
4	Perfluorodecanesulfonic acid (PFDS)	C10
5	Perfluorodecanoic acid (PFDA)	C10
6	Perfluorooctanoic acid (PFOA)	C8
7	Perfluorobutanesulfonic acid (PFBS)	C5
8	Perfluorobutanoic acid (PFBA)	C4
9	Perfluorohexanesulfonic acid (PFHxS)	C6
10	Perfluorohexanoic acid (PFHxA)	C6
11	Perfluoroheptanoic acid (PFHpA)	C6

Table 1. Ranking of predicted toxicities for the 11 PFAS with highest potential to alter heme conformation based on initial QSAR models. Chain length = number of fluorinated carbons.

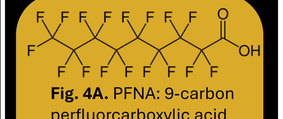

Fig. 4A. PFNA: 9-carbon perfluorcarboxylic acid

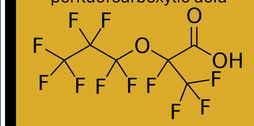

Fig. 4B. GenX or HFPO-DA: Hexafluoropropylene propanoic dimer acid

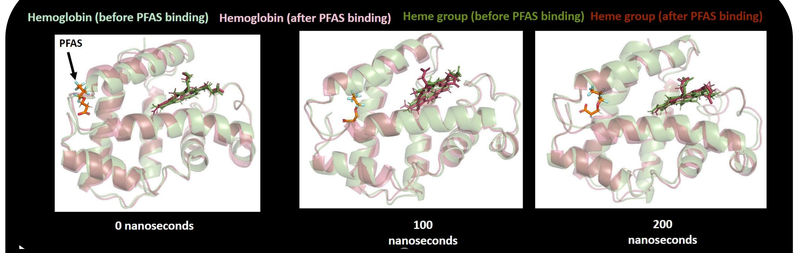

Fig. 5. Example of binding pose metadynamic model output, showing how PFAS binds to a *C. dilutus* Hb and the resultant shift in protein and heme conformations. The magnitude of this shift is used to rank potential toxicity via alteration of oxygen carrying capacity.

Next Steps

- *In vitro* testing in progress for top 5 most toxic PFAS based on models and all resultant binary mixtures.
- Performed PFAS exposures in vivo for two PFAS that should be toxic based on models: PFOS and GenX; analysis is currently underway.
- Will continue to refine models as new *in vitro* and *in vivo* data are generated.

Discussion & Implications

- With over 14,000 PFAS in the environment, we need models that can predict toxicity of mixtures for which experimental data are absent: our project is one step toward this important goal.
- Our approach provides a more efficient, cost-effective, and feasible alternative to experimentally testing PFAS mixtures as they are discovered.
- Ultimately, we hope to create a user friendly, web dashboard with a simple graphical user interface, where users could input PFAS mixtures and use our trained and validated models to predict toxicities of mixtures of interest.

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DUIRI Discovery Undergraduate Interdisciplinary Research Internship

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Works Cited