PFOS Bioaccumulation and Bioconcentration Factors in Multi-Generational Zebrafish Exposures

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Disclaimer Statement

The contents of this presentation neither constitute nor necessarily reflect DOD policy.

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- Sediment Management Work Group (SMWG)*

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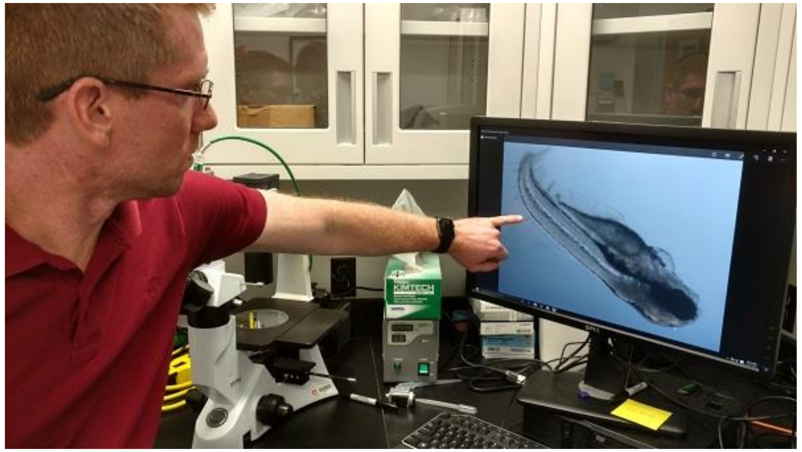
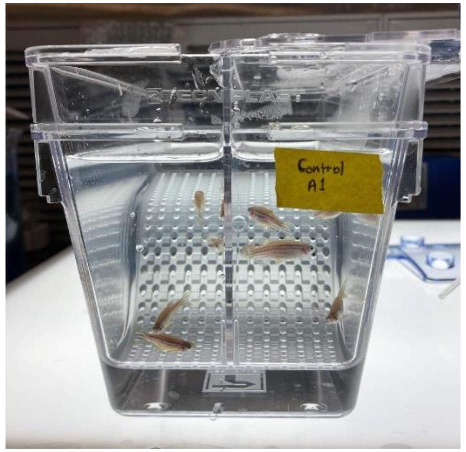




Background

Per- and polyfluoroalkyl substances (PFAS) pose exposure risks to diverse ecological systems and to humans.

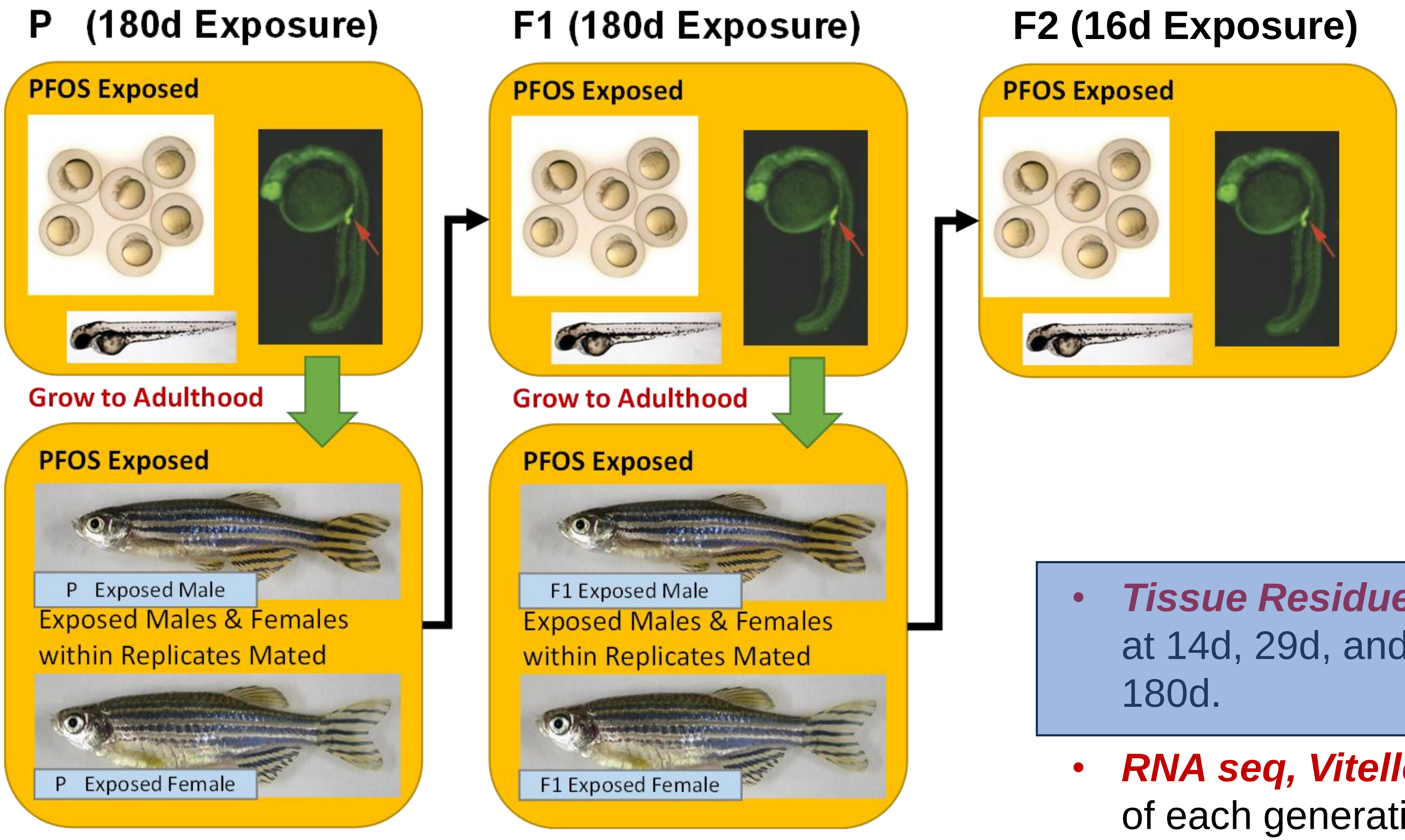
The PFAS, perfluorooctane sulfonic acid (PFOS), is broadly distributed in surface waters and is toxic to a variety of aquatic organisms including fish.

A need exists to understand how PFOS bioaccumulates in aquatic exposures.

In the Present Study

- We determined how exposure concentrations, exposure durations, and developmental life stages affected PFOS accumulation in zebrafish.
- Maternal transfer of PFOS to eggs was quantified.
- A toxicokinetic model was developed that accurately predicts PFOS body burdens in multiple zebrafish life stages spanning broad exposure durations.

Multi-Generational Exposure (Conceptual Overview)



- ***Survival*** – Measured Daily
- ***Growth*** – Measured at 30d, 60d, 90d, and 180d.
- ***Reproduction*** – Beginning at 4 months and measured 8 times for both P and F1.

- ***Tissue Residue Analysis*** – P Generation at 14d, 29d, and 180d and F1 Generation at 180d.
- ***RNA seq, Vitellogenin & Histopath.*** – End of each generation.

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Experimental Design (Continuous Multi-Gen Exposure)

Control 0.1 µg/L 0.6 µg/L 3.2 µg/L 20 µg/L 100 µg/L

Design Details:

- 5 PFAS Concentration + Control
- 5 Replicate Tanks / Treatment
- 50 Fish per Replicate
- Separation of Breeding Groups
- Minimum Statistical Power: > 0.8

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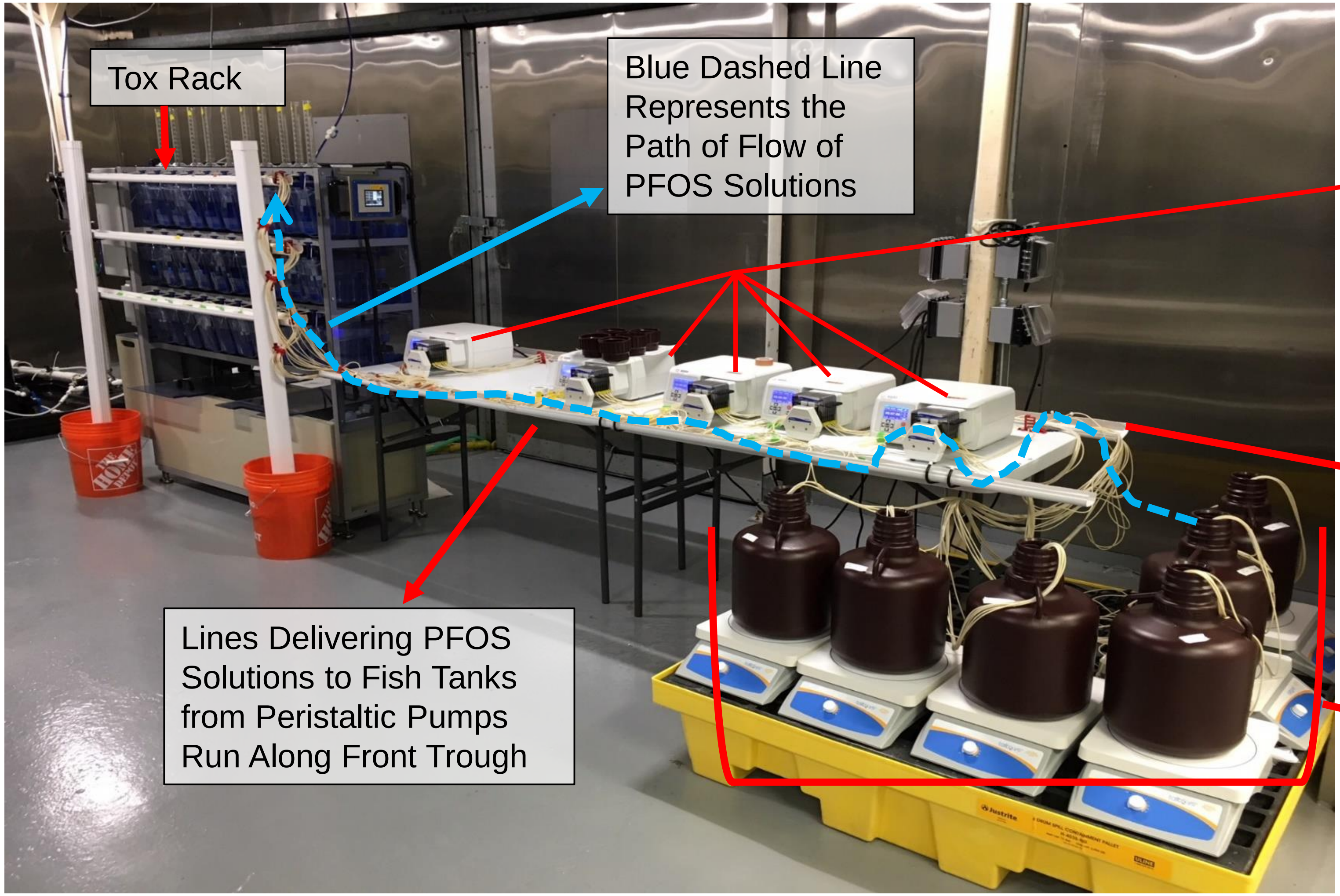
Exposure System



Peristaltic Pumps for Delivering PFOS Stock Solutions to Fish Tanks. Two Pumps Per Row to Compensate for Differential Timing of Water to Tanks on Tox Rack

Lines Delivering PFOS 10x Stock Solutions to Peristaltic Pumps Run Along Back Trough

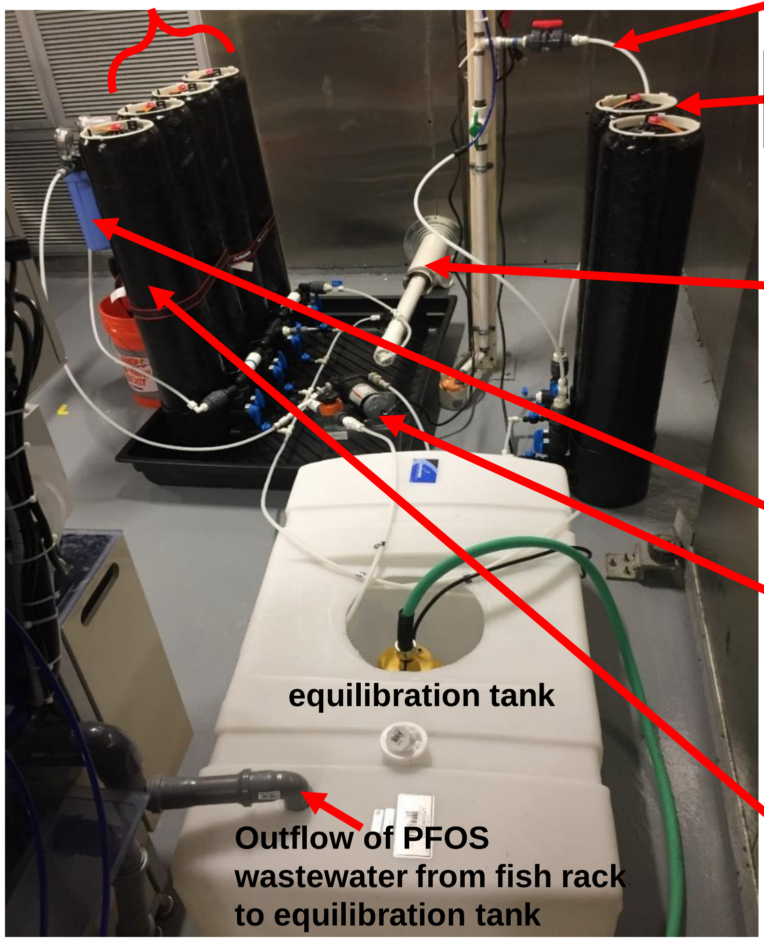
20L Amber HDPE Carboys Containing PFOS 10x Stock Dosing Solutions

PFAS Treatment Systems (pre- and post-treatment)

3 canisters of Purofine PFA694 resin for treatment of PFOS waste from equilibration tank

RO water line

2 canisters of Purofine PFA694 resin for pretreatment of RO water to fish racks to remove any residual PFASs

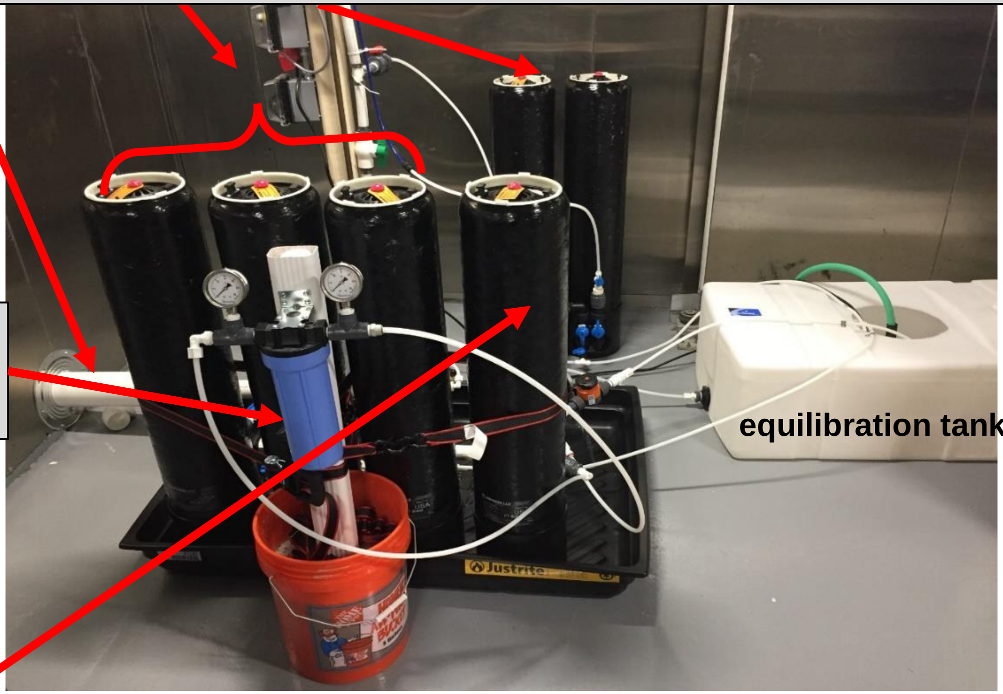


drain line of treated PFOS waste to large outside holding tank

Particulate filter to remove any debris (fish food, feces, etc.)

pump

Activated charcoal canister



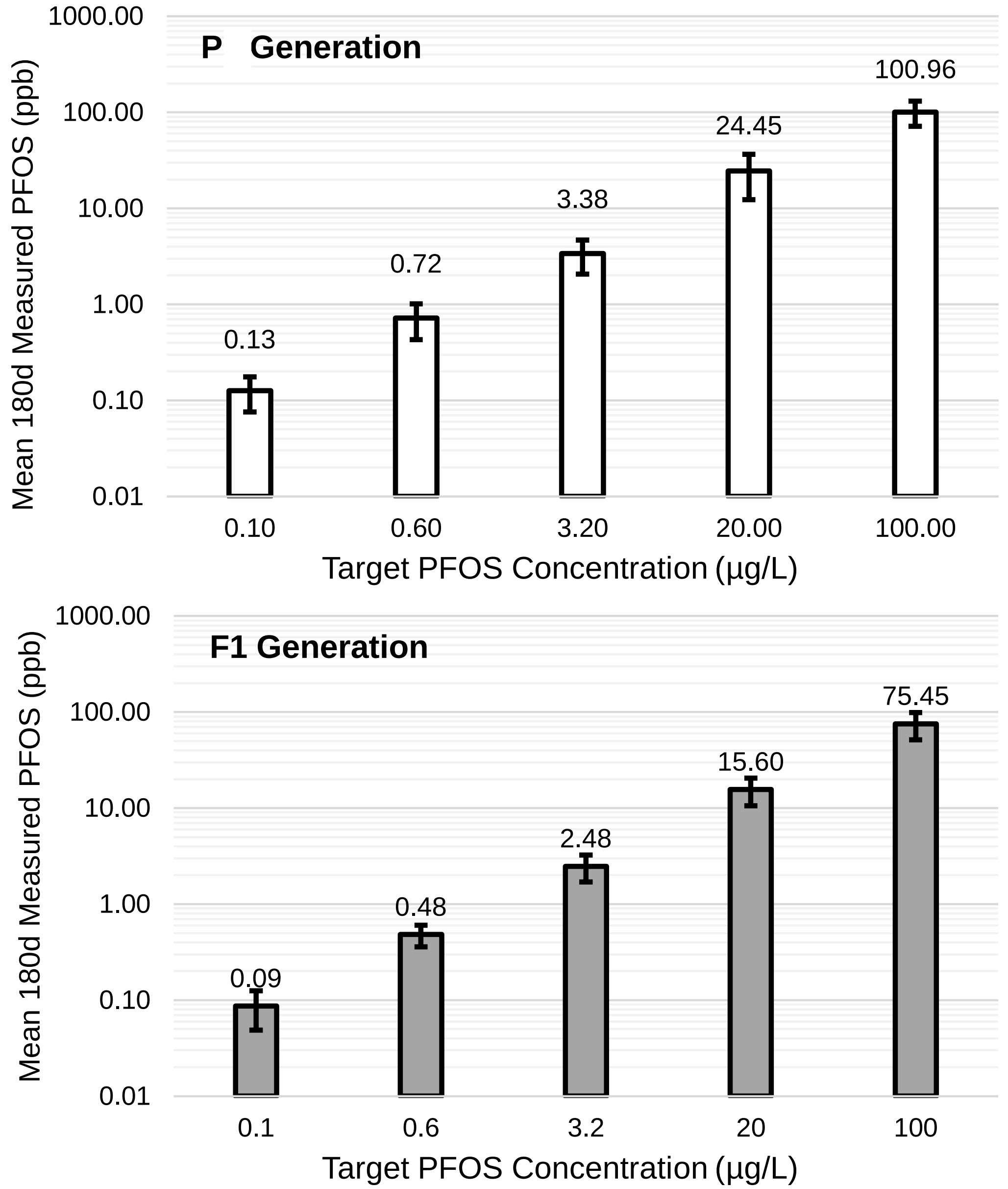
Measured PFOS in Exposure Water

PFOS concentrations measured using LC-MS/MS.

Two random tanks at each target concentration were sampled weekly through the first 4 weeks of exposure and biweekly thereafter through 180 days post-fertilization (dpf).

The figures display mean exposure concentrations across all samples taken through 180dpf.

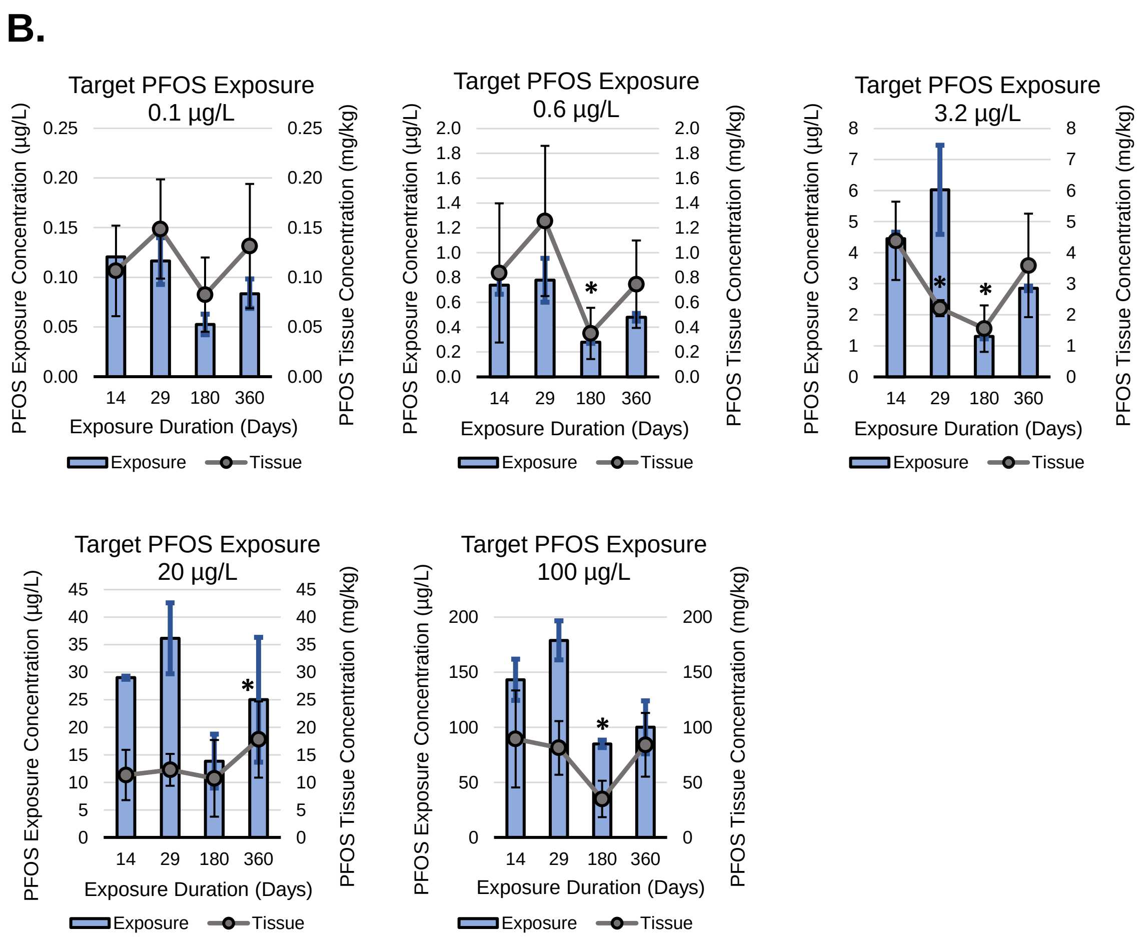
In the following slides bioaccumulation plots reflect exposure concentrations at the sampling date when tissues were sampled.

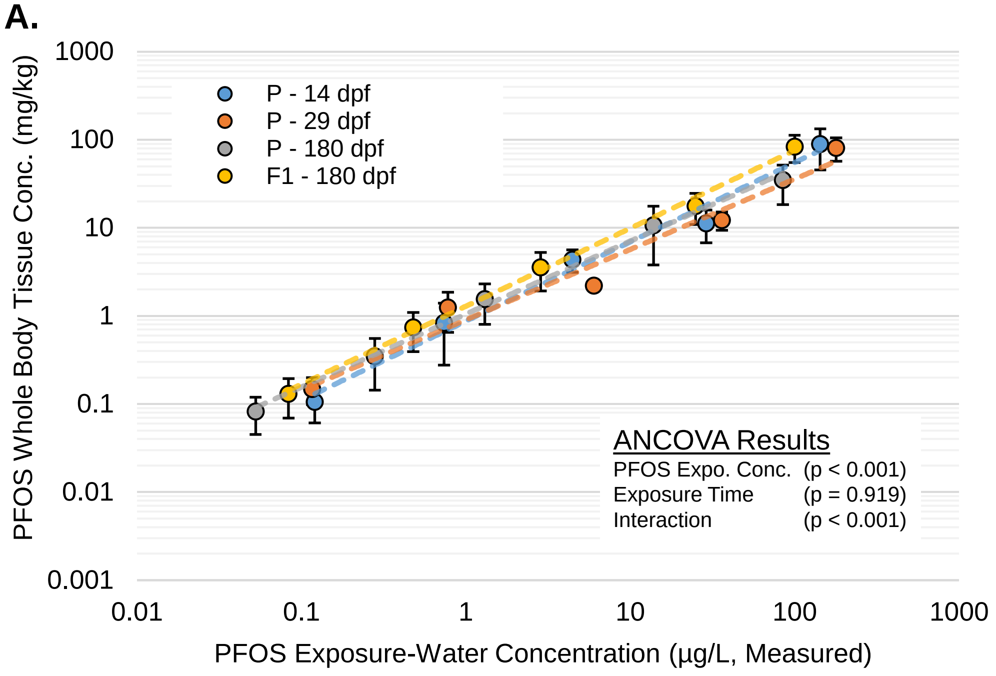


PFOS Bioaccumulation (All Values, P and F1)

PFOS body burdens in zebrafish remained relatively constant in the P generation through 14, 29 & 180dpf and F1 through 180dpf.

This result suggests **apparent steady state in tissues reached by 14dpf**.





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PFOS Bioaccumulation
(Males vs Females at 180dpf)

Significantly Increased PFOS bioaccumulation in males vs females across all PFOS exposure concentrations.

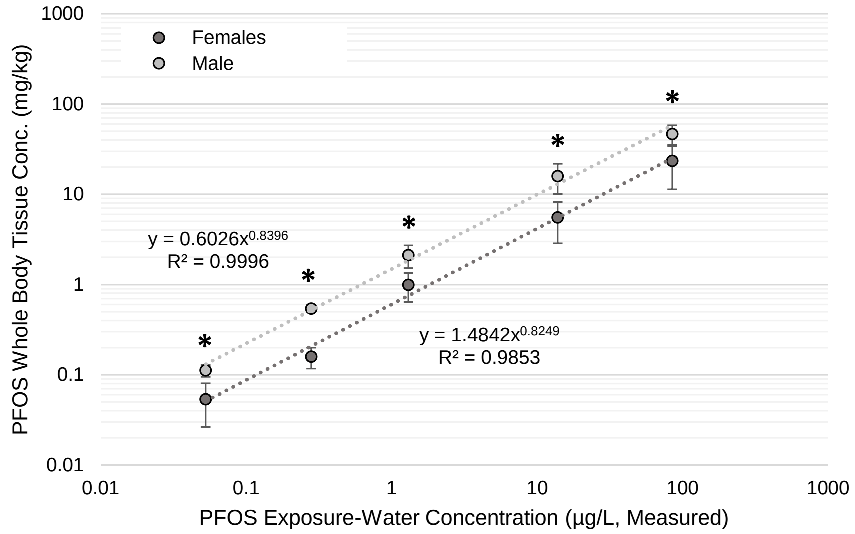
2.5-fold and 1.9-fold increases in PFOS body burdens for males vs. females in **the P1 and F1** generations, respectively.

Possible explanations:

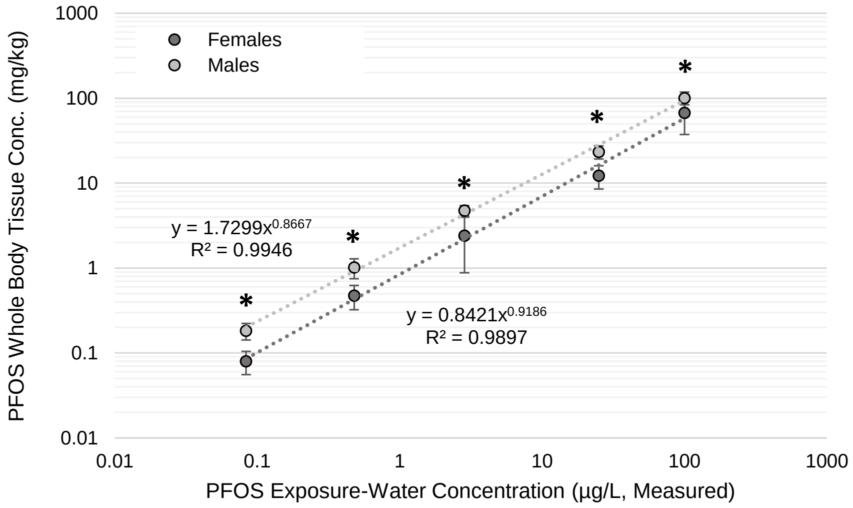
Zebrafish began breeding trials on day 131 with egg release representing a potential PFOS depuration mechanism *(tested – Results in upcoming slide)*.

Male and female fish have differences in body composition which may affect PFOS bioaccumulation potential.

A. Parental (P) - 180 dpf PFOS Accumulation



B. F1 - 180 dpf PFOS Accumulation



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PFOS Bioconcentration Factor (BCF)

PFOS bioconcentrated in zebrafish whole-body tissues:

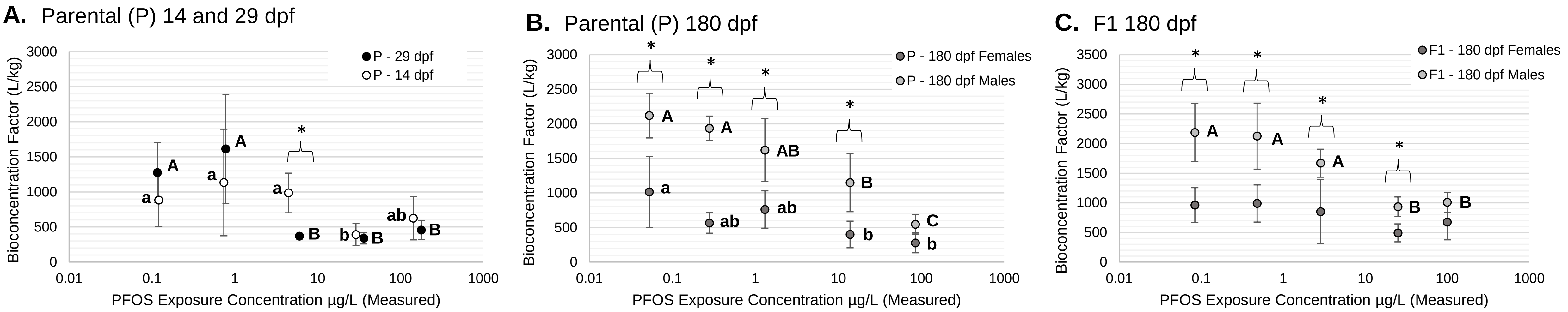
- 960 L/kg (mean BCF)
- 934 L/kg (median BCF)
- 276 to 2,187 L/kg (range of BCF observations)
- ± 443 L/kg (standard deviation of BCF observations)

Significantly Increased BCF in males vs females – 2.5- & 2.0-fold increases P and F1, respectively.

BCF **Decreased Significantly** with increasing PFOS exposure concentrations.

Possible explanations:

- Limited transport surfaces affecting bioaccumulation dynamics at higher PFOS concentration.
- Competition for molecular binding sites for PFOS at the cellular level.



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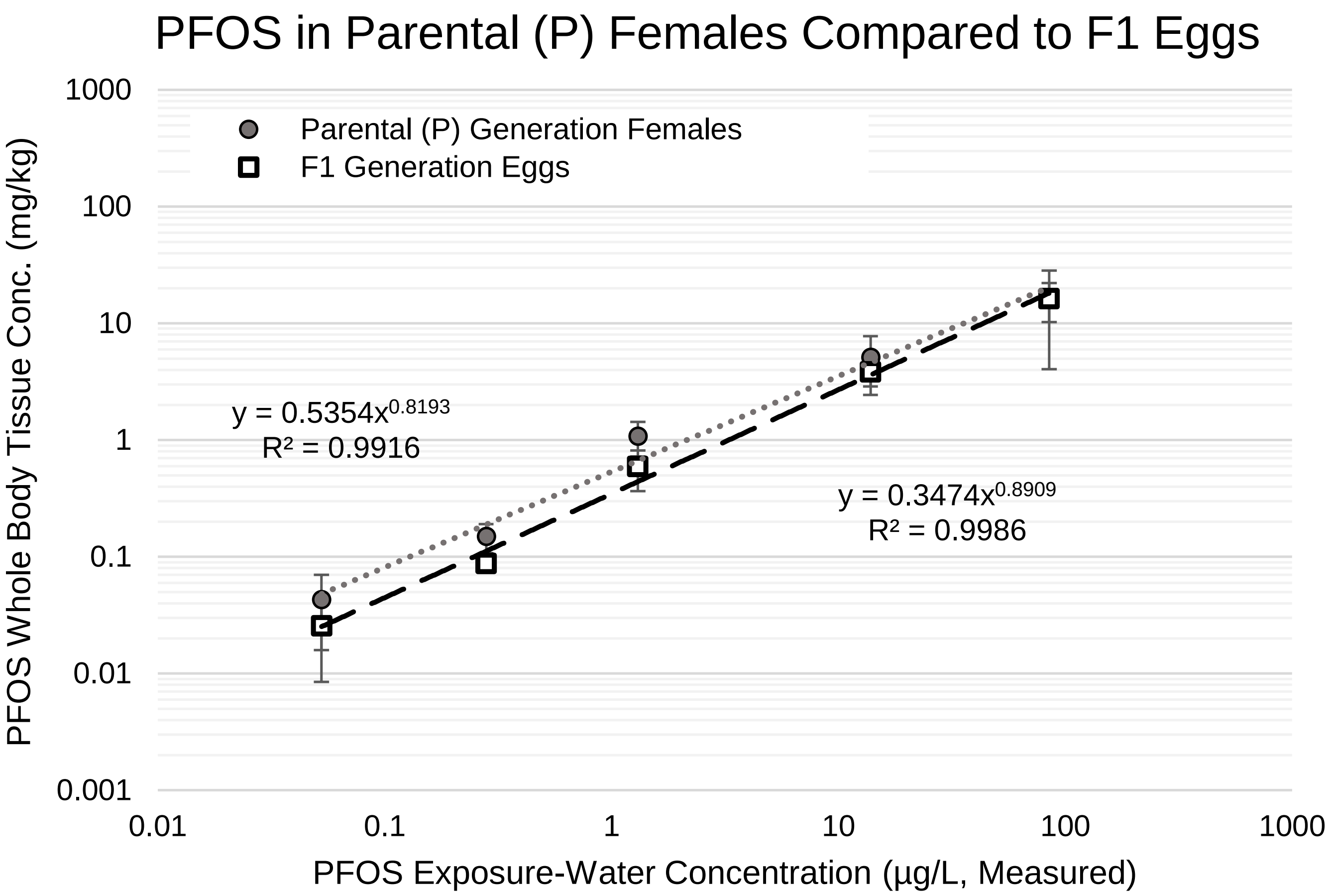
Maternal Transfer of PFOS to Eggs

PFOS was maternally transferred from P generation females to F1 eggs.

PFOS burdens in eggs and maternal whole-body tissue were statistically indistinguishable.

Based on this finding, PFOS off-loading to eggs does not appear to be a PFOS depuration mechanism for females.



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Toxicokinetic Model of PFOS Accumulation from Water

A one-compartment toxicokinetic model was developed to represent all life stages of zebrafish from embryos through adulthood.

$$h(t) = \frac{\left(\frac{t}{T}\right)^{\alpha}}{1+\left(\frac{t}{T}\right)^{\alpha}}. \quad (1)$$

$$V(t|s) = \frac{V_0(s) + V_\infty\left(\frac{t-s}{\tau}\right)^2}{1+\left(\frac{t-s}{\tau}\right)^2}. \quad (2)$$

$$\langle V\rangle(t) = \int_0^t ds\, p(s)\, V(t|s)\,, \quad (3)$$

$$S_{\mathrm{gill}} = \frac{\rho V}{\sigma}. \quad (4)$$

$$\frac{d}{dt}(V(t)C(t)) = kS_{\mathrm{gill}}(t)\frac{C_{\mathrm{exp}}}{K + C_{\mathrm{exp}}} - k_d S_{\mathrm{gill}}(t)C(t). \quad (5)$$

$$\langle C\rangle(t) = \int_0^t ds\, p(s) C(t-s)\,. \quad (6)$$

Symbol	Value	Unit	Description
T	62.23	hr	Characteristic hatching time (hpf), scale parameter of the log-logistic distribution function
α	19.25	—	Hatching exponent, shape parameter of the log-logistic distribution function
V_∞	0.51148	mm^3	Asymptote for the volume of adult zebrafish
τ	4282.1	hr	Characteristic growth time of zebrafish growth and development
ρ	1	$\frac{g}{mm^3}$	Unit density of water
σ	0.00265	$\frac{g}{mm^2}$	Scale parameter for the allometric relationship between gill surface area and mass of adult fish species
K	1.17	µM	Scale parameter for aqueous exposure concentrations
k	1.604	$\frac{\mu mol}{mm^2\ hr}$	PFOS mass uptake per unit exchange surface area and time
k_d	0.00151	$\frac{L}{mm^2\ hr}$	PFOS elimination constant in units of tissue volume per unit exchange surface area and time

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Hatching fraction

$$V(t|s) = \frac{V_0(s) + V_\infty \left(\frac{t-s}{\tau}\right)^2}{1+\left(\frac{t-s}{\tau}\right)^2} \,. \qquad (2)$$

Embryo/larval volume

$$\langle V\rangle(t) = \int_0^t ds\, p(s)\, V(t|s)\,, \qquad (3)$$

Embryo/larval volume averaged over hatching

$$S_{\text{gill}} = \frac{\rho\, V}{\sigma} \,. \qquad (4)$$

Gill surface areas

$$\frac{d}{dt}\big(V(t)C(t)\big) = kS_{\text{gill}}(t)\frac{C_{\text{exp}}}{K + C_{\text{exp}}} - k_d S_{\text{gill}}(t)C(t). \qquad (5)$$

Accumulation in fish

$$\langle C\rangle(t) = \int_0^t ds\, p(s)C(t-s)\,. \qquad (6)$$

Fish concentrations averaged over hatching periods

Symbol	Value	Unit	Description
T	62.23	hr	Characteristic hatching time (hpf), scale parameter of the log-logistic distribution function
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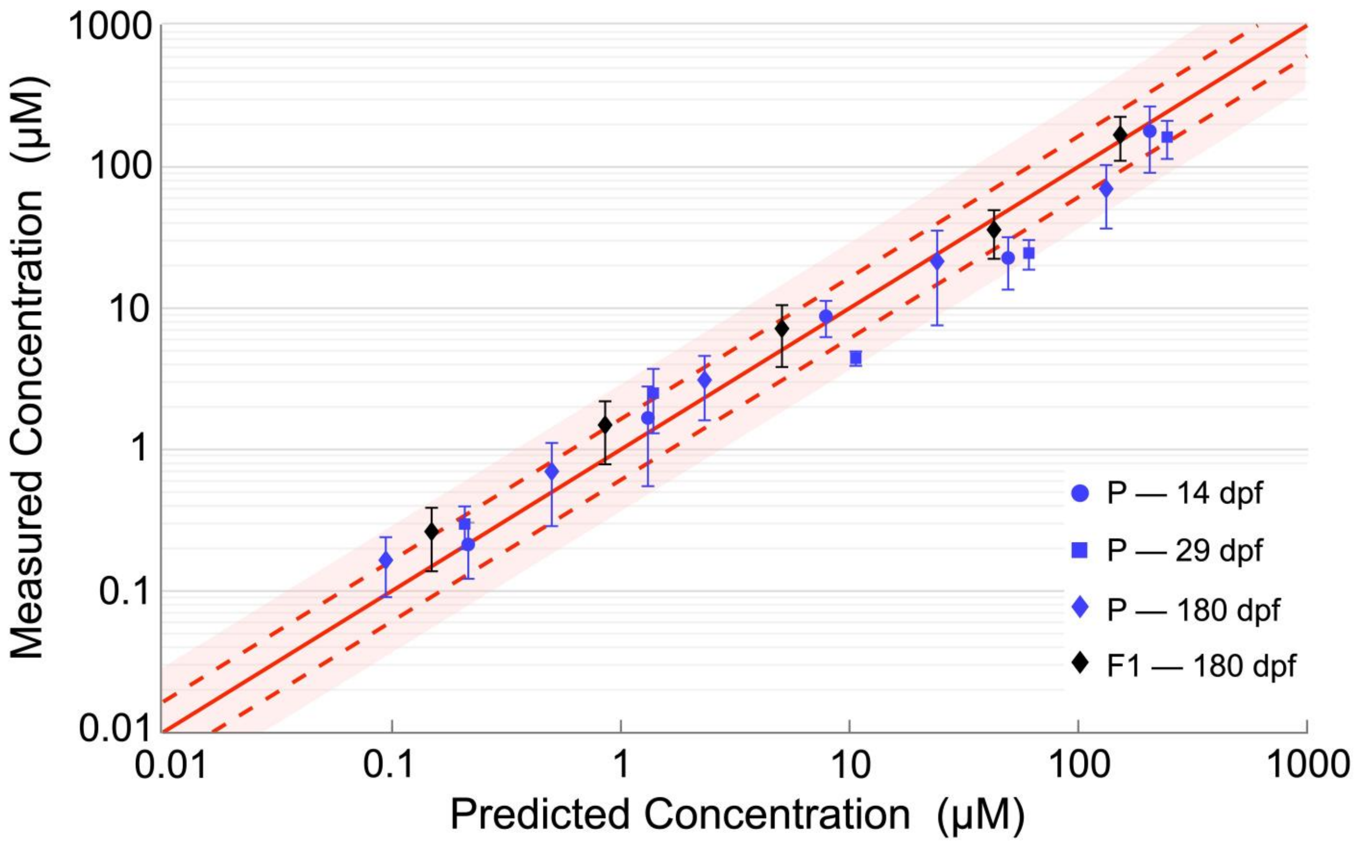
Toxicokinetic Model – Prediction vs. Measured Values

The toxicokinetic model reliably reproduced PFOS whole-body burdens (data within 1.64-fold of predicted values - dashed red lines).

Toxicokinetic model predictions fell within acceptable error tolerances (+/- 2-fold) for application to PFOS risk assessment for **larval, juvenile, and combined sexes of adult zebrafish**.



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Summary & Conclusions

PFOS Bioaccumulation: PFOS concentrations in zebrafish tissue reached apparent **steady state within 14 dpf.**

Equivalent Body Concentrations at 14, 29 & 180 dpf in P generation and 180 dpf in F generation despite zebrafish developmental maturation.

PFOS Bioconcentration Factor (BCF): PFOS bioconcentrated in zebrafish whole body tissue with **Median BCF of 934 L/kg.**

BCFs were significantly **higher in Males vs. Females (≥ 2-fold).**

BCF values were **significantly higher** in PFOS exposures <1.0 ppb relative to higher exposure levels in the 20 to 100 ppb range.

Summary & Conclusions (cont.)

Maternal Transfer: PFOS was **maternally transferred to eggs** at levels equivalent to maternal whole-body burdens.

Maternal Transfer does not appear to be a PFOS depuration mechanism explaining decreased PFOS burdens in females.

Toxicokinetic Model: A novel 1-compartment TK model accurately **predicts PFOS body burdens within 2-fold error.**

TK model predictive across zebrafish life stages.

Predictive of PFOS burdens in **lifetime chronic and multi-generational** exposures.

Current Events - Global Influence

Results from **Gust et al (2024)*** were acknowledged during open review of Australian regulatory standards for PFOS.

The technical rigor of the study was acknowledged & influenced the removal of a less robust earlier Multi-Gen PFOS exposure study by Keiter et al (2012).

Specifically, PFOS effective concentrations were higher than reported in Keiter et al (2012) and at levels consistent with the broader peer-reviewed literature.

Therefore, effects data for Keiter et al (2012) were replaced with data reported in Gust et al. (2024) for derivation of the new risk standard for PFOS.

*Gust et al. 2024 *Environ Toxicol Chem* 43(1):115-131

Keiter et al. 2012. Aquat Toxicol 118-119:116-129

Questions?

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