



# Developmental Toxicity Assessment of a Library of Per- and Polyfluoroalkyl Substances in Zebrafish (*Danio rerio*) Embryos/Larvae

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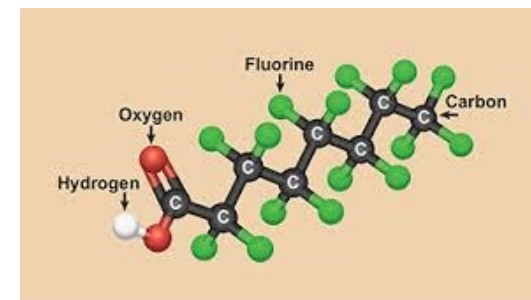
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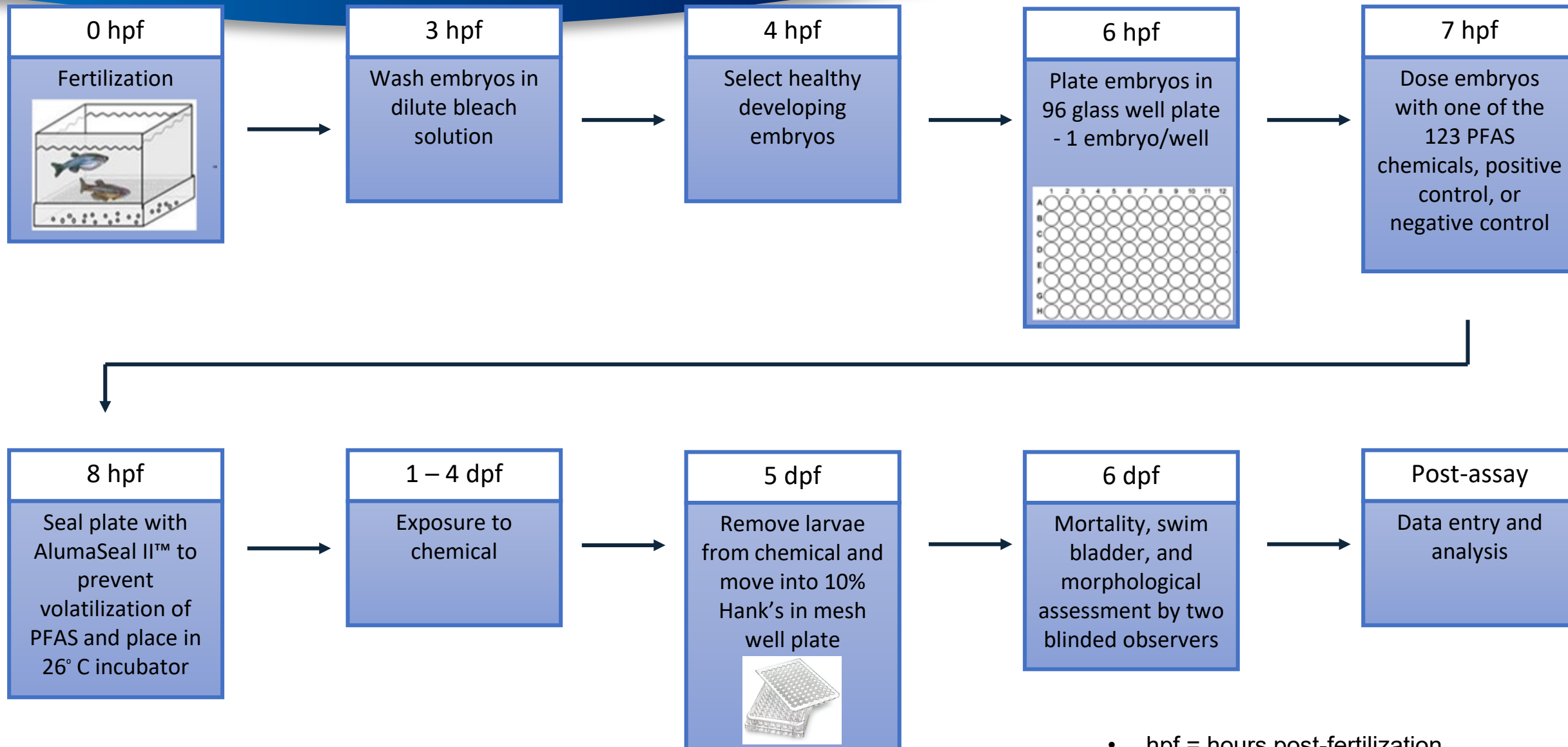


# Background

- The US Environmental Protection Agency (EPA) is leading an effort to understand per- and polyfluoroalkyl substances (PFAS)
  - PFAS are chemical compounds made up of one or more carbons and fluorides
  - There are currently ~1223 PFAS chemicals that are included in the Toxic Substances Control Act (TSCA) inventory; 602 are currently active with an unknown number of degradation and manufacturing byproducts
  - These chemicals are found in many consumer and industrial products
- We tested the developmental toxicity effects of 123 PFAS chemicals in a larval zebrafish vertebrate model
- Our objective was to determine the developmental toxicity of a group of PFAS chemicals in a zebrafish model to understand the structure/activity of this class of chemicals



# Methods



- hpf = hours post-fertilization
- dpf = days post-fertilization

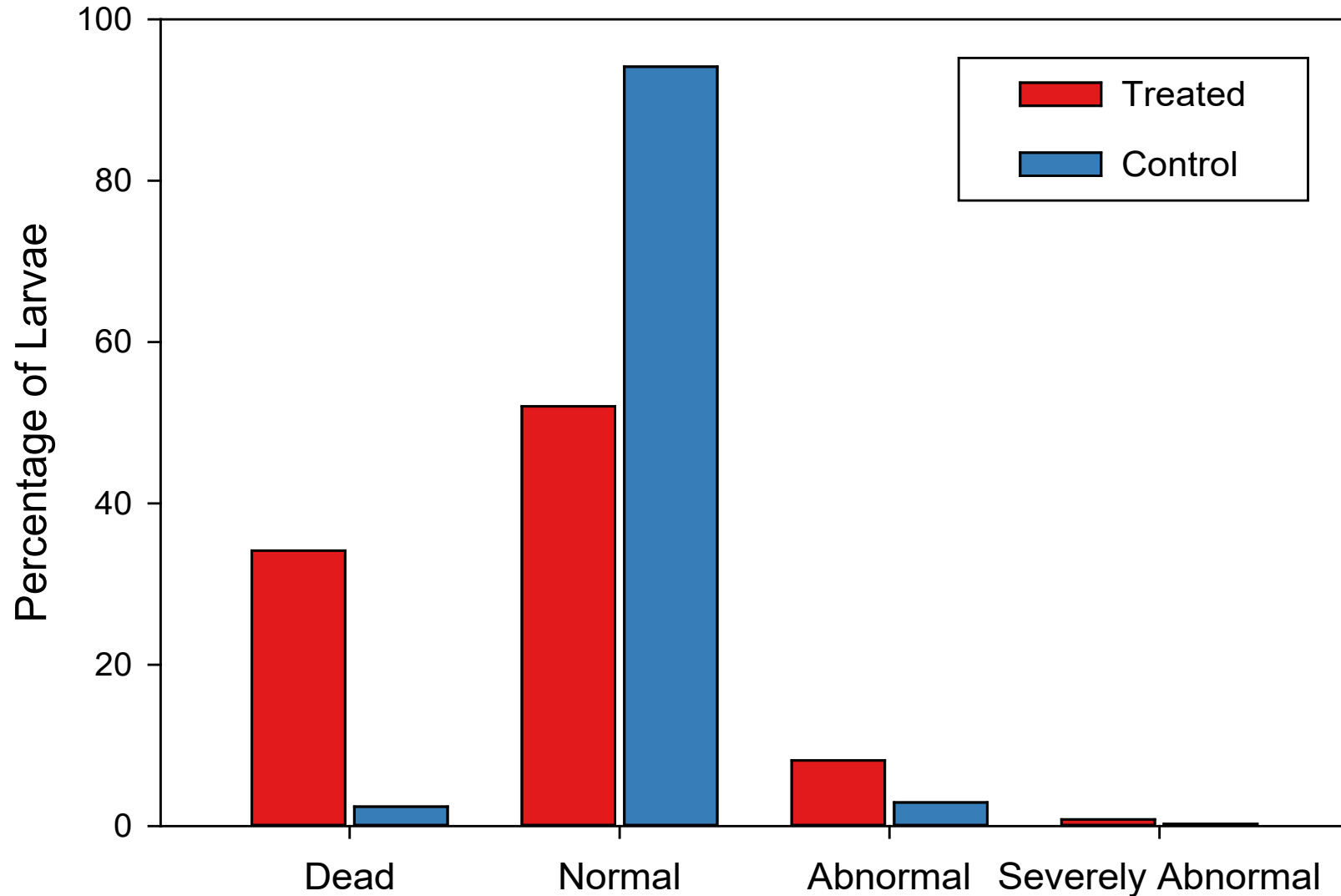
# Data

Plate #	Stock Plate #	Chemical ID	EPA_SAMPLE_ID	Final Concentration (uM)	Assessor	Empty ?	Transfer Damage ?	Living Live (L), Dead (D)	Hatched (DNH=did not hatch)	General Normal (N), Abnormal (A), Severely Abnormal (S)	Swim Bladder (NI=not inflated)	Cranio facial (usually small eyes)	Edema (1=mild edema; 2= severe edema)	Spine (N=not present; Y=curved spine)	Pigmentation (M=mild decrease; S=Severe decrease)	Position (upside down, lying on side)	Tail kinked (K), shortened (Sh)	Blood pooling/ Hemorrhage? Y= yes	QAPP# E-ISTD-003145-QP-1-1 Comments
PFAS-185	341	110	1210314483	100	Britton			L		SA	NI		1	Y		LS	K		Curved spine and curve in middle
PFAS-185	341	110	1210314483	100	Jarema			L		SA	NI		1	Y			K		Dip in tail, slight abdominal edema
PFAS-186	341	110	1210314483	100	Britton	Yes		D											
PFAS-186	341	110	1210314483	100	Jarema	Yes		D											
PFAS-187	341	110	1210314483	100	Britton			L		N									
PFAS-187	341	110	1210314483	100	Jarema			L		N									

- Two blinded assessors conducted morphological evaluations on every individual larva
  - Determined if larva was alive
    - Presence of movement and heartbeat
  - Examined extent of abnormalities if any were visible
    - Abnormal – larvae with a few abnormalities but can still survive
    - Severely abnormal – larvae with a greater extent of abnormalities and likely would not be able to survive long
- Data for each larva was entered based on plate #, location in plate, chemical ID #

# Results

Percentage of Affected Larvae By Group



- Higher percentage of dead, abnormal and severely abnormal larvae in the group of larvae treated with PFAS
- Higher percentage of normal larvae in the control group

# Results

- Treated group
  - 44 of the 123 (36%) chemicals tested produced developmental abnormalities in at least 3 of the 6 total larvae
- Control group
  - Only 22 out of 375 (6%) of the larvae had any abnormality

Side View

Top View



A. Control Larva - **Normal**



B. Treated Larva – **Abnormal**: uninflated swim bladder, curved body axis



C. Treated Larva – **Severely abnormal**: uninflated swim bladder, craniofacial abnormality, edema, spine/tail abnormalities,

## Initial Findings/Next Steps

- Some of the PFAS chemicals may be potential vertebrate developmental toxicants
- Future studies
  - Perform concentration response for all of the tested PFAS to assess the potency for each chemical
  - Determine the structure/activity relationship for the PFAS library, i.e., which molecular structures are associated with toxicity



## Acknowledgements



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