COMPARATIVE TOXICITY OF LEGACY AFFF & PFOS

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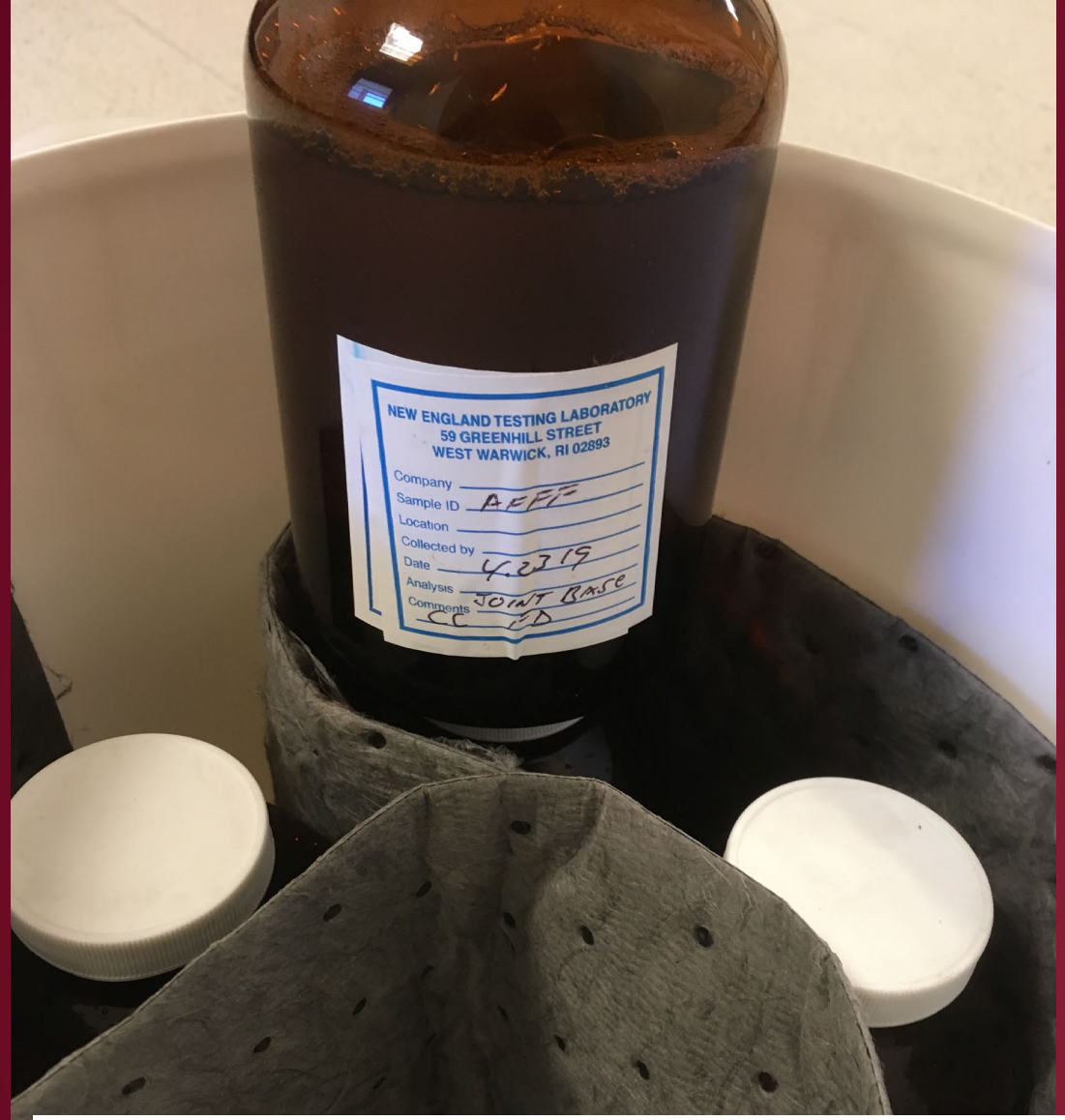

LEGACY AFFF

What's in it?

What components drive toxicity?

Is PFOS concentration a good predictor of mixture toxicity?

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Chemical Characterization of a Legacy Aqueous Film-Forming Foam Sample and Developmental Toxicity in Zebrafish (*Danio rerio*)

Kate M. Annunziato,[1] Jeffery Doherty,[2] Jonghwa Lee,[2] John M. Clark,[2] Wenle Liang,[1] Christopher W. Clark,[1] Malina Nguyen,[1] Monika A. Roy,[1,3] and Alicia R. Timme-Laragy[1]

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PART 1: WHAT'S IN IT?

Dr. John Clark

Dr. Jeff Doherty

UMASS Pesticide Analysis Laboratory

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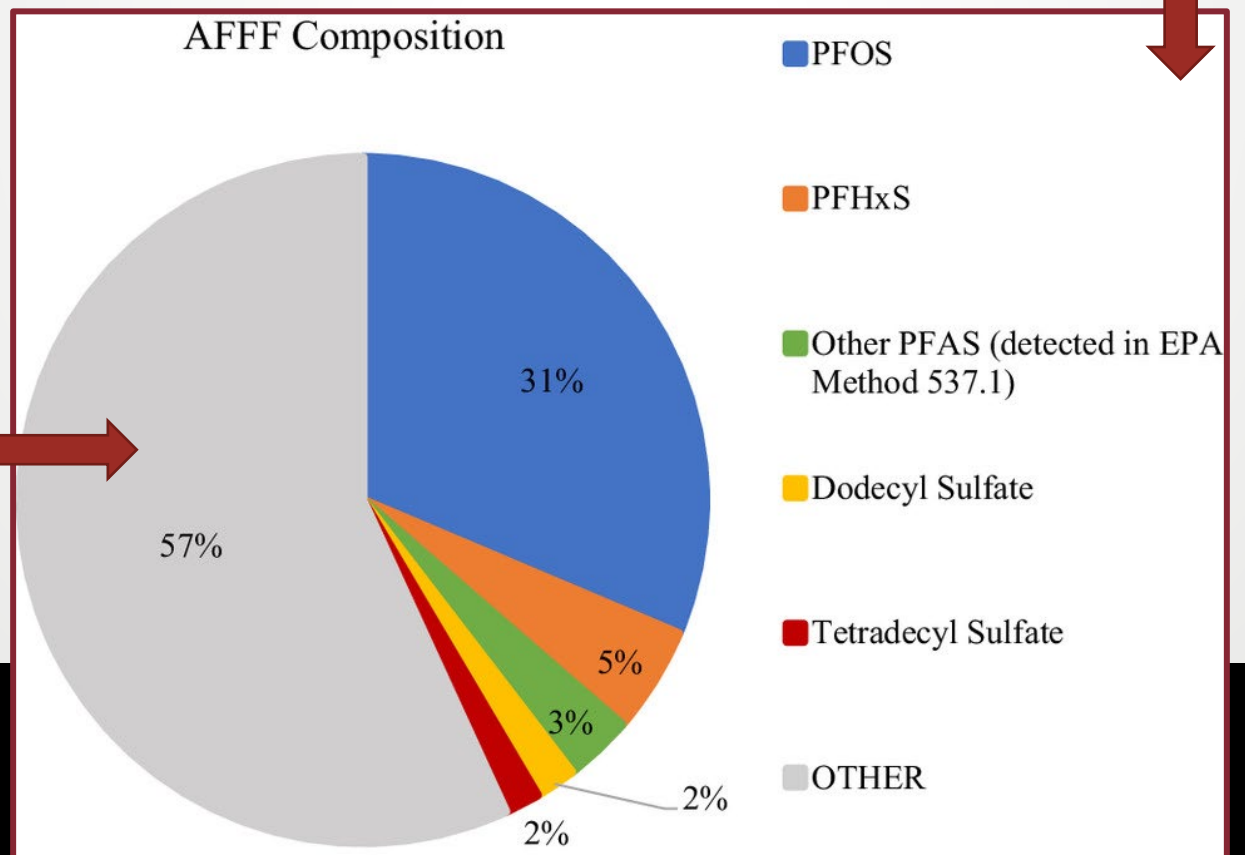
Abbreviation	CAS RN®	Compound discoverer™ Found	mzCloud match (%)[a]	MDL (ng/L)	PFAS in 3% AFFF (mg/L)	Relative % contribution to total PFAS content	Relative % contribution to total AFFF content
PFOS	1763-23-1	Yes	99	30,500	9,410	79.2	31.4
PFHxS	355-46-4	Yes	99.8	20,000	1,500	12.6	5.0
PFPeS	2706-91-4	Yes	99.5	7,500	223	1.9	0.7
PFBS	375-73-5	Yes	100	12,300	220	1.9	0.7
PFHpS	375-92-8	Yes	98.6	23,800	157	1.3	0.5
PFHxA	307-24-4	Yes	55.4	19,000	130	1.1	0.4
PFOA	335-67-1	Yes	97.3	15,800	108	0.9	0.4
PFPeA	2706-90-3	Yes	99.5	15,800	44.9	0.4	0.1
PFHpA	375-85-9	Yes	ND	22,800	43.6	0.4	0.1
PFBA	375-22-4	Yes	98.2	25,000	38.9	0.3	0.1

100 PFAS structures at or above 0.01% of PFOS peak intensity,+ non-fluorinated compounds



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Annunziato et al., EHP 2020

Table 2. Non-PFAS compounds detected in AFFF mixture using Orbitrap HRMS.

No.	Name	CAS RN®	Molecular weight (g/mol)	RT (min)	mzCloud best match (%)[a]	Relative peak intensity	Concentration (mg/L)	Industrial uses
1	Tetradecyl sulfate	1191-50-0	294.18642	8.363	100	6950653	496.4	Wetting agent, emulsifier
2	Dodecyl sulfate	151-41-7	266.15529	7.133	100	6229696	574.8	Wetting agent, emulsifier
3	Octyl gallate	1034-01-1	282.1500	6.85	88.6	3848182	NA	Antioxidant
4	Lauric acid	143-07-7	200.17761	7.340	99.9	649775	NA	Soap production
5	Decanoic acid	334-48-5	172.14613	6.531	99.9	236499	NA	Lubricant
6	Oleic acid	112-80-1	282.25596	9.386	99.7	240571	NA	Soap emulsifier
7	3,4-Dihydroxyphenyl propionic acid	71693-95-3	182.0612	5.088	99.2	218594	NA	Antioxidant
8	Pentadecanoic acid	1002-84-2	242.22646	8.522	100	224956	NA	Corrosion inhibitor, water repellant, plastic production
9	Nonanoic acid	112-05-0	158.13043	6.111	99.9	198824	NA	Plasticizer production
10	Disperse orange 3	730-40-5	242.08246	4.813	94	142701	NA	Indicator dye (Type I aviation deicing fluid)
11	4-Dodecylbenzene sulfonic acid	121-65-3	326.1916	7.50	86.2	575934	NA	Wetting agent, emulsifier
12	Δ2-cis-Hexadecenoic acid	2825-68-5	254.22479	8.362	99.8	68528	NA	Pesticide production
13	4-methyl benzotriazole_2	29878-31-7	133.06364	4.331	9.79	34981	NA	Corrosion inhibitor
14	4-methyl benzotriazole_1	29878-31-7	133.06364	4.708	98.5	30637	NA	Corrosion inhibitor

Note: AFFF, aqueous film-forming foam; HRMS, high-resolution mass spectrometry; min, minutes; NA, not analyzed; non-PFAS, non–per- and polyfluoroalkyl substances; RT, retention time.

[a]mzCloud match is percent match to compounds in the mzCloud mass spectral database.

PART 2: WHAT DRIVES TOXICITY?

Dr. Kate Annunziato

Wenle Liang

Malina Nguyen

Chris Clark

Dr. Monika Roy

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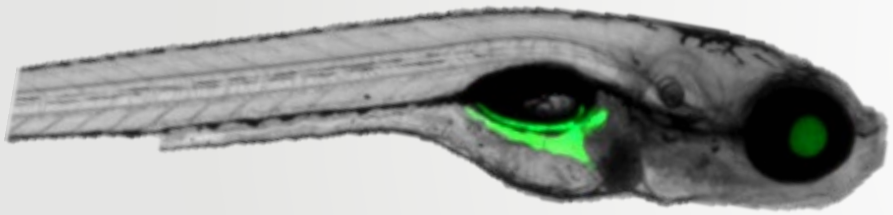
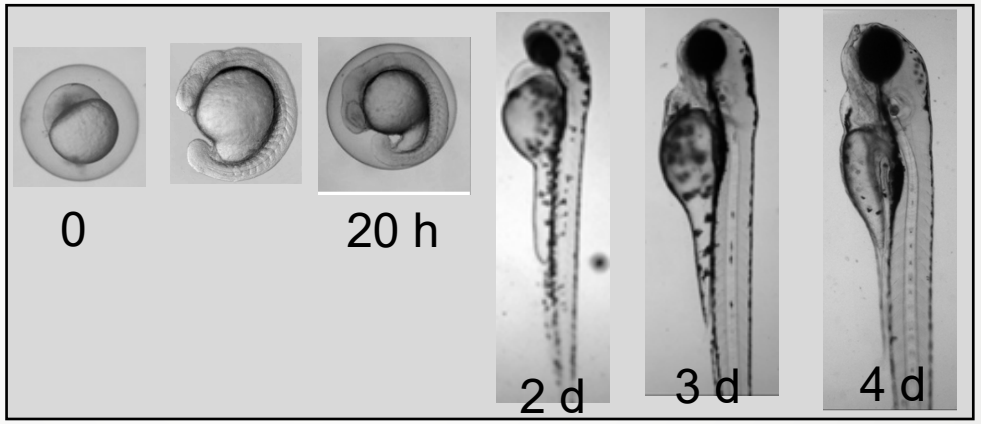
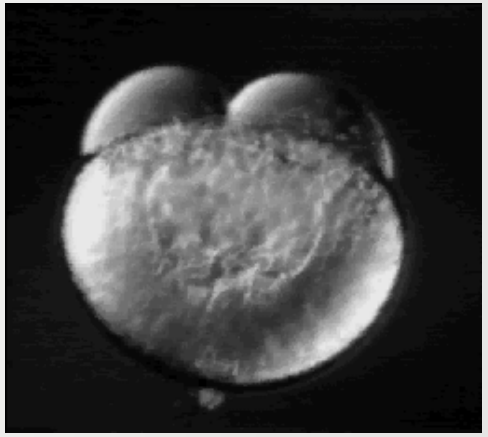
ZEBRAFISH

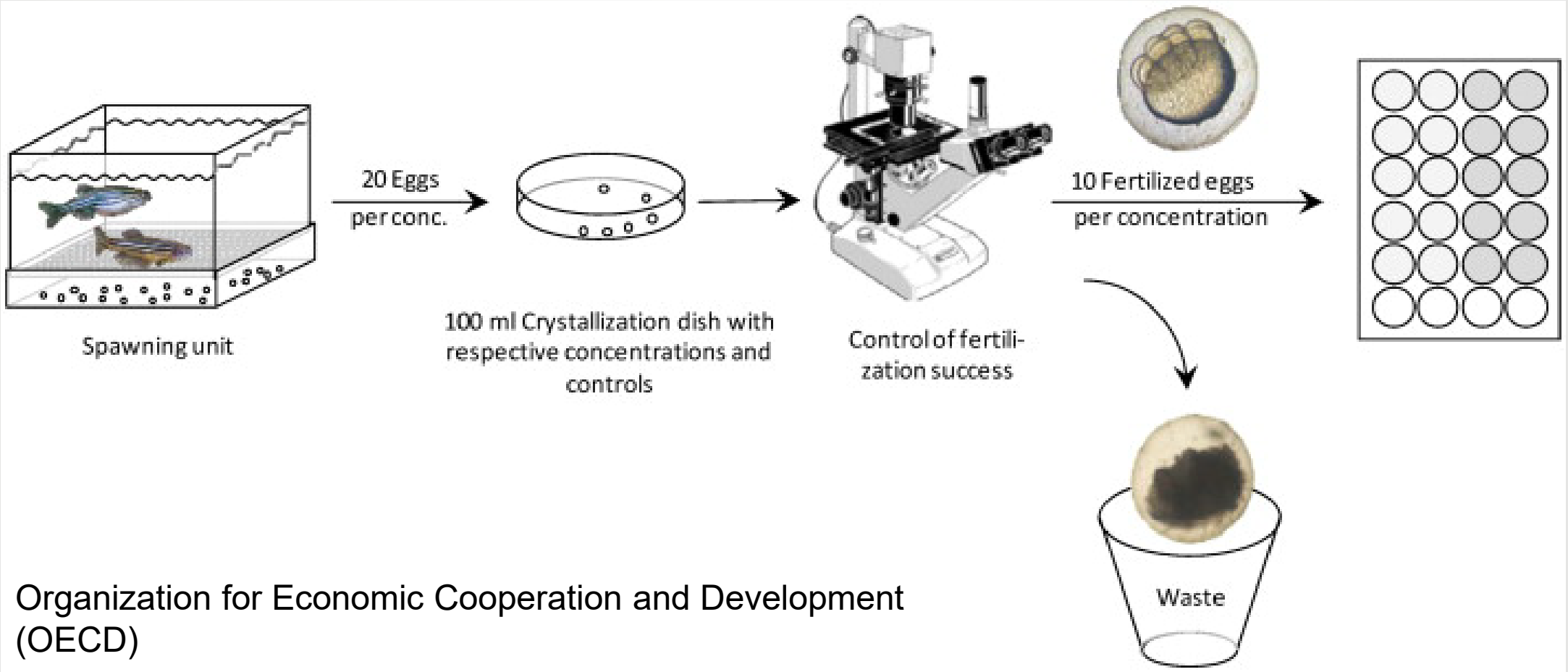






Video by Dr. Rolf Karlstrom (UMASS)

OECD 236 FET (FISH EMBRYO TOXICITY) TEST



Organization for Economic Cooperation and Development (OECD)

WHAT DRIVES LETHALITY?

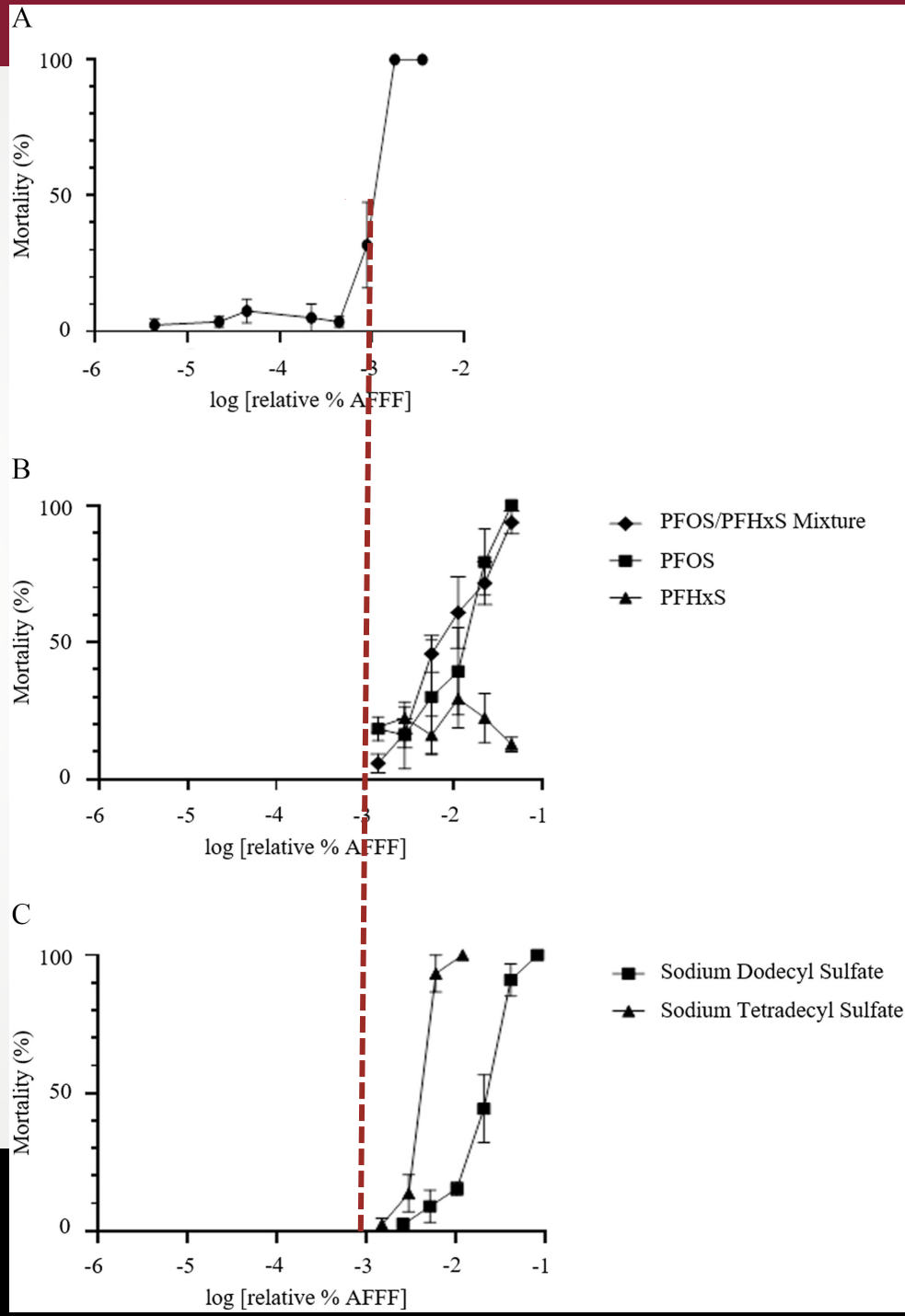
- AFFF Mortality curve most closely resembled the detergents.
- Dilutions of AFFF based solely to match PFOS concentrations was 100% lethal
- PFHxS did not reach a LC50 at concentrations found in the legacy AFFF
- PFOS+PFHxS mixture mortality was similar to PFOS alone*

Table 5. Comparison of LC_{50}s in 96 hpf larvae following exposure to AFFF and its main constituents.

	PFOS (mg/L)	PFHxS (mg/L)	PFAS mixture (total PFOS & PFHxS mg/L)	SDS (mg/L)	TDS (mg/L)
Amount of each in AFFF LC_{50}: 7.41×10^{-4}% AFFF	2.32	0.37	2.69	0.14	0.12
LC_{50} per compound	31.03	N.D.	29.63	3.67	0.66
95% Confidence interval	(23.15, 42.99)		(24.18, 36.14)	(3.09, 4.38)	(0.59, 0.74)

Note: The first row lists the concentrations of perfluorooctanesulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS), PFOS/PFHxS in combination, dodecyl sulfate and tetradecyl sulfate found in the LC_{50} of AFFF (7.41×10^{-4}% AFFF) are listed. The second and third rows list the LC_{50}s and 95% confidence intervals, determined in the present study, of each compound. AFFF, aqueous film-forming foam; ND, not determined in this study; PFAS, per- and polyfluoroalkyl substances; PFOS, perfluorooctanesulfonic acid; PFHxS, perfluorohexanesulfonic acid; SDS, sodium dodecyl sulfate; TDS, sodium tetradecyl sulfate.

3- 4 experimental replicates, 15 embryos exposed individually per concentration and chemical



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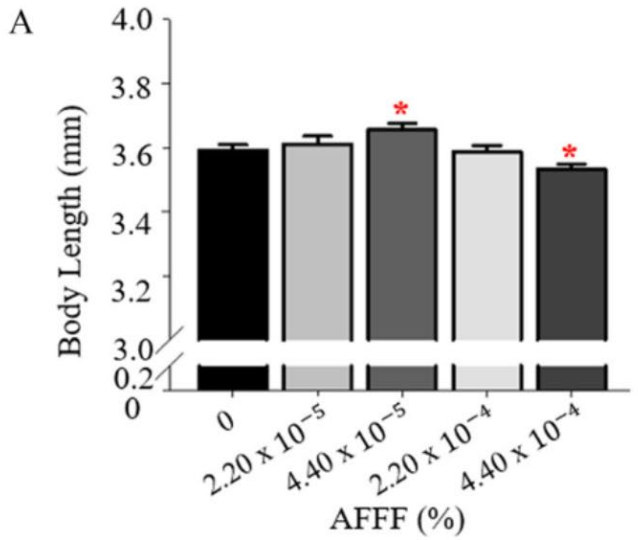
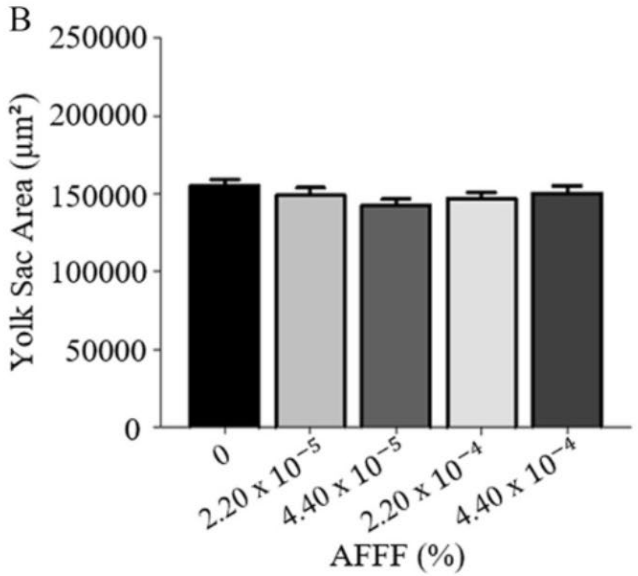
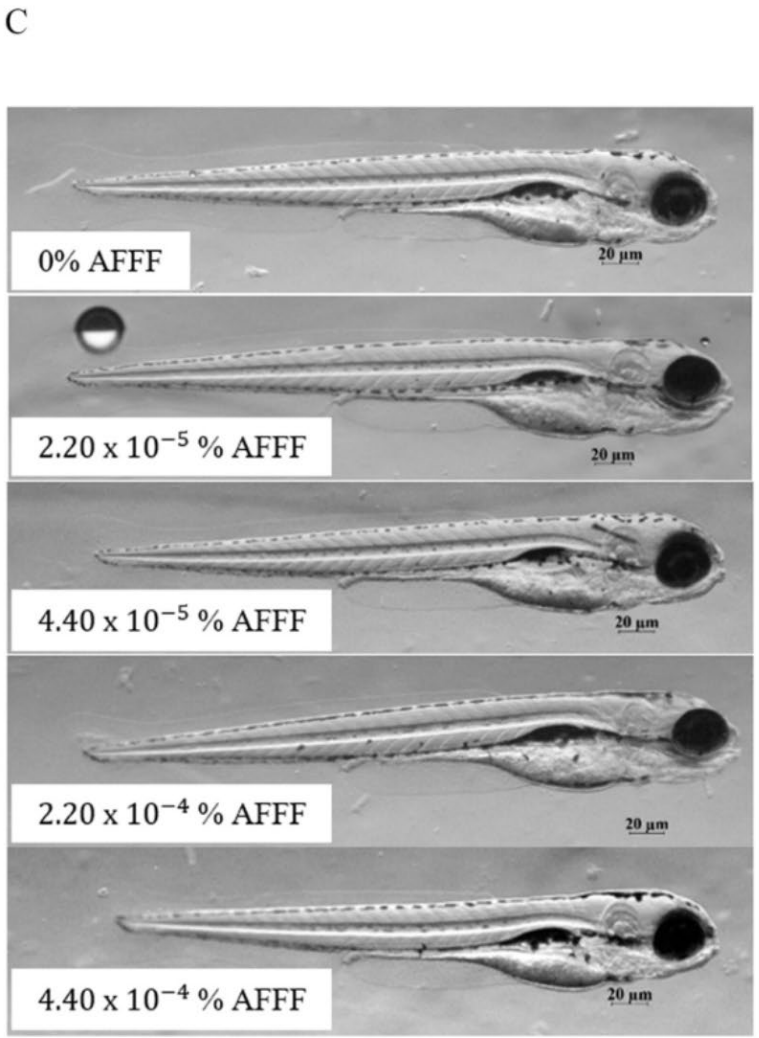
Annunziato et al., EHP 2020

SUBLETHAL EFFECTS

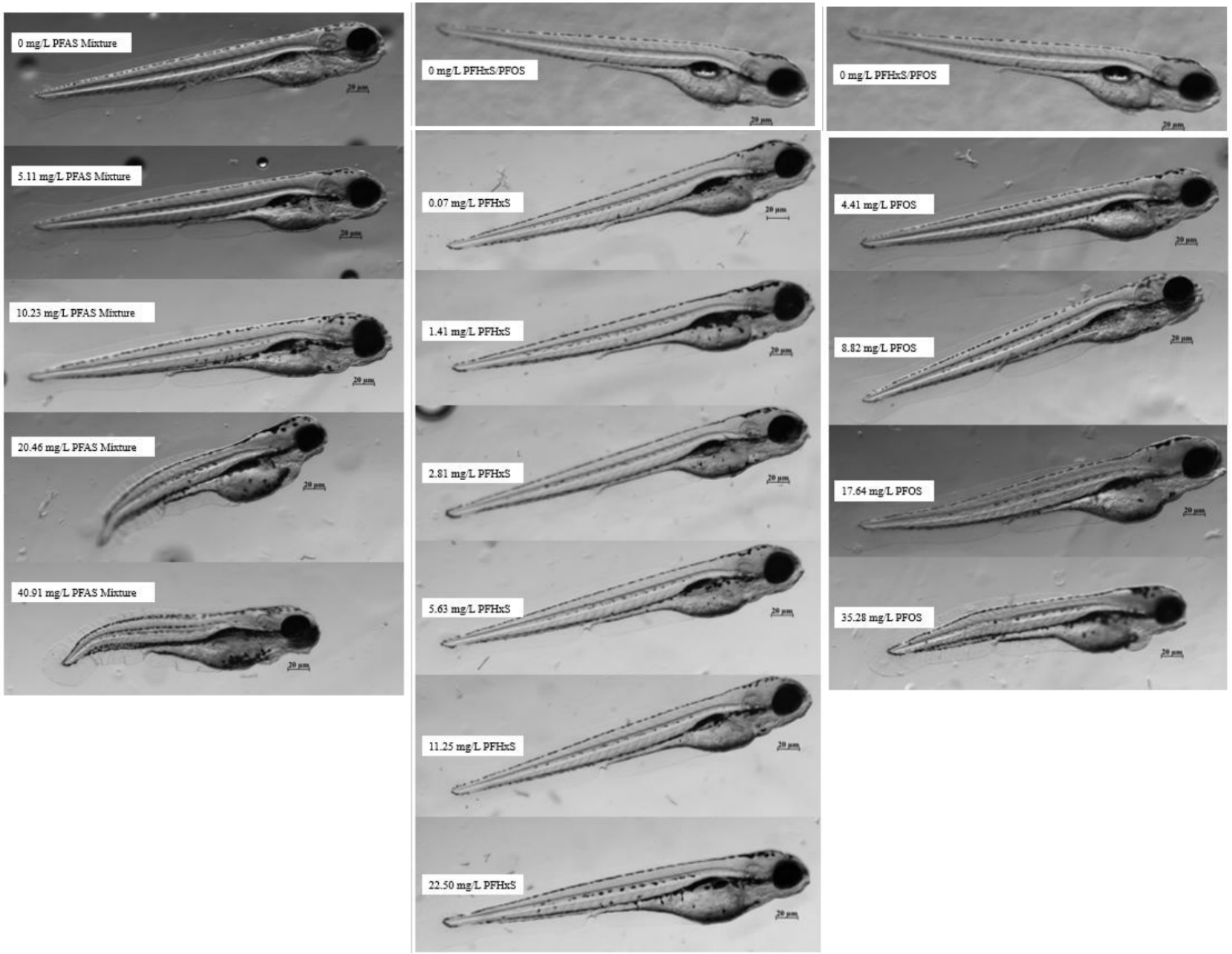
- Growth
- Yolk sac utilization
- Gross deformities
- Internal organ formation







Annunziato et al., EHP 2020



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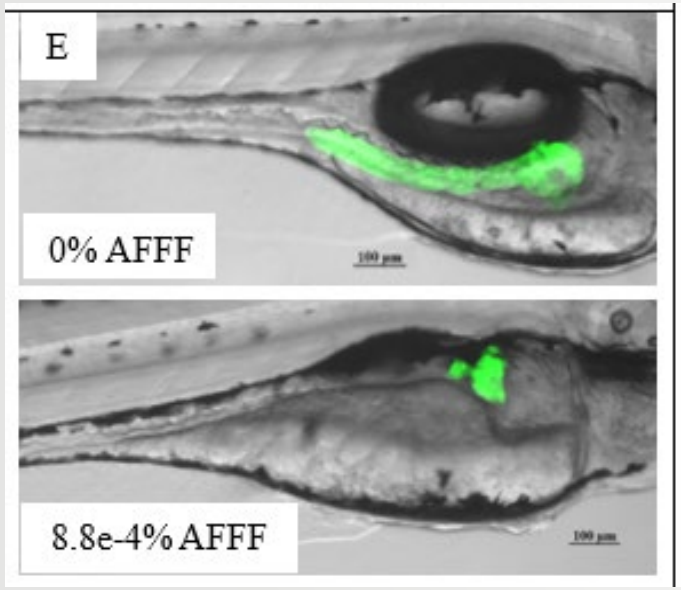
EXOCRINE PANCREAS TRUNCATION

AFFF



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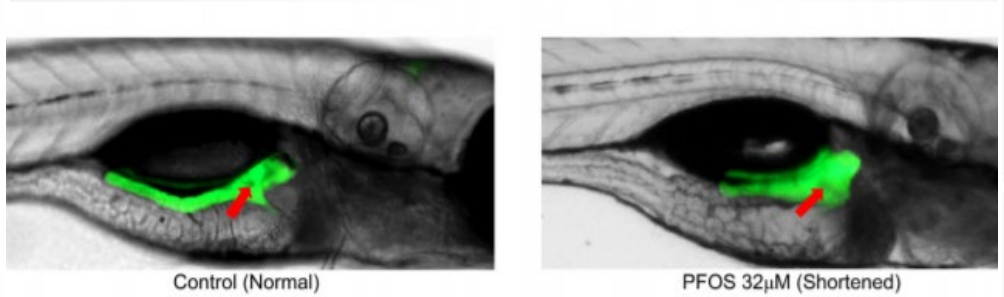
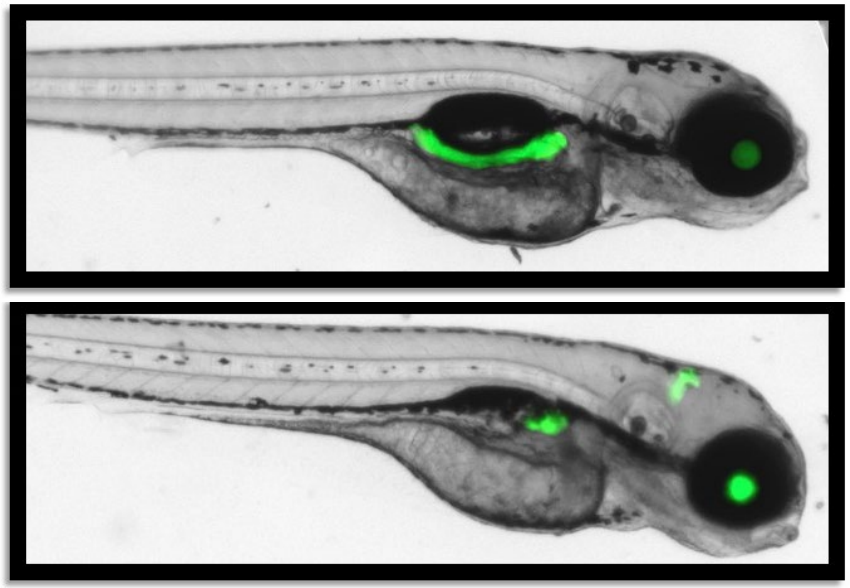
PFOS



Partial pancreatic agenesis

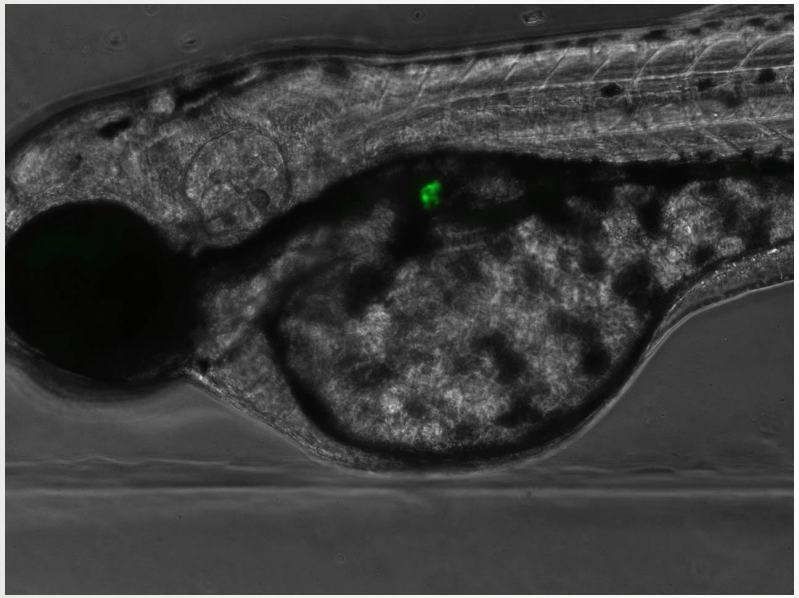
Sant et al., Env. Poll 2017

PFBS

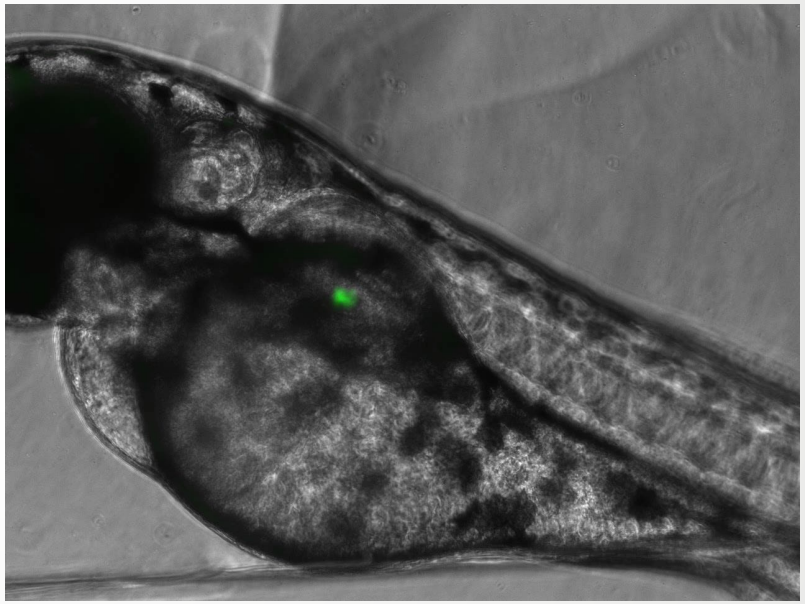


Sant et al., Tox Sci 2019

ABNORMAL BETA CELL DEVELOPMENT

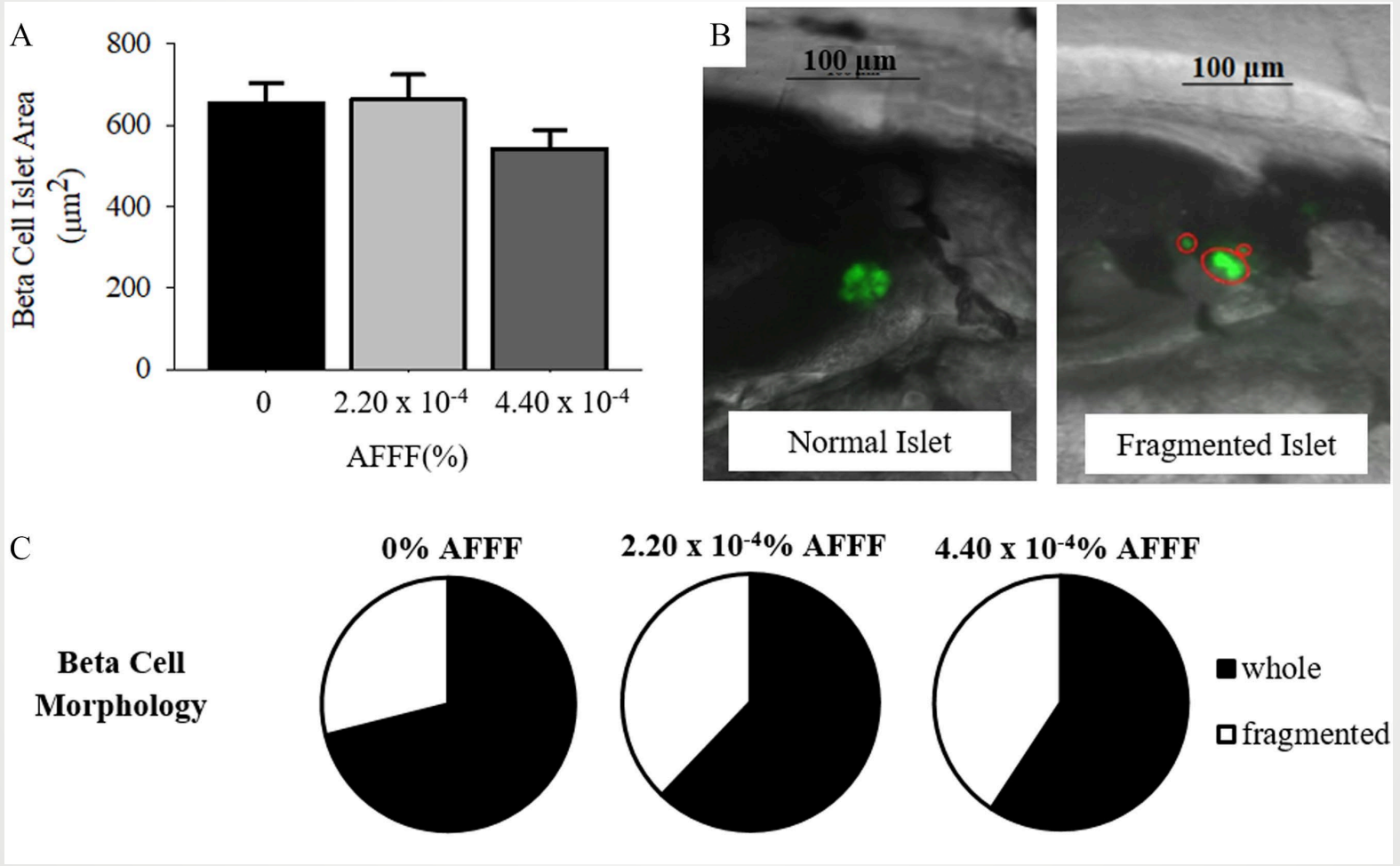


Normal



Fragmented

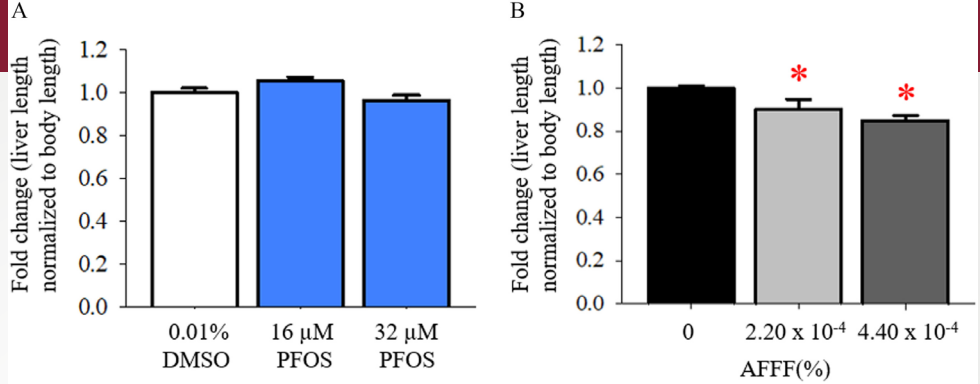
FRAGMENTED ISLET DEVELOPMENT

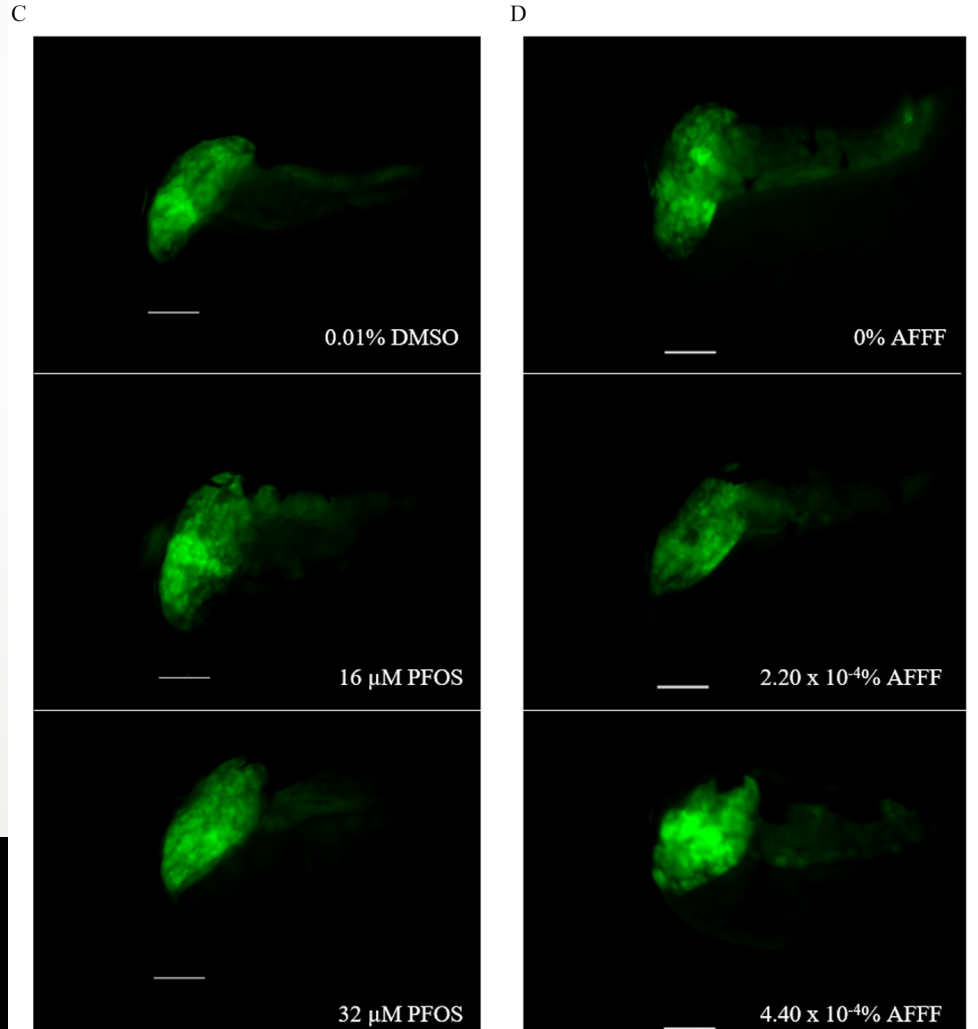


Annunziato et al., EHP 2020

LIVER DEVELOPMENT

- **AFFF impeded liver growth in embryos**
- **PFOS did not affect liver growth**





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Annunziato et al., EHP 2020

PART 3: IS PFOS CONCENTRATION A GOOD PREDICTOR OF AFFF MIXTURE TOXICITY?

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AFFF COMPARISON TO PFOS

	AFFF (%)			PFOS (µM)	
	2.20e-4 (1.38 µM PFOS)	4.40e-4 (2.76 µM PFOS)	8.80e-4 (5.52 µM PFOS)	16	32
Body Length	↔	↓	↓	↔	↓
Yolk Sac Area	↔	↔	↔	↓	↔
Liver Length	↔	↓		↔	↔
Beta Cell Islet Area	↔	↔		↓	↓
Beta Cell Aberrant Morphology	↔	↑		↑	↑
Stunted Exocrine pancreas	↔	↑	↑	↑	↑

TAKE-AWAYS

- **Subtle developmental effects can be informative (liver, pancreas) and differ from LC50s**
- **AFFF is more acutely toxic that PFOS alone**

- **Knowledge gaps:**
  - Mixture toxicity
  - Mechanisms
  - Critical exposure windows

Marjorie Marin

Dr. Kate Annunziato

PRECONCEPTION AS A CRITICAL WINDOW OF EXPOSURE

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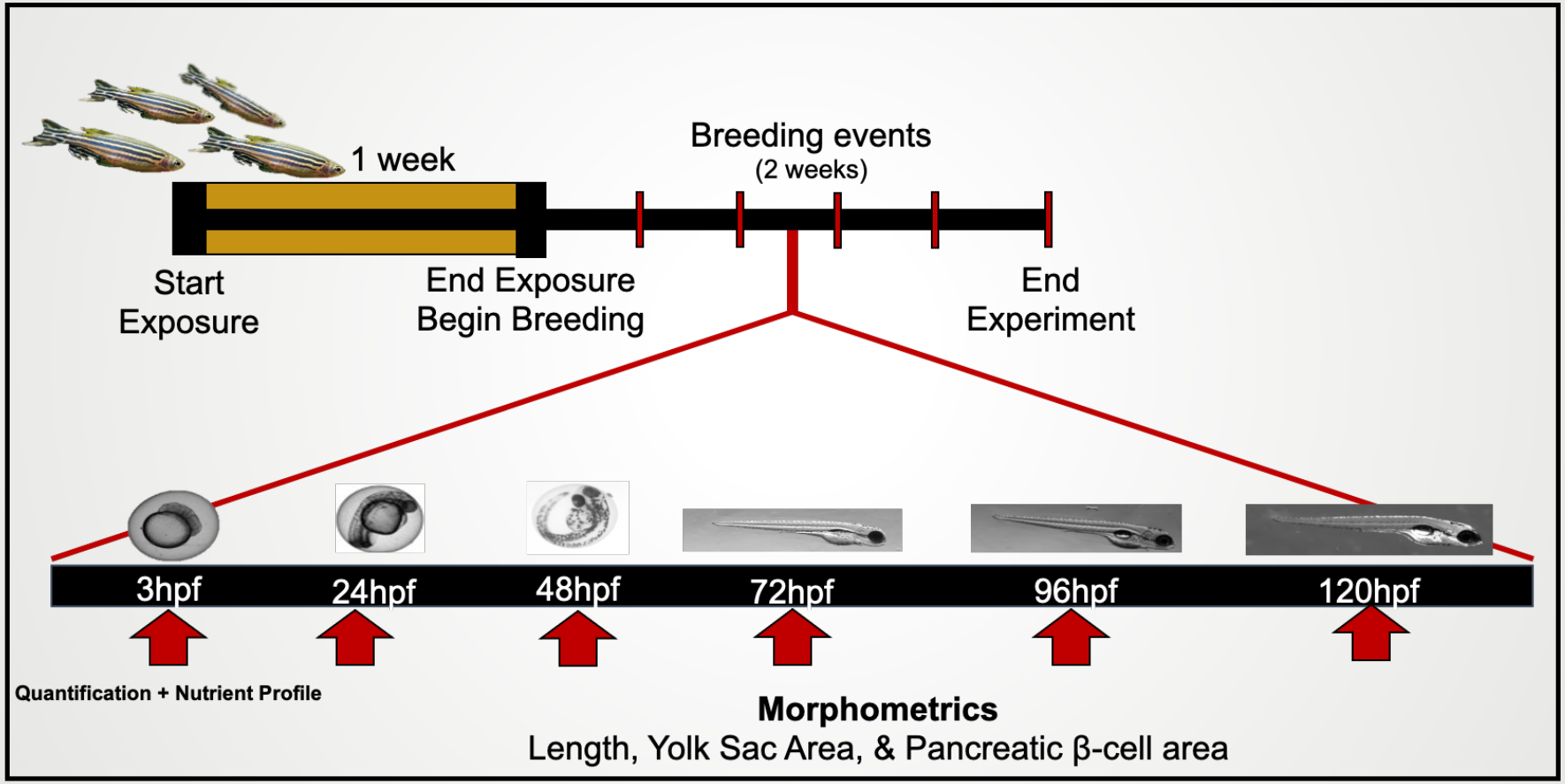
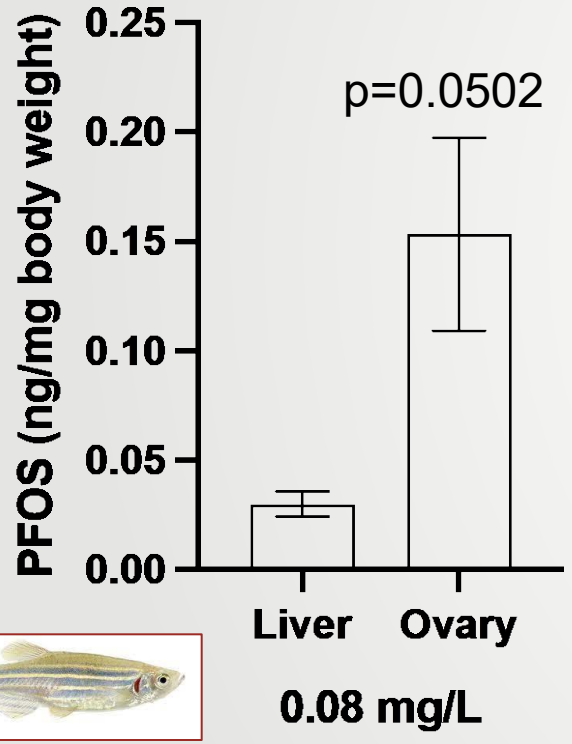
Exposure Paradigm



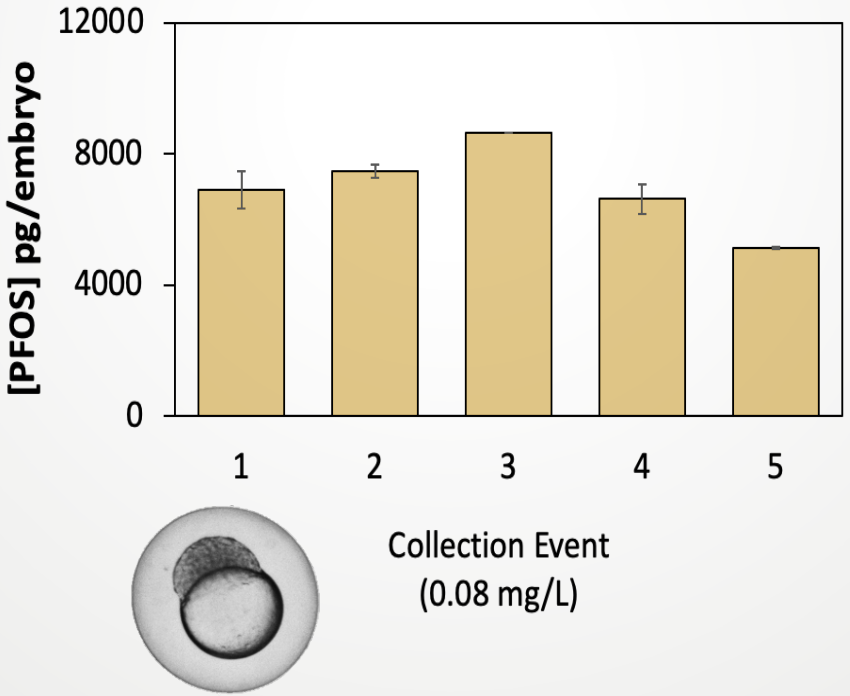
PFOS MEASUREMENTS

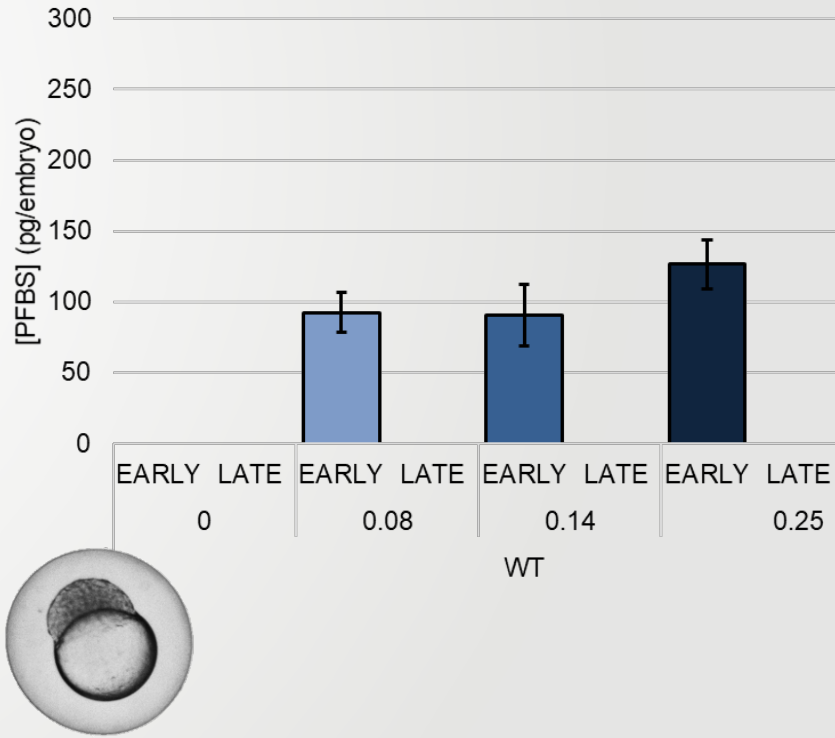
✓ **PFOS levels are higher in the ovaries than liver.**



Preliminary data, n= 3 females, t-test.

✓ **PFOS is loaded into the eggs across all breeding events but PFBS is depurated**



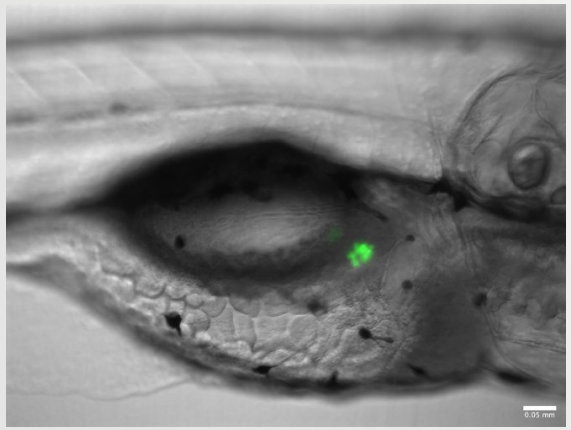


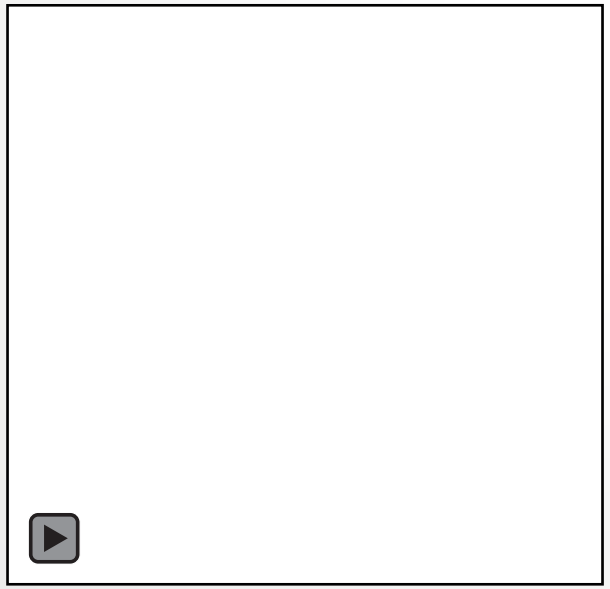
Marin et al., in prep
Annunziato et al., Chemosphere 2021

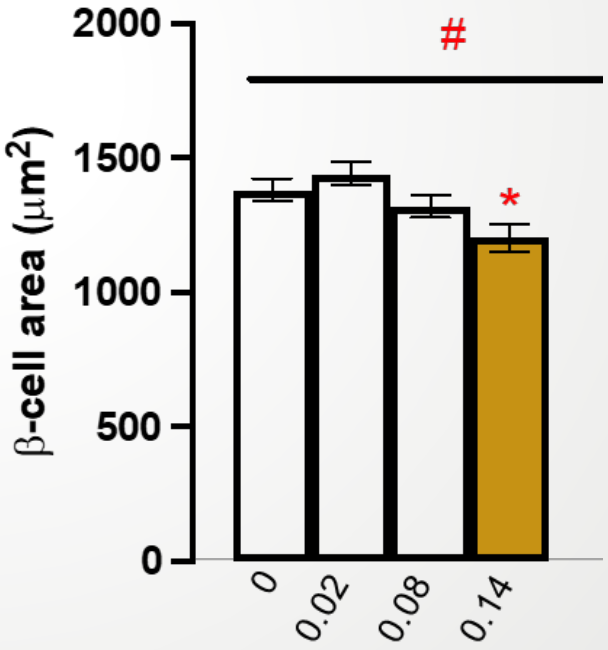
PANCREATIC β-CELL DEVELOPMENT

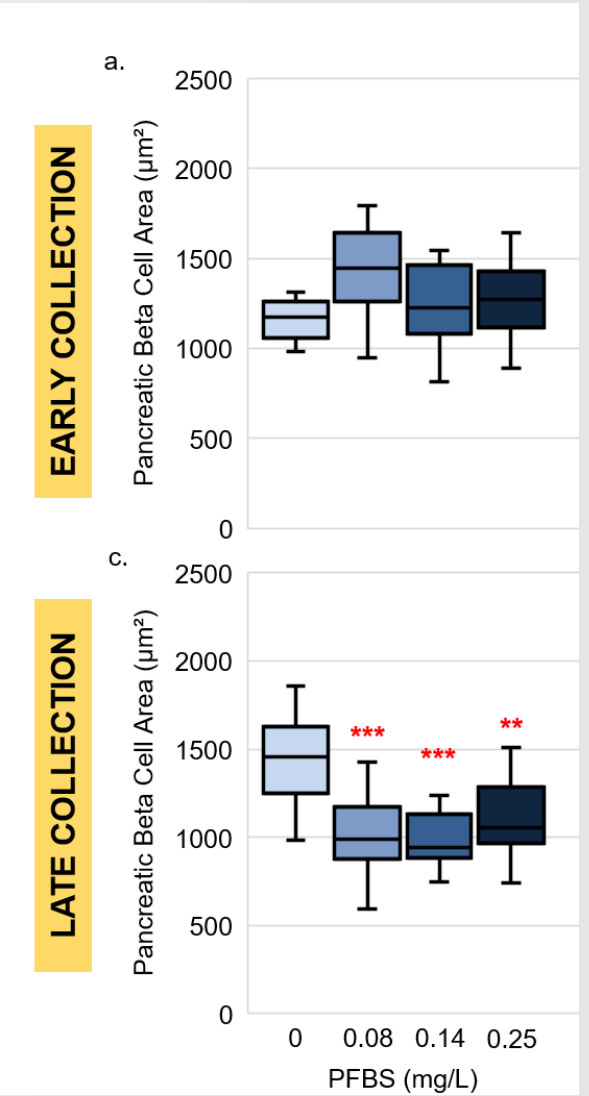
At 5DPF:

- ✓ **embryos from 0.14 PFOS group have a decreased islet area compared to controls.**
- ✓ **PFBS maternal exposure only affected islet growth after depuration had occurred- lasting metabolic impacts?**









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Marin et al., in prep
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TAKE-AWAYS

- When the exposure occurs matters
- PFAS with shorter half-lives can have lasting impacts on lipid health even after depuration

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IALS Models to Medicine Center

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Dr. Monika Roy
Chris Clark

Collaborators:

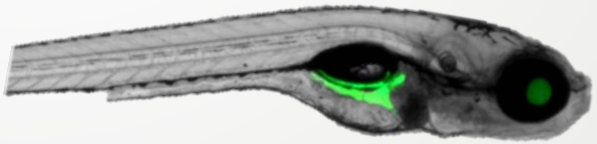
Dr. John Clark
Dr. Jeff Doherty
Dr. Yeonhwa Park

Community Partner:

WRAFT (Westfield Residents Advocating For Themselves)

NIH National Institute of Environmental Health Sciences

R01ES025748, R01ES028201, F32ES028085, F31ES03097, R21ES033532



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BTP UMass Biotech Training Program

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