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Sources, Transport, Exposure & Effects of PFASs

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PFAS Mixtures & Liver Adverse Outcomes: Finding PFAS Bad Actors

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Perfluoroalkyl substances (PFASs) and concerns

- Highly persistent in environment
- Long half-lives, especially for PFOA, PFOS, and PFHxS
- 7000+ PFAS – how do we assess safety? Toxicokinetics?
- The mechanisms that dictate tissue distribution, clearance, and half-life are not well understood – even for PFOA and PFOS.
  - Highly absorbed, yet slowly eliminated
  - Cross placental barrier, detectable in breast milk
- Do not occur individually. We are exposed to complex mixtures, which are challenging to recapitulate.

PFAS	Half-life in Humans	
PFBS	665 hours (3)	
PFHxS	8.5 years (1)	*
PFOS	5.4 years (1)	
PFBA	81 hours (2)	
PFHxA	32 days (5)	
PFHpA	1.2 years (4)	
PFOA	3.8 Years (1)	
PFNA	4.3 years (4)	*
PFDA	12 years (4)	**
PFUnDA	12 years (4)	**
PFDoDA	Unknown	
PFPrOPrA	Unknown	

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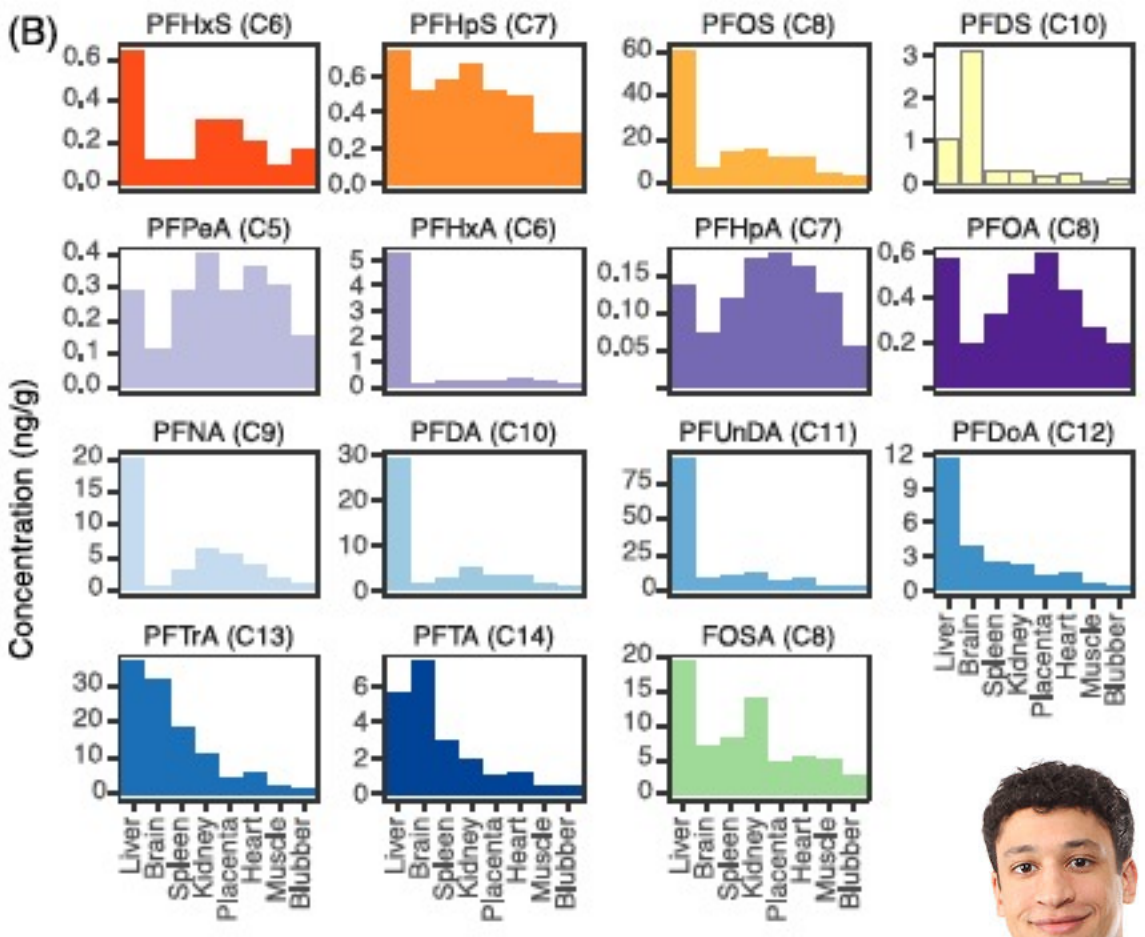
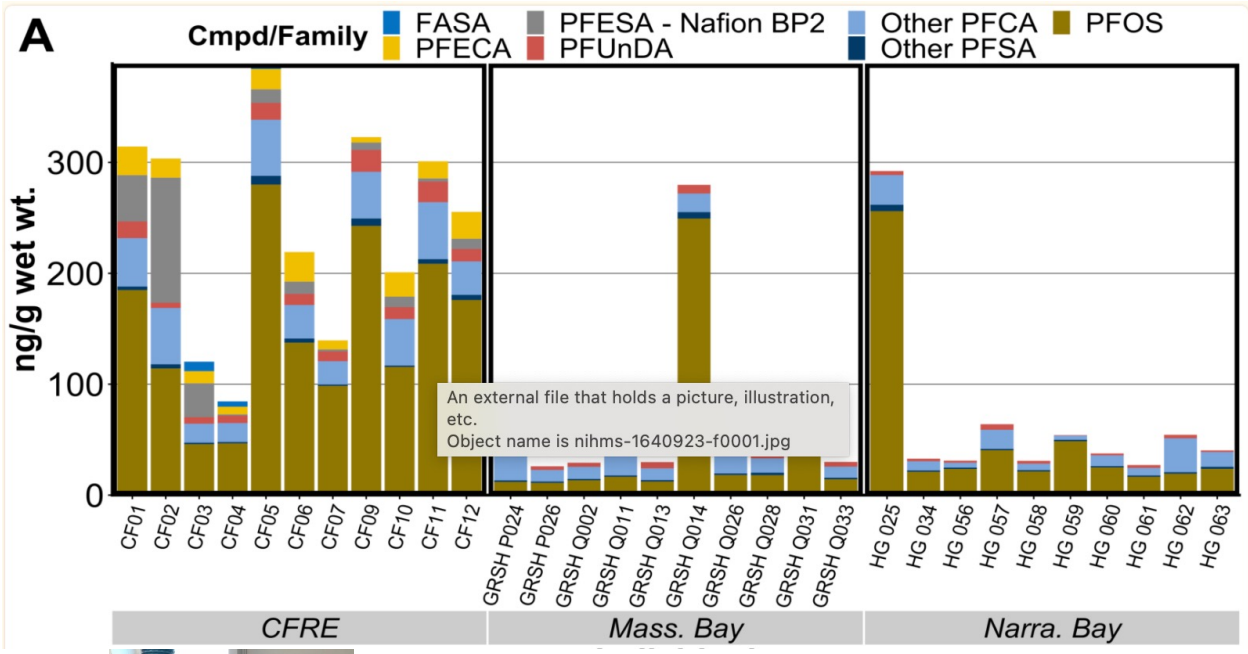
Emerging PFAS are also detected in pilot whale liver and livers of juvenile marine birds





Robuck, A. R . Et al., (2020). 54(20), 12938–12948. https://doi.org/10.1021/acs.est.0c01951

Dassuncao et al., (2019). *Environ. Sci. Technol. Lett.* 6, 119-125.

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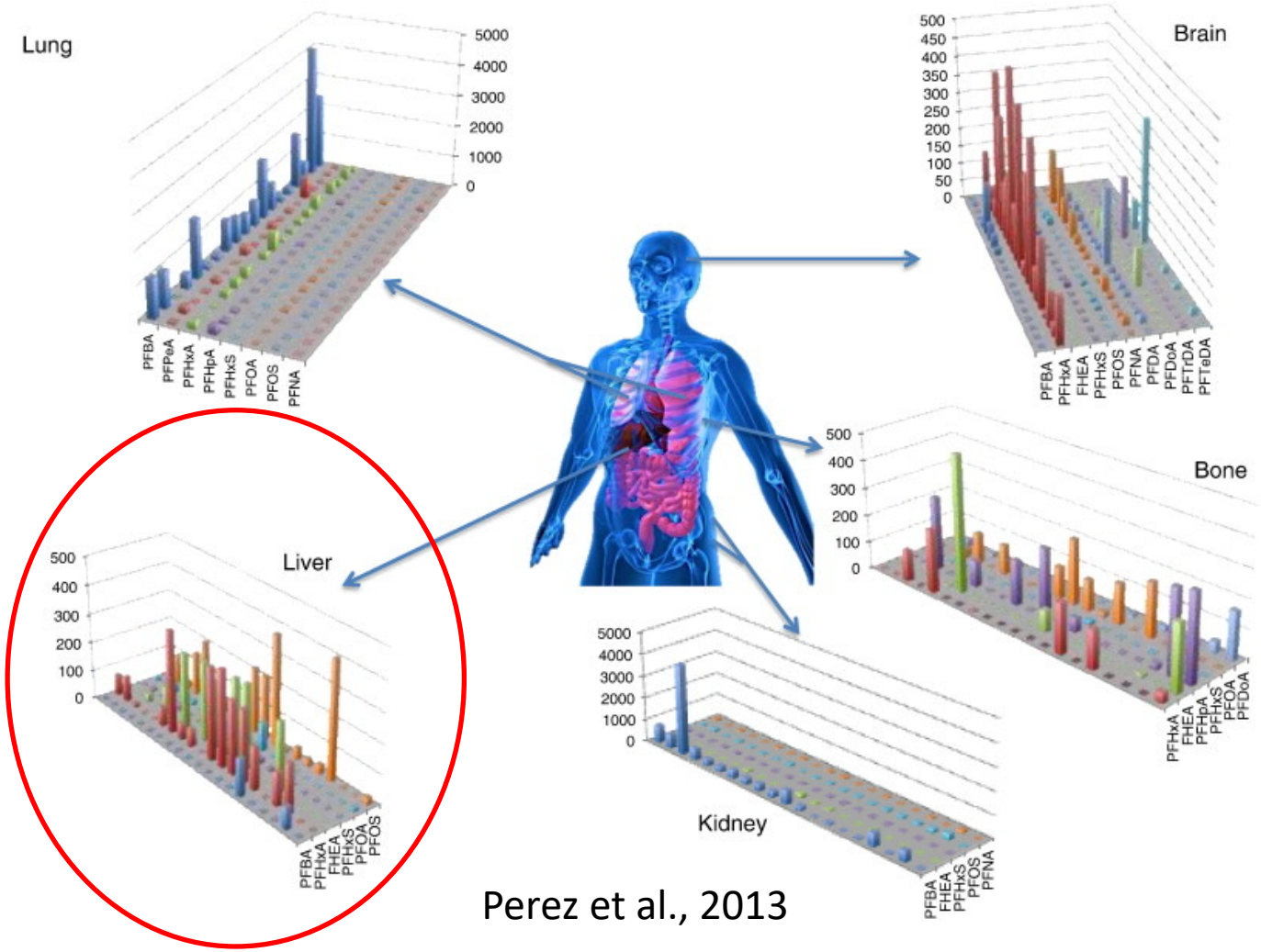
PFAS Effects and Liver

- Liver is the tissue that often is for distribution and accumulation. Relatively high concentrations, likely due to transporters and high tissue binding.
- Associations with slight elevation in serum liver enzymes (multiple studies – human and rodent).
- Rodents demonstrate hepatomegaly (liver enlargement and cancer). This endpoint is widely debated about relevance to humans because of species differences in PPAR-alpha signaling.
- Liver is a depot for **multiple** PFAS. In humans, ~400 ng/g PFOA and ~600 ng/g PFHpA observed in livers from residents of Catalonia, Spain.
- *Little is known about PFAS mixture and adverse liver effects.*



Perez et al., 2013

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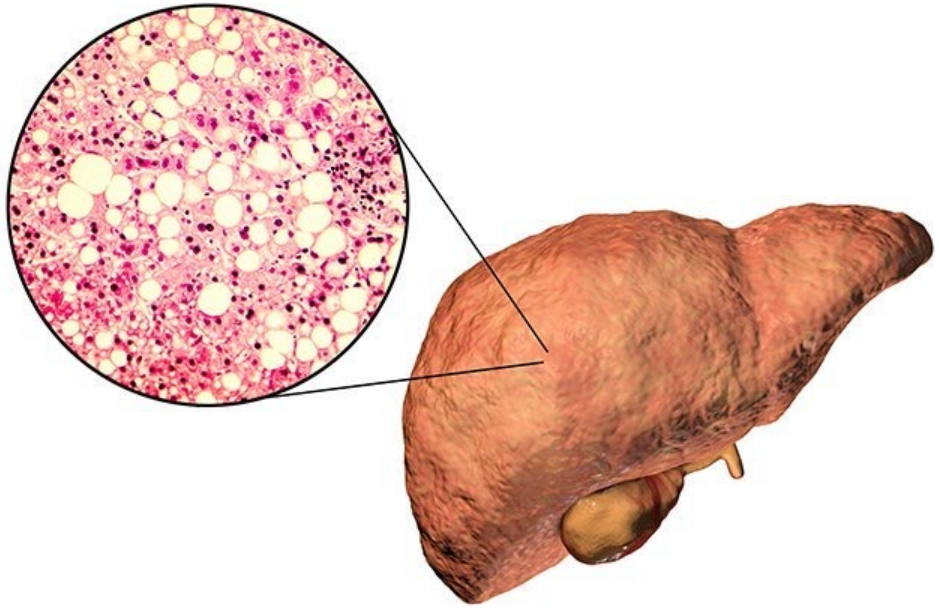
Some PFAS Increase Rick Liver Injury and Steatosis

- Non-alcoholic fatty liver disease (NAFLD) is a rising health issue globally. Aside from potential damage to the liver, hepatic steatosis and NAFLD can increase risk for cardiovascular disease.
- Epidemiological data has found positive associations between PFOS and PFOA and biomarkers of liver injury[1,2,3,4.]
- It is unclear if the liver diseases occur in humans, but associated with elevated markers for liver injury.
- Positive associate of PFAS association with CK18 (marker for NASH).
- Rodent models illustrate increased steatosis with PFOA, PFOS at high doses (> 1mg/kg) and increased liver injury.
- Higher PFAS exposure was associated with more severe disease in children with NAFLD[8]
- Little is known about how maternal exposure can affect liver outcomes, but higher exposure to PFAS during pregnancy has been associated with higher liver enzyme levels in children[9]



1. Gallo et al, Environ Health Perspect, 2012, 120(5); 655-661
2. Gleason et al.Environ Res 2015; 136, 8–14.
3. Lin et al. Am J Gastroenterol. 2010;105(6):1354-1363.
4. Darrow et al, Environ Health Perspect. 2016 Aug; 124(8): 1227–1233.
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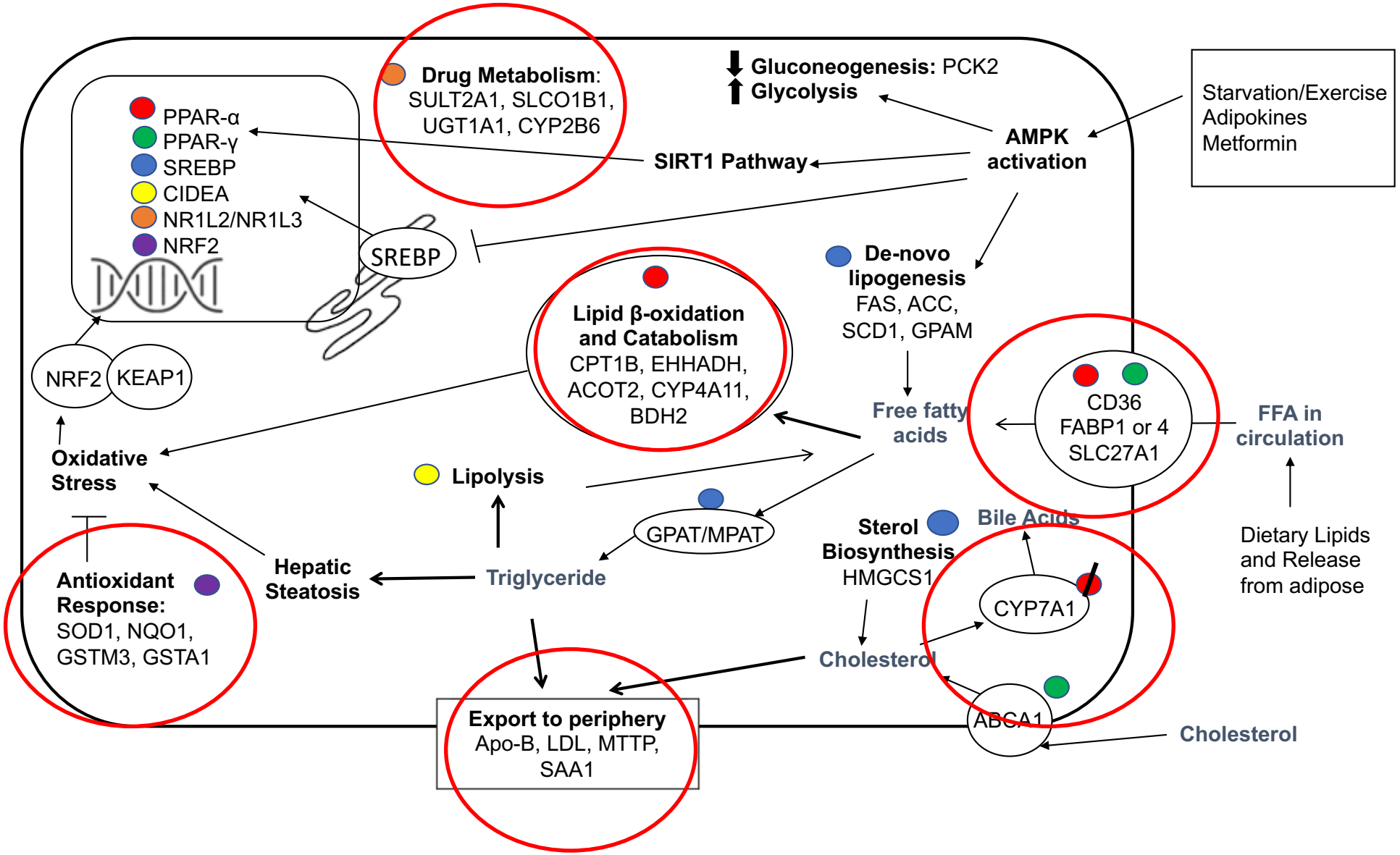
6. Botelho et al.Chemosphere 2015;*129*, 225–231.
7. Seacat et al, Toxicol Sci. 2002, 68(1):249-64.
8. Jin R et al., *Environ Int.* 2020 Jan;134:105220. doi 10.1016/j.envint.2019.105220.
9. Stratakis et al., Hepatology, 2020, 72(5):1758-177

Mechanisms of steatosis



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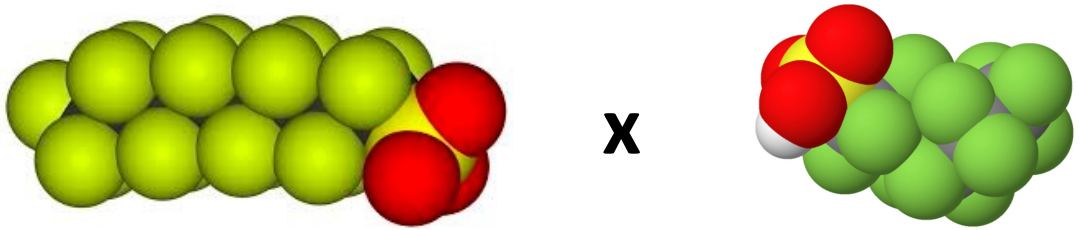
Hypotheses

- ✓ **PFAS mixtures will act synergistically to induce steatosis**
- ✓ **Maternal consumption of a high fat diet in combination with PFAS will induce adverse liver outcomes in pups**



PFAS x PFAS (PxP) Interaction

Diet x Exposure (DxE) Interactions

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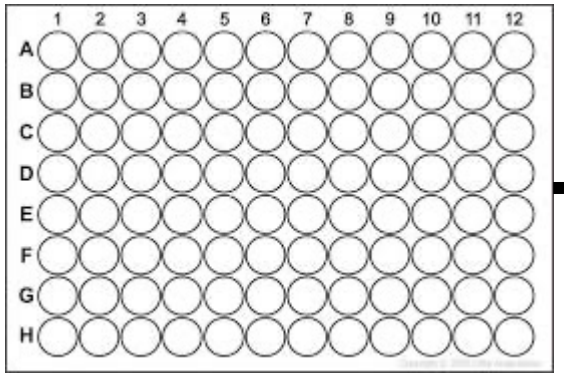
Testing PFAS in Human Hepatocytes

Cryostax 5-donor hepatocytes



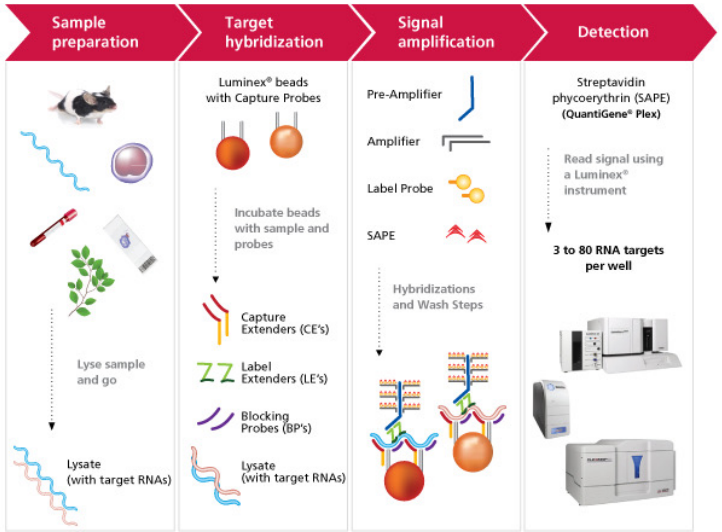
24h

Treatment with:
DMSO (vehicle), various PFAAs and mixtures of PFAAs at concentrations of 0.25 µM to 25 µM with daily media changes of treatments.

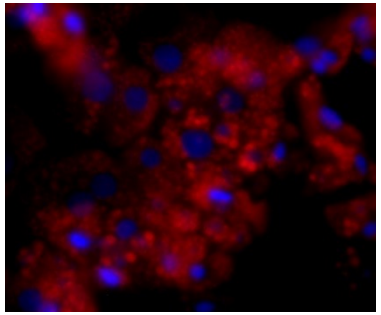
48h

72h

Gene expression analysis



Stain Nile-red



Targeted gene array from Invitrogen for 35 genes related to lipid and Drug metabolism

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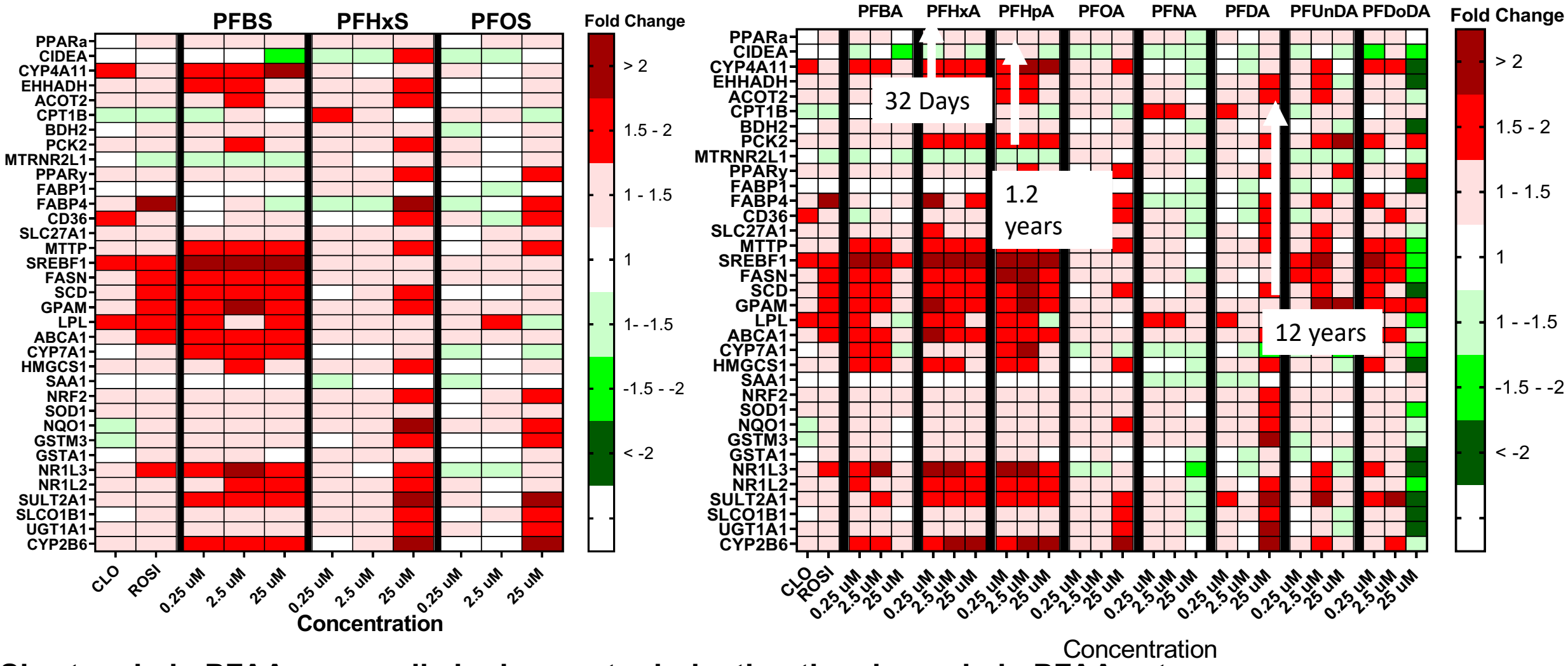
PFASs Induce Lipogenic Gene Expression in Human Hepatocytes



✓ **Shorter chain PFAAs generally had a greater induction than long chain PFAAs at lower concentrations in both sulfonates and carboxylic acids**

Marques et al., *Toxicol. Appl. Pharmacol.*, 2022

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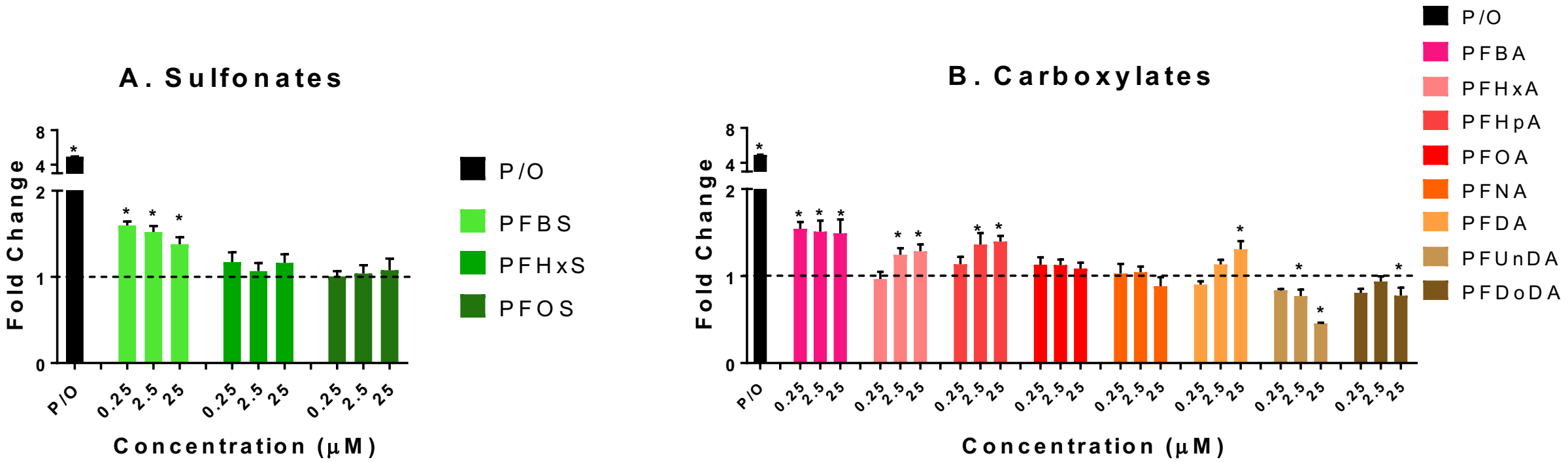
PFASs are capable of inducing lipid accumulation in human hepatocytes



- ✓ **Only short chain PFAAs (except PFDA) induced liver lipid accumulation in our assays.**
- ✓ **Lipid accumulation is limited by glucose content in the media**

Marques et al., *Toxicol. Appl. Pharmacol.*, 2022

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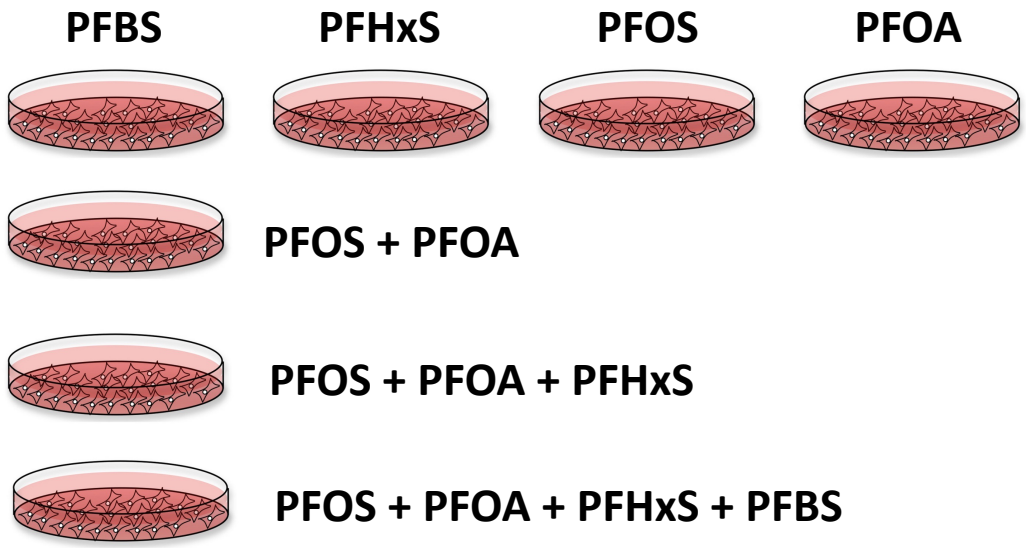
Testing PFAS mixtures at health advisory levels

- **Mix data suggested that the concentrations were too high or perhaps the PFAS negate each other's effects.**
- **Ultra low (pM) to high concentrations (um)**
- **Stack the treatments**
- **Selected PFAS commonly detected in Rhode Island drinking water and Cape Cod private well water.**



Slitt and Marques, *unpublished*

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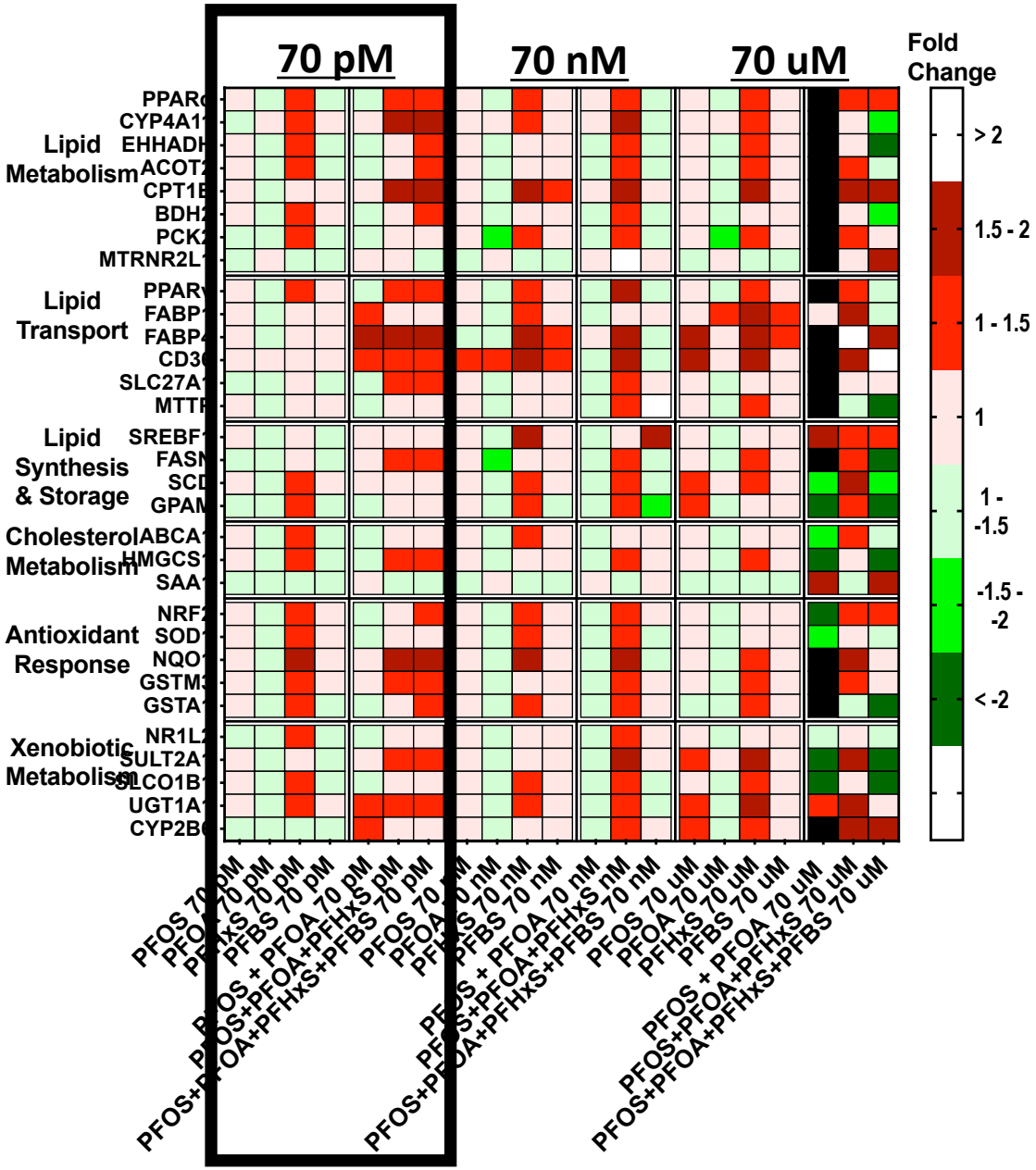
Additive effects are observed at exposure relevant concentrations in Human Hepatocytes

- Cryopreserved human hepatocytes. 70 pM-70 μM
- Treated 6 hours after plating (vs. ~16 hours)
- Treated for 48 hrs total
- Gene expression occurred at changes at 70 pM
- Gene expression changes were observed at 70 pM but not at 70 nM or μM.
- Strong activity for PFHxS



Slitt and Marques, *unpublished*

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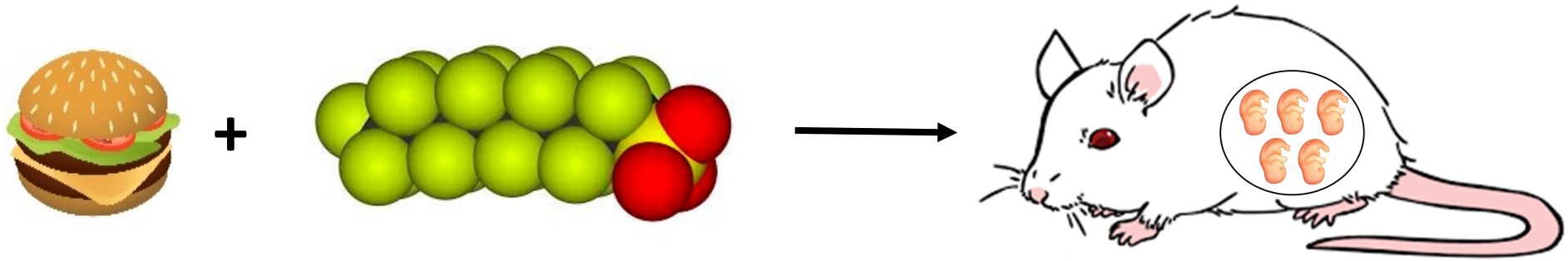
Conclusions – In Vitro

- PFHxS has the potential to be transcriptionally active in cryopreserved human hepatocytes and at exposure-relevant media concentrations.

- There is potential additivity at very low PFAS concentrations (pM)

- Human hepatocyte culture conditions seems to be important for detecting PFAS activity.

An in vivo approach to mixtures



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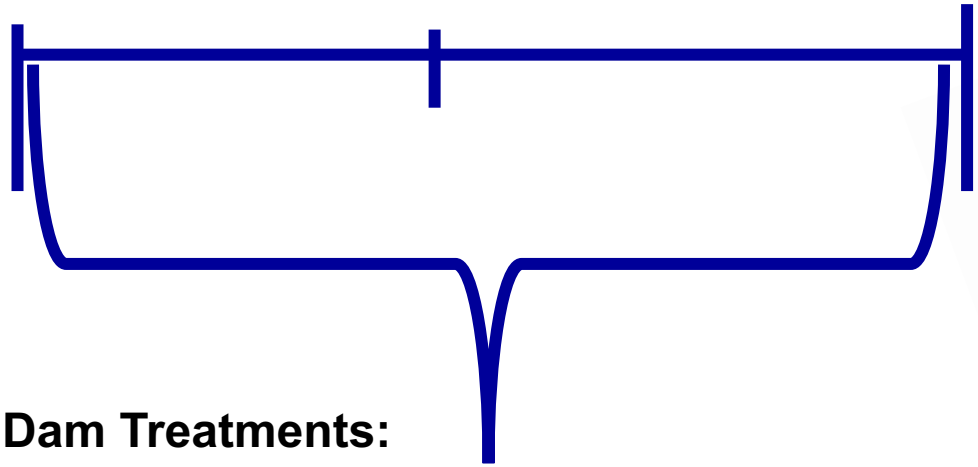
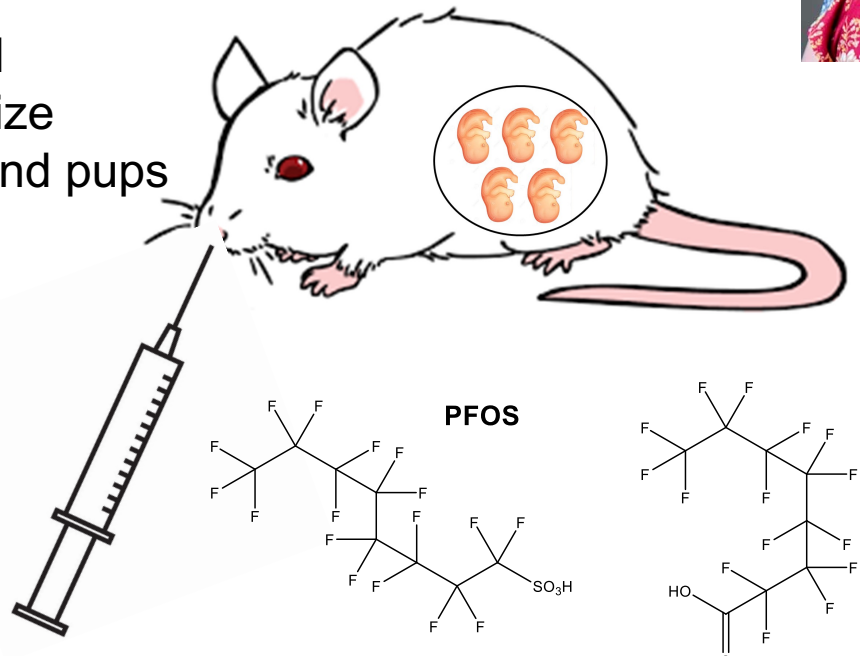
Study Design

Timed-pregnant
Dams arrive (GD1)
Assign Treatment groups
(n=5)

Birth
GD18 or 19
PND 0

PND 21
Euthanize
dams and pups





SD

HFD

Dam Treatments:
(oral gavage)

- Veh: 0.5% Tween 20
- PFOA- 1 mg/kg
- PFOS- 1 mg/kg
- PFHxS- 1 mg/kg
- PFAS mix

PFAS mix (1:1:1):
1 mg/kg of each
PFOA
PFOS
PFHxS

Marques et al., *Toxicology*, 2022

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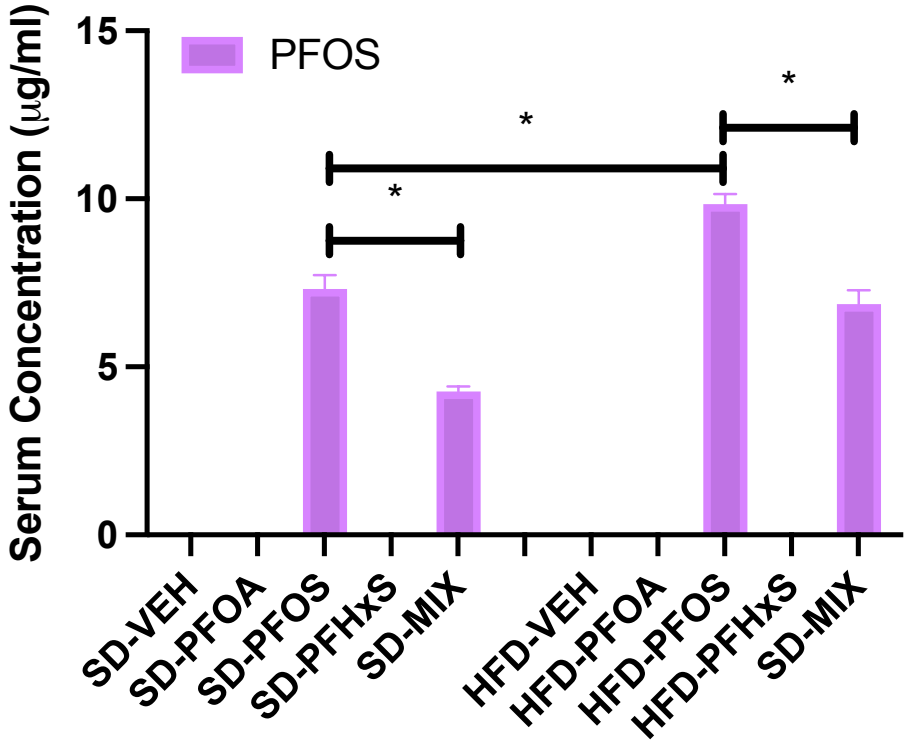
PFAS concentrations in Dams



PFHxS > PFOA > PFOS

HFD vs. SD

PFOS	**7.32 ug/ml ± 0.82 vs. 9.84 ug/ml ± 0.61***
PFOA	**115.2 ug/ml ± 09.23 vs. 139.2 ug/ml ± 8.43**
PFHxS	**212.9 ug/ml ± 16.2 vs. 225.6 ug/ml ± 25.2**

PFAS Mix versus PFAS

PFOS — **SD and HFD**

Marques et al., *Toxicology*, 2022

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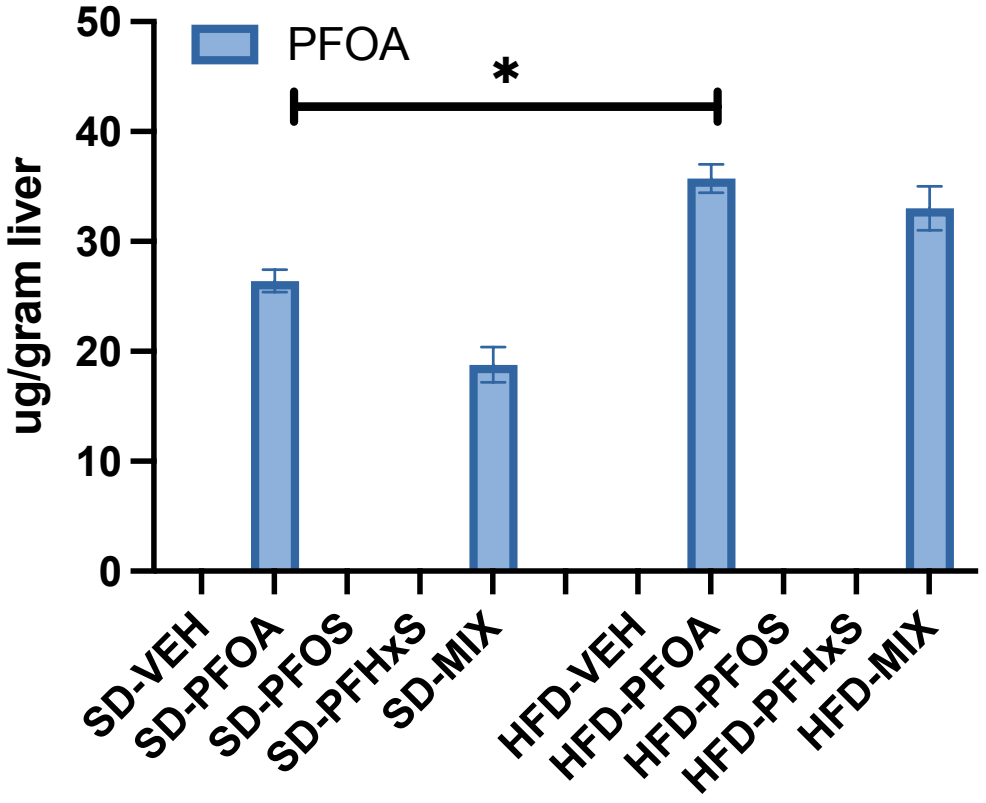
PFAS concentrations in PND21 Offspring Liver



PFOA > PFHxS > PFOS

HFD vs. SD

PFOS ⟷

PFOA ↑

PFHxS ⟷

PFAS Mix versus PFAS

PFOS **SD** ↓

Marques et al., *Toxicology*, 2022

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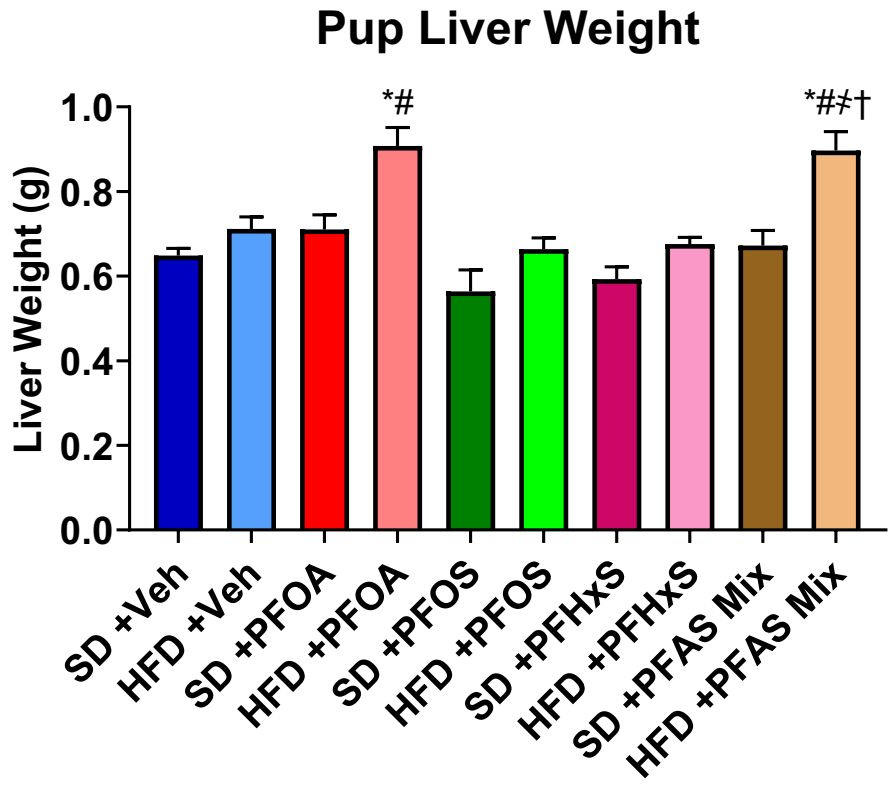
Dam and Pup Liver Weights



- **PFOA and PFAS mix treatments increased liver weights in pups**

* = p<0.05 versus SD+Veh
# = p<0.05 versus HFD+Veh
§ = p<0.05 versus PFOA and PFAS mix within each diet
≠ = p<0.05 versus PFOS and PFAS mix within each diet
†= p<0.05 versus PFHxS and PFAS mix within each diet

Marques et al., *Toxicology*, 2022

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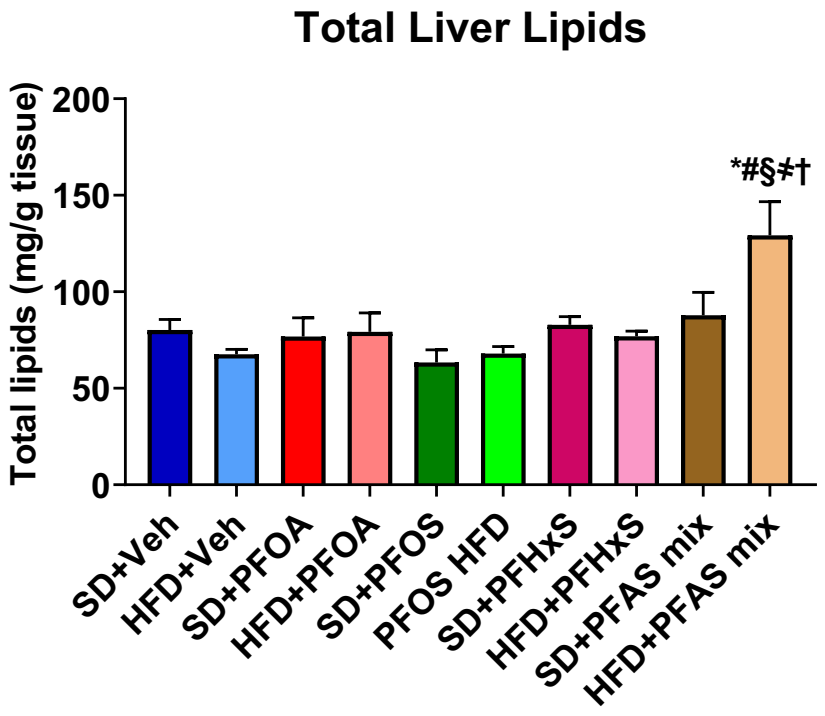
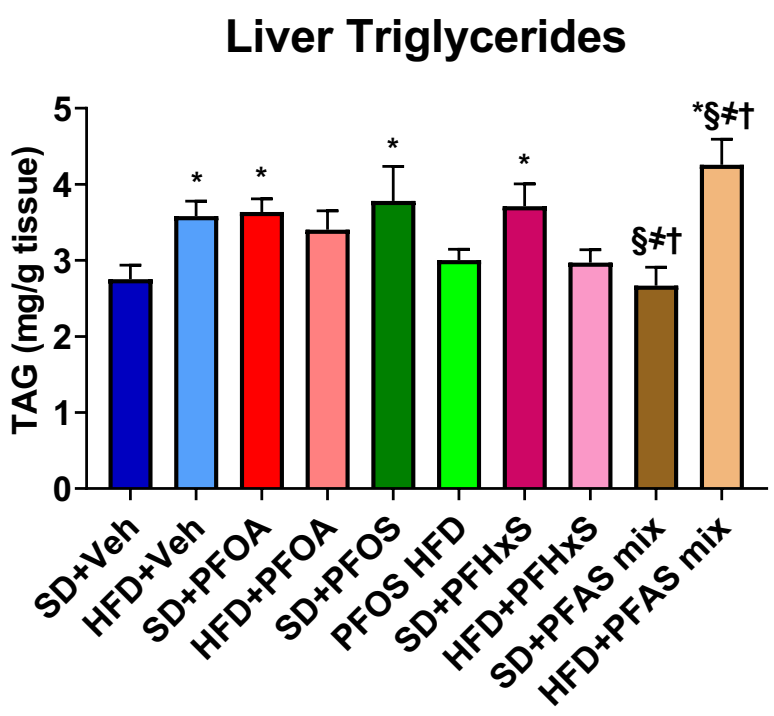
The PFAS mix increased liver total lipid content and TAGs in pup livers





- **Livers of HFD-PFAS mix offspring had the highest total lipid content**
- **SD-PFOA, -PFOS, and -PFHxS offspring had higher livers TAGs, which was not observed in the HFD offspring**
- **HFD-PFAS mix offspring had the highest liver TAGs**

* = p<0.05 versus SD+Veh
\# = p<0.05 versus HFD+Veh
§ = p<0.05 versus PFOA and PFAS mix within each diet
≠ = p<0.05 versus PFOS and PFAS mix within each diet
†= p<0.05 versus PFHxS and PFAS mix within each diet

Marques et al., *Toxicology*, 2022

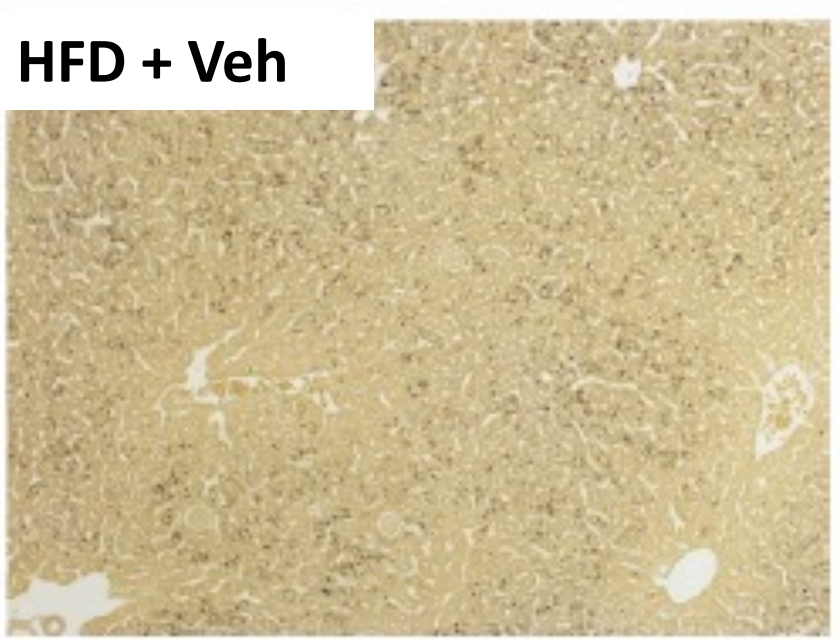
PFAS Mix increased hepatic steatosis in pups

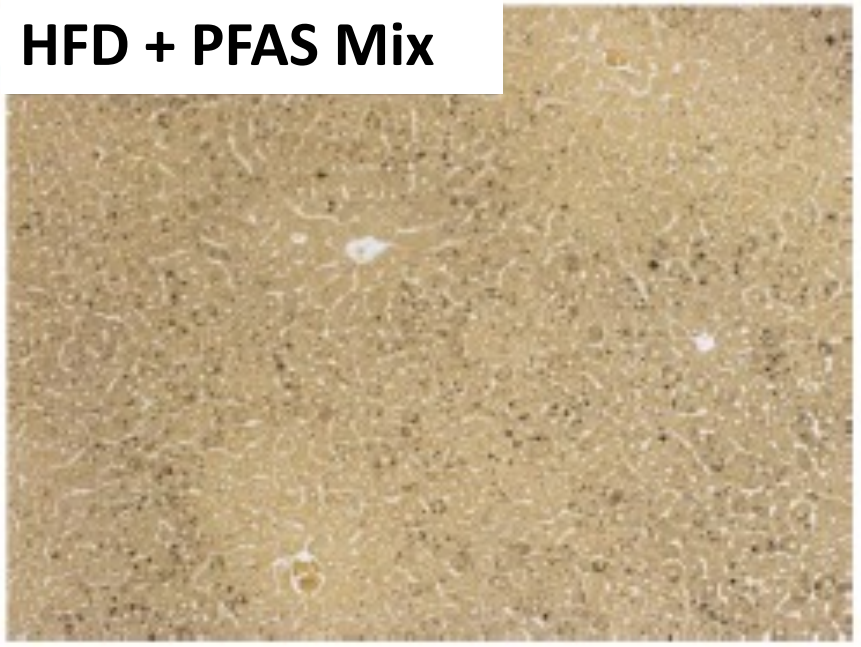
HFD + PFOA ↓

HFD + PFOS ↔

HFD + PFHxS ↓

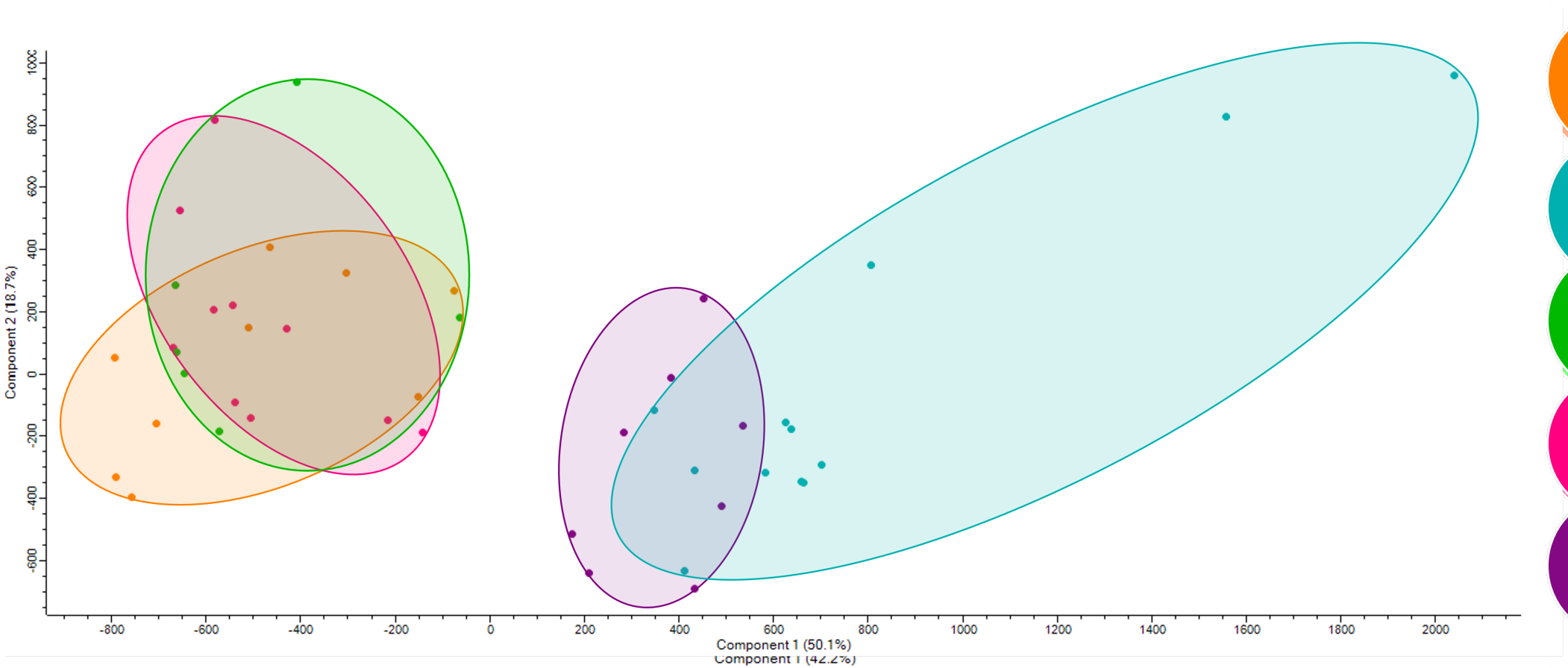
HFD + Mix ↑





Marques et al., *Toxicology*, 2022

PFOA and PFAS Mix markedly shift the proteome in offspring livers



Kaye et al., *In preparation.*

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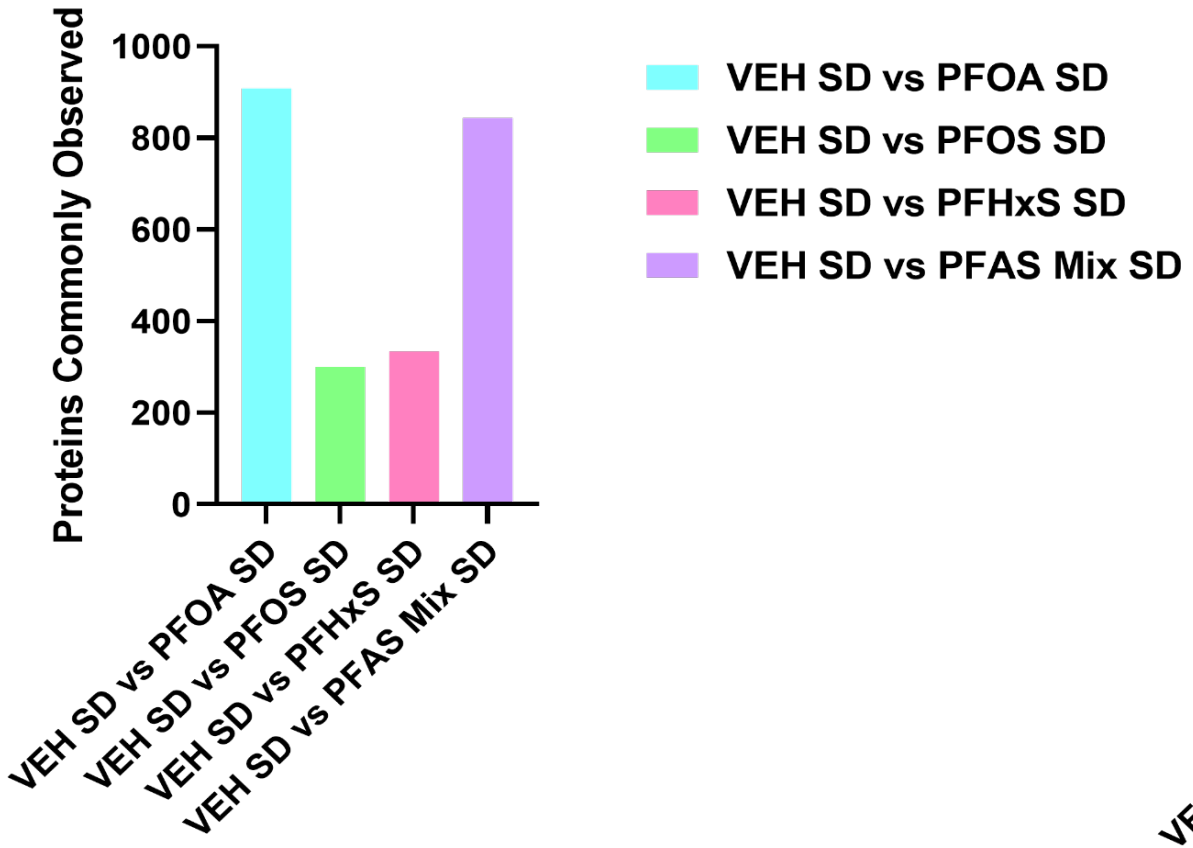
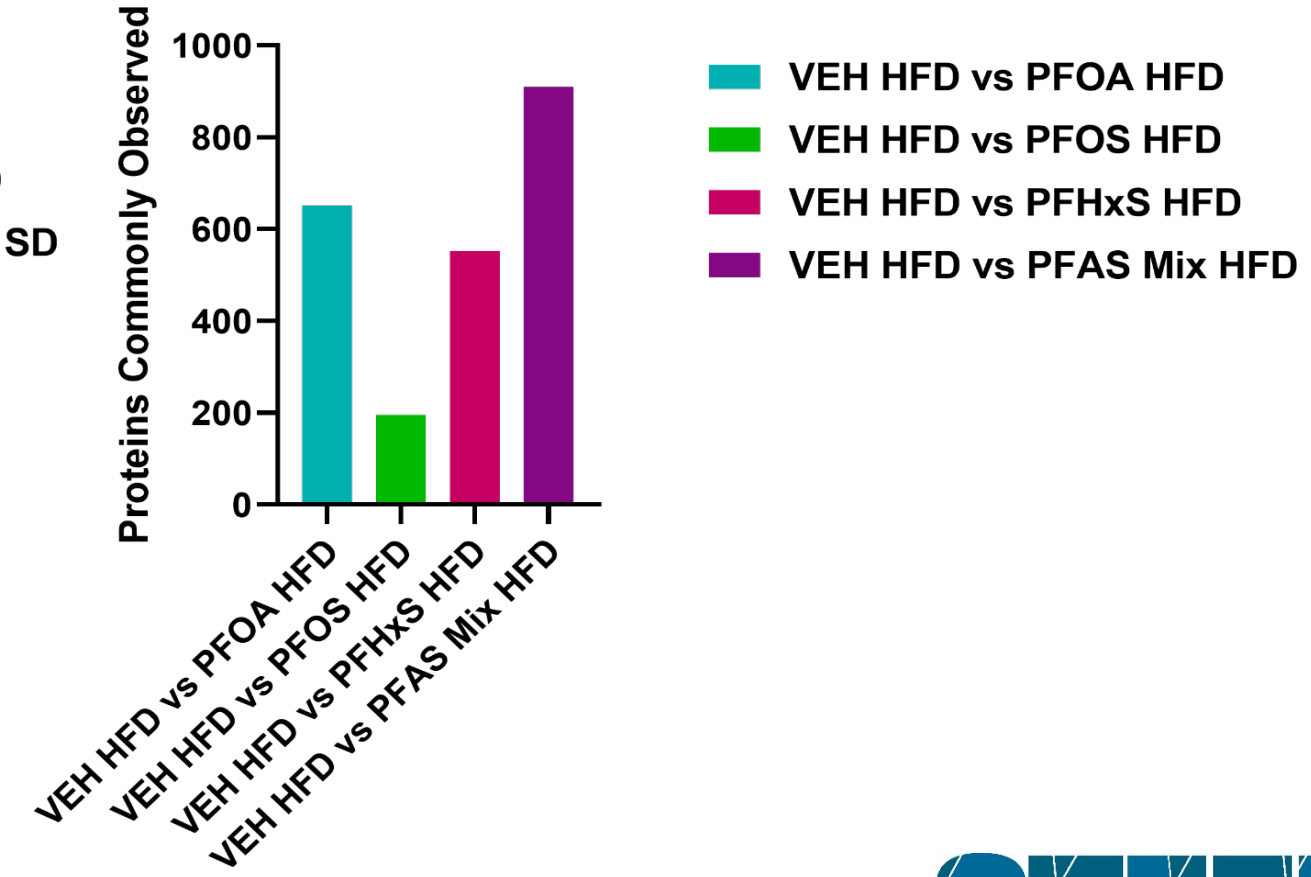
PFOA and a PFAS Mix induce the highest number of protein changes in offspring liver





Kaye et al., Abstract #3080 , *In preparation.*

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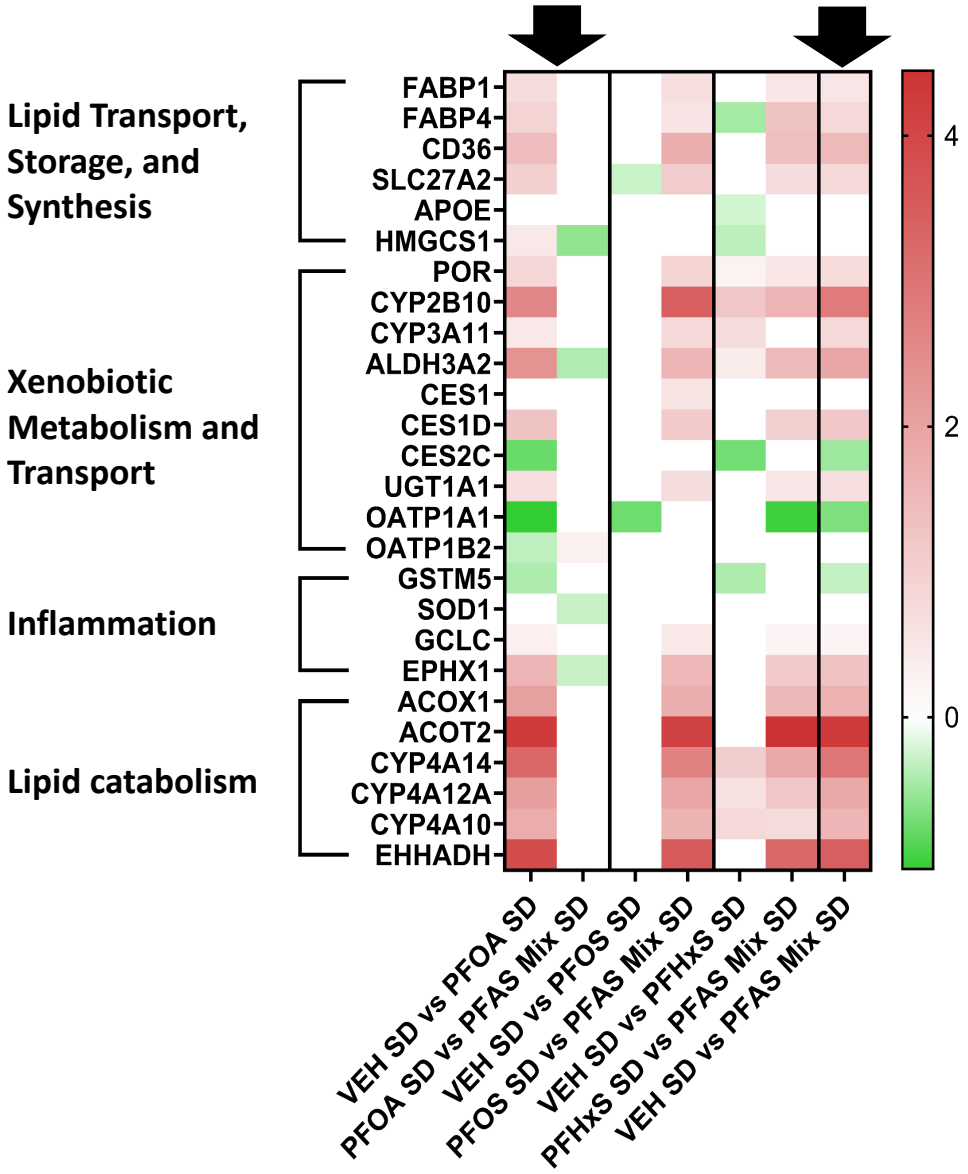
PFOA and a PFAS Mix induce the highest number of protein changes in offspring liver

- PFOA and PFAS mix increase lipid catabolism, transport, and synthesis pathways.
- PFHxS some activity
- PFOS least activity
- PFOA driver?



Kaye et al., *In preparation.*

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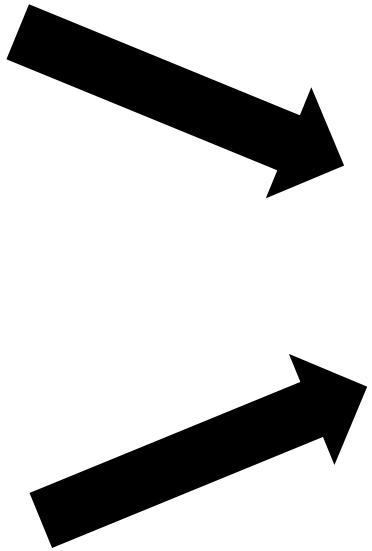
What were the top hits for liver proteins?

PFOA vs. STD

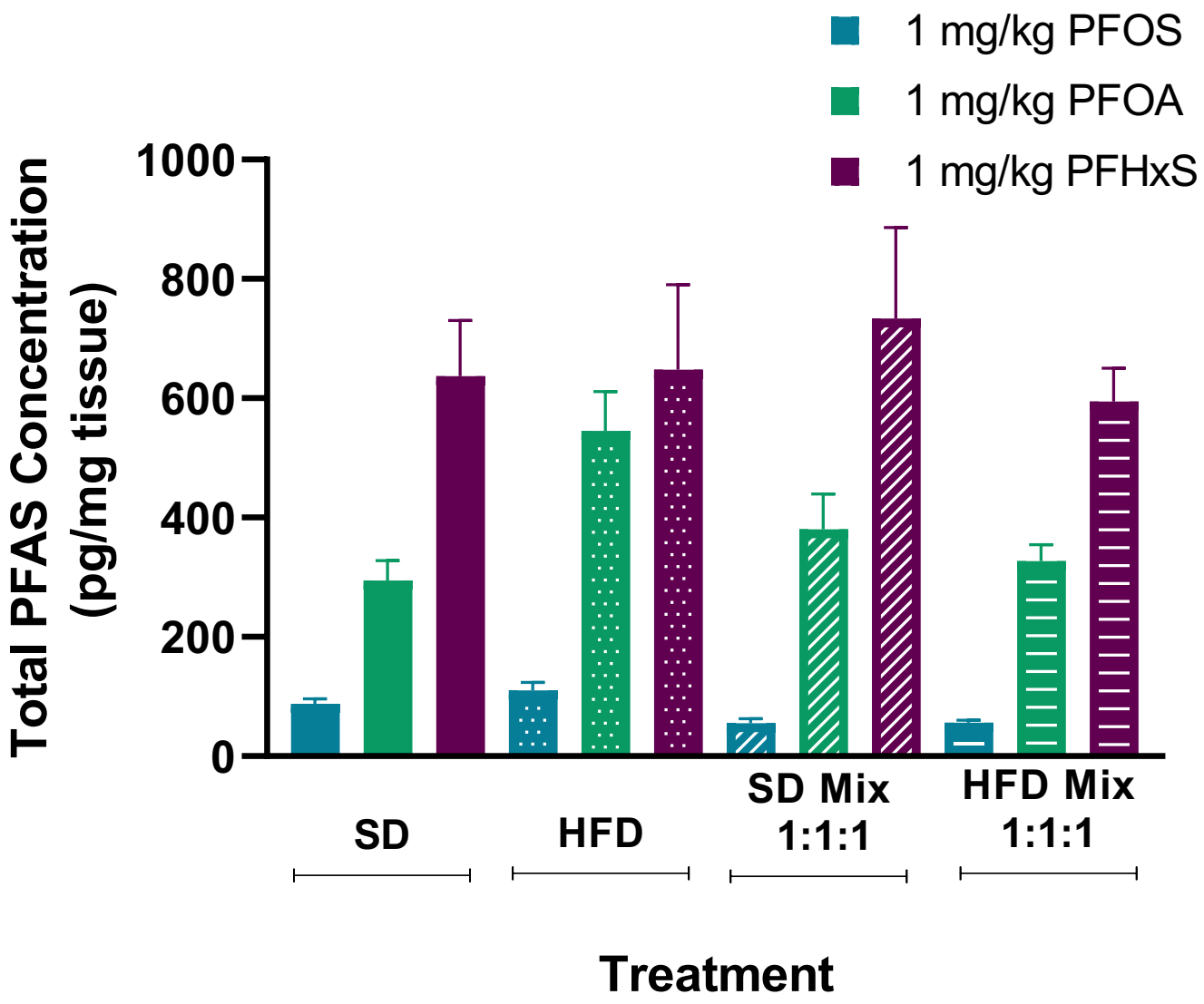
PFAS MIX vs. STD



- **Acyl-CoA thioesterase 1 (Acot 1)**
- **Acyl-CoA thioesterase 2 (Acot 2)**
- **Enoyl-CoA Hydratase And 3-Hydroxyacyl CoA Dehydrogenase (Ehhadh)**

Kaye et al., *In preparation.*

Brain Response Differed Significantly from Liver



- **PFHxS>PFOA>PFOS (Brain) verus PFOA>PFHxS>PFOS (liver)**
- **PFOA levels increased in HFD-PFOA**
- **PFOA levels decreased with mix**
- **PFHxS unchanged with diet or PFAS mix**

Agudelo et al.*, In preparation.*

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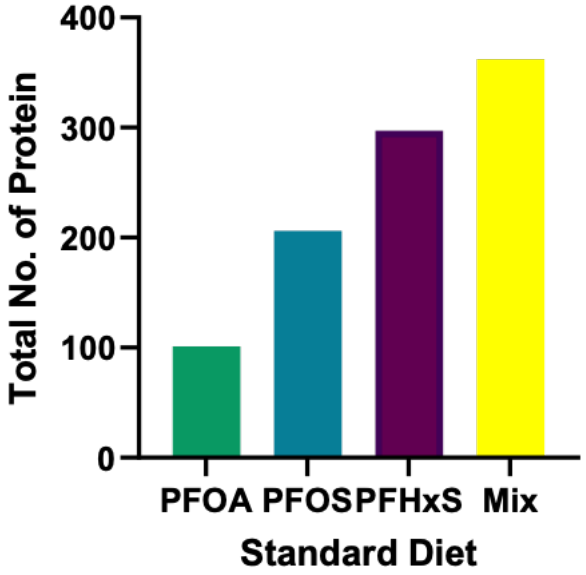
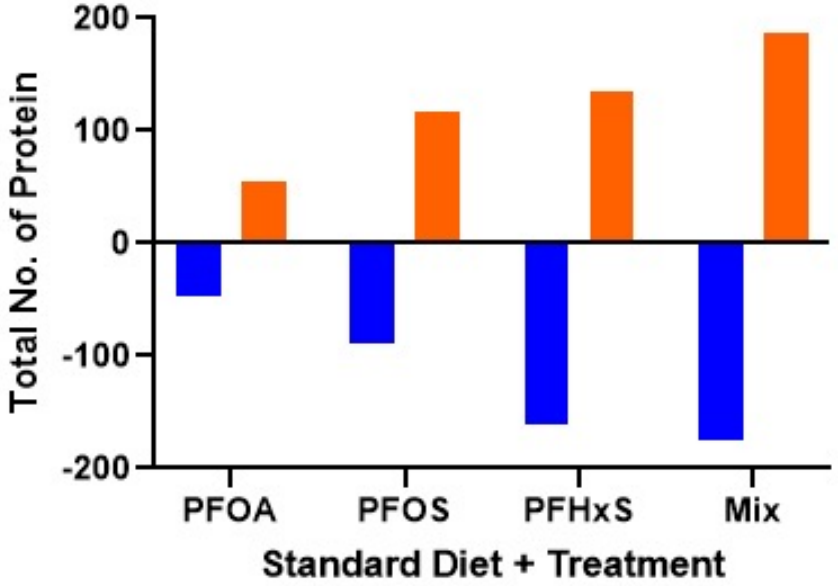
Unlike liver, PFHxS was the most active modulator of the brain proteome





- The largest # of protein expression changes was observed in the SD-PFAS mix offspring
- PFHxS most active

- PFOS > PFOA

Agudelo et al., Abstract #3807, *In preparation.*

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Conclusions – In Vivo

- Maternal treatment with a PFAS Mix in combination with exposure to a high fat diet increased measures of hepatic steatosis in mice offspring.
- Diet and presence of other PFAS influenced serum and liver concentrations (very important to measure tissue concentrations!!)
- The offspring proteome was most changed by PFOA and PFAS Mix
- Brain concentrations and proteomic response differed from liver

Bad Actors??

SOME Bad Actors

- PFOA appeared to drive much of the response in liver
- PFHxS very active in human hepatocyte model

SUM Bad Actors

- We do observe examples of synergistic behavior (PFAS mix induced liver pathologies and proteome changes in offspring)
- PFAS mix seemed to shifted brain and liver proteome the most

Acknowledgments

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  - Dr. Geoff Bothun, URI, COE
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- Rohitash Jamwal

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Thoughts for the PFAS field

- Cell culture conditions can be critical for evaluating PFAS
- Diet-PFAS interactions are complex
- PFAS-PFAS measures are complex. Could have PFAS-PFAS competition for certain tissues, especially at high concentrations.
- Measuring tissue concentrations is an absolute ***must*** to related to tissue outcomes.
- Robust discussion about models, concentrations, etc. needed to design experiments that are informative.
- Evidence that PFAS in a mix has more activity than the PFAS alone.

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Thank You!

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