

Examining modes of action for PFAS using transcriptomics

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Some current ideas about MOA for PFAS:

PFAS are known to activate PPARα and the activation of this receptor and gene expression changes may be associated with toxic responses.

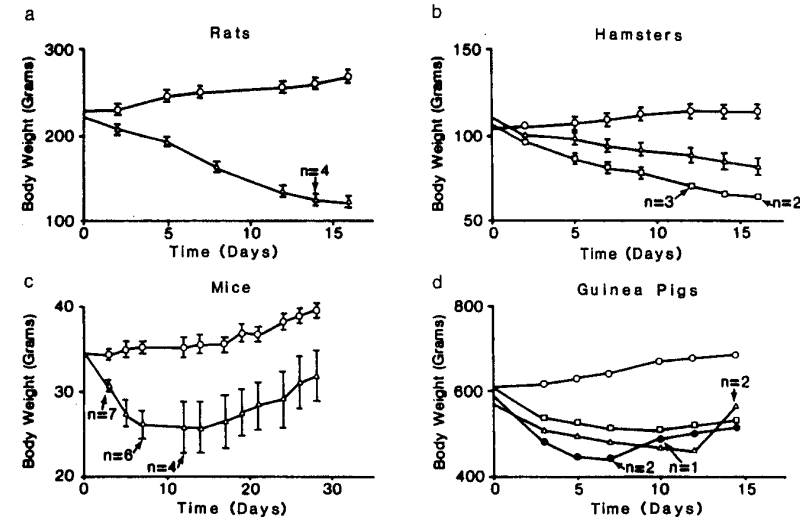
Studies with PPARα-KO animals are not consistent with activation of this receptor as the sole reason for responses to PFAS.

Some reproductive and liver responses were seen in both WT and KO animals and some 11 to 24% of genes affected by PFAS were independent of PPARα.

What other MOAs are supported and should all PFAS be regarded as having similar MOAs?

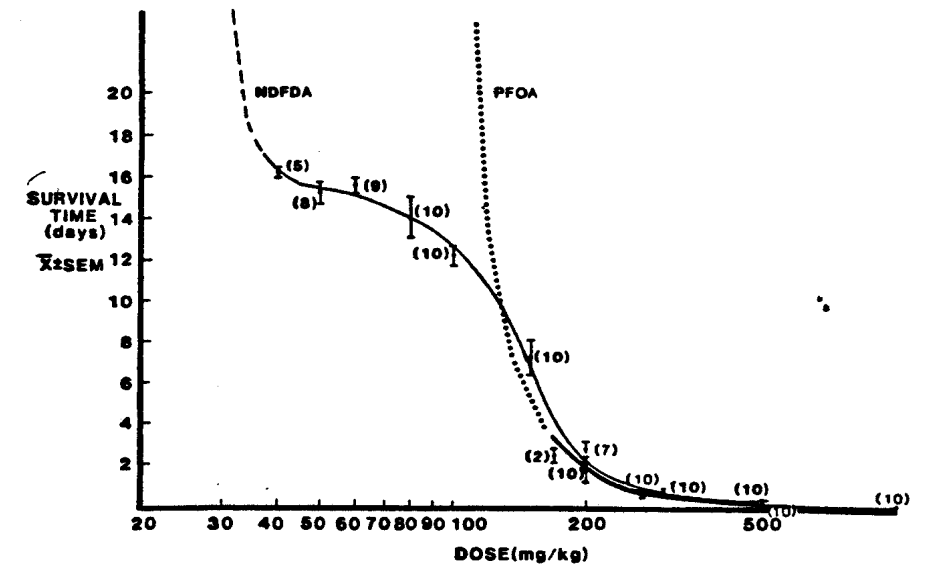
Wasting in rodents seen with PFDA in work in the early 1980's.

Responses like those seen with TCDD.



EFFECTS OF PFDA ON THE RELATIVE LIVER WEIGHT^{a,b} OF RATS, HAMSTERS, MICE, AND GUINEA PIGS AT THE APPARENT LD50 DOSE OF PFDA FOR EACH SPECIES

Species	Dose (mg PFDA/kg)	Time after dosing (days)	Relative liver weight	
			Control (Percentage of body weight)	PFDA-dosed
Rat ^c	50	14	3.33 ± 0.08 (n = 23)	6.02 ± 0.28 ^d (n = 21)
Hamster	50	16	4.26 ± 0.24 (n = 4)	7.47 ± 0.18 ^d (n = 4)
Mouse	150	28	5.34 ± 0.11 (n = 9)	14.19 ± 1.34 ^d (n = 4)
Guinea pig	150	14	3.84 ± 0.19 (n = 3)	4.30 ± 0.09 (n = 3)

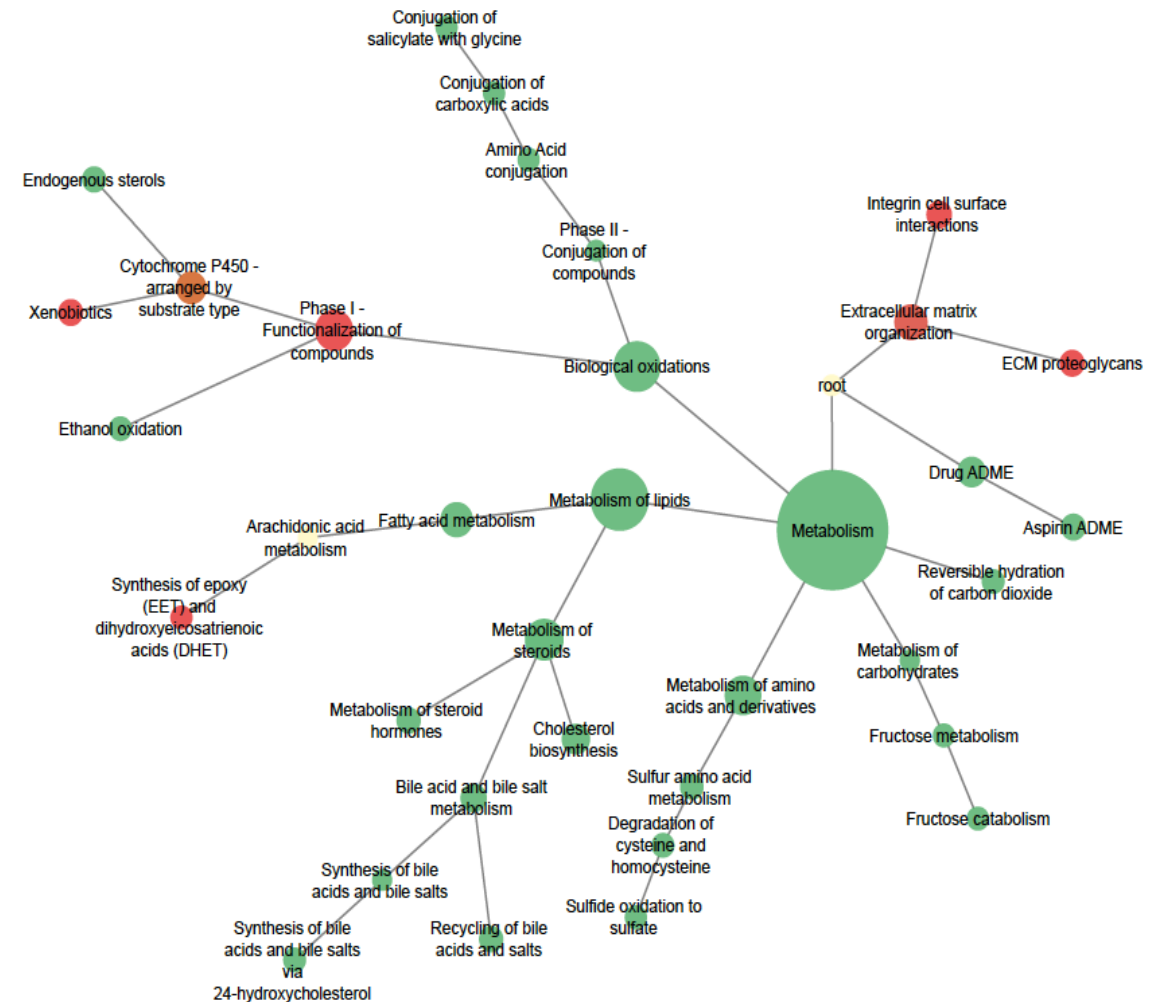


Examining Differential Gene Expression for TCDD after doses causing wasting-like responses in rats

- Treated rats with different daily doses of TCDD for 4 weeks
- Remove liver; prepare RNA (both CL and PP regions sampled)
- Determine DEGs
- Use Reactome Ontology to assess pathway enrichment.
- Display relationship using CytoScape
- Color represent up(red) or down (green) regulated pathways - MOAviz
- Size represents relative number of genes in the category
- **Profound downregulation of pathways of fatty acid and steroid metabolism at doses causing wasting**

B

Wildtype PP 1000 ng/kg/day TCDD



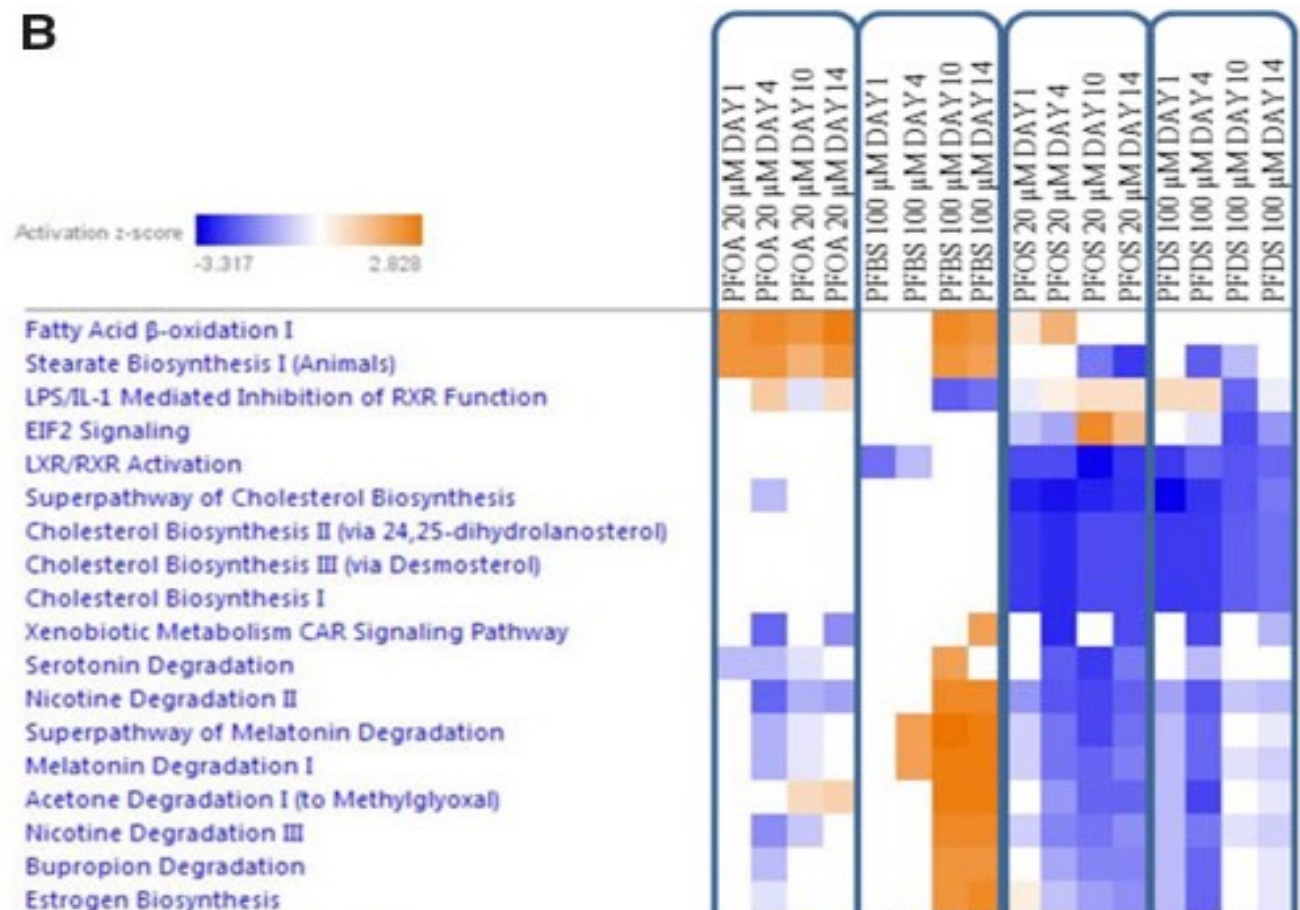
Gene Expression changes caused by PFAS

Studies conducted with various PFAS to study DEG using human primary hepatocyte spheroids.

Ingenuity pathway enrichment showed qualitative differences between PFOA and PFOS.

No inferences offered about MOAs.

We downloaded gene expression results for 11 straight chain PFAS to examine enrichment with with MOAviz platform tools.

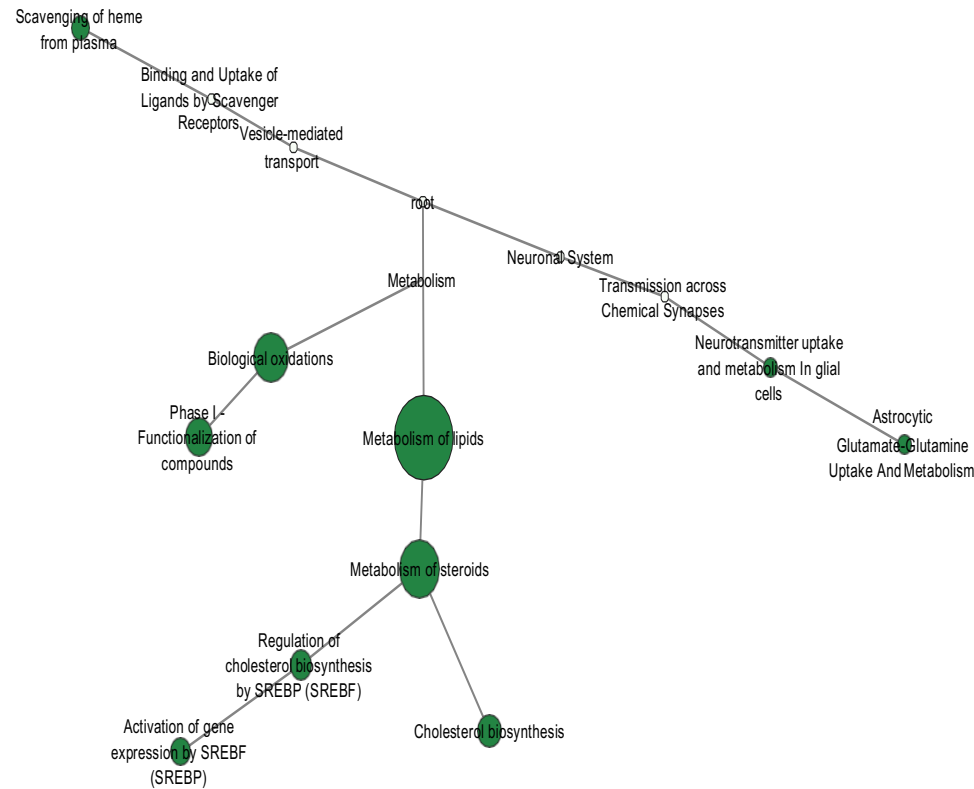


Rowan-Carroll et al. (2021).

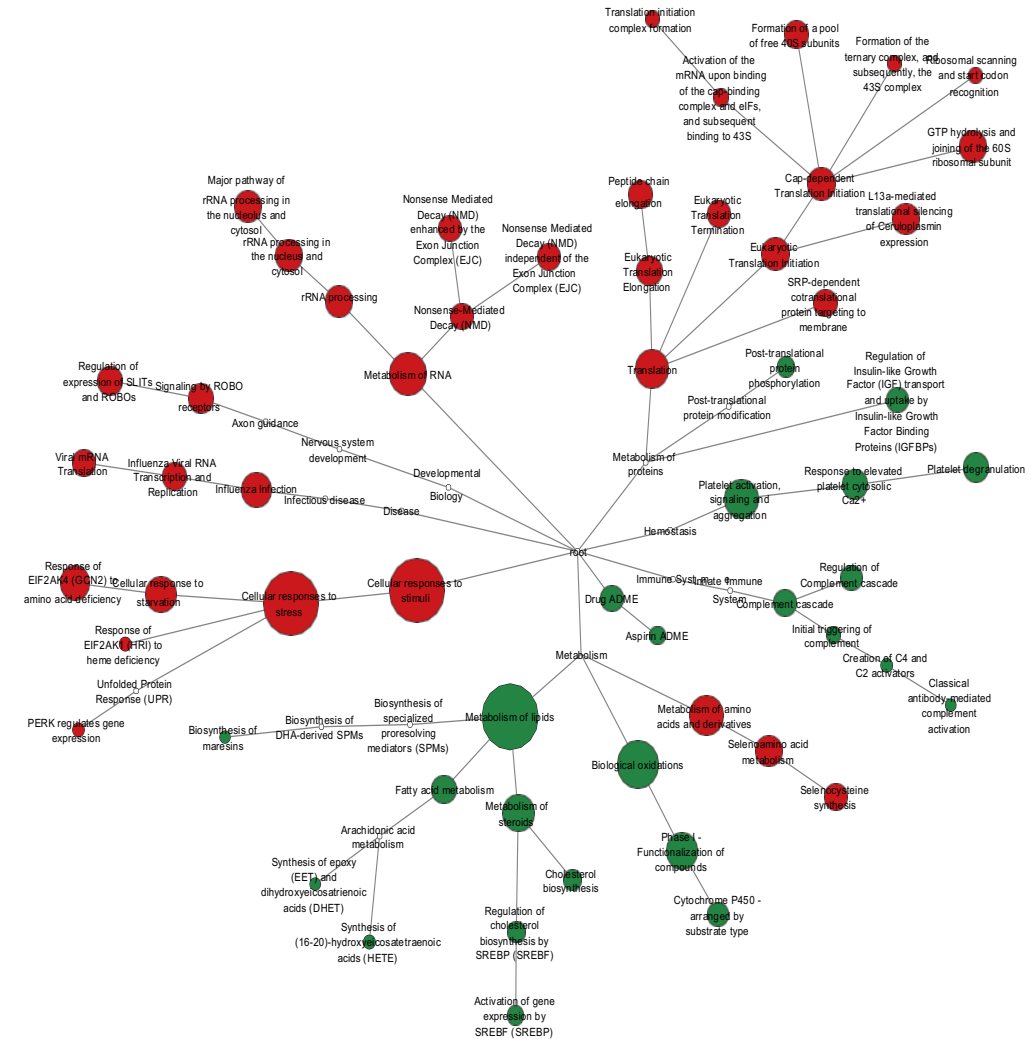
Compounds evaluated and exposure conditions

PFAS	Treatment Concentration	Duration of exposure (days)	Differentially Expressed Genes
Perfluoropentanoic acid (PFPeA)	100 uM	10 days	18 (30)
Perfluorohexanoic acid (PFHxA)	100 uM	10 days	42 (46)
Perfluoroheptanoic acid (PFHpA)	100 uM	10 days	48 (51)
Perfluorooctanoic acid (PFOA)	20 uM	10 days	40 (57)
Perfluorooctanoic acid (PFOA)	50 uM	10 days	285 (363)
Perfluorononanoic acid (PNAA)	50 uM	1 day	147 (179)
Perfluorodecanoic acid (PFDA)	50 uM	1 day	170 (196)
Perfluoroundecanoic acid (PFUnA)	13 uM	1 day	82 (95)
Perfluorobutane sulfonate (PFBS)	100 uM	10 days	36 (21)
Perfluorohexane sulfonate (PFHxS)	100 uM	10 days	43 (24)
Perfluoroheptane sulfonate (PFHpS)	50 uM	10 days	110 (140)
Perfluorooctane sulfonate (PFOS)	10 uM	10 days	50 (131)
Perfluorooctane sulfonate (PFOS)	20 uM	10 days	188 (300)
Perfluorodecane sulfonate (PFDS)	50 uM	14 days	65 (57)

PFOS – 10 and 20 uM

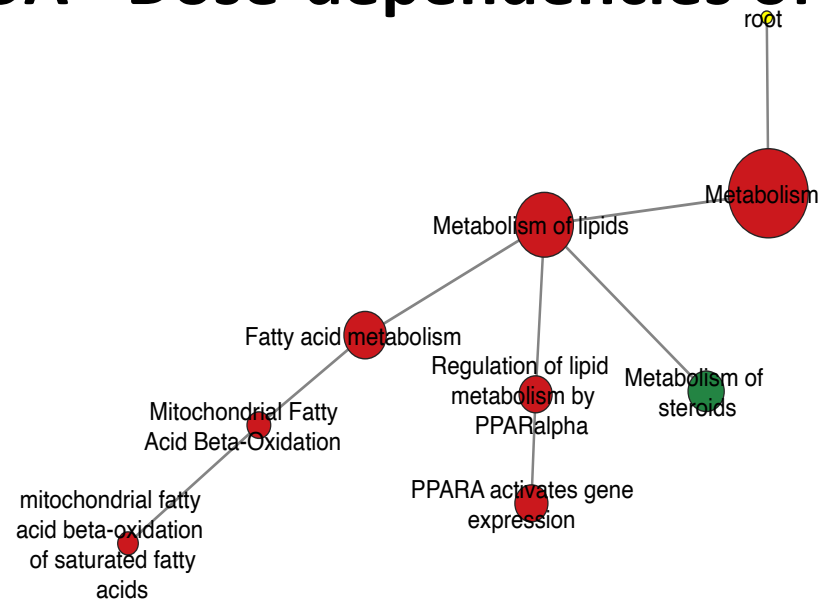


Lower concentration – downregulation of metabolism of steroids and biological oxidations.

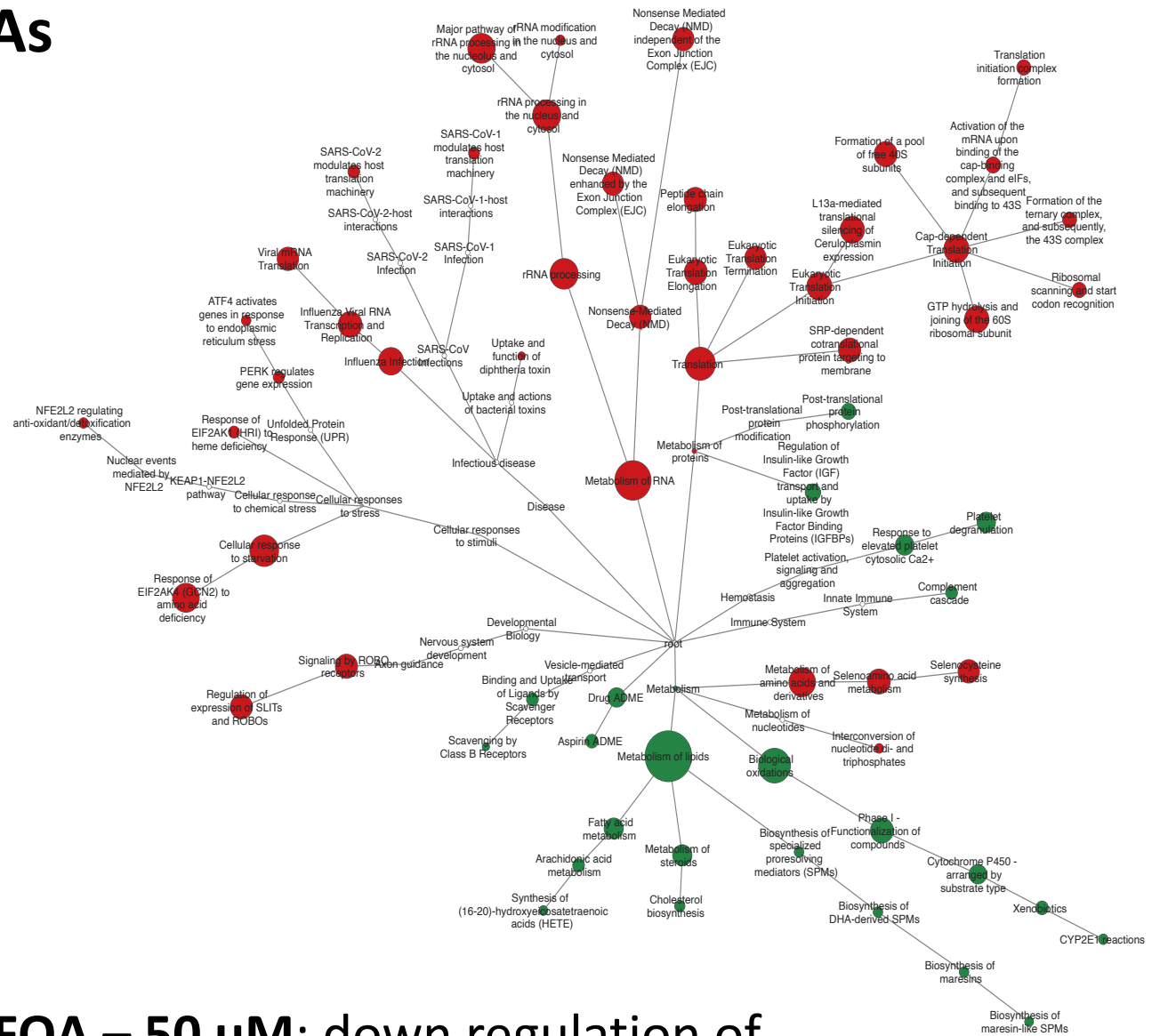


Higher treatment – downregulation of steroid and fatty acid metabolism, biological oxidations and upregulation of pathways associated with toxicity

PFOA - Dose-dependencies of MOAs

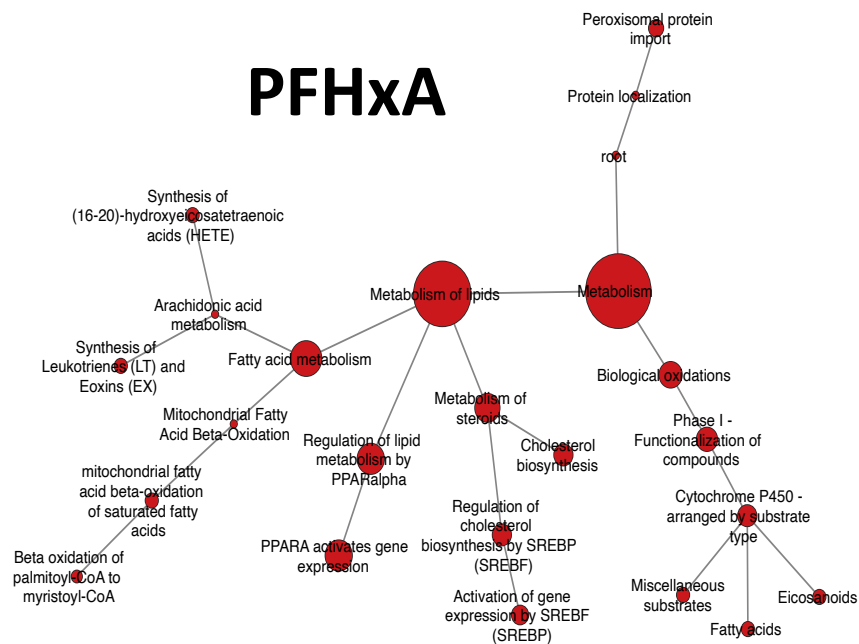


PFOA – 20 μ M: upregulation
fatty acid metabolism,
downregulation of
metabolism of steroids

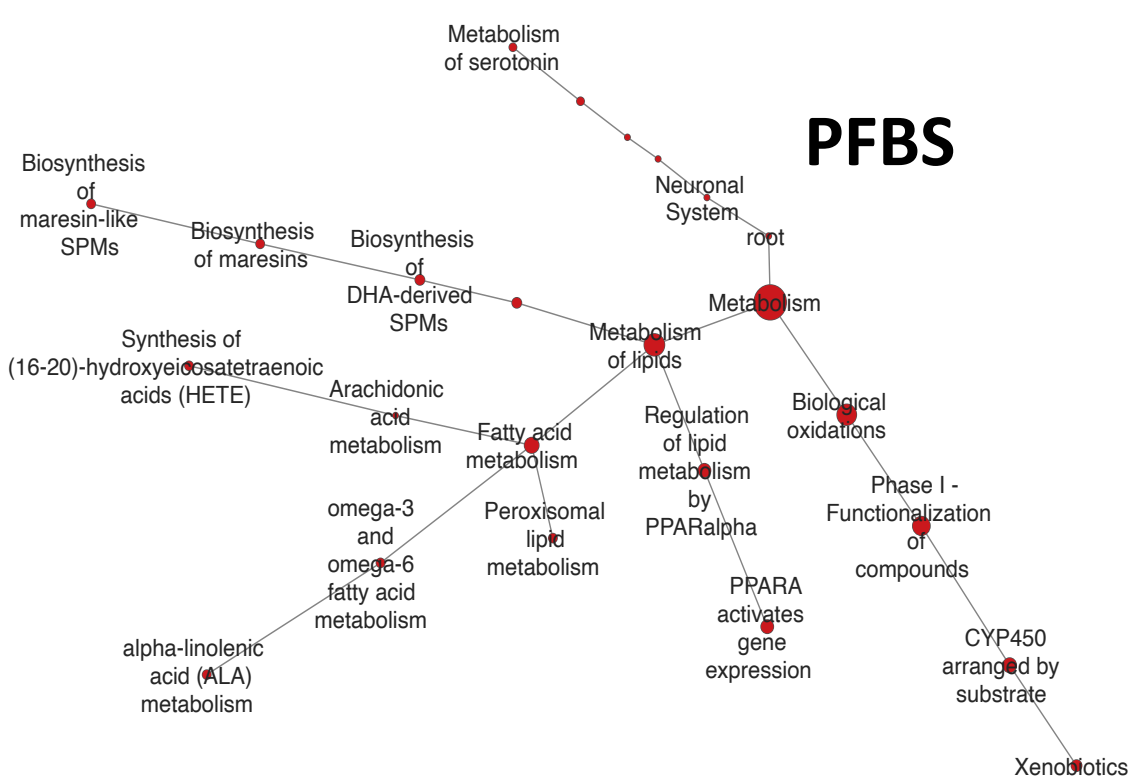


PFOA – 50 μ M: down regulation of both fatty acid and steroid metabolism and pathways related to toxicity

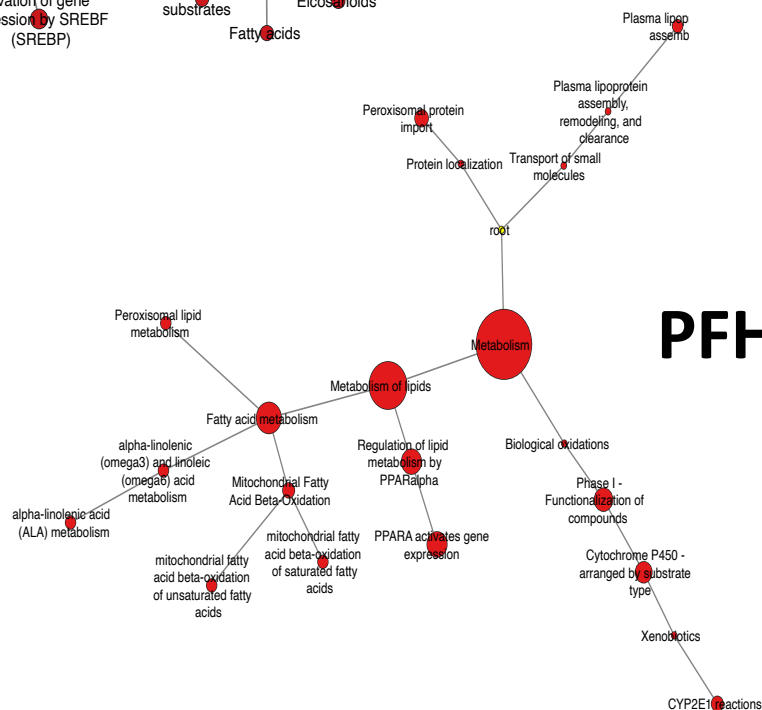
PFHxA



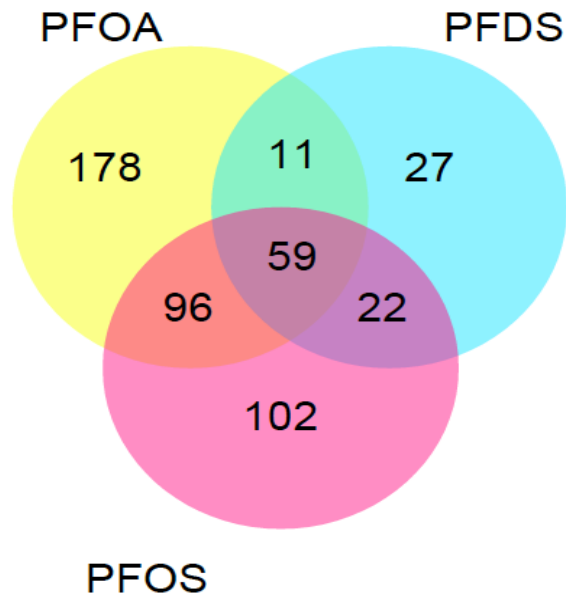
PFBS



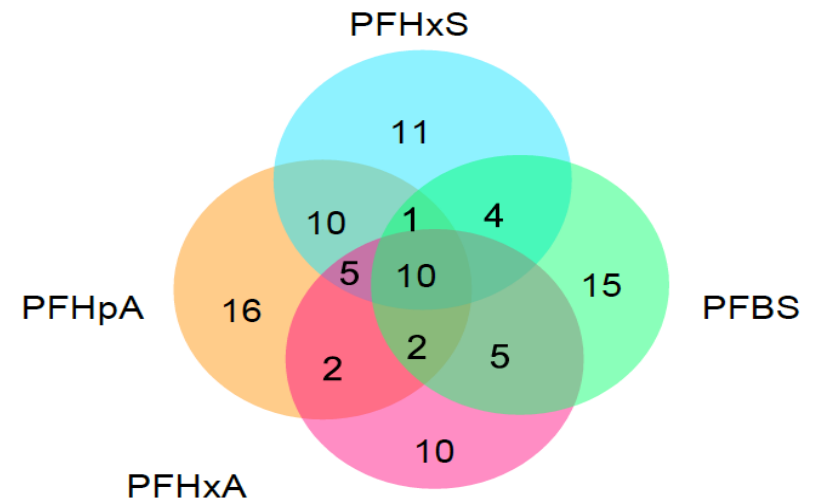
PFHpA



Different patterns of enrichment for different short-chain PFAS

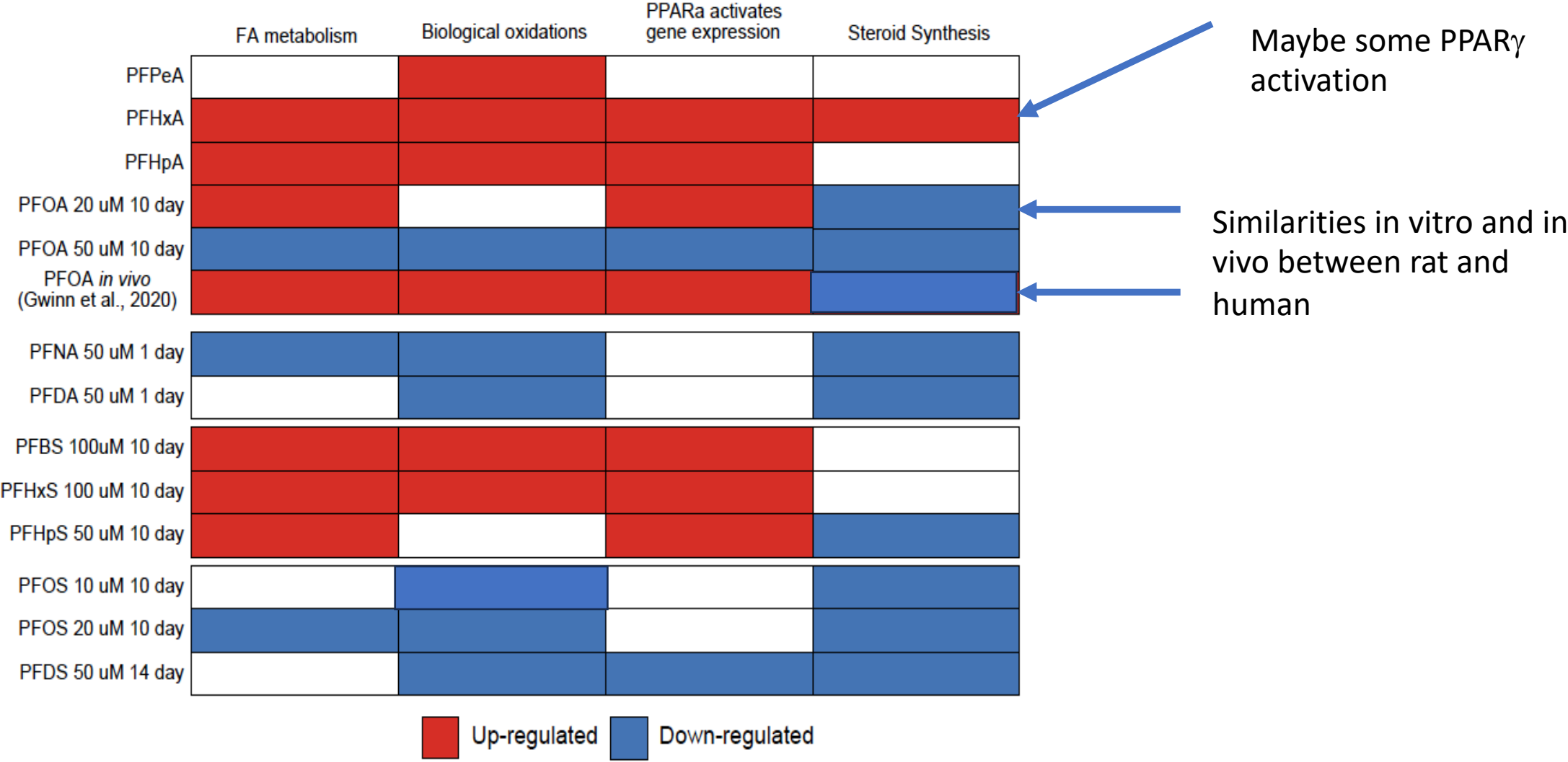


Venn diagram for downregulated genes with the three longer chain PFAS: very similar patterns of enrichment of metabolism of fatty acids and steroids



Venn diagram for upregulated genes for the shorter chain PFAS: more consistent with enrichment of metabolism of fatty acids, but with significant variability between these PFAS.

Patterns Pathway Enrichment based on DGE for Various PFAS



General Summary:

Gene expression changes with the spheroids show considerable differences in pathway enrichment for these PFAS.

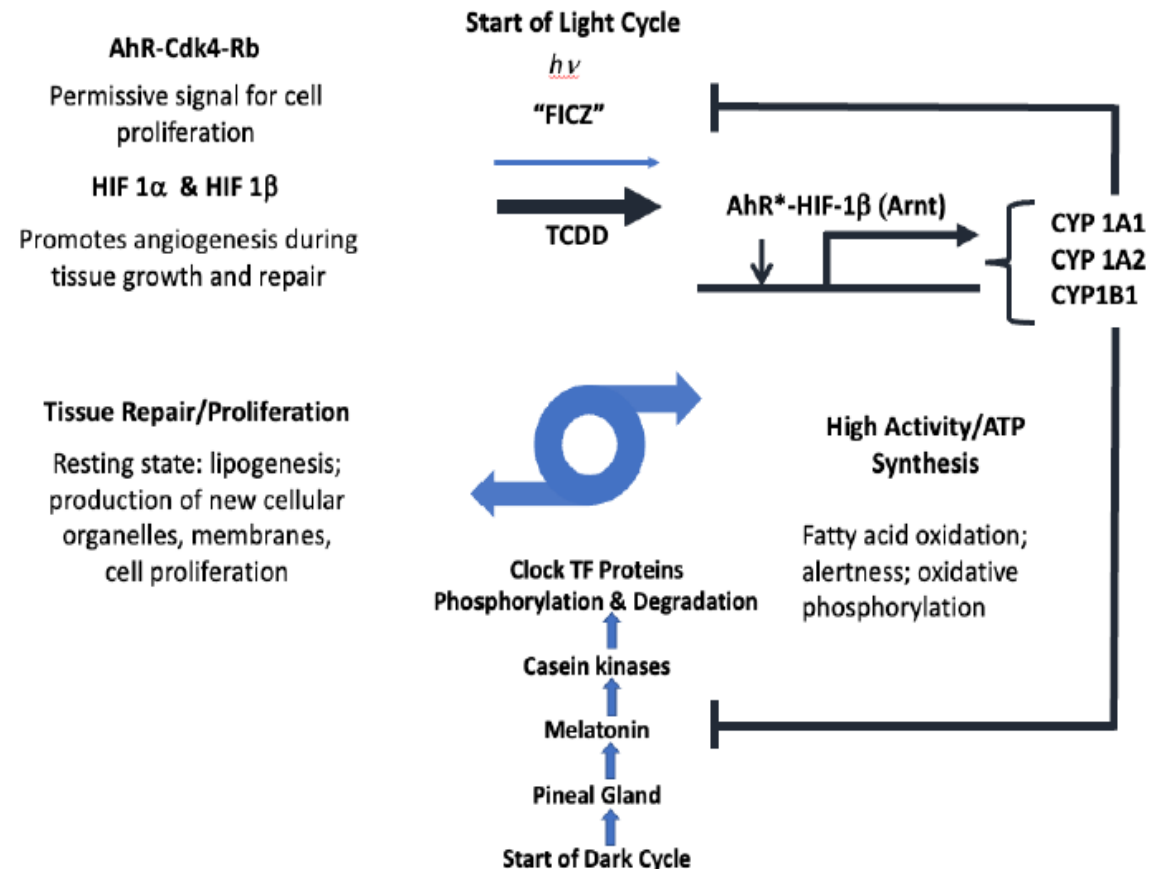
Wasting with TCDD and the longer chain PFAS is associated with profound downregulation of lipogenesis (**Step 1 in MOA**)

PPARα pathways appear more important for biological responses with the shorter chain PFAS (i.e., upregulation of fatty acid metabolism pathways). Other MOAs would be at work with downregulation seen at higher dose, longer-chain length, and wasting.

Risk assessment approaches for mixtures of PFAS should not assume additivity.

MOA Hypothesis – Step 2: Mechanisms to account for downregulation of lipogenesis with TCDD.

Our hypothesis: TCDD blocks the ability of cells to shuttle between the resting cycle part of the circadian cycle using acetylCoA for lipogenesis and the activity and the activity portion with use of AcetylCoA in Krebs cycle and ATP produ



MOA Hypothesis –Step 2: Mechanism that would account for downregulation of lipogenesis with longer-chain PFAS

Our hypothesis: Longer chain PFAS are recognized (in an unknown manner) as a signal that the cell has too much long chain fatty acids generating signals to downregulate steroid and fatty acid synthesis.

PPAR β,δ has been shown to have activity as a transcription repressor of PPAR α and PPAR γ and to integrate PPAR receptor activity.

Inhibition of fatty acid and steroid synthesis may arise due to longer chain PFAS binding to PPAR β,δ to inhibit both fatty acid metabolism and lipid synthesis.

Differential effects of PFAS would depend on binding affinity to PPAR β,δ

Useful Next steps:

Do more complete dose-response modeling of pathway alterations with the 11 straight chain compounds. Are there dose-dependencies similar to those seen with PFOA?

Apply MOAviz analysis to the broader suite of PFAS compound in Reardon et al. and Rowan-Carroll et al papers and see whether the compounds might be classified based similarity of MAOViz pathway signatures.

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