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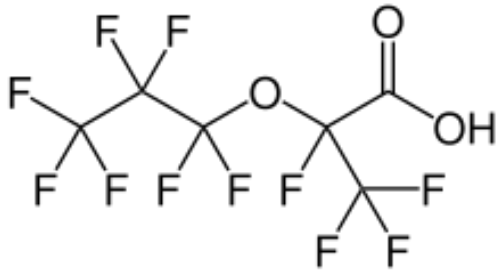
The Role of PPAR-alpha in the Toxicity of HFPO-DA

BelTox 2022

HFPO-DA



- HFPO-DA is used by Chemours as a polymerization aid in the manufacture of some types of fluorinated polymers
  - semiconductor fluid handling, high-purity chemical processing, aerospace and telecommunications cabling, renewable hydrogen production, and lithium-ion batteries in transportation
- Inaccurately referred to broadly as a PFOA replacement
  - fluoropolymer manufacturers have developed their own replacement polymerization aid technologies
- Chemours does not use HFPO-DA in firefighting foam, carpets, textiles, paper, or other consumer products
- HFPO-DA is rapidly eliminated from mammals

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PPAR-alpha and relevance to human risk assessment

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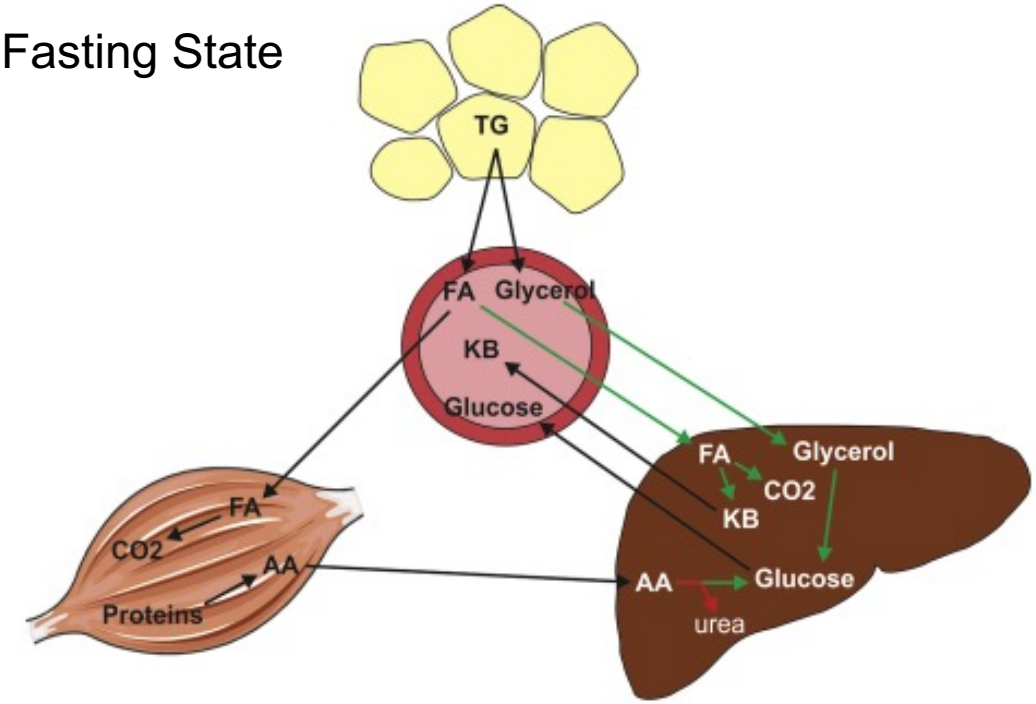
PPAR-alpha

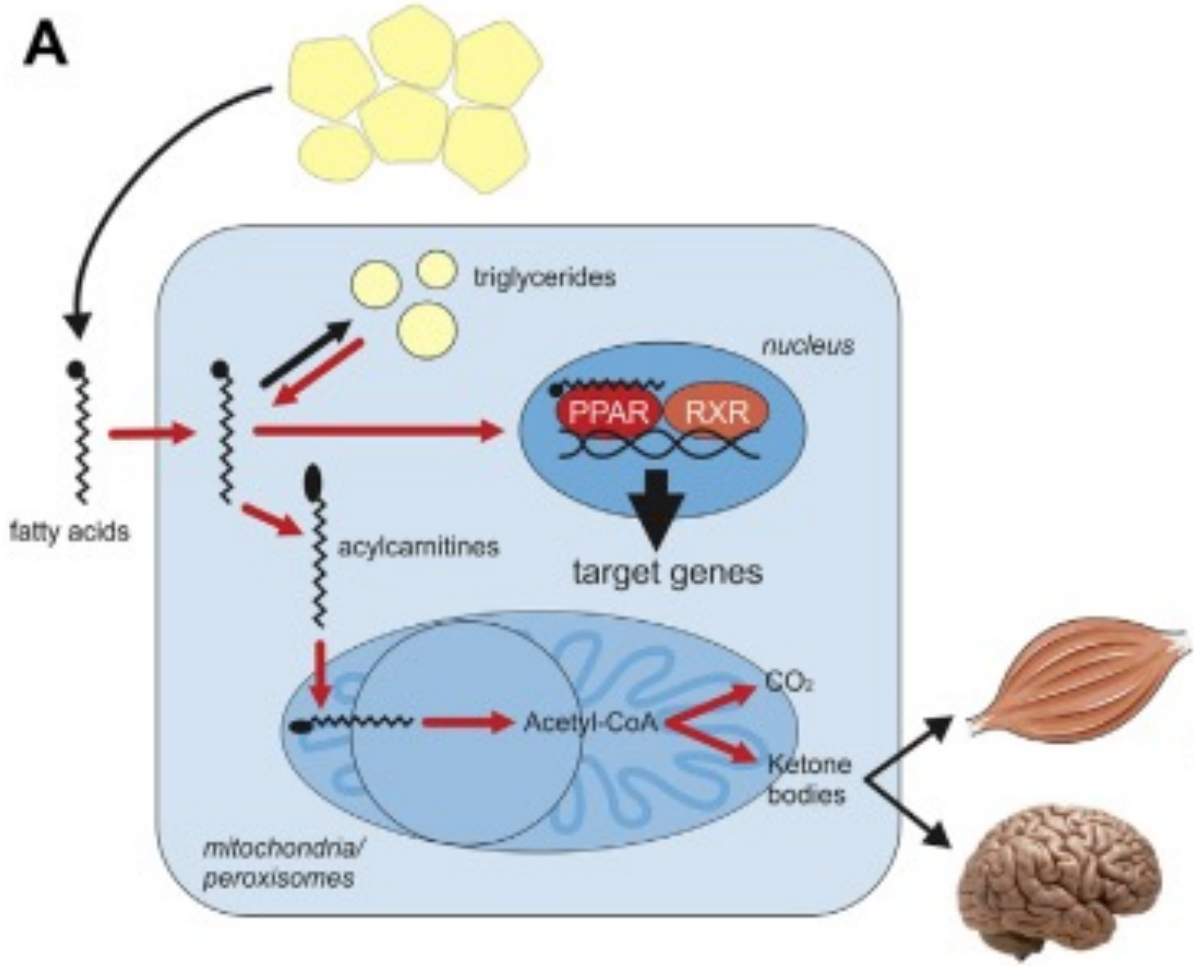
- What is it?
  - Nuclear receptor found in many animals, particularly well studied in rats, mice, monkeys, and humans
  - Three isoforms
    - **Alpha: highest abundance in liver, some in brown fat**
    - Delta: found in most tissues
    - Gamma: mostly limited to adipocytes, present in macrophages
- What does it do?
  - Important for lipid metabolism, particularly in response to fasting
    - Knock-out rodents are generally phenotypically normal so PPAR not essential for life
  - Endogenous ligands appear to be free fatty acids
  - Activation leads to increases in fatty acid oxidation
    - In rats and mice there is a concurrent increase in both lipid metabolism and proliferation (peroxisomes and cells)
    - In other species activation leads to changes in lipid metabolism only

PPAR-alpha

Fasting State



Green = induced by PPAR-alpha activation



Red = induced by PPAR-alpha activation

Figures from Kersten, S. Molecular Metabolism. 2014; 3(4):354-371

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PPAR-alpha

Metabolites changes in PPARα−/− mice.

Intracellular	
Increased	**Decreased**
Triglycerides	Short chain acyl-carnitines
Long chain acyl-carnitines	Free carnitine
Free fatty acids	Glucose
Glycogen (fasted state)	Glycogen

Blood plasma	
Increased	**Decreased**
Triglycerides (in fed state)	Short chain acyl-carnitines
Long chain acyl-carnitines	Free carnitine
Free fatty acids	Ketone bodies
Urea	Glucose
	Arginine
	Tyrosine
	Alanine

Kersten, S. Molecular Metabolism. 2014; 3(4):354-371

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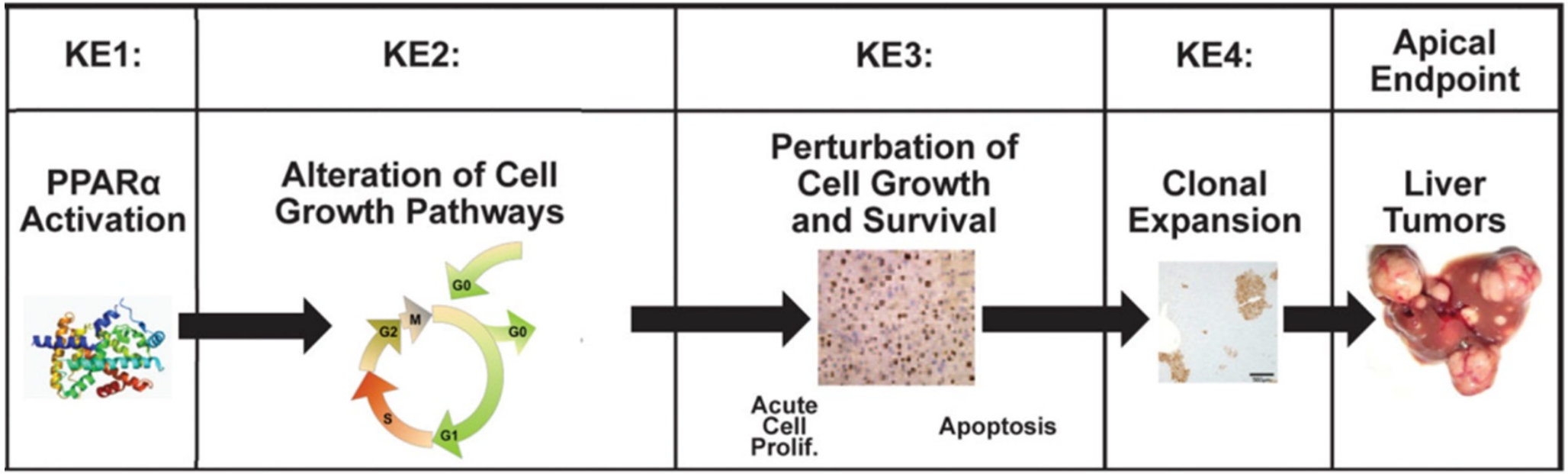
PPAR-alpha

- Why is it important to toxicology?
  - Peroxisome proliferating substances are structurally diverse group that share the characteristic of binding to this receptor
  - In mice and rats, binding to this receptor induces the proliferation of peroxisomes which leads to dramatically increased liver weights
    - Mice are particularly strong reactors - potent activator can lead to doubling of liver weight over several days
    - Does not occur in guinea pigs, non-human primates, or humans
  - PPAR-alpha is a pharmacological target for some drugs
    - No indication of increased liver weights or proliferation from humans who take these drugs
  - Increased liver proliferation in rodents can lead to neoplasms which may proceed to carcinomas

PPAR-alpha

- PPAR-alpha activation leading to liver tumors in rodents is well understood and widely accepted as not relevant to humans
- Tumors are last stage in a series of events



Corton et al. Arch Toxicol. 2018 Jan; 92(1):83-119

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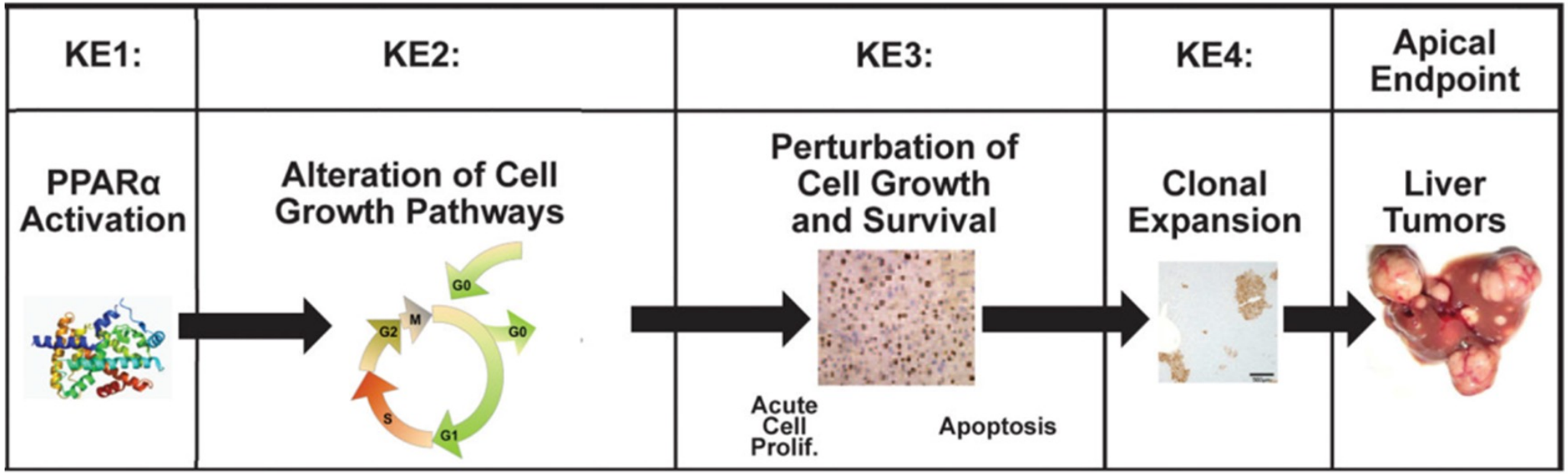
PPAR-alpha

- PPAR alpha activation leading to liver tumors in rodents is well understood and widely accepted as not relevant
- Tumors are last stage in a series of events that are detectable via histopathology



Corton et al. Arch Toxicol. 2018 Jan; 92(1):83-119

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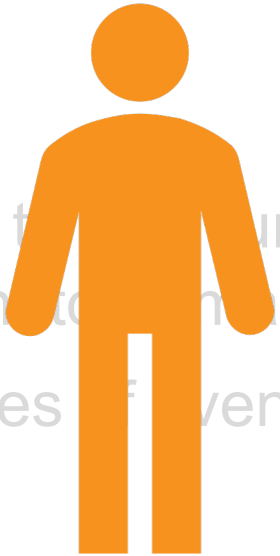
PPAR-alpha



- PPAR alpha activation leading t umors in rodents is well understood and widely accepted as not relevan ns
- Tumors are last stage in a series f vents that are detectable via histopathology

KE1:	KE2:	KE3:	KE4:	Apical Endpoint
PPARα Activation	Alteration of Cell Growth Pathways	Perturbation of Cell Growth and Survival	Clonal Expansion	Liver Tumors

Corton et al. Arch Toxicol. 2018 Jan; 92(1):83-119

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PPAR-alpha

- For general knowledge of PPAR-alpha :
  - Kersten, S. Integrated physiology and systems biology of PPARα. Molecular Metabolism. 2014; 3(4):354-371. PMID: 24944896
  - https://en.wikipedia.org/wiki/Peroxisome_proliferator-activated_receptor_alpha
- For background on rodent liver neoplasms and their human relevance:
  - Klaunig et al. PPAR-alpha agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol. 2003; 33(6):655-780. PMID: 14727734.
  - Felter et al. Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regul Toxicol Pharmacol. 2018 Feb; 92:1-7. PMID: 29113941.
  - Corton et al. The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. Arch Toxicol. 2018 Jan; 92(1):83-119. PMID: 29197930.

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HFPO-DA and PPAR-alpha

- General agreement that the observed toxicity is consistent with PPAR-alpha, but is it only PPAR-alpha?
- ECHA raised this during an appeal to a CoRAP Board of Appeals
- US EPA raised this during a Reference Dose determination and identified four potential modes of action
  - PPAR-alpha
  - PPAR-gamma
  - Cytotoxicity
  - Mitochondrial dysfunction*
- Important because mechanisms other than PPAR-alpha may be more relevant for risk assessment

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HFPO-DA and PPAR-alpha

- Tools to confirm PPAR-alpha and refute other potential mechanisms:
  - Reevaluation of the histopathology
    - Apoptosis versus necrosis can help discriminate between PPAR-alpha and other MOA
    - The definition that pathologists used to described some types of necrosis changed in the mid-2010s
    - Different staining techniques can differentiate apoptosis from necrosis
  - Transcriptomics of stored samples
    - Available repeated dose study samples
    - Prepared from saved tissue blocks
    - Focus on liver, other tissues available

Study	Rat	Mouse
28-day repeated dose	X	X
90-day repeated dose	X	X
Chronic	X	In progress
Carcinogenicity	X	In progress
1-Generation Reproduction	NA	X
Prenatal Development	X	NA

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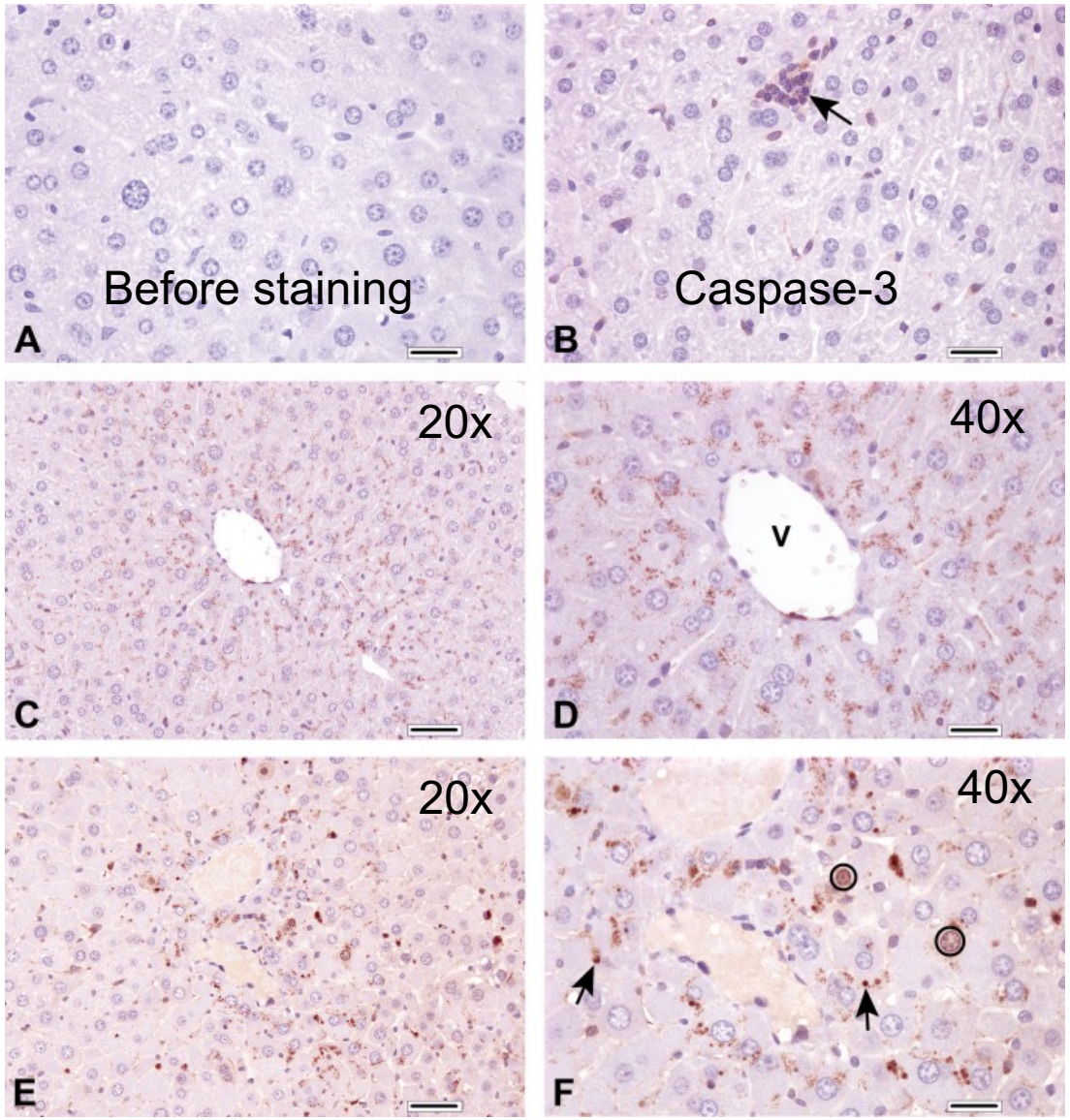
HFPO-DA and PPAR-alpha: Histopathology



Untreated

0.5 mg/kg/day HFPO-DA

5 mg/kg/day HFPO-DA

- Caspase-3 staining identifies apoptotic cells
- Distinguishes necrosis from apoptosis

Chappell et al. 2020

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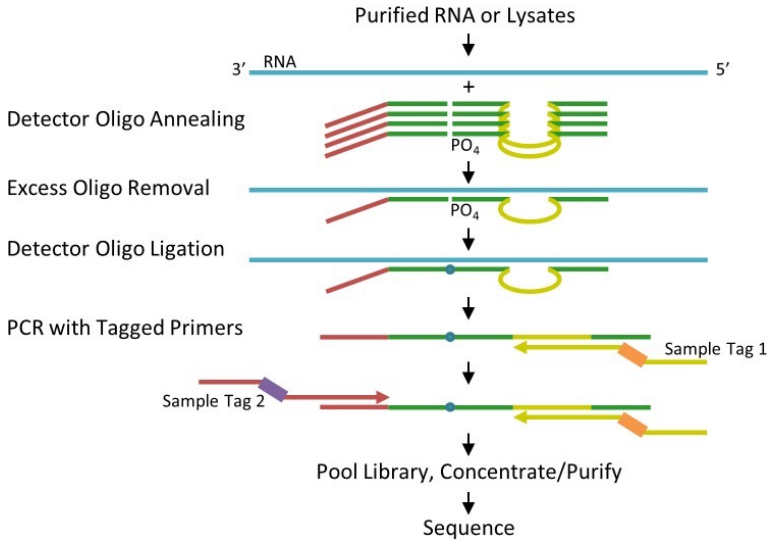
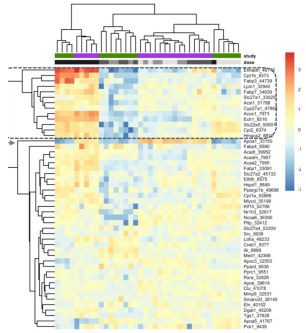
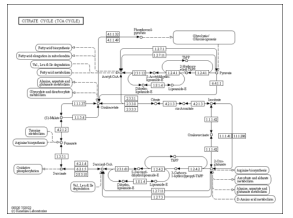
HFPO-DA and PPAR-alpha: Transcriptomics

- Start with paraffin-embedded liver from previous study
- Send to BioSpyder for whole transcriptome expression profiling (TempO-Seq®)





Differential Gene Expression (DGE)



Gene Set Enrichment Analysis (GSEA)

Yeakley et al. PLoS One. 2017;12(5):e0178302. PMID: 28542535.

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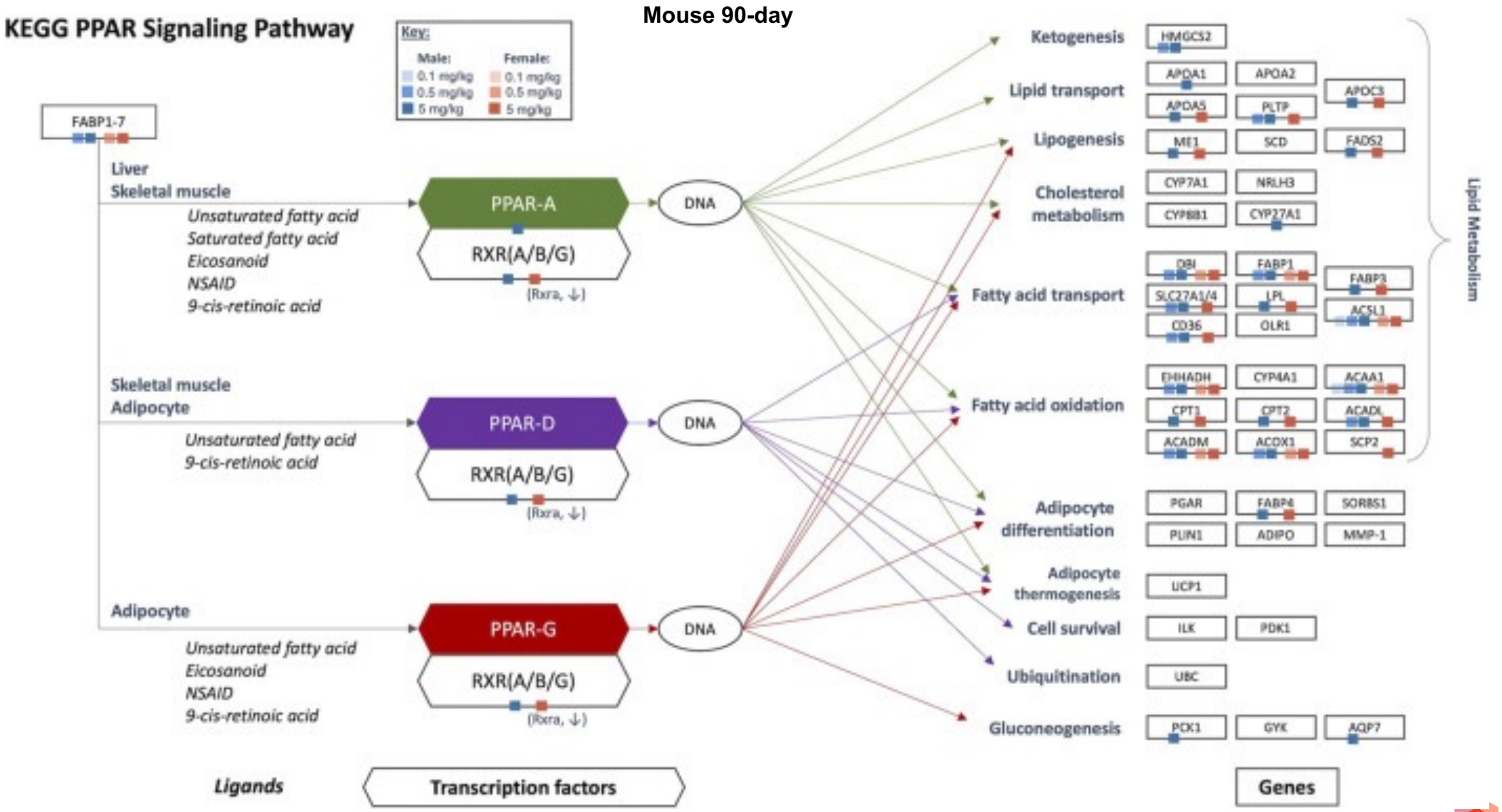
HFPO-DA and PPAR-alpha: Transcriptomics (DGE)



Heintz et al. 2022

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HFPO-DA and PPAR-alpha: Transcriptomics (DGE)

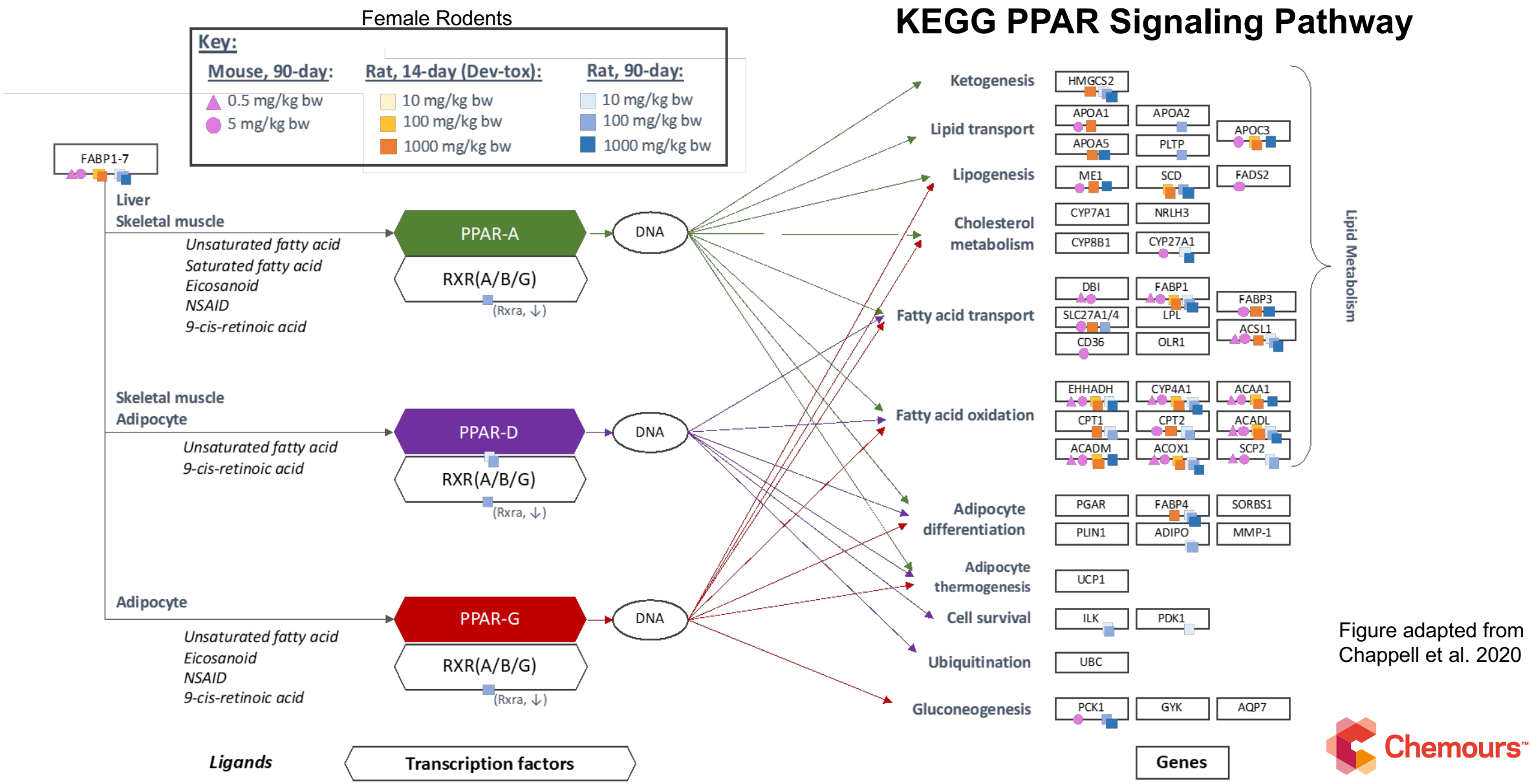

Figure adapted from Chappell et al. 2020

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HFPO-DA and PPAR-alpha: Transcriptomics (GSEA)

- Most significantly enriched gene sets for male and female rats and mice:
  - up-regulated
    - fatty acid metabolism
    - PPAR signaling (KEGG PPAR Signaling Pathway and WP PPAR Signaling)
    - mitochondrial and peroxisomal fatty acid β-oxidation
  - down-regulated
    - complement and coagulation cascades
- Gene sets specific to **PPAR-alpha significantly enriched**
- Gene sets specific to **PPAR-gamma not enriched**

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HFPO-DA and PPAR-alpha: Transcriptomics

- Publications
  - Heintz et al. Responses in Livers of Mice Exposed to the Short-Chain PFAS Compound HFPO-DA. Front Toxicol. 2022 Jun 27;4:937168. doi: 10.3389/ftox.2022.937168. PMID: 35832492
  - Chappell et al. Assessment of the Mode of Action Underlying the Effects of GenX in Mouse Liver and Implications for Assessing Human Health Risks. Toxicol Pathol. 2020 Apr;48(3):494-508. doi: 10.1177/0192623320905803. Epub 2020 Mar 6. PMID:32138627.
  - Thompson et al. Development of an oral reference dose for the perfluorinated compound GenX. J Appl Toxicol. 2019 Sep;39(9):1267-1282. doi: 10.1002/jat.3812. Epub 2019 Jun 18. PMID: 31215065
- Submitted
  - Heintz et al. Assessment of the mode of action underlying development of liver lesions in mice following oral exposure to HFPO-DA and relevance to humans

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Is this MOA applicable to other Substances?

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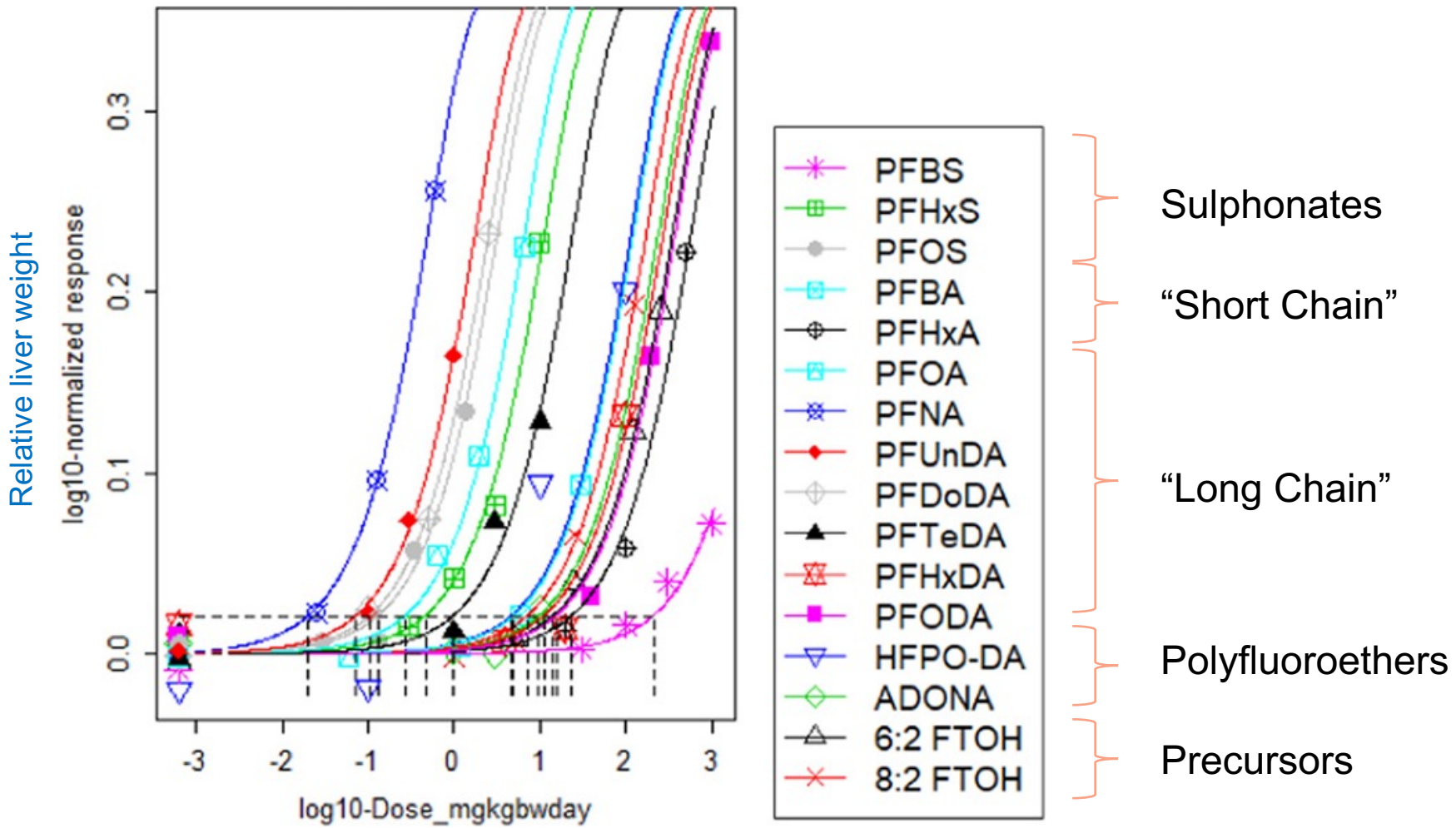
Is this MOA relevant to other Substances?



Bil et al. Enviro Toxic and Chemistry, Volume: 40, Issue: 3, Pages: 859-870, First published: 30 July 2020, DOI: (10.1002/etc.4835)

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Is there a way to confirm “PPAR-alpha only” or exclude other MOA for other PFAS?

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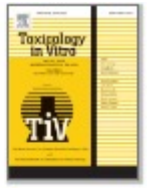
MOA assessment

ELSEVIER

Toxicology in Vitro
Volume 64, April 2020, 104463



Identifying qualitative differences in PPARα signaling networks in human and rat hepatocytes and their significance for next generation chemical risk assessment methods

Patrick D. McMullen [a], Sudin Bhattacharya [b], Courtney G. Woods [c], Salil N. Pendse [a], Mary T. McBride [d], Valerie Y. Soldatow [e], Chad Deisenroth [a], Edward L. LeCluyse [e], Rebecca A. Clewell [f], Melvin E. Andersen [a]

- Key findings
  - Greater number of genes altered in rat then in human hepatocytes
  - Overlapping response in fatty acid metabolism
  - Differences between rat and human changes were associated with cell cycle and differentiation genes
  - The difference in response elements between rat and mouse genomes are the likely explanation for the expression differences

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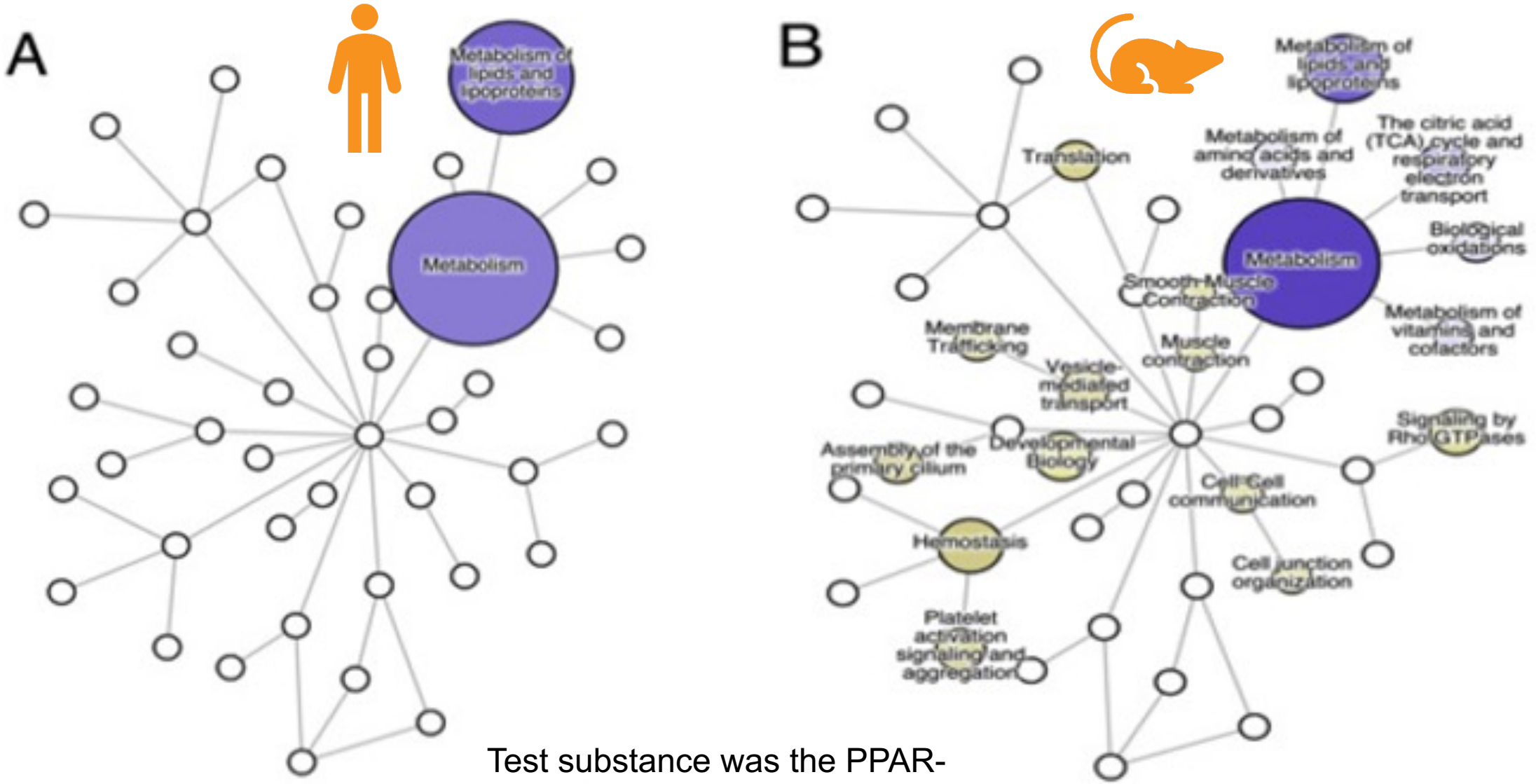
MOA assessment



Test substance was the PPAR-alpha activator GW7647

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MOA assessment

Positive Control

Hepatocyte

Transcriptomics

GW7647: known PPAR-alpha activator
Acetaminophen: known cytotoxic agent
Rosiglitazone: known PPAR-gamma activator

Wildtype Mouse
PPAR-null Mouse
Rat
Human

Whole transcriptome (TempO-Seq)
Fingerprint for

- PPAR-alpha
- PPAR-gamma
- Cytotoxicity

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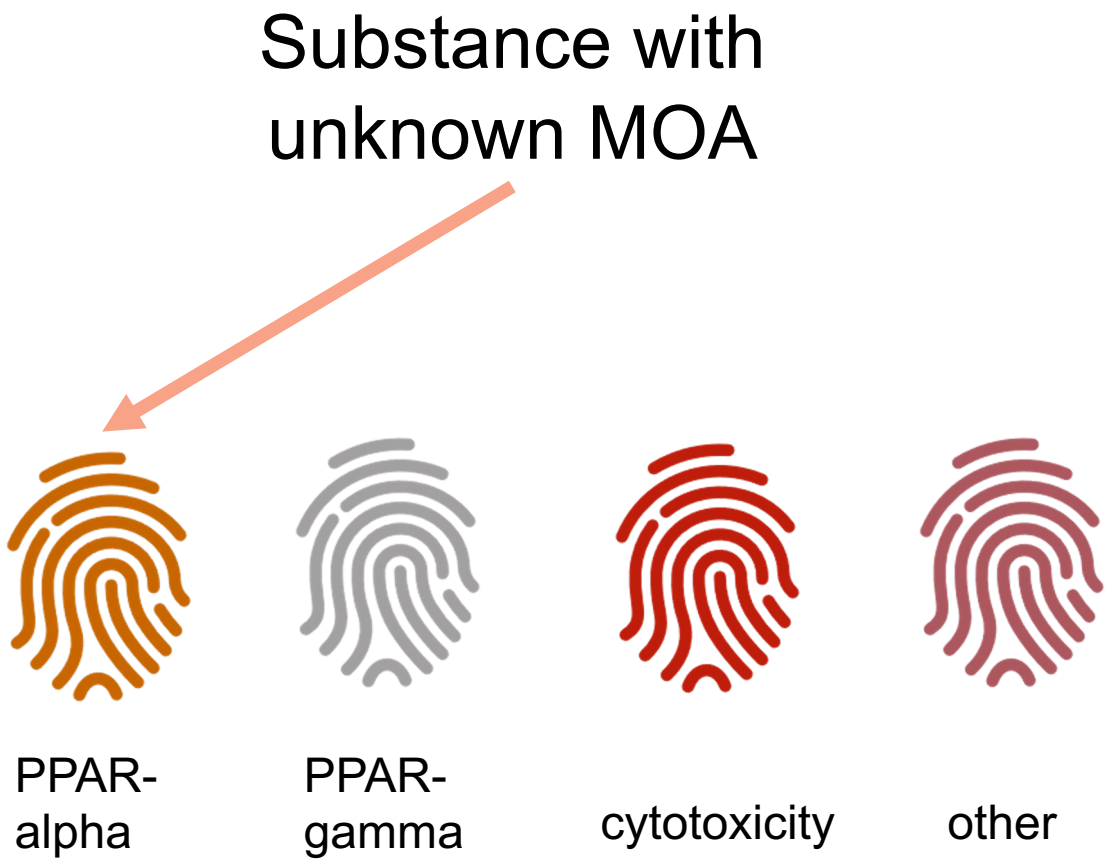
MOA assessment



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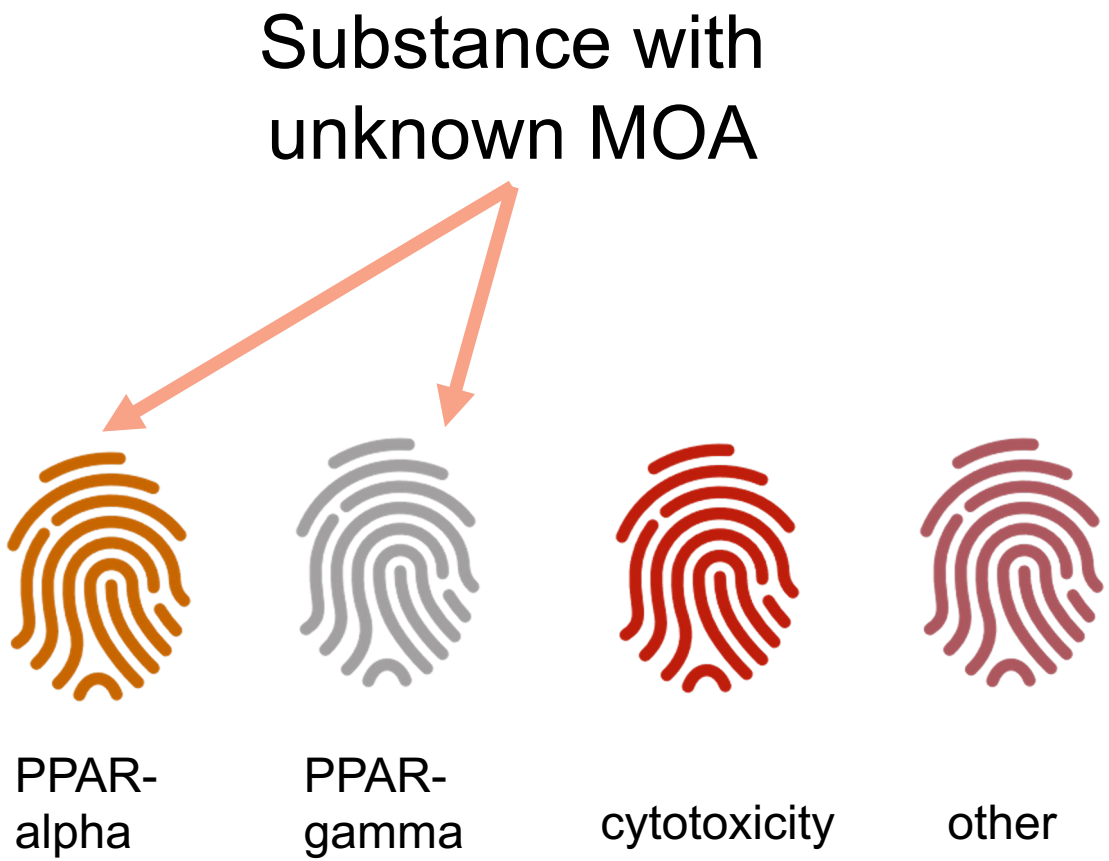
MOA assessment



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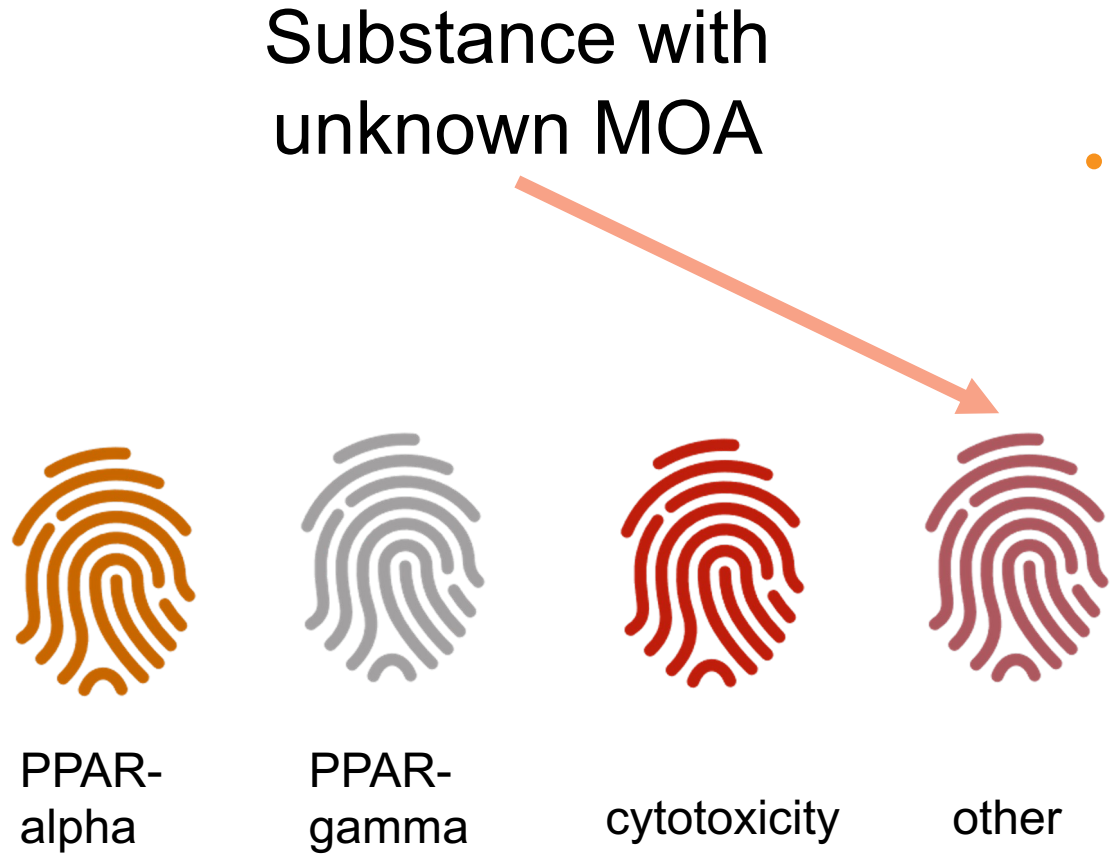
MOA assessment

- Total transcriptomics looks at everything
- Other nuclear receptors
  - CAR, PXR, etc.
- Unexpected, novel MOA



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MOA Assessment

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Summary

- All of the data shows that HFPO-DA induces liver changes in rodents solely through PPAR-alpha activation
- The liver changes induced by PPAR-alpha are not relevant to humans and should not be used for risk assessment
- PPAR-alpha is important for endpoints other than liver
- The increased liver weight resulting from PPAR-alpha activation seems to be the most sensitive endpoint for many PFAS substances
  - Other PFAS substance likely function solely through PPAR-alpha, or via mixed-mode MOA
- Transcriptomics is a powerful tool to understand the MOA of PFAS substances
  - Can both confirm a mode of action and rule out others
  - Can be done *in vitro* and with a higher throughput than animal studies

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Thank you!

Special thanks to:

- BelTox Organizers
- Tox Strategies