NOVEL FLAME RETARDANTS AND HEPATIC STEATOSIS: ELUCIDATION OF MECHANISMS TO DEVELOP (QUANTITATIVE) ADVERSE OUTCOME PATHWAYS, (Q)AOPS

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BACKGROUND

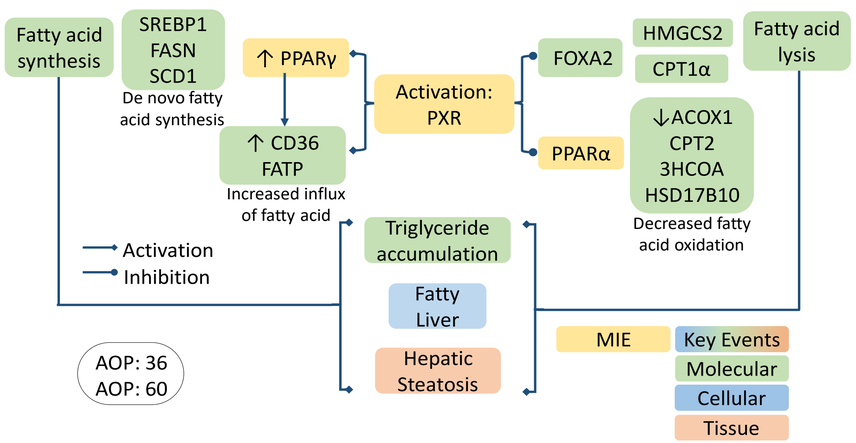
- Following the ban of polybrominated diphenyl ethers (PBDEs), a wide range of novel flame retardants (nFRs) are used as a replacement[1].
- Despite their increasing use and widespread presence, risks, especially hazards such as hepatic effects remain unknown[2].
- Evidence suggests potential steatotic effects of few nFRs but the exact mechanisms are not well-documented, toxicological data is insufficient for human health risk assessment of these replacement products.
- The issue of mixture toxicity is highly relevant to flame retardants but is still an understudied area of research.

RATIONALE

- Development of steatosis is a major health concern as it leads to more severe liver diseases such as cirrhosis and hepatocellular carcinoma[3].
- Several studies have shown a strong correlation between chemical exposure and steatosis in humans, exposure to nFRs might be one of the contributing factors among other multifactorial pathogenesis.
- Nuclear receptors such as PXR, PPAR, a major regulator of lipid metabolism have been shown to participate in hepatic steatosis induction and progression, moreover, in-vivo evidence and TOXCAST data suggest nFRs modulate PXR and PPARγ activity[4].

EXPERIMENTAL HYPOTHESIS

NOVEL FLAME RETARDANTS (AND THEIR MIXTURES) COULD CONTRIBUTE TO HEPATIC STEATOSIS THROUGH YET NOT FULLY EXPLORED MECHANISMS AND PATHWAYS



Prioritised Chemicals		TDICPP	TPhP	TMPP	TBBPA	TCEP	TNBP	TBOEP	TCIPP	EHDPP
Nuclear Receptors	PPARγ	✓	✓	✓	✓		✓	✓		✓
	PXR	✓	✓	✓	✓	✓	✓	✓	✓	✓

Figure 1. Schematic of crosstalk of PXR and associated genes in hepatic lipid metabolism, Table represents nuclear receptor activity data from TOXCAST (reported active)

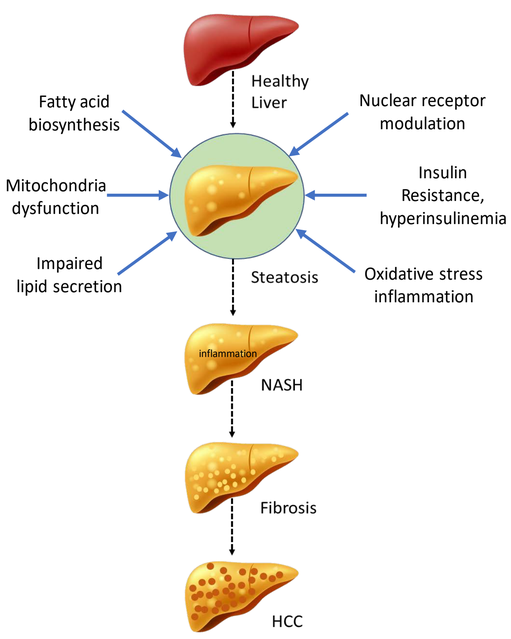


Figure 2: The progression of liver disease: potential role of nFRs in hepatic steatosis

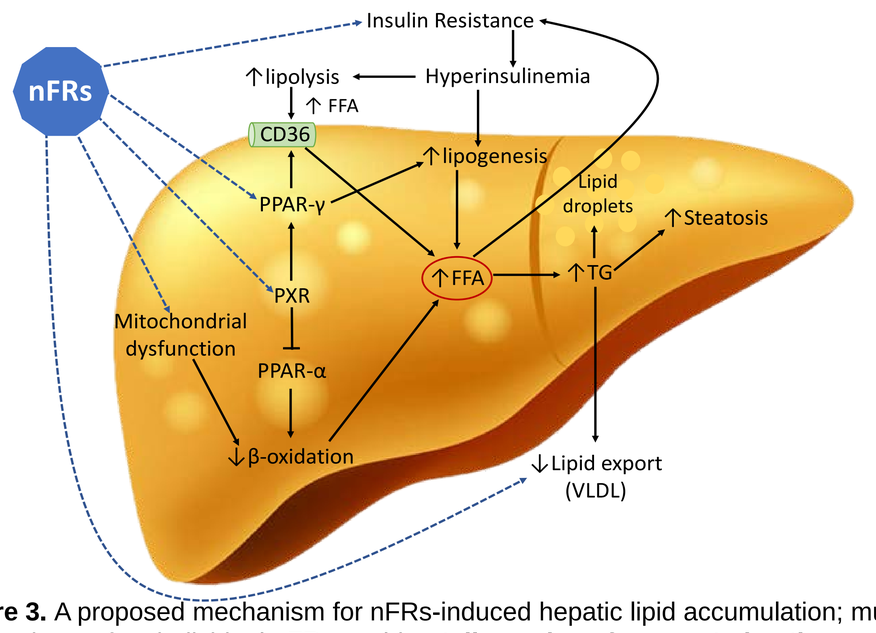


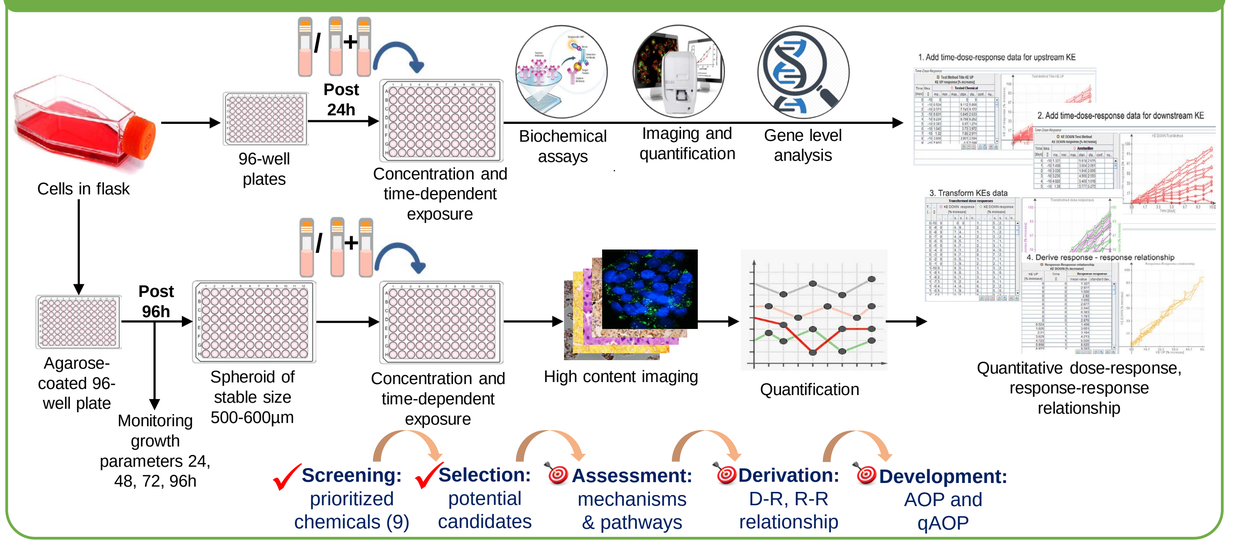
Figure 3. A proposed mechanism for nFRs-induced hepatic lipid accumulation; multiple mechanisms of an individual nFRs could **entail a series of connected pathways** causing biochemical disruption leading to accumulation of lipids (steatosis) eventually fatty liver disease **(dotted line represents proposed pathways)**

RESEARCH OBJECTIVES

This research project aims to determine whether exposure to emerging contaminants such as nFRs cause fatty liver using the mechanistic and predictive toxicology approach

I. To explore the mechanistic pathways for nFRs mediated hepatic steatosis and to identify different key events
II. To study the mixture effects of different nFRs on hepatic steatosis and to identify the mixture of highest concern
III. To develop a quantitative relationship between different identified key events contributing to hepatic steatosis
IV. To develop a putative AOP & (q)AOP to be submitted to OECD endorsed platforms OECD AOP wiki, Effectopedia

EXPERIMENTAL DESIGN



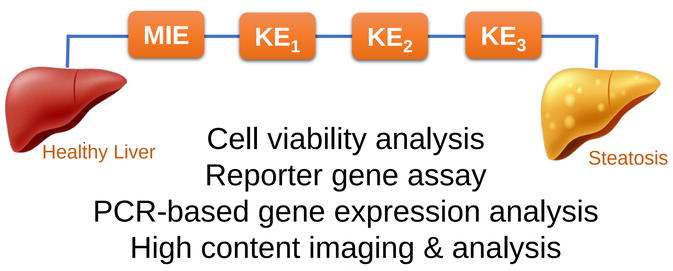
MATERIALS AND METHODS

HepG2: human hepatocyte-derived cell line:

A widely used relevant model to investigate mechanisms leading to steatosis
Similarities to human hepatocyte especially in **lipid & glucose metabolism.**

HepG2 cells spheroids:

More reflective of in vivo cellular responses, retain a functional metabolic competence which is comparable to primary human hepatocytes[5]



Cell viability analysis
Reporter gene assay
PCR-based gene expression analysis
High content imaging & analysis
Lipid specific staining
Immunoblot analysis
Statistical analysis
Dose−response and temporal relationships

PRELIMINARY OBSERVATION RESULTS

I. Screening for the evaluation of steatosis induction by nFRs in HepG2 Cells

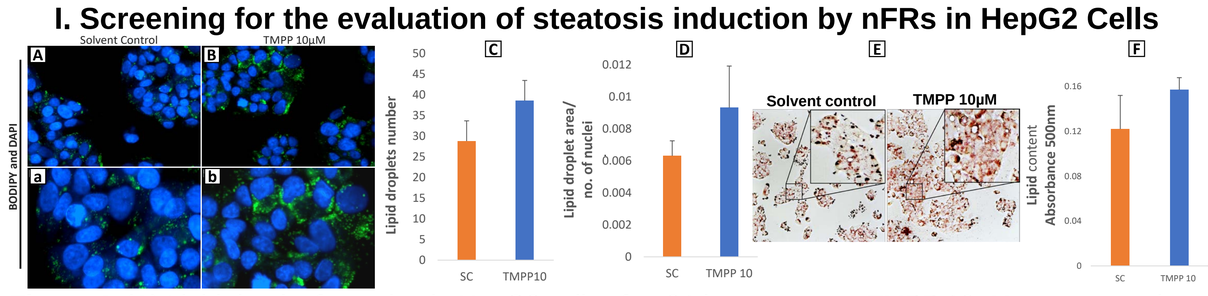
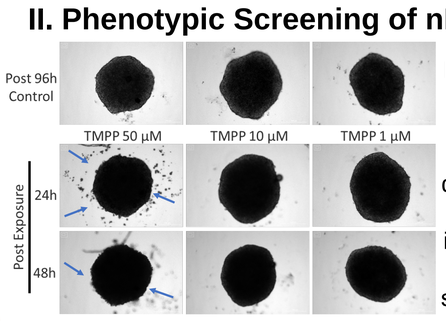


Figure 4. (A) Lipid droplet formation in HepG2 cells after 24-h exposure to TMPP 10µM as assessed using **BODIPY 493/503 (green),** stains neutral lipids & **DAPI (blue),** stains nuclei, scale 100µm (A,B), 20µm (a,b); (C-D) Quantitative analysis of image data; (E) Oil Red O staining images of HepG2 cells treated with 10µM TMPP for 24h, scale bar 100µm, (lipids dyed red); (D) Spectrophotometry-based quantification of Oil Red O staining. Preliminary results indicate potential lipid accumulation in HepG2 cells after exposure to different nFRs at sub cytotoxic concentrations, analysis of genes involved in hepatic steatosis is ongoing. **Results shown are for TMPP (Tris(methylphenyl) phosphate) only.**

II. Phenotypic Screening of nFRs for toxicity prediction: Imaging hepatic toxicity



4000 cell/96h

Figure 5. Bright field images of HepG2 in the presence of different conc. of TMPP. Arrows indicate diffused cell outer layer, scale bar 200µm

FUTURE DIRECTIONS

- Mechanistic study to unravel the molecular mechanisms of nFRs-induced hepatic steatosis.
- Derivation of D-R and R-R relationship.
- Design of environmental relevant mixture of nFRs and assessment of mixture effects.
- High content imaging & analysis of 3D spheroids with fluorophores, stains for viability, oxidative, mitochondrial markers and Immunostaining.
- Development of AOP and (q)AOP.

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