FLAME RETARDANTS - WIDELY USED MATERIAL ADDITIVES - AND HEPATIC STEATOSIS: MECHANISMS AND ADVERSE OUTCOME PATHWAYS

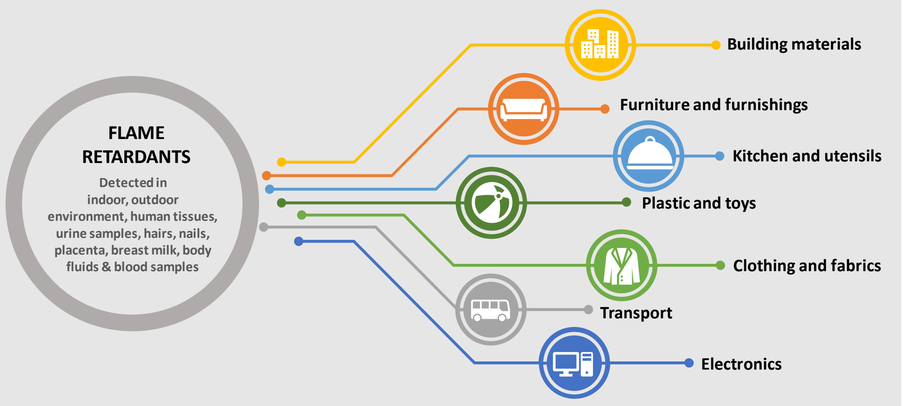
Chander K. Negi, Lola Bajard, Luděk Bláha

Faculty of Science, RECETOX, Masaryk University, Kamenice 5, CZ62500 Brno, Czech Republic

CONCLUSION

- **Several tested novel flame retardants-induced lipid accumulation in human liver cell culture.**
- **TMPP, TPHP, EHDPP and TDCIPP induced the highest lipid accumulation by altering the expression of genes encoding hepatic lipogenesis and mitochondrial dysfunction.**
- **In vitro data from ToxCast and in silico molecular docking suggests PXR and PPARγ could be the potential molecular initiating events**

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 metabolic and reproductive effects are poorly understood[2].
- Accumulating evidence, including epidemiological studies, suggests potential endocrine disruptive effects of several nFRs, nevertheless, the molecular mechanisms associated with endocrine-mediated metabolic effects remain elusive.
- The toxicological data is insufficient for environment and human health risk assessment.

RATIONALE & HYPOTHESIS

- Hepatic steatosis is a major health concern as it leads to more severe liver diseases such as 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.
- Nuclear receptors such as PXR, PPAR, are major regulators of lipid metabolism and have been identified as molecular initiating events (MIEs) in the adverse outcome pathways for hepatic steatosis.

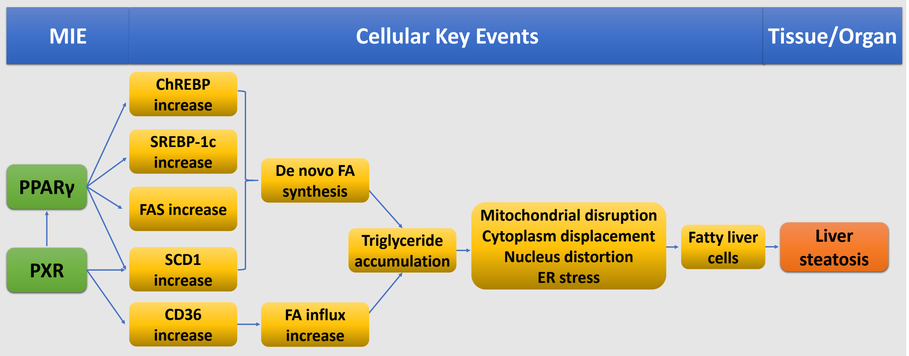


Figure 1. Adverse outcome pathway for hepatic steatosis with PXR and PPARγ activation as molecular initiating events

RESEARCH OBJECTIVES

This research aims to determine whether exposure to emerging contaminants such as nFRs cause metabolic disruption using the mechanistic and predictive toxicology approach to aid in environmental and human health risk assessment

- To unravel the molecular mechanisms for nFRs-induced hepatic steatosis and to identify the associated MIEs & KEs.

METHODS

- In vitro: Human liver cell lines (HepG2 cells)
- Cell viability analysis, high content imaging and analysis, RT-qPCR-based gene expression analysis, lipid specific staining, etc.
- In silico: Molecular docking

REFERENCES

1. Greaves et al. Environ Sci Technol (2014) 48:7942-50.
2. Bajard et al. Environ Sci Eur (2019) 31:14.
3. Younossi et al. Hepatology (2016) 64(1):73-84.

RESULTS

I. In vitro screening and assessment of hepatic steatosis induction by novel flame retardants

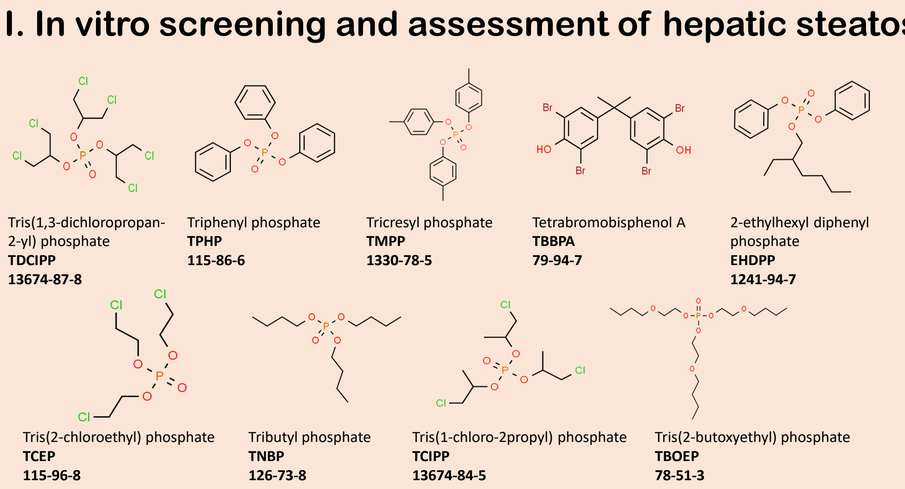


Figure 2. Structure and CAS number of studied nFRs

Cytotoxicity assessed using
Neutral red uptake (lysosomal activity)
CFDA-AM assay (cytoplasmic esterase activity)
Resazurin assay (mitochondrial activity)
Non cytotoxic concentrations (2 µM and 10 µM) selected for further studies

II. nFRs enhanced lipid accumulation in HepG2 cells and induced lipotoxicity

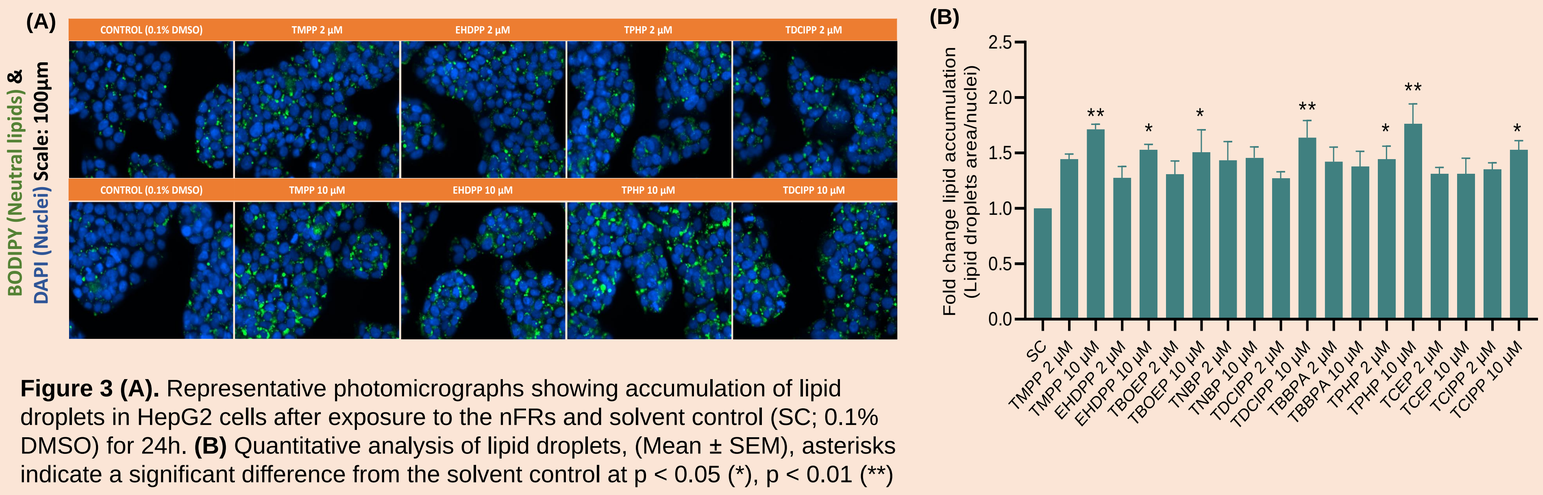


Figure 3 (A). Representative photomicrographs showing accumulation of lipid droplets in HepG2 cells after exposure to the nFRs and solvent control (SC; 0.1% DMSO) for 24h. **(B)** Quantitative analysis of lipid droplets, (Mean ± SEM), asterisks indicate a significant difference from the solvent control at $p < 0.05$ (*), $p < 0.01$ (**)

III. nFRs affected the expression of lipid metabolism-related gene

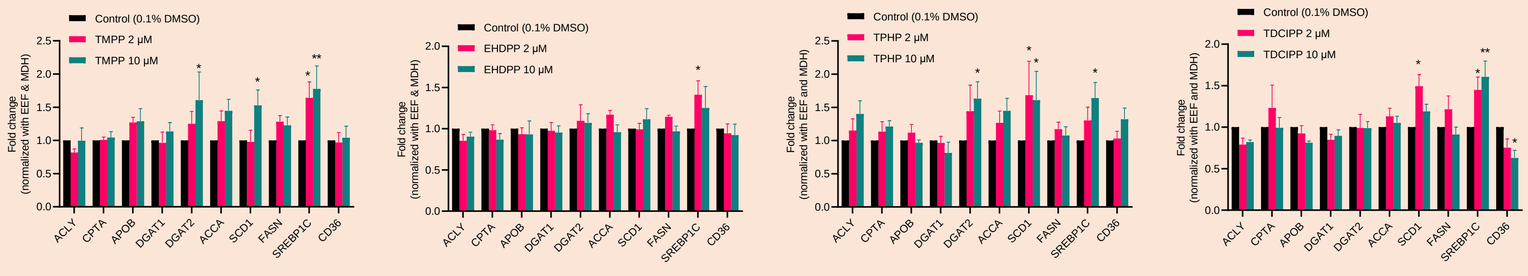


Figure 4. Regulation of expression of lipid metabolism related genes by nFRs in HepG2 cells treated with TMPP, TPhP, EHDPP, and TDCIPP for 24h as analyzed using RT-qPCR. (Mean ± SEM), asterisks indicate a significant difference from the solvent control at $p < 0.05$ (*), $p < 0.01$ (**).

IV. nFRs induced mitochondrial dysfunction depicted by decreased intercellular ATP production

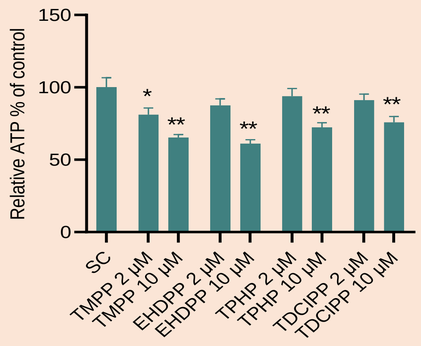


Figure 5. Effects of TMPP, EHDPP, TPHP, and TDCIPP on cellular ATP levels in HepG2 cells. (Mean + SEM percentage of controls). The asterisks indicate a significant difference from the solvent control at $p < 0.05$ (*), $p < 0.01$ (**).

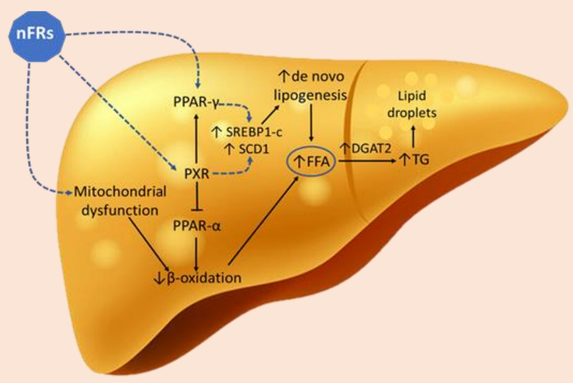


Figure 8. Schematic of the proposed role of nFRs mediated increased hepatic lipogenesis and steatosis induction in human primary hepatocytes via SREBP1c-lipogenic pathway (*de novo* lipogenesis).

V. Identification of potential molecular initiating events

nFRs	TDCIPP	TPHP	TMPP	TCEP	TNBP	TCIPP	TBBPA	TBOEP	EHDPP
PXR	++	++	++	+	++	++	++	++	++
PPARγ	+	++	++	-	+	-	++	+	++

Figure 6. Binding of several nFRs to nuclear receptors as per human fluorescence reporter assay in HepG2 cells from the ToxCast database.
(++) indicate AC50 <10 µM, (+) indicate AC50 >10 µM, (-) indicate not active.

nFRs	CASN	Binding energy	
		PXR	PPARγ
TMPP	1330-78-5	-8.6	-7.4
TPHP	115-86-6	-8.3	-6.7
EHDPP	1241-94-7	-7.7	-7.4
TDCIPP	13674-87-8	-5.5	-5.2

Figure 7. The binding energy (kcal/mol) for PXR, PPARγ, and selected nFRs
PXR: TMPP<TPHP<EHDPP<TDCIPP
PPARγ: TMPP<EHDPP<TPHP<TDCIPP

FUTURE DIRECTIONS

- Elucidation of molecular mechanisms and signalling pathways for nFRs-induced metabolic dysfunction.
- Assessment of ecotoxicological effects of nFRs in aquatic species (zebrafish).

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