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MUNI | RECETOX

Elucidation of the impact of Poly-and perfluorinated compounds (PFAS) on the liver metabolome and associated diseases using a 3D advanced *in vitro* model

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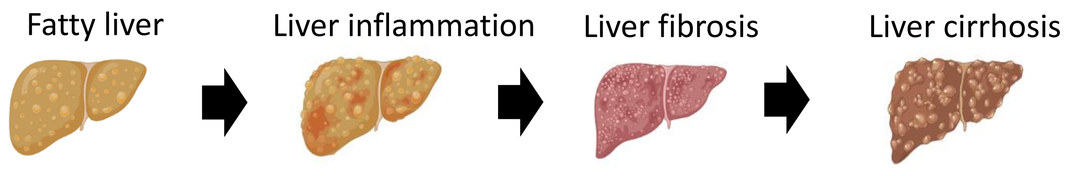
INTRODUCTION AND BACKGROUND

- **Poly- and perfluorinated compounds (PFAS)** are highly stable substances extensively employed in applications such as textiles, medical devices or firefighting foams. Due to their global ubiquity, persistence, bioaccumulation and recognized toxic potential, the whole class of PFAS have been proposed to be restricted in the European Union [1].
- **Exposures to PFAS** are **associated with endocrine and metabolic dysfunctions** [2]**.** These effects are elicited primarily through non-genotoxic perturbations, including inhibition of metabolic cooperation facilitated via gap junctions in the liver cells, that may contribute to **acute or chronic liver diseases** such as metabolic dysfunction-associated fatty liver disease (**MAFLD**) and promotion and progression of **hepatocellular carcinoma (HCC).**
- MAFLD is a term referring to liver conditions, e.g., a **build-up of fat in the liver (steatosis)** to **permanently damaged liver (cirrhosis)** which can contribute to the development of **HCC.**



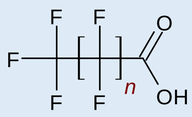
- This research aims to investigate the **impact of PFAS mixtures on the liver metabolome**, employing an advanced **scaffold-free 3D HepG2 *in vitro* model** in **long-term dynamic cultivation** and combining it with **^{1}H nuclear magnetic resonance spectroscopy (NMR),** followed by targeted analyses of selected genes, proteins and markers by **qPCR,** immunocytochemistry (**ICC**), biochemical assays.

GOAL OF THE STUDY: Investigating the impact of a reconstituted real-life PFAS mixture on the liver metabolome using a more physiologically relevant *in vitro* system in combination with NMR-based metabolomics.

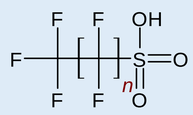
In vitro* system:** ***>Human cells >3D architecture >Dynamic conditions >Long-term culture & exposure >Larger sample size >Feasibility to combine with NMR

METHODOLOGY

Compounds of interest in the PFAS mixture



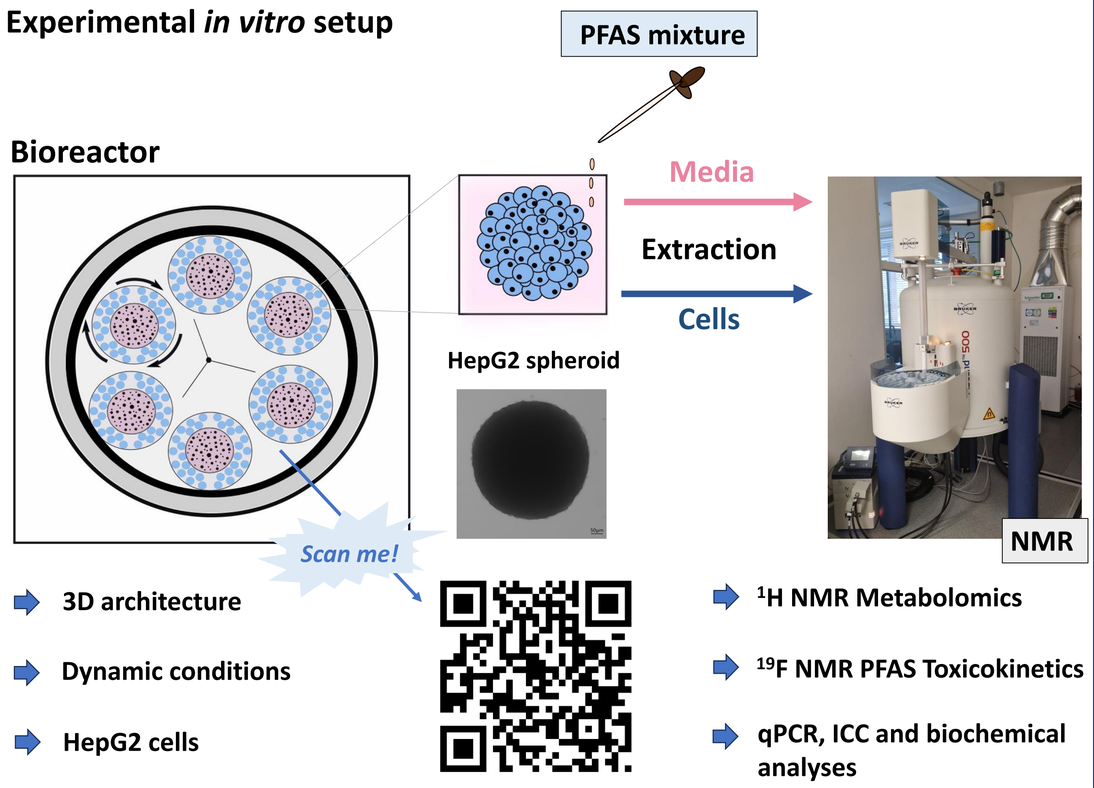
PFHpA: Perfluoro-**hepatoic**-acid (n=4)
PFOA: Perfluoro-**octanoic**-acid (n=5)
PFNA: Perfluoro-**nonanoi**c-acid (n=6)
PFDA: Perfluoro-**decanoic**-acid (n=7)
PFUnDA: Perfluoro-**undecanoic**-acid (n=8)



PFHxS: Perfluoro-**hexanesulfonic**-acid (n=5)
PFOS: Perfluoro-**octanesulfonic**-acid (n=7)

Reconstructed mixture *represents PFAS composition found in the seminal plasma of occupationally exposed firefigthers: [4.72 : 36.05 : 2.15 : 6.44 : 3 :16.74 : 30.9 %] [PFHpA:PFOA:PFNA:PFDA:PFUnDA:PFHxS:PFOS]*

Experimental *in vitro* setup



- **3D architecture**
- **Dynamic conditions**
- **HepG2 cells**
- **^{1}H NMR Metabolomics**
- **^{19}F NMR PFAS Toxicokinetics**
- **qPCR, ICC and biochemical analyses**

- **3D HepG2 spheroids** were formed from a cell suspension seeded into the ClinoStar™ system. After 28 days, maturated spheroids were exposed for 7 days to a reconstituted real-life PFAS mixture (total PFAS conc. 10 µM & 100 µM, solvent control).
- **Post-exposure extraction** of **non-polar** and **polar** metabolites, from both **media** and **cells** was conducted, using a multiple-solvent extraction method (Methanol/H_2O/MTBE).
- **NMR measurement** was performed on a 500 MHz spectrometer (Bruker Ascend™ 500) using a 1D nosey pulse sequence for the ^{1}H NMR and ^{1}H-decoupled ^{19}F NMR.
- **qPCR, ICC and biochemical analyses** of markers relevant for MAFLD/HCC are in progress.

MTBE (Methyl tert-butyl ether)

RESULTS

Formation and growth of human liver spheroids during 35 days of dynamic culture in the Clinostar™ system

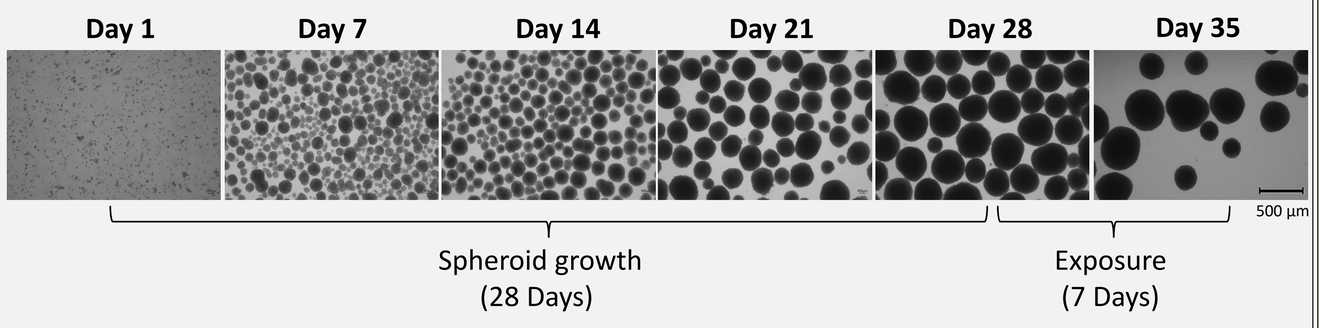
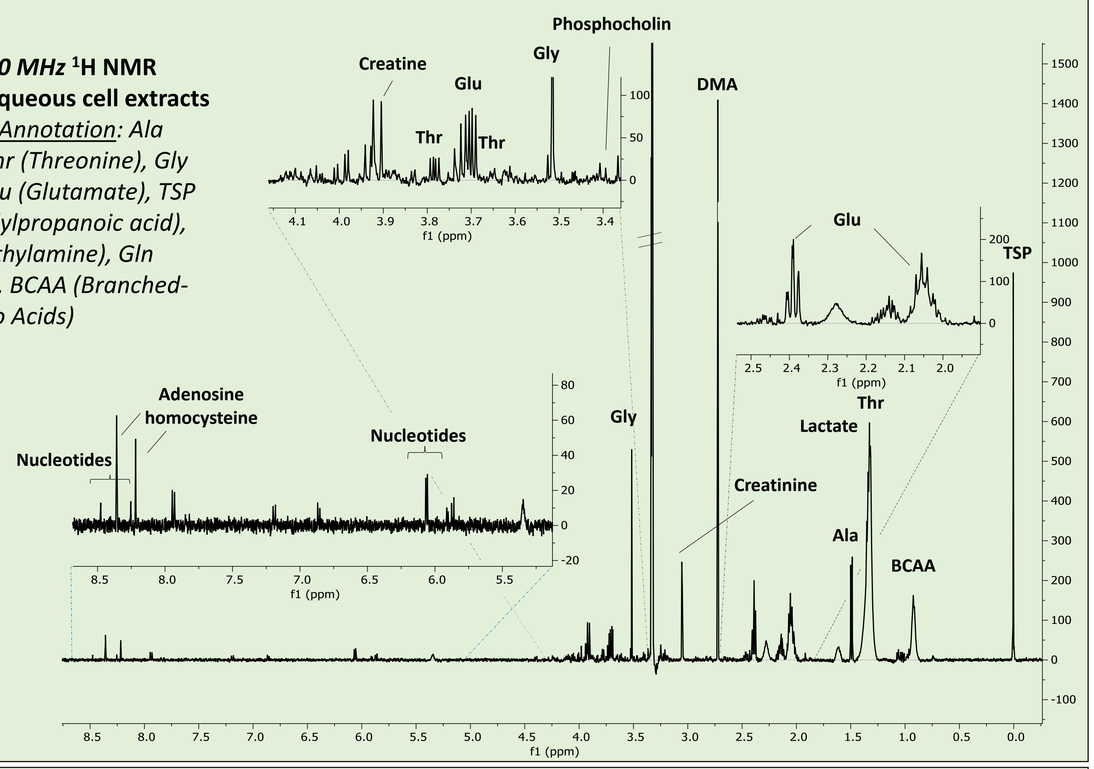


Figure 1: *Microphotographs of 3D HepG2 spheroids growing for 35 days in the dynamic culture of ClinoStar™ bioreactors (CelVivo). After 28 days spheroids were exposed for 7 days to a total concentration of 10 µM and 100 µM PFAS mixture as well as a negative control (solvent-treated cells).*

- **Clinostar™ is well-suited** for long-term studies.
- **Large sample quantities** (600-1000) of spheroids, and ~8 mL of media could be harvested from each bioreactor, which **provided enough sample material for NMR** based metabolomics and other downstream analyses such as qPCR and ICC.

^{1}H NMR-cell spectra of the aqueous extract metabolome of 35-day-old spheroids

Figure 2: 500 MHz **^{1}H NMR spectra of aqueous cell extracts** *Preliminary Annotation: Ala (Alanine), Thr (Threonine), Gly (Glycine), Glu (Glutamate), TSP (Trimethylsilylpropanoic acid), DMA (Dimethylamine), Gln (Glutamine), BCAA (Branched-Chain Amino Acids)*



^{1}H NMR spectra of aqueous extract resulted in a metabolic fingerprint of 30–40 metabolites including amino acids, carbohydrates, nucleotides, carboxylic acids and lipids.

PFAS could be detected by ^{19}F NMR in the media and in 100 µM exposed cell extracts.

Human cells ✓ ***3D architecture*** ✓ ***Dynamic conditions*** ✓ ***Long-term culture & exposure*** ✓ ***Larger sample size*** ✓ ***Feasibility to combine with NMR*** ✓

CONCLUSION: Combining the a Clinostar™ system with NMR based metabolomics enabled the performance of sophisticated *in vitro* analyses exploring the impacts of PFAS mixtures on the liver metabolome, with ongoing evaluations currently underway.

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Brno Ph.D. Talent

Faculty of Agrobiology, Food and Natural Resources

References:

[1] European Chemicals Agency (ECHA). (last time accessed 17.01.24). All news - ECHA (europa.eu)
[2] S. Fragki et al.: Crit. Rev. Toxicology, 51 (2021) 141–164.