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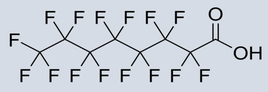


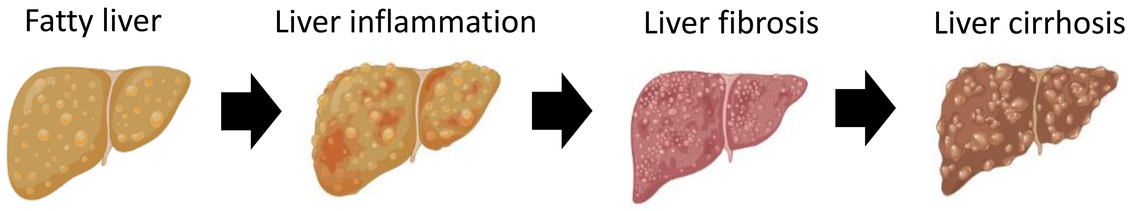
Perfluorooctanoic acid (PFOA)

Metabolomic and toxicokinetic *in vitro* approach for studying chemically-induced chronic liver diseases

Perfluorooctanesulfonic acid (PFOS)

INTRODUCTION AND BACKGROUND

- Acute or chronic exposures of the human body to chemicals can lead to **acute or chronic liver diseases** such as nonalcoholic fatty liver disease (**NAFLD**).
- NAFLD is an umbrella term covering a range of liver conditions, from a **build-up of fat in the liver (steatosis)** to **permanently damaged liver (cirrhosis)** [1][2].



- Underlying mechanisms of NALFD development include disruption of lipid metabolism in the liver cells. It is strongly associated with metabolic and cardiovascular disorders, such as obesity and type 2 diabetes [3].
- **Perfluorooctanesulfonic acid (PFOS)** and **perfluorooctanoic acid (PFOA)** have been repeatedly **associated with metabolic disruptions**, manifested e.g., as **increased concentrations of cholesterol** in the blood as well **as elevated triglyceride levels** [4].
- Additionally, PFAS has an association with **worsening NAFLD** and the **development of hepatic steatosis** [5].
- Our preliminary *in vitro* results suggest a **structure-dependent effect on lipid homeostasis** since **PFOS did induce lipid accumulation** in the 2D HepG2 cell-based model, but **PFOA did not** (**Figure 1**).

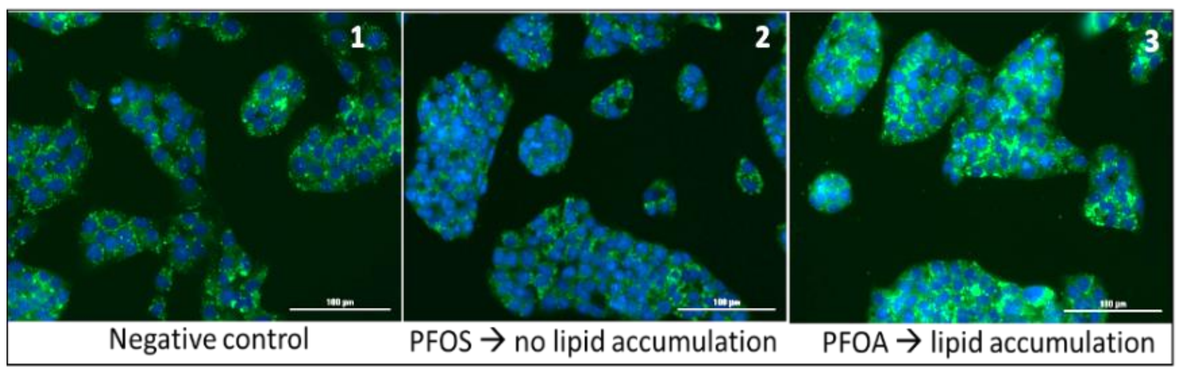


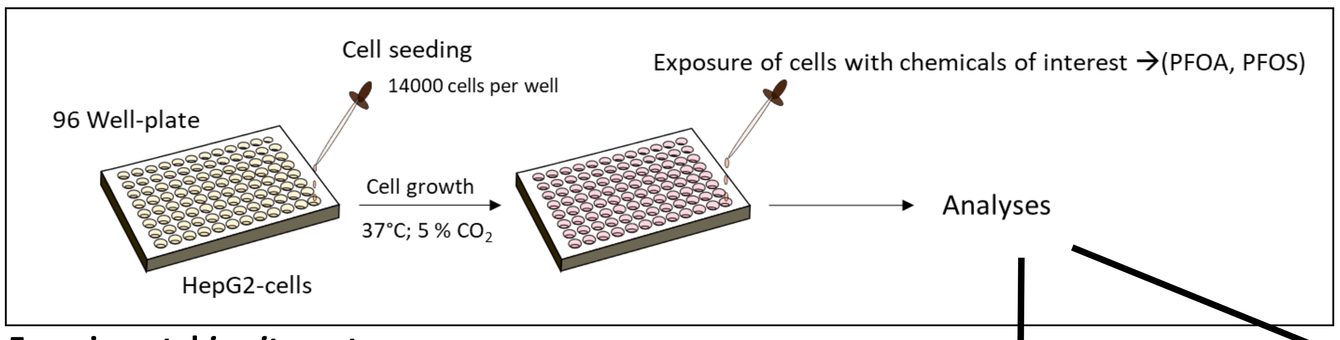
*Figure 1: Preliminary data showing 2D HepG2 cells after the 48-h exposure to 25 µM PFOA **(3)** and PFOS **(2)** as well as a negative control (solvent-treated cells) **(1)**. The exposed cells stained with BODIPY 493/503 (**green**) and DAPI (**blue**) (scale bar = 100 µm)*

- Thus, these substances are great candidates to investigate chronic liver diseases of PFASs **as direct comparison of structurally similar substances**

RESEARCH QUESTION

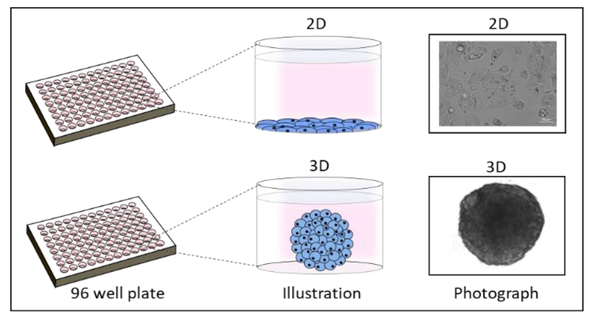
What is the contribution of the perfluorinated compounds to the development of nonalcoholic fatty liver disease (NAFLD) and what are the cellular and molecular mechanisms involved?

METHODS AND PLANS



Experimental *in vitro* setup

I will use HepG2 cell-based traditional 2D and advanced 3D *in vitro* models for this project.



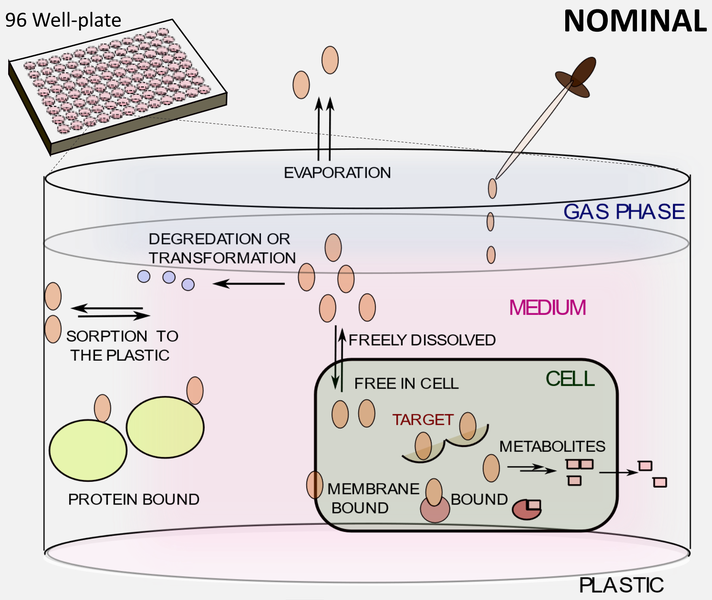
TOXICOKINETIC APPROACH (ongoing)

Evaluation of the behavior and toxicokinetic distribution of the chemicals within the 2D and 3D HepG2 cell-based in vitro systems (*in vitro* toxicokinetic assessment).

Using the $C_{NOMINAL}$ for risk assessment can lead to **FALSE RESULTS!**

→ **UNDERESTIMATION OF THE TOXIC POTENTIAL OF A COMPOUND.**

Factors which can affect the nominal concentration:
Protein or plastic binding, transformation, metabolism and evaporation of the chemical from the *in vitro* system → **Nominal concentration ≠ effective dose at the target site in cells!**



NOMINAL CONCENTRATION ($C_{NOMINAL}$)
Amount of a chemical added to the *in vitro* system divided by the exposure medium volume **[6]**.

BIOLOGICALLY EFFECTIVE DOSE
The dose at the target site in cells or tissues that cause a toxicological effect **[6]**.

Biologically effective concentration *in vitro* ??

Computational determination in collaboration with INERIS (France). VIVD [7]

Experimental determination in 2D and 3D *in vitro* liver models

METABOLOMIC APPROACH (starting this year)

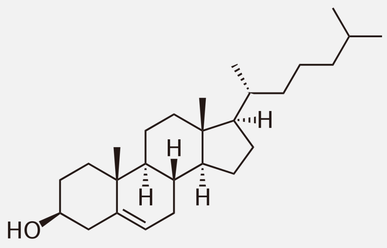
What are the effects of PFOS and PFOA on the 3D HepG2 *in vitro* models and what are the mechanisms involved (*in vitro* toxicodynamic assessment)?

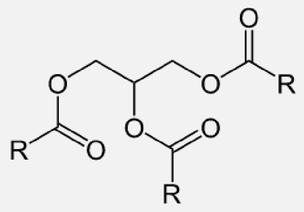
- Study the impact of the chemicals on the metabolome on the 3D HepG2 model by **NMR-based metabolomics**.

NMR

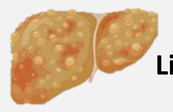
Collaboration with the lab of doc. Ing. Jaroslav Havlík, Ph.D. from the Dpt. of Food Science from the Czech University of Life Sciences Prague. Faculty of Agrobiology, Food and Natural Resources

- Study the impact of the chemicals on lipid homeostasis in the 3D HepG2 model by **HPLC-MS-based metabolomics**.





- Study the impact of the chemicals on the lipid droplet proteins (e.g. perilipins) using targeted **HPLC-MS proteomics**.



Liver inflammation

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Acknowledgments: This work was funded by the European Union's Horizon 2020 research and innovation program under grant agreement no 825712 - OBERON project (https://oberon-4eu.com), that is part of the European EURION cluster (http://eurion-cluster.eu/).

Brno Ph.D. Talent