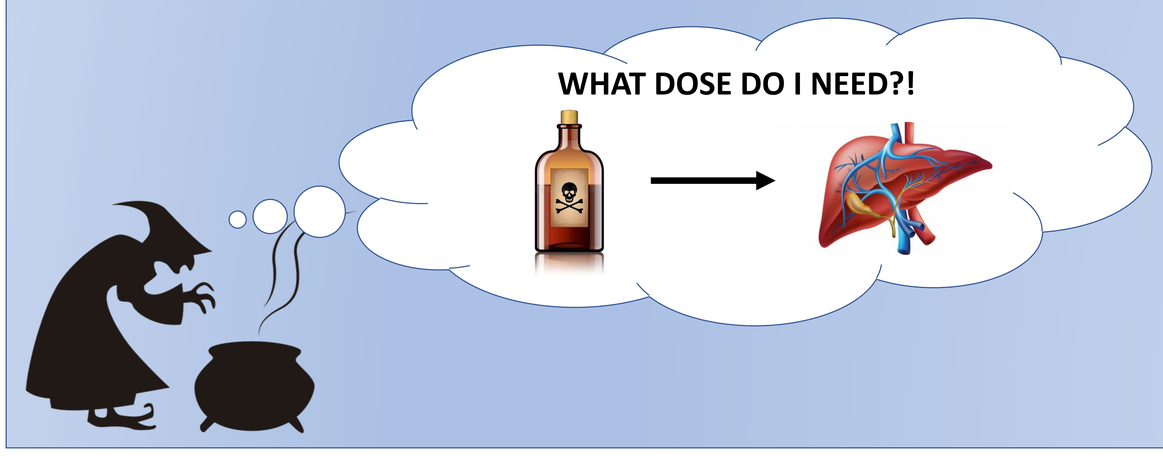
Daniela Brenner E-mail: daniela.brenner@recetox.muni.cz
Supervisor: Dr. Iva Sovadinová **Consultant:** Dr. Pavel Babica
Collaborators: Marina Felipe Grossi, Ishita Virmani, Jana Navrátilová, Ph.D., Eliška Sychrová, Ph.D.
Group: Cell and Tissue Toxicology; **Web:** http://secantox.weebly.com/; **Twitter:** @ToxCell

MUNI | RECETOX



Toxicokinetic assessment of metabolism-disrupting chemicals in 2D and 3D *in vitro* liver models to improve quantitative *in vitro* to *in vivo* extrapolation (QIVIVE)

OBJECTIVES

- **Toxicokinetic approaches using computational modelling**
- **Direct toxicokinetics in 2D & 3D *in vitro* models of HepG2 cells**

} **to determine the effective concentrations of 7 selected metabolism-disrupting chemicals (MDCs)**

- **Quantitative extrapolation of the *in vitro* and computational results to biologically effective concentrations *in vivo*.**

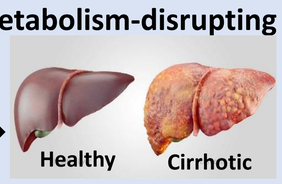
BACKGROUND

What is the problem I need to solve?

- ***In vitro* human cell-based models** play a crucial role in the 21st century toxicology → replacement of **animal-based methods;** more suitable **for high-throughput screening**
- So far, toxicity assessment of a tested chemical is mostly based on **nominal concentrations** (the amount of a chemical added divided by the exposure medium volume).
- However, the **added chemical concentration can be affected by several factors!!**
- Using the $C_{NOMINAL}$ for risk assessment can lead to **FALSE RESULTS!!** → **UNDERESTIMATION OF THE TOXIC POTENTIAL OF A STUDIED COMPOUND.**
- Consequently, **target (intracellular),** or at least **free, *in vitro* concentrations** should be used for a precise quantitative *in vitro* to *in vivo* extrapolation (**QIVIVE**).

Which chemicals I am going to analyze?

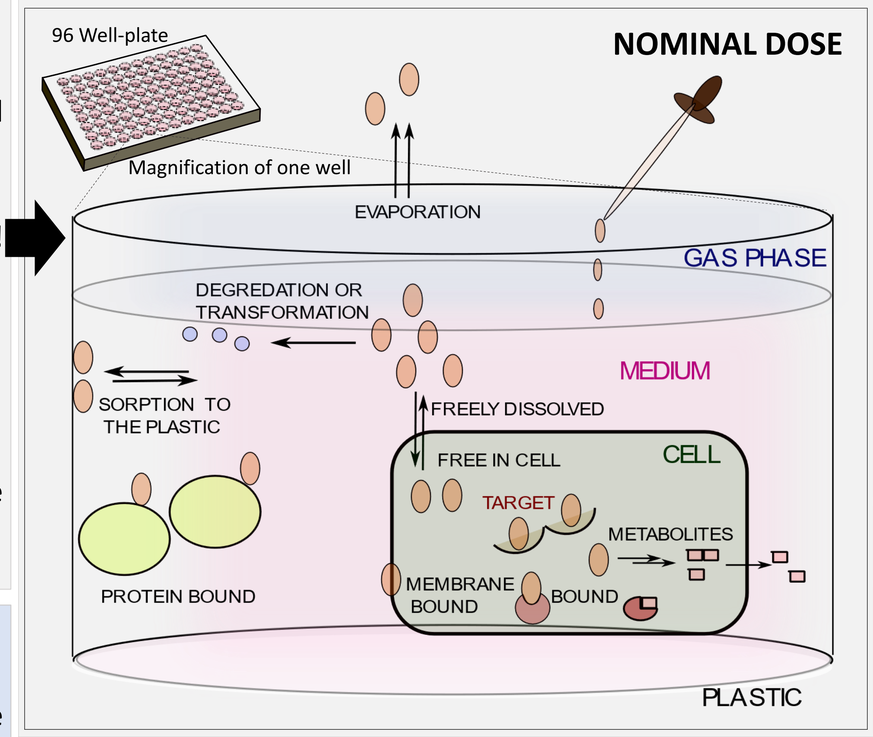
- Endocrine-disrupting chemicals (**EDCs**) are suspected of causing, beside reproductive abnormalities. **metabolic disorders**; these EDCs are called **metabolism-disrupting chemicals (MDCs)**.
- MDCs exposures are associated with **chronic liver diseases [2]**.



- Our Cell and Tissue Toxicology Group (SECANTOX) is part of the EU-funded H2020 **OBERON project**, which aims to establish Integrated **Approaches to Testing and Assessment (IATA)** to detect EDCs-related metabolic disorders where *in vitro* toxicokinetics and QIVIVE should play an indispensable role.

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 **[1]**.

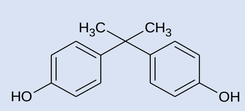
TOTAL CONCENTRATION (C_{TOTAL})
Concentration in the exposure medium. This includes compounds which are freely available in medium + compounds bound to medium constituents **[1]**.

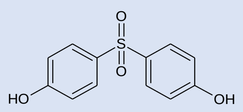
FREEE CONCENTRATION (C_{FREE})
The unbound concentration of a compound in the exposure medium **which is available for uptake into the cells [1]**.

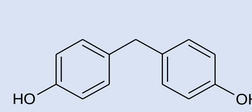
INTRACELLULAR CONCENTRATION (C_{CELL})
Concertation in the cell **[1]**.

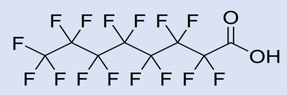
BIOLOGICALLY EFFECTIVE DOSE
The dose at the target site in cells or tissues that cause a toxicological effect **[1]**.

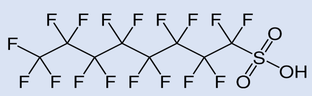
***In vitro* toxicokinetics research is being conducted for 7 MDCs from the OBERON project.**

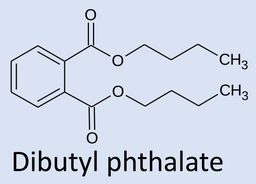

Bisphenol A (**BPA**)

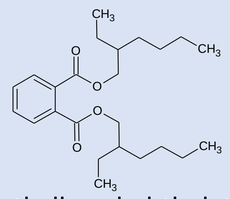

Bisphenol S (**BPS**)


Bisphenol F (**BPF**)


Perfluorooctanoic acid (**PFOA**)


Perfluorooctanesulfonic acid (**PFOS**)

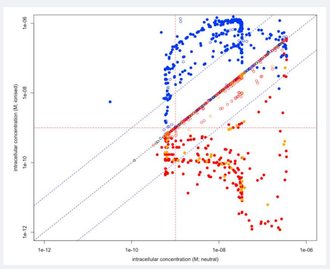

Dibutyl phthalate (**DBP**)


Diethylhexyl phthalate (**DEHP**)

METHODS

A multi-pronged approach to determine the biologically effective concentration (BED) of MDCs.

1. Computational determination

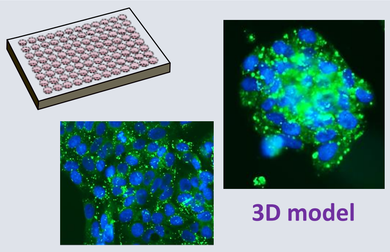


Computational modeling of C_{FREE}, C_{CELL}, C_{TOTAL} using the VIVD model [3]

This step will proceed in collaboration with Dr. Celline Brochot, the unit "Models for Ecotoxicology and Toxicology", INERIS, France.

Best approach

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



Direct determination of C_{FREE}, C_{CELL}, C_{TOTAL}

Effective concentration *in vitro*

Indirect determination of C_{FREE}, C_{CELL}, C_{TOTAL}

Experimental plan and methods in 2D *in vitro* liver models

- 96-well plate design
- Usage of **HepG2 cell line** (human liver cancer cell line purchased from ATCC® HB-8065™)
- 48-h exposure with the respective MDC →conc.: 0.01; 0.1; 1; 10; 25 µM solution in 0.2 % DMSO

- **Determination of C_{FREE}**
  → e.g., solid phase microextraction (SPME)
- **Determination of C_{CELL}**
  → e.g., removing of medium followed by cell extraction with acetonitrile:water solution (1:2)
- **Determination of C_{TOTAL}**
  → e.g., extraction from the exposure medium with acetonitrile

- **Determination of $C_{PLASTIC}$, $C_{EVAPORATED}$, C_{BOUND} for indirect determination of C_{FREE} and C_{CELL} and C_{TOTAL}**
  → e.g., DC assay (C_{BOUND}),…

$$C_{TOTAL} = C_{NOMINAL} - C_{PLASTIC} - C_{EVAPORATED} - C_{DEGREDATION}$$
$$C_{FREE} = C_{TOTAL} - C_{BOUND} - C_{CELL}$$
$$C_{CELL} = C_{TOTAL} - C_{BOUND} - C_{FREE}$$

- **Measurements with HPLC-MS or GC-MS**

Quantitative in vitro in vivo extrapolation (QIVIVE) i.e., physiologically based toxicokinetic modelling (PBTK) leads to:

Biologically effective concentration *in vivo*

PRELIMINARY RESULTS

- **Experimental work**: The first analysis of BPA, BPF and BPS for direct determination of C_{TOTAL} and C_{CELL} showed **high blind values (BV)** → possible **sources** which led to contamination can be plastic **lab equipment** and contaminated **water** → first attempts were performed to discover sources to reduce BV:
  1. Measurement of water blanks (2 water sources)
  2. Usage of more glass ware and less plastic equipment
  3. Method adaptation to reduce the contamination of BPA, BPF, BPS from the plastic
- **Literature research**: Reviewing of available methods for the evaluation of C_{FREE} and C_{BOUND} .

OVERVIEW & PLANS FOR THE NEXT YEAR

- Usage of more sophisticated **3D *in vitro* models** → for increased number of target sites; for better analyses of metabolism.
- **Planned research stay abroad** in the unit "Models for Ecotoxicology and Toxicology" INERIS (France). →**Training to use *in vitro* kinetics models**
- Poster presentations at the **EUROTOX Congress 2021** and **the 10th Annual Meeting of the ASCCT 2021**

INERIS

REFERENCES

[1] F. A. Groothuis, *et al.*: Dose metric considerations in *in vitro* assays to improve quantitative *in vitro-in vivo* dose extrapolation. *Toxicology* 332 (2015) 30-40
[2] R. Cano, *et al.*: Role of Endocrine-Disrupting Chemicals in the Pathogenesis of Non-Alcoholic Fatty Liver Disease: A Comprehensive Review. *International Journal of Molecular Sciences* 4807 (2021) 1-22
[3] C. Fisher, *et al.*: VIVD: virtual *in vitro* distribution model for the mechanistic prediction of intracellular concentrations of chemicals in *in vitro* toxicity assays. *Toxicology in Vitro* 58 (2019) 42-50

Acknowledgments: This work is 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/).

OBERON