In vitro relative potencies of perfluoroalkyl substances (PFASs) based on gene expression changes in human HepaRG liver cells

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Aims of the study

- To obtain insight into the cellular effects of PFASs on the human liver by studying concentration-dependent whole genome gene expression changes in PFOS-exposed HepaRG cells.
- To determine in vitro relative potencies of a series of PFASs (**Figure 1**) based on concentration-dependent changes in expression of selected genes in PFAS-exposed HepaRG cells.

Methods

Test system and exposure: Human HepaRG liver cells were exposed for 24 hours to increasing concentrations of PFASs (**Figure 1**).
Readouts: Microarray (Affymetrix Human Gene 2.1 ST arrays) for PFOS and RT-qPCR for selected genes for all PFASs.
Data analysis: BMDE express microarray data, PROAST BMD modelling RT-qPCR data.

Results

- BMDExpress analysis of PFOS microarray data point to various affected cellular processes, of which several are related to cholesterol biosynthesis, lipid metabolism pathways, or endoplasmic reticulum (ER) stress (**Figure 2**).
- Based on the PFOS microarray data, 10 genes were selected (**Figure 3**) to study the concentration-dependent effects of 18 PFASs (**Figure 1**). Concentration-response data of a selection of PFASs on *OAT5* expression are shown in **Figure 4.**
- Data are being analysed using PROAST software to determine in vitro relative potency factors (RPFs; compared to PFOA). Preliminary results for the *OAT5* gene are presented in **Figure 5**. Comparison of these in vitro RPFs with reported RPFs obtained from in vivo data (Bil et al., 2021 (doi: 10.1002/etc.4835); 2022 (doi: 10.1002/etc.5236)) is presented in **Figure 6**.

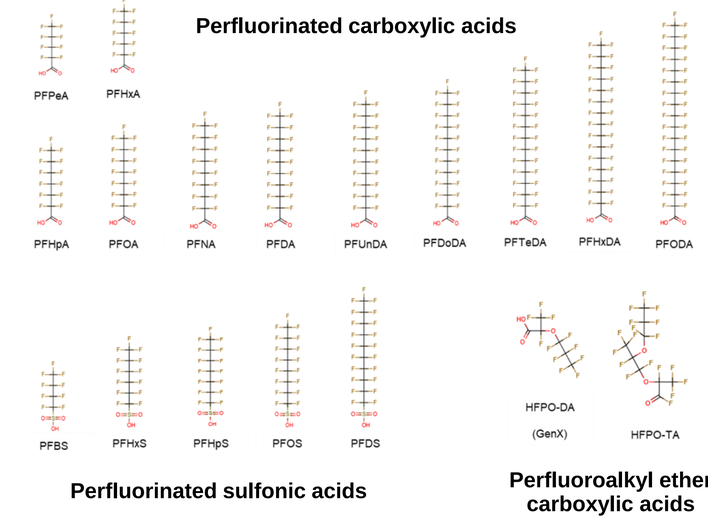

Figure 1. PFASs tested in the present study.

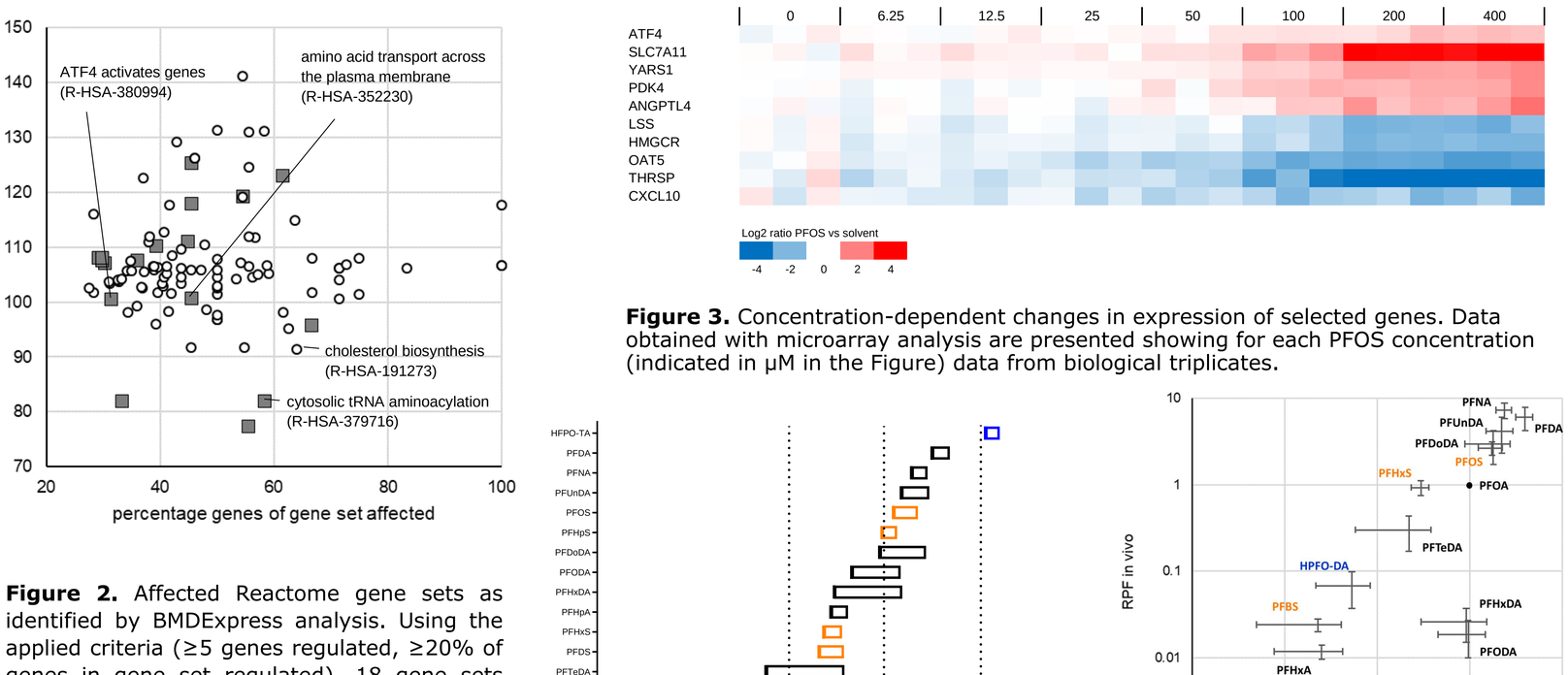

Figure 2. Affected Reactome gene sets as identified by BMDExpress analysis. Using the applied criteria (≥5 genes regulated, ≥20% of genes in gene set regulated), 18 gene sets were upregulated (≥ 60% of regulated genes upregulated; grey squares) and 90 gene sets were downregulated (≥ 60% of regulated genes downregulated; open circles). Gene sets related to processes that were previously found to be affected in HepaRG cells by 100 µM PFOA, PFOS and PFNA (Louisse et al., 2020; doi: 10.1007/s00204-020-02808-0) are indicated.

Figure 3. Concentration-dependent changes in expression of selected genes. Data obtained with microarray analysis are presented showing for each PFOS concentration (indicated in µM in the Figure) data from biological triplicates.

Figure 5. In vitro RPFs (90% confidence intervals) of PFASs based on gene expression changes of *OAT5* in HepaRG cells, using PFOA as index chemical (RPF=1; 90% confidence interval BMC10 PFOA: 1.4-3.2 µM).

Figure 6. Comparison of in vitro RPFs (Figure 5) with reported in vivo RPFs obtained from data on PFAS-induced relative liver weight increase in male rats (Bil et al., 2021; 2022). 90% confidence intervals of RPF values are presented.

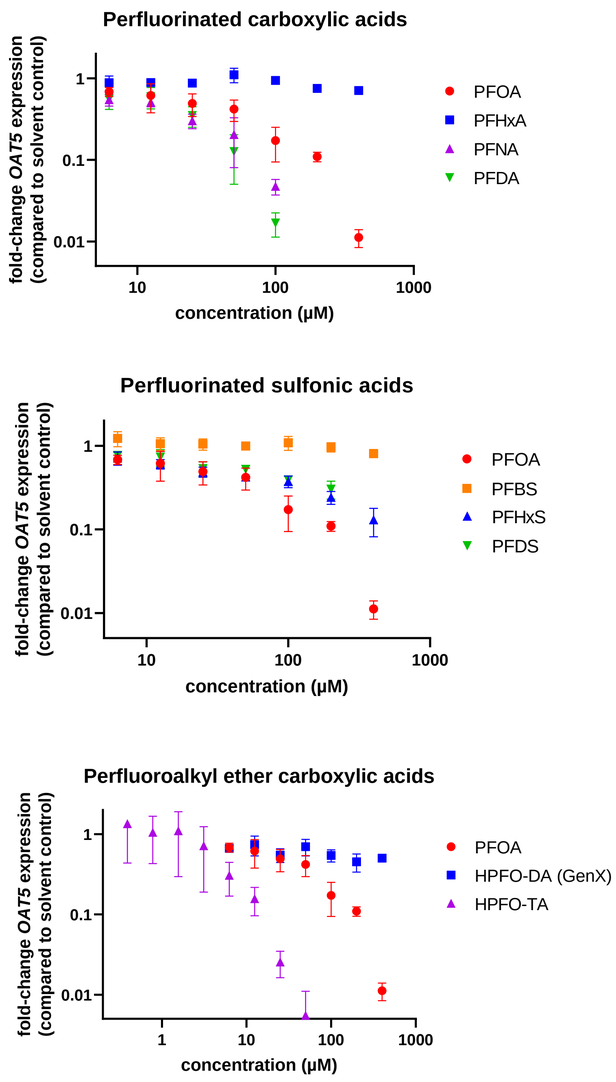

Figure 4. Concentration-dependent changes in expression of *OAT5* by selection of PFASs.

Conclusions

- The PFOS microarray data from HepaRG cells provide mechanistic insights in the effects of PFASs on the liver.
- Decrease in *OAT5* expression in HepaRG cells can be used as readout to estimate toxic potencies of PFASs.

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