Toxicological activity profiling and potency ranking of per- and polyfluoroalkyl substances (PFAS) using ToxProfiler®

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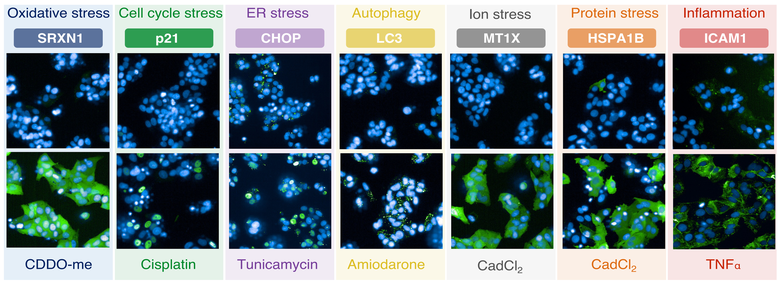
Introduction

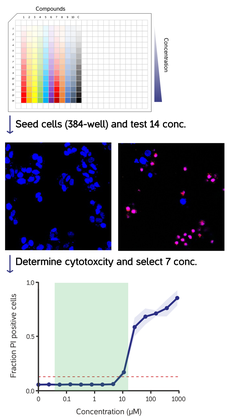
- **Per- and polyfluoroalkyl substances (PFAS)**, also known as "the forever compounds", have the tendency to accumulate in organisms and their environment. Despite extensive *in vitro* and *in vivo* testing of PFAS, there is a remaining concern about the human safety of these chemicals.
- **ToxProfiler** is a unique human-based reporter assay that provides an extensive quantitative toxicological profile of novel chemicals and drugs. The assay contains seven fluorescent reporter genes to visualise the major cellular stress response pathways responsible for cellular/organ toxicity.
- **In this study, we have applied ToxProfiler to gain insight into:**

1. Biological activity of 13 often used PFAS.
2. Potency ranking and identification of the primary toxicological mode-of-action of PFAS.
3. Relationship between *in vitro* toxicity and structure-activity relationship (carbon chain length) of PFAS.

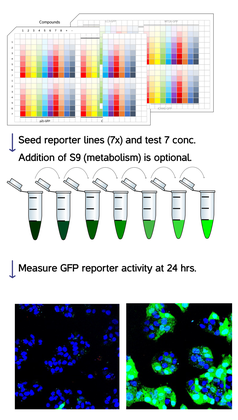
ToxProfiler: Experimental design



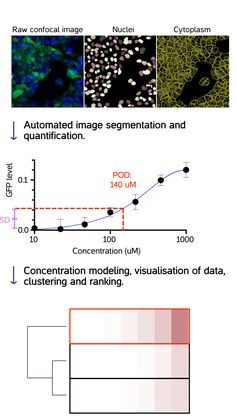
1. Concentration range finding
Goal: Determine appriopriate concentration range
Endpoint: Propidium iodide (cytotoxicity)



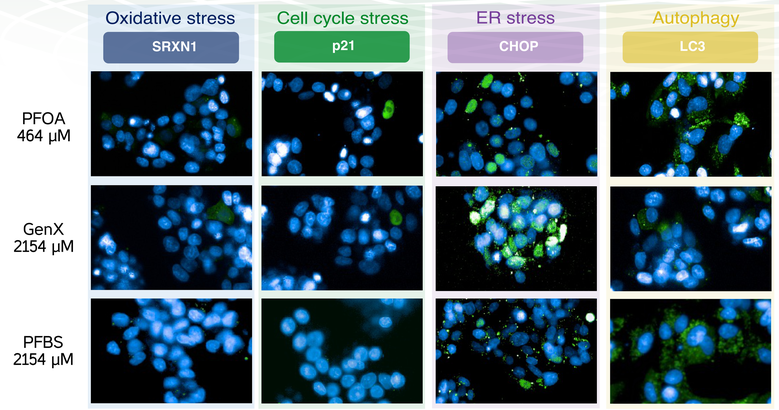
2. ToxProfiler reporter assay
Goal: Visualize ToxProfiler reporter inductions
Endpoint: Live cell confocal imaging



3. Image analysis and data processing
Goal: Quantify GFP responses on single cell level
Output: Concentration response plots/heatmaps/ clusterings/potency ranking.

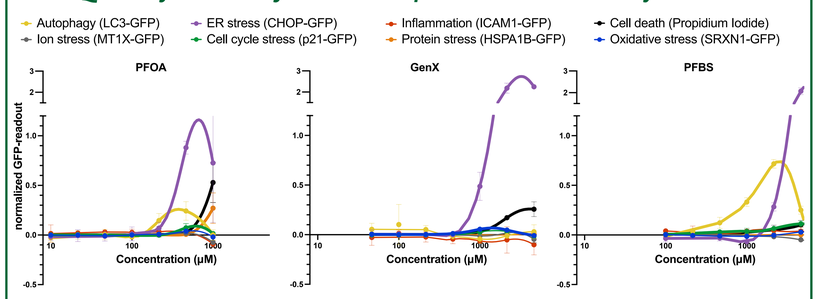


Cellular stress induction by PFAS



Representative ToxProfiler confocal images of three example PFAS. Pictures were generated using the automated Operetta CLS microscope setup at 24 hours after the exposure. PFOA, GenX and PFOS were chosen because these compounds are often used as model PFAS.

Quantification of stress response activation by PFAS

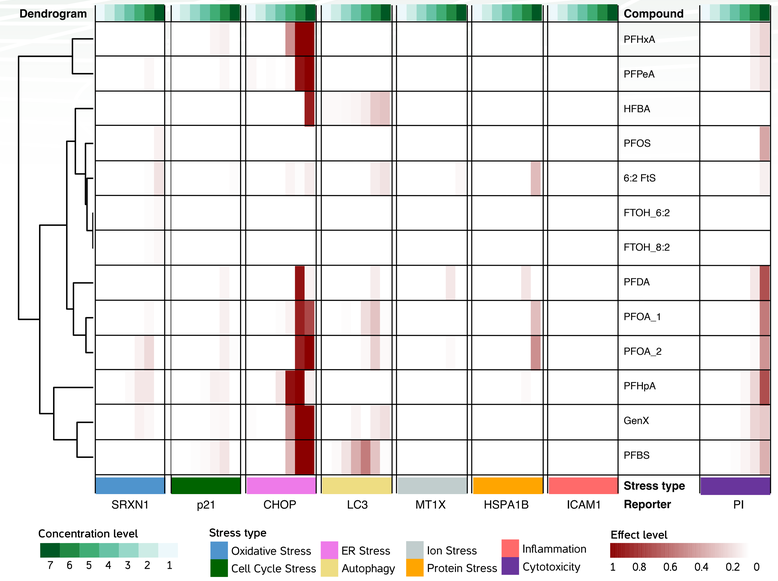


Example of ToxProfiler concentration response curves. Concentration response plotting allows to compare amplitide of the reporter activations (shift along y-axis) as well as the potency (shift along x-axis.)

Point of departures and potency ranking of PFAS

Compound name	CAS #	Carbon Length	Hepatotox RPF (Bil, 2021)	Concentration range ToxProfiler	Oxidative stress (SRXN1)	Cell cycle stress (p21)	ER stress (CHOP)	Autophagy (LC3)	Ion stress (MT1X)	Protein stress (HSPA1B)	Inflammation (ICAM1)	Cytotoxicity (PI)	ToxProfiler rank
FTOH_8:2	678-39-7	C10	0,04	1 - 100 µM	***68***								***1***
PFDA	335-76-2	C10	4 < RPF < 10	5 - 464 µM		3000	150	***93***	215	150		150	***2***
PFOA_1	335-67-1	C8	1	10 - 1000 µM	***120***	300	215	215		720		660	***3***
PFOA_2	3825-26-1	C8	1	10 - 1000 µM	***150***	300	300	215	300	710		500	***4***
PFBS	375-73-5	C4	0,001	100 - 10000 µM		900	1500	***150***				1500	***5***
6:2 FtS	2706-90-3	C5	0,01 < RPF <0,05	10 - 1000 µM	560	650	830	***170***	580	790		740	***6***
PFHpA	375-85-9	C7	0,01 < RPF < 1	46 - 4642 µM	418	511	***232***			1500		464	***7***
GenX	27619-97-2	C8	ND	46 - 4641 µM	557	603	***278***	696				1000	***8***
FTOH_6:2	647-42-7	C8	0,02	5 - 464 µM	***330***								***9***
PFOS	1763-23-1	C8	2	10 - 1000 µM	***600***							750	***10***
PFHxA	307-24-4	C6	0,01	50 - 5000 µM		750	***700***					1077	***11***
PFPeA	13252-13-6	C6	ND	100 - 10000 µM	1300	***900***	1200					1600	***12***
HFBA	375-22-4	C4	0,05	100 - 10000 µM			6800	***2154***				7400	***13***

Heatmap clustering of PFAS toxicity finger prints



Heatmap with hierarchical clustering of ToxProfiler reporter activation profiles of PFAS. The intensity of the red color of the heatmap is representative for the GFP reporter induction. The dendrogram on the left represents similarity scoring of the different chemicals. Note that PFAS are screened in a equitox level and not necessary on an equimolar fashion.

Conclusions

- ToxProfiler is a novel reporter assay that can be applied in early mechanistic toxicity testing which can unravel the toxicological MoA and help with the risk assessment of chemicals (e.g. PFAS).
- In general, ToxProfiler data suggests that PFAS with longer carbon chains (FTOH 8:2 and PFDA) are more potent than PFAS with shorter carbon lenghts (HFBA). This is concordant with the Calculated Relative Potency Factors (RPF) of PFAS regarding their hepatotoxicity.
- Majority of the tested PFAS strongly activated ER stress. Autophagy, oxidative stress and cell cycle stress were also induced by most of the tested PFAS. Activation of these ToxProfiler readouts is correlated with Drug Induced Livery Injury (DILI).
- PFAS with shorter carbon lengh (C4; PFBS and HFBA) strongly induced autophagy.
- GenX technology was developed to replace PFOA. A GenX derived compound was indeed found to less potently induce stress signalling. Nevertheless, the tested GenX derived compound is a strong ER stress inducer at high concentrations (>250 µM).

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