The Final EUTOXRISK Symposium 29

INDUSTRY CASE STUDY with Syngenta

How to de-risk a developmental pipeline for active substances using NAMs

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Industry Question & Case Study approach

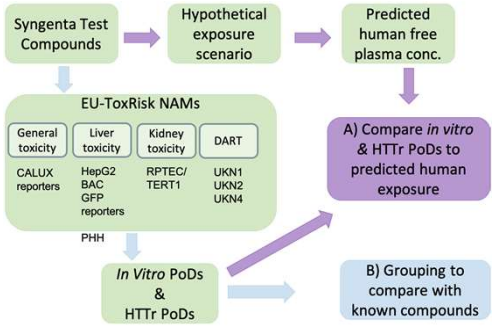
Introduction

Fast and reliable identification of unacceptable toxic effects is crucial in the early development of new active substances.

- NAMs can provide insights into underlying mechanisms of toxicity.
- Syngenta provided selection of discontinued research compounds that showed toxic effects in *in vivo* tests.

Aim: To apply a selection of the EU-ToxRisk *in vitro* test battery, relevant to DART and repeated dose toxicity (RDT), and determine if they can be used in screening of new active substances.

Case Study Strategy & Methods

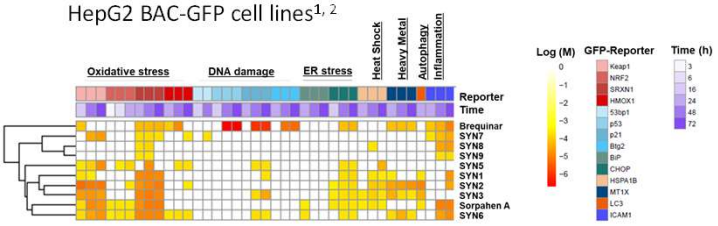


Schematic overview of case study testing approach with two ways to analyse the data.

Case Study Toxicodynamics NAM Toolbox

HepG2 BAC-GFP reporter cells

- Heatmap of PoDs from all 10 CS compounds in 14 different 2D HepG2 BAC-GFP cell lines[1, 2]



Compounds	Test. conc. range	HepG2 2D reporter results Brief Overall Conclusion
SYN3	0.03 – 300 µM	Induces an oxidative stress, heavy metal stress, unfolded protein and an autophagy response The cell cycle is inhibited (p21 induction and cell count reduction).
Soraphen A	0.03 – 300 µM	Induces an oxidative stress response at low concentrations. At higher concentrations, autophagy and unfolded protein response is activated. Some cell death is induced at the highest concentration.
SYN6	0.03 – 300 µM	Induces a strong oxidative stress response at low concentrations. At higher concentrations, an unfolded protein response is activated. The cell cycle is inhibited at the highest concentration.

Neurodevelopmental toxicity assays

- **Migration assay (UKN2)** – migration inhibition of neural crest cells (NCC)[3]
- **Neuritox (UKN4)** – neurite outgrowth in LUHMES cells[4]
- **UKN1** – impairment differentiation iPSC to neuroectoderm progenitors[5]

	Compounds	UKN2				UKN4				UKN1	
MoA	SYN/Name	BMC10 (V)	BMC25 (M)	BMC10(V)/ BMC25(M)	Specific (Y/N)	BMC25 (V)	BMC25 (NA)	BMC25(V)/ BMC25(NA)	Specific (Y/N)	BMC10 (V)	% DEG
ACCase inhibitors	Soraphen A	-4.7	-4.0	0.2	N	-3.7	-4.4	4.6	Y	-9.4	1.3
	SYN8	N/A	N/A	N/A	N	N/A	N/A	N/A	N	-4.5	1.6
	SYN5	-4.2	-4.5	2.0	Y	-3.7	-4.1	2.8	N	-5.4	18
	SYN6	N/A	N/A	N/A	N	N/A	N/A	N/A	N	-5.4	2.2
	SYN7	N/A	N/A	N/A	N	N/A	N/A	N/A	N	-4.5	0.3
	SYN9	N/A	N/A	N/A	N	N/A	N/A	N/A	N	-4.0	0.4
DHODH inhibitor	Brequinar	-4.0	-4.7	4.5	Y	-3.7	-4.2	3.1	N	-9.4	0.2
UNKNOWN	SYN1	-5.0	-5.4	2.2	Y	-5.0	-5.1	1.2	N	-5.4	28
	SYN3	-4.6	-5.1	3.0	Y	-4.4	-4.5	1.3	N	-5.0	19
	SYN2	-4.6	-4.5	0.8	N	-4.6	-4.7	1.1	N	-5.0	34

Definition of endpoint-specific effects: UKN2: BMC10(V)/BMC25(M) ≥ 1.3 UKN4: BMC25(V)/BMC25(NA) ≥ 4

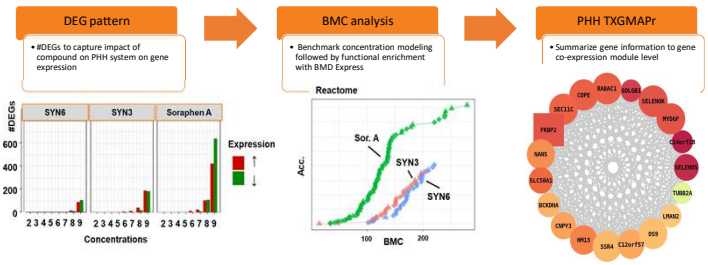
Compound SYN6 has no effect on NCC migration and LUHMES neurite outgrowth.

Soraphen A specifically inhibits the neurite outgrowth and is cytotoxic to both NCC and differentiating NEP.

Compound SYN3 specifically inhibits NCC migration and is cytotoxic to both differentiating LUHMES and NEP cells.

Soraphen A and **Brequinar** were extremely potent in the UKN1 viability assay.

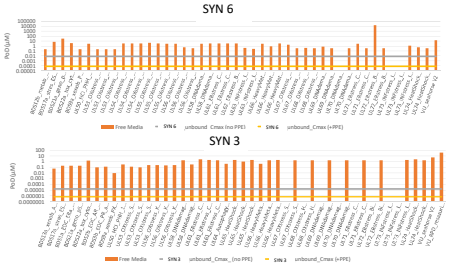
PHH transcriptomic analysis - an in depth look at SYN3 & SYN6



Compound SYN6 *induces concentration dependent gene expression changes. This is reflected in the BMC accumulation plots (blue line). BMC functional enrichment highlights DNA Damage responses. Early BMC modules for SYN6 indicate gene changes similar to those induced by Doxorubicin (DNA damager) and Diclofenac (liver toxicant). Module functional enrichments links this behavior to ncRNA processing (ncRNA have been shown to be players in the DNA Damage response)*

Compound SYN3 *also induces dose dependent gene expression changes. This is reflected in the BMC accumulation plot (pink line). BMC functional enrichment highlights inflammation-related responses. Early BMC modules for SYN3 indicate gene changes similar to those induced by acetominophen, nimensulide and Valproic acid (potential liver toxicants). Module functional enrichments links this behavior to PPARa gene expression regulation and fatty acid metabolism alterations.*

Conclusion of Case Study



Overall conclusions

- NAMs are able to detect adverse effects in the tested conc. range
- *In vitro* PoDs grouped in accordance with known MoA of CS compounds
- *In vitro* test methods and HTTr provided insights into toxic mechanisms
- Calculated unbound medium PoDs were all below the unbound concentrations using historical class specific exposure scenarios for SYN6 and SYN3.

References: **1.** Wink S et al. 2018, Arch Toxicol. Dynamic imaging of adaptive stress response pathway activation for prediction of drug induced liver injury. doi: 10.1007/s00204-018-2178-z. **2.** Wink S et al. 2017, Arch Toxicol. High-content imaging-based BAC-GFP toxicity pathway reporters to assess chemical adversity liabilities. doi: 10.1007/s00204-016-1781-0 **3.** Nyffeler J et al. 2017, ALTEX. Design of a high-throughput human neural crest cell migration assay to indicate potential developmental toxicants. doi: 10.14573/altex.1605031 **4.** Delp J et al. 2018, ALTEX. A high-throughput approach to identify specific neurotoxicants/ developmental toxicants in human neuronal cell function assays. doi: 10.14573/altex.1712182 **5.** Dreser N et al. 2019, Arch Toxicol. Development of a neural rosette formation assay (RoFA) to identify neurodevelopmental toxicants and to characterize their transcriptome disturbances. doi: 10.1007/s00204-019-02612-5

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www.eu-toxrisk.eu/