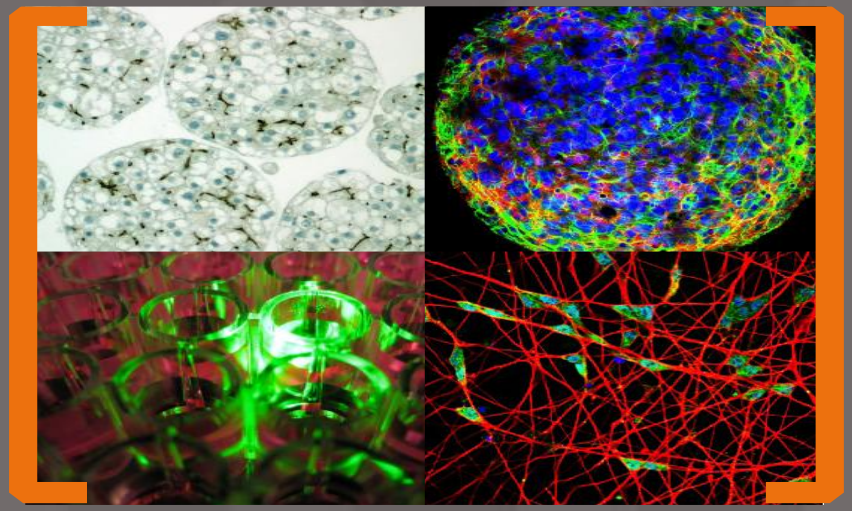
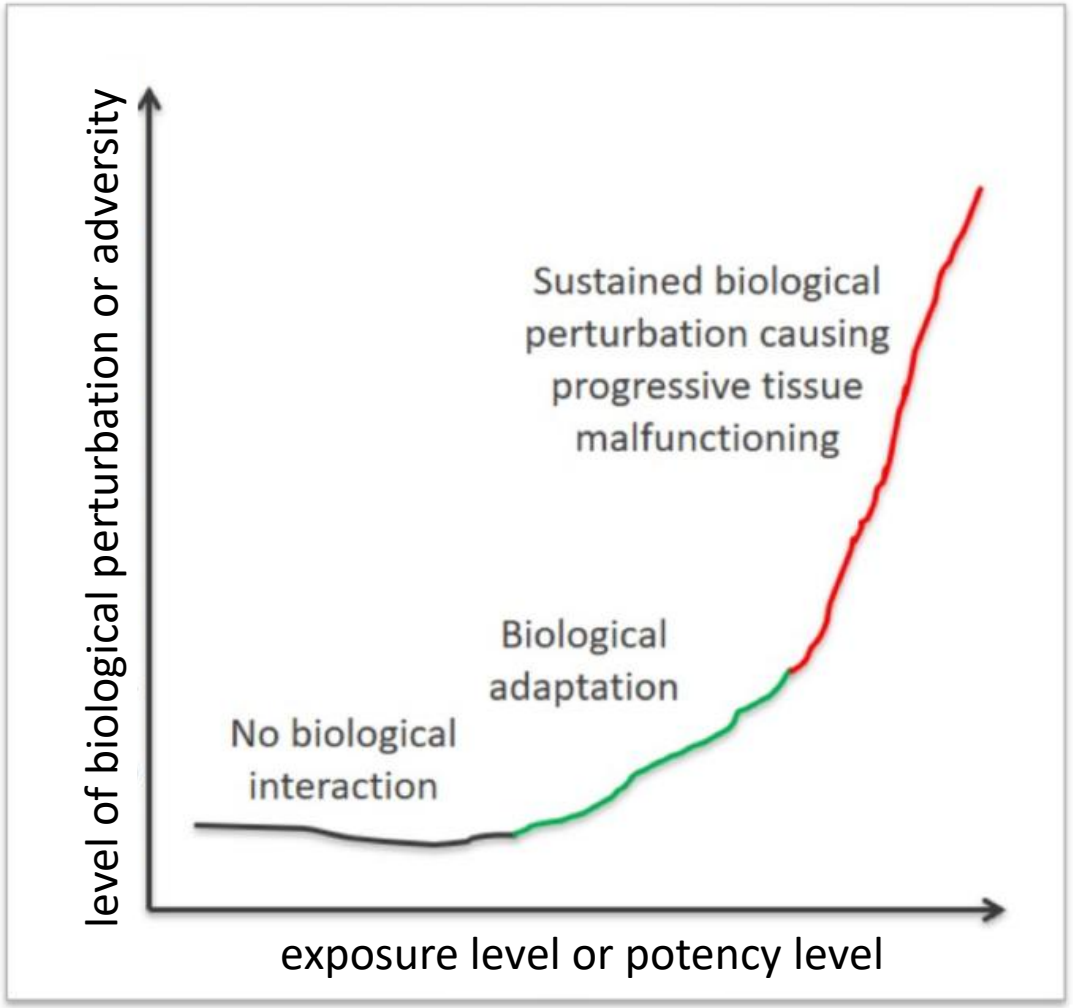
The case of a high-concern toxicity profile screening

Bob van de Water

Division of Drug Discovery and Safety
Leiden Academic Centre for Drug Research
Leiden University



Integration of NAMs into prioritization of low tonnage compounds



Low tonnage compounds do have a limited set of in vivo studies.

How can we best use NAM data to identify those that are of concern for human health?

EUTOXRISK

Case study hypothesis

Main hypothesis:

High throughput NAMs do properly **rank order ECHA substances** based on **mode-of-action** in relation to **CMR/ED** as well as overall potency on **perturbation of human biological systems** and can thereby add in the **prioritization of ECHA chemicals** for further testing.

Impact:

Ability to **classify compounds** and determine a **performance metric on concordance** would lead to further **confidence on the application of NAMs**.

Objective

Demonstrate the overall feasibility to identify substances with a liability for a high toxicity profile based on high throughput NAM hazard information.

Hazard information based on qualitative and quantitative mechanistic mode-of-action assessment using both *in silico* approaches and *in vitro* human test systems.

Overall approach

- **TRAINING** set: available pools of chemicals (high tonnage compounds/in vivo safety data) → selected from high tonnage ECHA list

- **Toolbox:** EU-ToxRisk *in silico* and *in vitro* NAMs with focus on high throughput

- **TEST** set: substances based on Annex 8 compounds (10-100 tonnes) that will have 28 day repro and basic mutagenicity *(optional)*

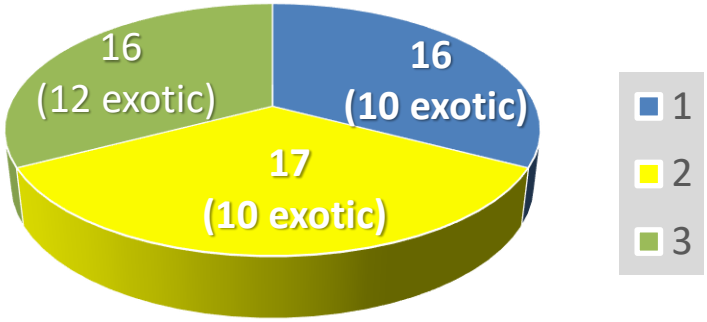
Proof of concept study chemical selection

150 ECHA compounds
From high tonnage ECHA list
(data rich)

Filter for compounds with available high quality repeated dose toxicity data

44 compounds with
RDT data in high quality databases analysed

Classification based on high quality databases



1. (blue) = toxic (NOEL < 10 mg/kg bw/d)
2. (yellow) = moderate
3. (green) = low toxic (NOEL >100 mg/kg bw/d)

Toxicodynamics

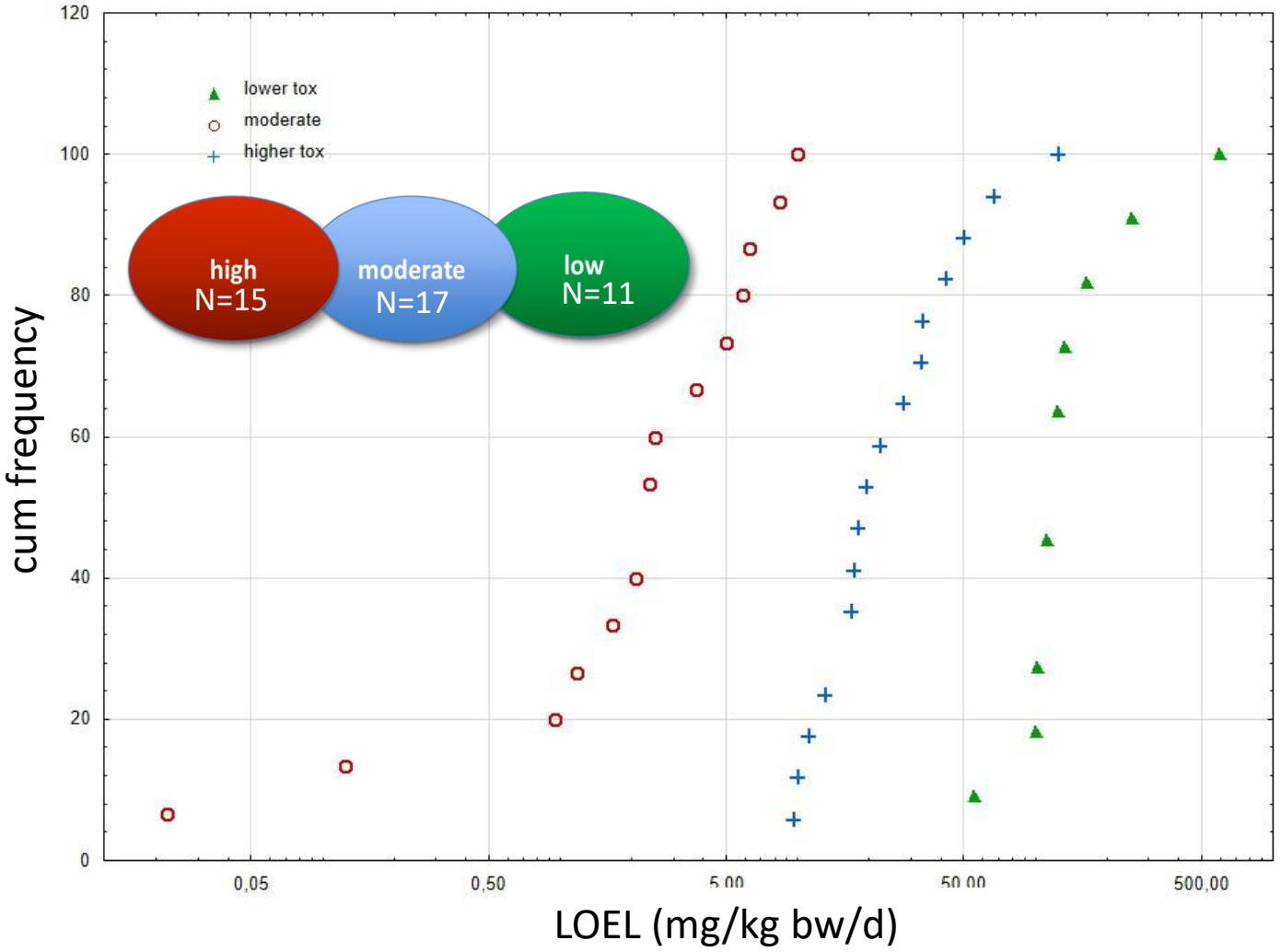
- *In silico*
- *In vitro*

Toxicokinetics

- *In silico*

EUTOXRISK

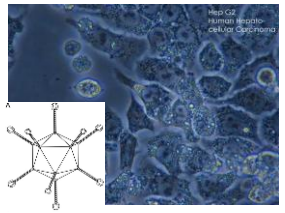
Case study chemicals from different severity classes.



EUTOXRISK

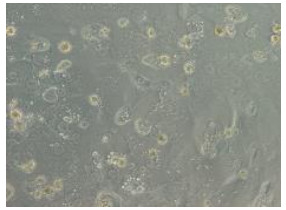
Test systems involved in the case study to assess toxicodynamics.

HepG2 AdvCYP



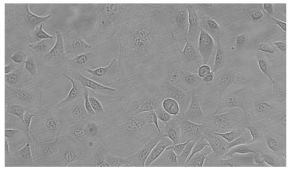
Organ system liver (metabolism)
Partner: HULAFE

PHH



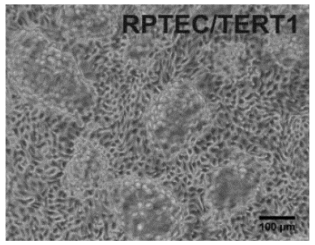
Organ system liver
Partner: Cyprotex

PBEC



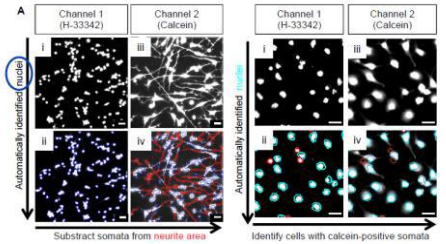
Organ system lung
Partner: LUMC

RPTEC



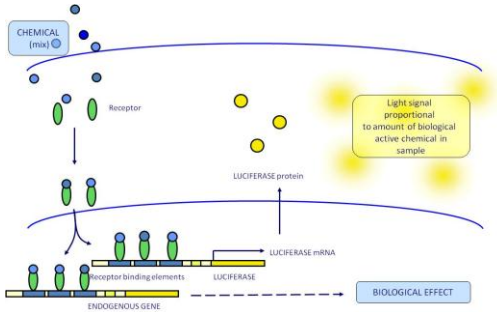
Organ system kidney
Partner: VUA

LUHMES

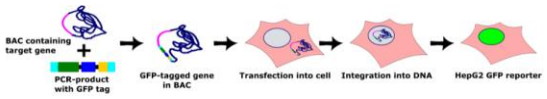


Organ system neuron
Partner: UKN

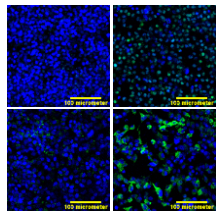
CALUX



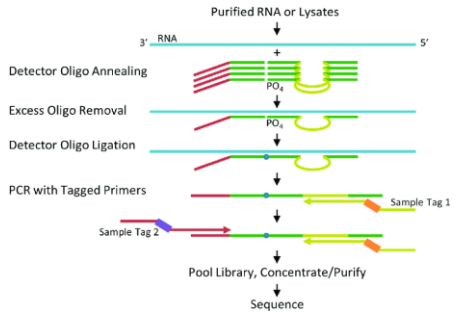
HepG2 BAC-GFP



Oxidative stress *SRXN1
DNA damge
ER stress
Inflammatory stress
Heat shock



High throughput transcriptomics TempO-Seq (HTTr)



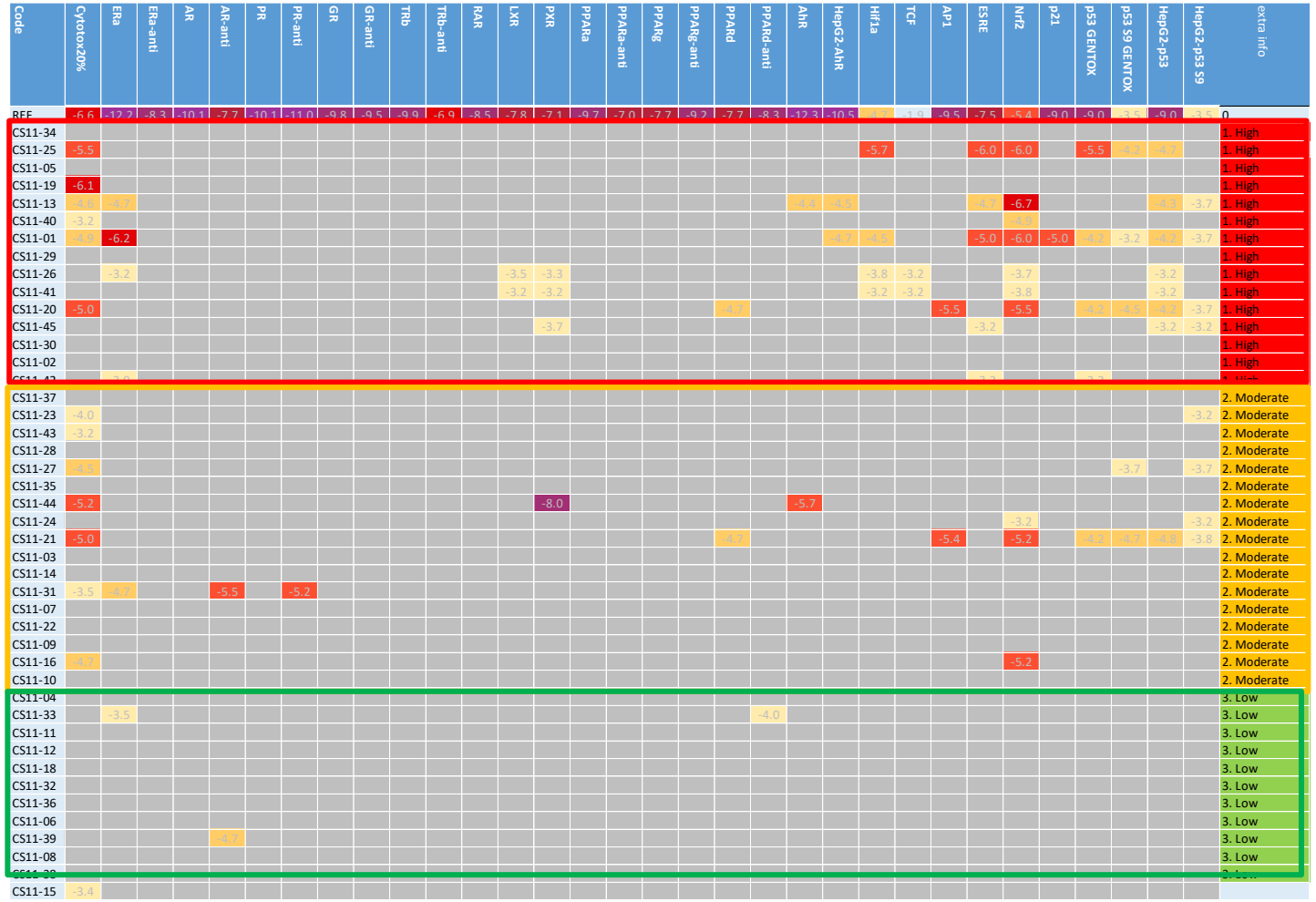
1. HepG2
2. PHH
3. PBEC
4. RPTEC
5. LUHMES

EUTOXRISK

CALUX – reporter assays

- Lowest observed effect concentrations (LOECs) in LogM

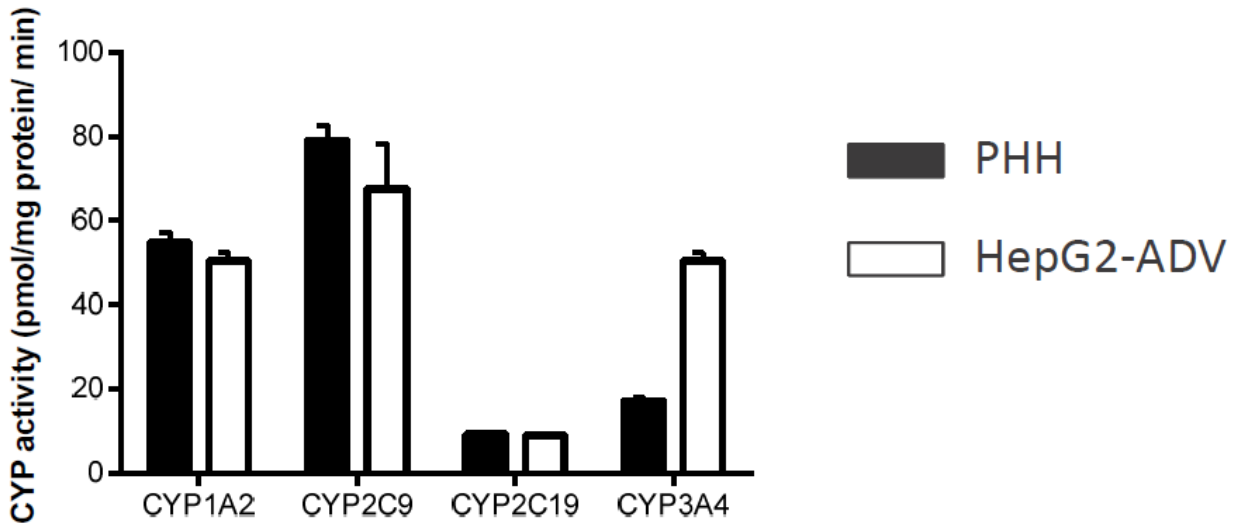
Category	Hits/c	% active
Toxic	3.8	66.7 %
Moderate	1.5	47.1 %
Low	0.3	18.2 %



EUTOXRISK

Metabolic retrofitting of HepG2 HCI assay

- We have successfully transfected simultaneously HepG2 cells with 4 different ADV encoding for CYP1A2, CYP2C9, CYP2C19 and CYP3A4



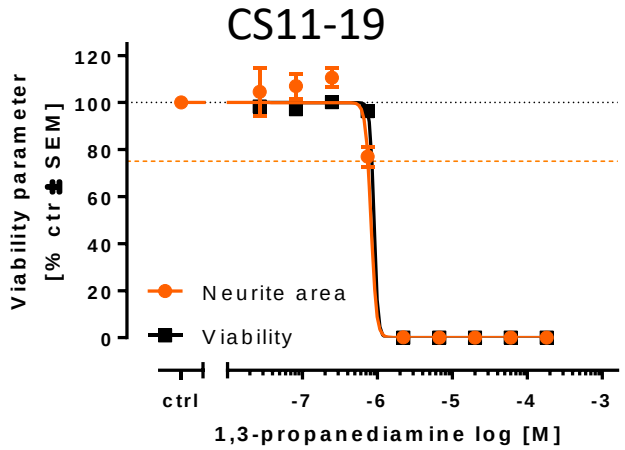
EUTOXRISK

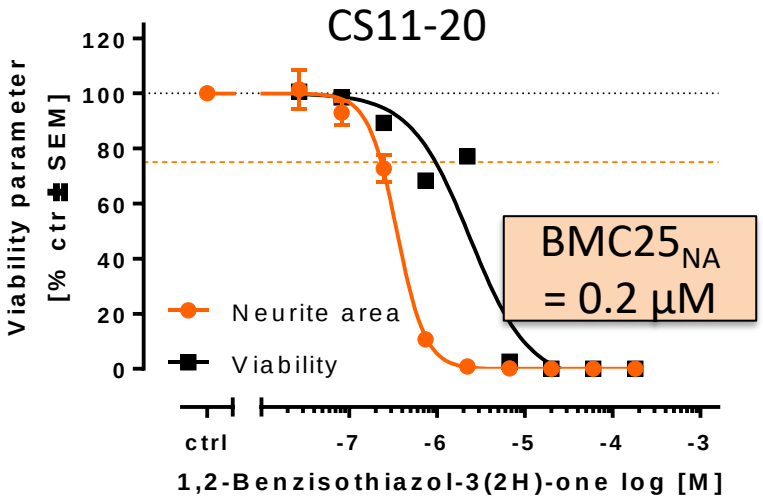
Metabolic retrofitting of HepG2 HCI assay

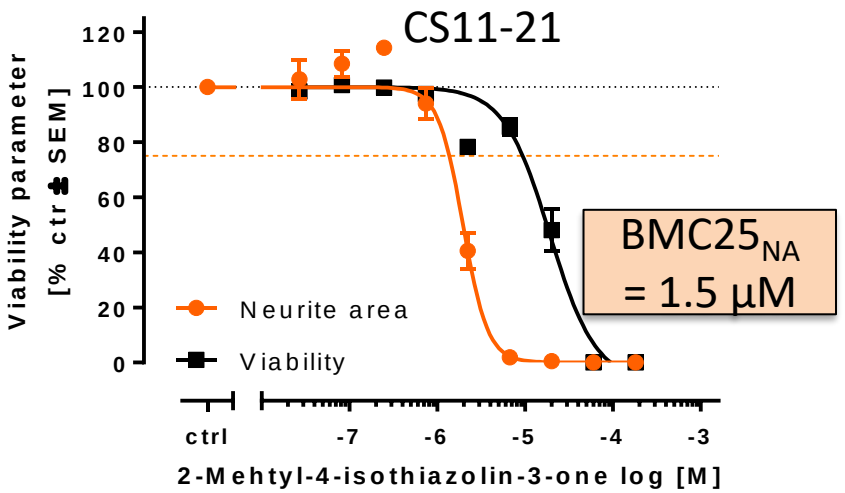
			HepG2	HepG2 + Adv-CYP
CS11-19	2372-82-9	1,3-propanediamine, N-(3-aminopropyl)-N-dodecyl-	3	3
CS11-20	2634-33-5	1,2-Benzisothiazol-3(2H)-one	60	180
CS11-21	2682-20-4	2-Methyl-4-isothiazolin-3-one	60	180
CS11-22	2695-37-6	sodium 4-vinylbenzenesulphonate	>180	>180
CS11-23	2855-13-2	(5-Amino-1,3,3-trimethyl-1-cyclohexylmethyl)amine	180	180
CS11-24	2871-01-4	2-(4-amino-2-nitroanilino)ethanol	>180	60
CS11-25	3811-73-2	1-Oxide-2-pyridinethiol, Sodium salt	10	>180
CS11-26	3926-62-3	2-chloroacetic acid sodium salt (1:1)	>180	>180
CS11-27	4719-04-4	1,3,5-Triazine-1,3,5(2H,4H,6H)-triethanol	180	>180
CS11-28	552-30-7	1,2,4-benzenetricarboxylic anhydride	>180	60
CS11-29	56-93-9	benzyltrimethylammonium chloride	>180	>180
CS11-30	58-55-9	1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione	>180	>180

Neurite outgrowth effects CS11 compounds

Example of cytotoxic classified compound:



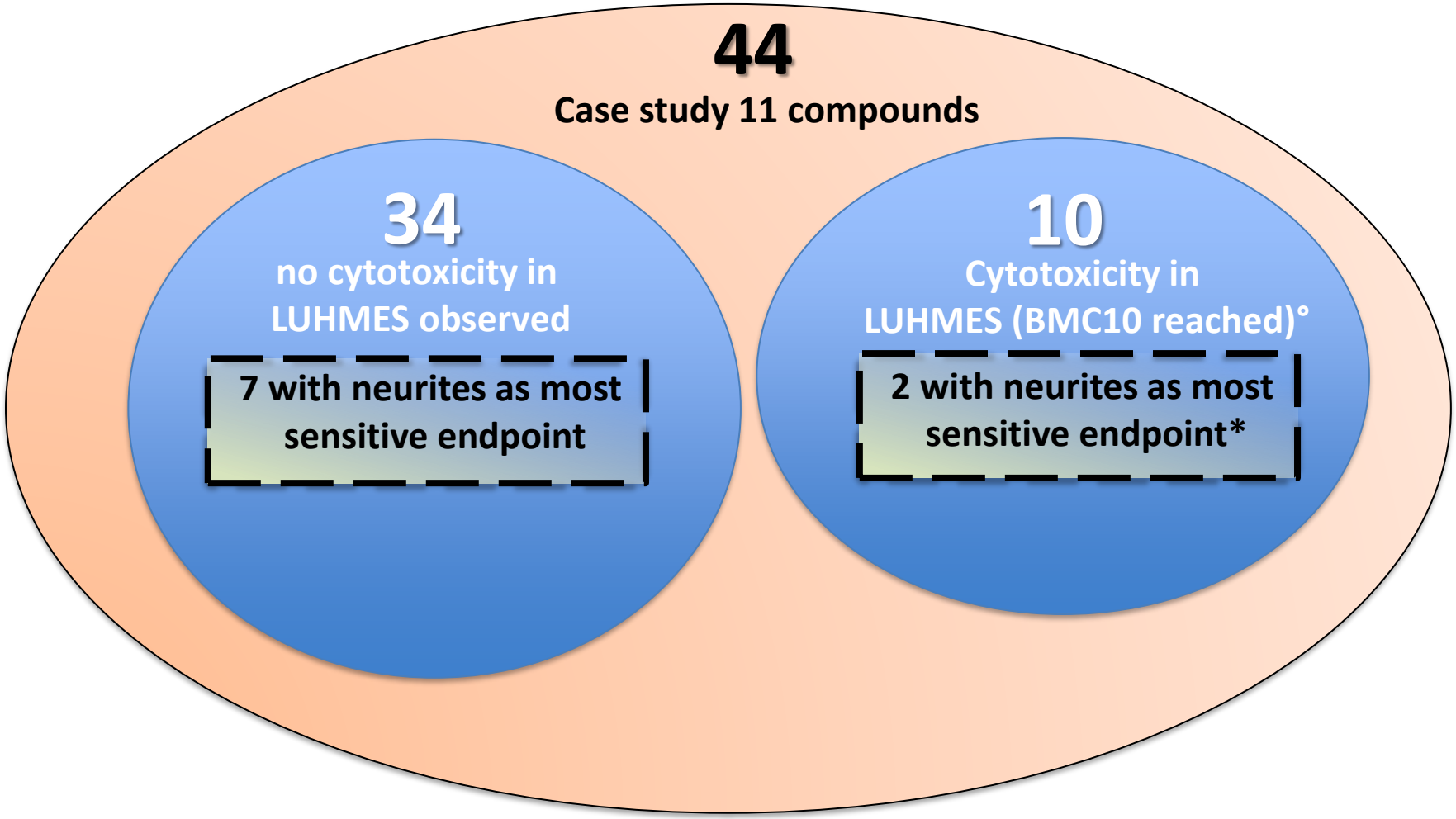




Neurite endpoint ~5x more sensitive than viability for CS11-20 and CS11-21

EUTOXRISK

Summary neurite outgrowth phenotypic assay



High throughput transcriptomics

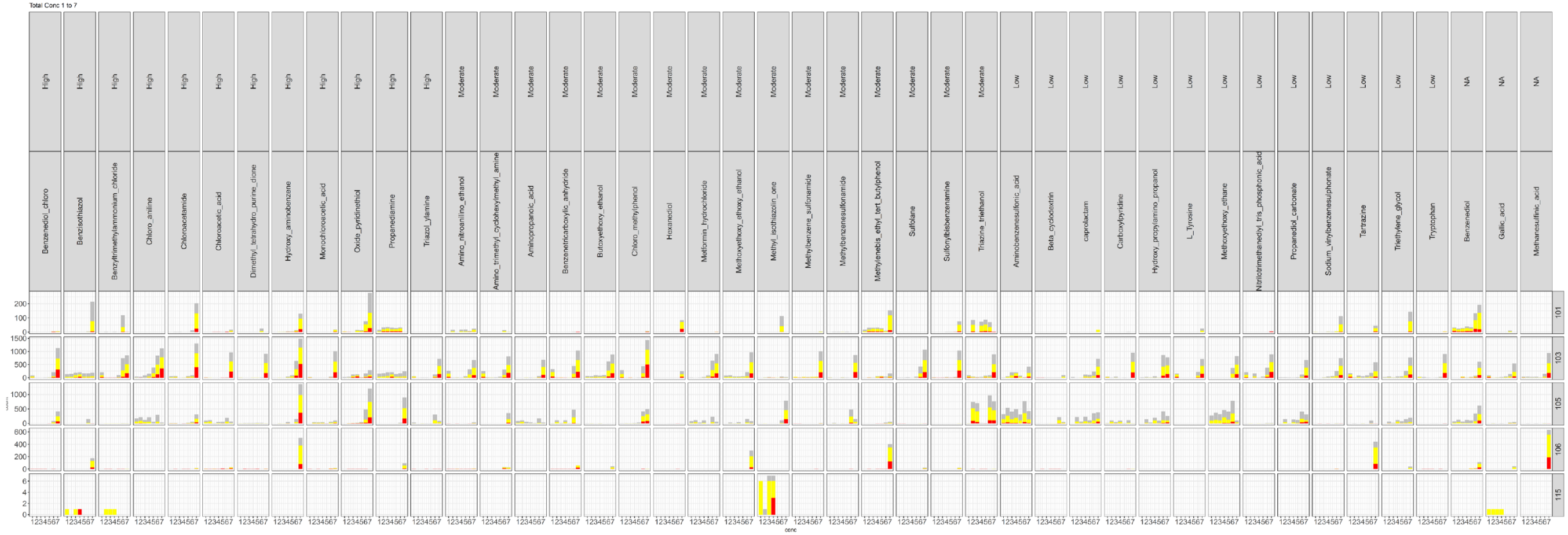
Toxicodynamics - Hazard characterization

Detailed MoA analysis

- High throughput transcriptomics (HepG2, PHH, PBEC, RPTEC, LUHMES)
- TempO-seq EU-ToxRisk gene panel (~3500 sentinel genes)

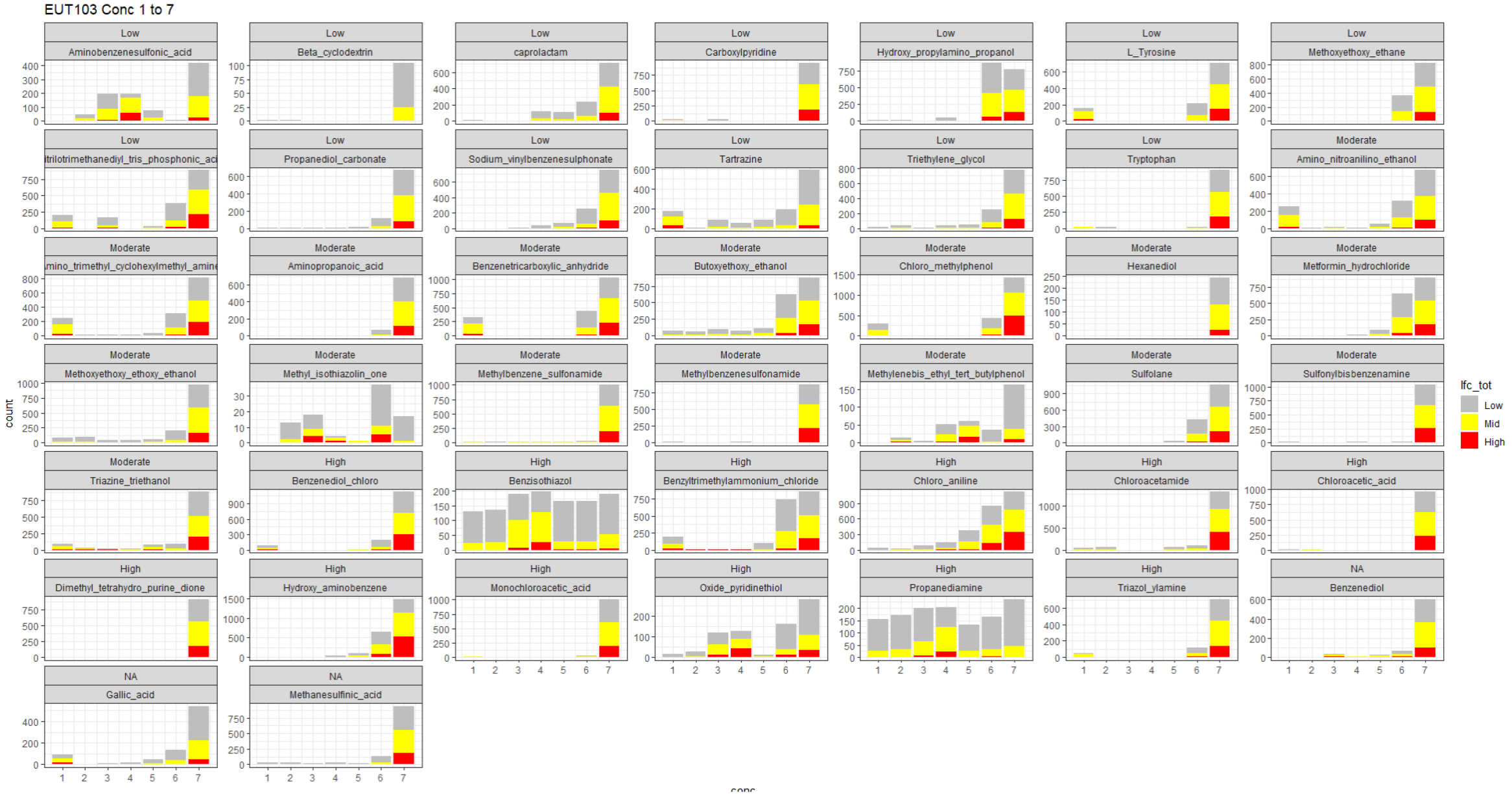
EUT	Partner	Model	Status	Samples
101	VU	RPTEC/TERT	Data analysis	1855
103	LUMC	PBEC	Data analysis	1923
105	CPX	PHH	Data analysis	1839
106	UL	HepG2	Data analysis	2064
115	UKN	LUHMEs	Data analysis	945

High throughput transcriptomics: CS11 compounds



EUTOXRISK

High throughput transcriptomics: lung PBEC dose response



Conclusions

- Target prediction models can identify candidates for some chemicals
- Metabolic retrofitting critical to determine toxicity liability / potency.
- Highly toxic chemicals show more profound effects in reporter assays.
- Phenotypic assays (e.g. neurite outgrowth assay) are more sensitive than cytotoxicity.
- HTTr is a sensitive method to determine biological perturbations.

➢ *Preliminary conclusion:* HT NAMs can contribute to prioritization of highly toxic chemicals.

EUTOXRISK

Thanks to entire Case Study 11 team!!!

Thank you!!