The Final EUTOXRISK Symposium 17

Chemical-induced Mitochondrial Toxicity

Mode of action based biological read-across

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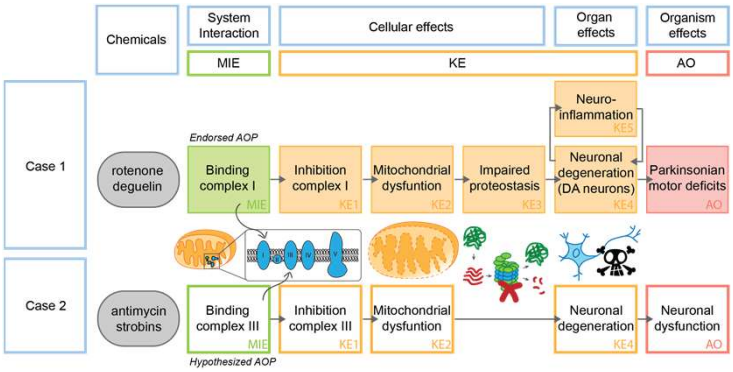
Regulatory Question

Biology-driven read-across

- pesticide regulations demand mammalian studies in risk assessment
- read-across approaches support a shift to NAM-based risk assessment
- can an AOP-based NAM testing support read-across for pesticides?
- two AOP-based read-across cases: 1) rotenoids and 2) strobilurins
- *Rotenoids case study:* hazard assessment based on anticipated mitochondrial complex I driven occurrence of parkinsonian defects
- *Strobilurins case study:* flagging no-neurotoxicity for related chemicals based on toxicity observed in by a reference chemical with similar mode-of-action (mitochondrial complex III inhibition)

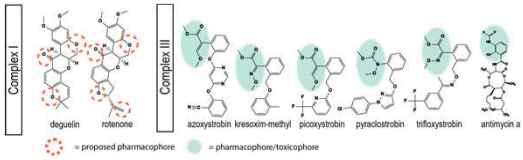
AOP-driven toxicity assessment



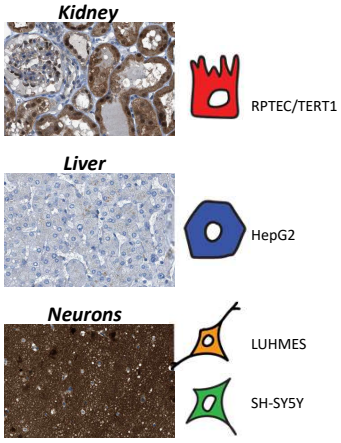
Overview on the Case Study Approach

Chemicals

Panel compounds	ETC complex inhibited	Read across	Inhibitor type	Putative binding site
Deguelin	I	Target		Quinone binding pocket
Rotenone	I	Source	Type B	Quinone binding pocket
Antimycin A	III	Reference	Qi	Q-cycle
Azoxystrobin	III	Target	Qo, Pm	Q-cycle
Kresoxim-methyl	III	Source	Qo, Pm	Q-cycle
Picoxystrobin	III	Source	Qo, Pm	Q-cycle
Pyraclostrobin	III	Source	Qo, Pm	Q-cycle
Trifloxystrobin	III	Source	Qo, Pm	Q-cycle



Cell models



Methods

Replace *in vivo*	NAMs available						Difficult to replace
Key event	MIE	KE1	KE2	KE3	KE4	AO	
Assay	PK studies	Docking studies	Seahorse assay	MMP assay	Protease activity assay	Viability assays Cell death assay	In vivo outcome (NA)
	Bioavailability	Similarity studies			Proteosome activity markers	Neuronal health	
Key event						KE5	
Assay						No assay included	

Assay	Type	Measurement	Technique
Pharmacokinetics (PK)	*in silico/ in vitro*	Chemical distribution within a species	Plasma and tissue concentrations were predicted using physiologically based pharmacokinetic (PBPK) models.
Bioavailability	*in silico*	Amount of free chemical in a system	Model predictions (2D steady-state biokinetic model [Fisher 2019])
Docking studies	*in silico*	Probability prediction of binding to a receptor	Model predictions (using Schrödinger software)
Similarity studies	*in silico*	Structural similarity of chemicals	Predictions (2D = Tanimoto score and 3D = USR-CAT)
Seahorse assay (intact cells)	*in vitro*	Mitochondrial respiration	In a seahorse machine: Fluorescent detection of O2 and H+
Seahorse assay (perm. cells)	*in vitro*	Complex specific mitochondrial respiration	In a seahorse machine: Fluorescent detection of O2 and H+
Mitochondrial membrane potential (MMP) assay	*in vitro*	Mitochondrial integrity	Confocal/ fluorescent imaging of potential sensitive dye
Protease activity assays	*in vitro*	Degradation of protease substrate	Fluorescent measurement of substrate which becomes visible upon cleavage
Proteosome activity markers	*in vitro*	Expressio of CHOP	Confocal imaging of CHOP coupled to GFP
Viability assay	*in vitro*	Viability based on resazurin reduction and ATP level	Assessment of resazurin or ATP levels based respectively on fluorescence and luminescence
Neuronal health	*in vitro*	Neuron outgrowth: length of axons and dendrites	Fluorescent imaging of neurons
	in vitro	Neuron degradation: neuronal mass	Brightfield imaging of neurons

Outcome of the Case Study

Uncertainty assessment

Factor		Read across complex I inhibition: rotenoids		Read across complex III inhibition: strobilurins	
		Uncertainty	Comment	Uncertainty	Comment
Structural boundary of the read across		Low/Medium	Hig chemical similarity - but based on an analogue approach with only two compounds	TD = Low TK =Medium	Common toxophore and binding site Substitutions could affect ADME, but *in vivo* and simulations comparable
Mode of action/AOP		Low	OECD endorsed AOP	MoA = Low AOP = Medium	strobilurins are desgined to target CIII as their MOA/ AOP putative
Hypothesis		Medium	Based on endorsed AOP but predictions based on *in vitro* and *in silico* data	Low	Neurotoxic potential established/CII inducing NT is high. NAMs covering TK/TD support hypothesis
Similarity of source chemicals for read-across		Low	Common toxophore	Low	High 3D similarity with common toxophore
Physical/Chemical properties		Low	Structurally highly similar compounds	Medium	Molecular weight & H-bond similar. Target chemical has a lower logP indicating that less reaches the brain
Toxicokinetic		Low	Metabolism similar/PBPK predictions similar	*in vivo* = Low *in silico* = low	*In vivo* data similar PBPK predictions similar
Similarity of supportive data	*in vivo*	Low	Regulatory *in vivo* data & human data	Low	Regulatory *in vivo* data
	in vitro	Medium	Not OECD validated – but routine assays in the labs	Medium	Not OECD validated – but routine assays in the labs
Number of analogues		*Not relevant*		Low/Medium	Source compounds demonstrating similar negative results. However, only 1 positive compound which was not similar in structure
Quality of end point data	Uncertainty of the assay	Medium	Reported according to ToxTemp/DB-ALM. *In silico* models not validated externally	Low/Medium	Reported according to ToxTemp/DB-ALM. *In silico* models not validated externally
	Following proposed AOP	Low/Medium	AOP endorsed but with the described uncertainties	High	AOP – putative
	KEof the AOPs reflected by assays	Low/Medium	Low uncertainty for all, except proteosomal activity	Medium	Uncertainty whether assays can predict neurotoxicity
Similarity of endpoint data (among source compounds))		Low/Medium	Similar for all assays except one	Low	No signs of in vivo neurotoxicity of source chemical. High similarity *in vitro* between source and target
Concordance and weight of evidence for justifying the hypothesis		Low	Combined data support that rotenone and deguelin have similar behavior in the various test methods but different potency	Low	The target compound is biologically comparable to the source compounds
Overall		Low	Based on the uncertainty of the above factors and their impact on prediction	Low/Medium	Based on the uncertainty of the above factors and their impact on the prediction

Conclusions

- *Rotenoids case study:* deguelin has similar MoA as rotenone, but is less potent.
- *Strobilurin case study:* no evidence for a higher potency for azoxystrobin compared to the other strobilurins. Since other strobilurins did not induce neurotoxicity effects *in vivo* it is unlikely that azoxystrobin has a neurotoxicity potential.

Learnings

- AOP-based testing strategies support linking data to relevant endpoints
- multiple assays per KE increase weight of evidence
- accumulation of evidence from individual KEs strengthens a decision
- Read-across based on biological similarity requires broad coverage of toxicodynamics of late KEs and toxicokinetic data
- a low-toxicity hypothesis is more difficult and requires a well defined strategy and the addition of reference chemicals

The EU-ToxRisk project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002.

www.eu-toxrisk.eu/