The Final EUTOXRISK Symposium 22

Insecticides that function like nicotine

Application of a NAM battery to assess potential adverse effects

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Regulatory context: Pesticides

New approach methods used in case study

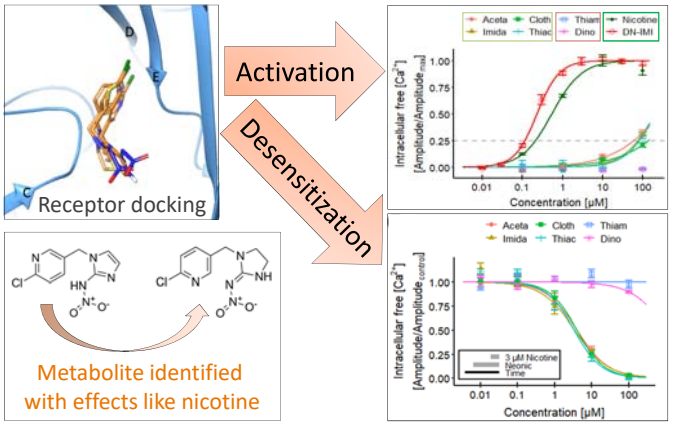
- Testing in a broad battery of new approach methods (NAM)
- Assessment of 6 neonicotinoids and 2 metabolites
- Nicotine as well-known neurodevelopmental toxicant is reference substance for testing
- Transcriptional profiling of test systems used for testing

	Key neurodevelopmental processes and cell survival					
Test Method	**UKN2**	**UKN4**	**UKN5**	**RoFA**	**TD42 iPSCs**	**SHSY5Y cells**
Test System	neural crest cells	LUHMES (CNS neurons)	hiPSC neurons (PNS neurons)	hiPSC differentiation into neuronal		
Endpoint 1	cytotoxicity	cytotoxicity	cytotoxicity	cytotoxicity	cytotoxicity	cytotoxicity
Endpoint 2	migration	neurite outgrowth	neurite outgrowth	rosette formation	-	-

	Biochemical/Signalling			Zebrafish	In silico	
Test Method	**CALUX assays**	**Calcium measurements**		**FET (OECD 236)**	**Docking**	**PBTK/QIVIVE**
Test System	reporter gene assays in U2OS	LUHMES	SHSY5Y	Fish embryos	IFD; Schrödinger Release 2020-2	in silico
Endpoint 1	cytotoxicity	Ca^{2+} whole well and single cell	Ca^{2+} whole well	Lethality	representative docking poses	Internal exposure
Endpoint 2	receptor- or stress pathway	patch clamp	-	developmental alterations	-	-

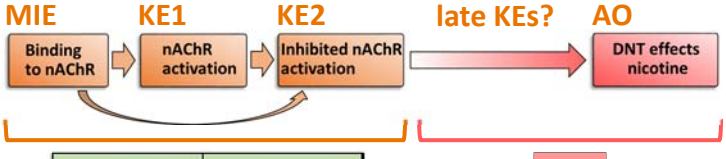
Neuronal signaling alterations via nicotine & neonicotinoids

- Key neurodevelopmental processes tested in NAM battery are not affected
- Receptor docking modeling confirmed interaction at human receptors
- Receptor signaling measurements indicated: **nicotine very potent activating and desensitizing; 4 (of 6) neonicotinoids are active and desensitizing**
- Identification of a metabolite with similar potency as nicotine (desnitro-imidacloprid)



Potential neurodevelopmental effects of tested pesticides

- Concern for developmental neurotoxicity based on molecular actions similar to those of nicotine
- We built a putative adverse outcome pathway (AOP) based on nicotine
- Case study provided:
  - Mechanistic understanding of molecular initiating event (MIE) & early key events (KE)
  - High evidence for early KEs - signaling data in two cell systems (LUHMES & SH-SY5Y)



Nicotine	high potency
Imidacloprid metabolite	high potency
Imidacloprid	active
Acetamiprid	active
Thiacloprid	active
Clothianidin	active
Thiamethoxam	inactive
Dinotefuran	inactive

No adversity in panel of test methods used

Further investigations

CS publications: Loser et al., https://doi.org/10.1007/s00204-021-03031-1 & Loser et al., https://doi.org/10.1007/s00204-021-03168-z

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www.eu-toxrisk.eu/