A Case Study to Establish a Standardized Read-across Process for Pesticide Active Substances and their Metabolites for Assessment of Genotoxicity

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BACKGROUND

- Guidance from the European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues defines a process for identifying pesticide residues and evaluating their potential risk based on toxicity and dietary exposure. (doi: 10.2903/j.efsa.2016.4549)
- Guidance on the use of a weight-of-evidence approach in scientific assessments was also published. (EFSA Journal 2017;15(8):497)

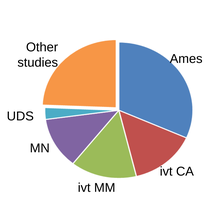
OBJECTIVES

- Develop *in silico* strategies to fill data gaps due to limited experimental data on toxicological properties of pesticide metabolites and impurities
- Critically evaluate the performance and reliability of existing *in silico* methods for predicting genotoxicity of pesticidal metabolites and impurities
- Evaluate the applicability and reliability of different methodologies for grouping and read-across
- Evaluate the impact of metabolic or degradation processes on the genotoxic potential of these substances.

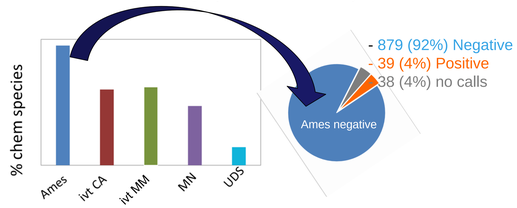
METHODS

Data Source

- EFSA Public Database: genetic toxicity of pesticides and their metabolites
  - Released in 2017 July (https://data.europa.eu/euodp/de/data/dataset/database-pesticide-genotoxicity-endpoints)
- Description
  - 956 chemical species comprised of parents and related components
    - Active substance: parents (350)
    - Components: metabolites, mixture components, degradants, etc.(560)
  - Data for 33 genetic toxicity endpoints in 17,927 studies
    - Fewer than 5% of the chemical species were missing 3 or more essential regulatory endpoints.
      - Bacterial reverse mutagenesis (Ames), *in vitro* chromosome aberration (ivt CA), *in vivo* micronucleus (MN), *in vitro* mammalian mutagenesis (ivt MM)
    - Other studies include *in vivo* chromosome aberration, *in vitro* micronucleus, DNA damage & repair, dominant lethal, cell transformations assays, unscheduled DNA synthesis (UDS), and sister chromatid exchange (*in vivo* and *in vitro*)



% in studies

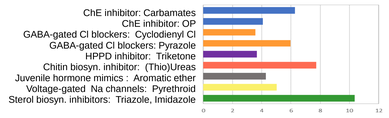


% in unique chemical species (Sub-Com pair)

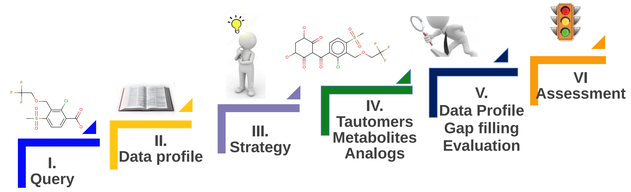
METHODS

Read-Across Approach

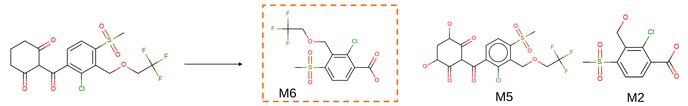
- Read-across predictions of metabolites based on data from active substances
  - full information usually received in the dossier for active pesticides, but not for metabolites
- Target endpoint
  - Ames reverse mutagenesis
  - *in vitro* chromosome aberration (not presented in the poster)
  - micronucleus with additional bioavailability information to interpret issues in exposure routes (not presented in the poster)
- Preliminary grouping
  - Pesticide mode of action[1,2]
  - ToxPrint chemotypes[3,4]
  - Molecular properties[4]



- Very diverse chemical space
  - 50% of the pair-wise similarities are ≤ 0.1
  - Only 10% of the pair-wise similarities are ≥ 0.26
  - The average Tanimoto similarity to the first nearest neighbor is 0.7.



CASE STUDY



Compound Detail	Description
Name	2-Chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoic acid
CMS ID	CMS-203791
CAS RN	335104-84-2
Form	M6 of Tembotrione (CMS-11340)
Use type (parent)	Pesticide: Herbicide
Mode of Action (parent)[1,2]	4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor. Leads to chlorophyll destruction by photooxidation and causes bleaching of emerging foliar tissue
Chemical class (parent)	Triketone class
ADME profile (parent)	Tembotrione was rapidly absorbed, extensively metabolized, and excreted.

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CASE STYDT RESULTS

Line Up & Sum Evidence Table

Search Results › Analog Structures › Analog Structures › Toxicity Data › Toxicity Prediction › Summary

Individual Evidence		T1	A1-T1	A2-T1	A3-T1	A4-T1
Structure						
CMS ID		CMS-11340-M6	CMS-62061	CMS-11340	CMS-6762	CMS-7202
Name		M6 of tembotrione	Sulcotrione	Tembotrione	Mesotrione	Isoxaflutole
Analog similarity	structure-based	1.00	0.40	0.70	0.30	0.40
	skyline plot correlation	1.00	-0.13	0.88	0.16	0.41
Analog profile	skyline profile					
Experimental Data						
AMES test		Assumed no data on M6	POSITIVE	NEGATIVE	POSITIVE	POSITIVE
	reliability score		0.90	0.90	0.60	0.80
	rationale		OECD 471: no deviation	OECD 471: no deviation	OECD 471 (equivalent) Deviation: No control info; incomplete replicates	OECD 471 (equivalent) Deviation: WP2 uvra not tested; Control info OK
AMES test			NEGATIVE		NEGATIVE	
	reliability score		0.60		0.90	
	rationale		OECD 471 (equivalent) Deviation: non-GLP; WP2 uvra not tested; Control info OK		OECD 471: no deviation	
General profile - triketone		No hits	Potential match for Michael acceptor	Potential match for Michael acceptor	Potential match for Michael acceptor and Aromatic nitro	Potential match for Michael acceptor and strained ring

Actions: search Analog Structures; generate Metabolites; generate Tautomers; predict Toxicity; export PDF File; export XLS File

System designed and developed by MN-AM

Weight of Evidence Combination[5]

WOE Assessment: Ames Reverse Mutagenesis		T	A1	A2	A3	A4
In vivo experimental data						
	Outcome	None Used	EQUIVOCAL	NEGATIVE	EQUIVOCAL	POSITIVE
	prob(POS) +/- uncertainty	NA	0.54 +/ 0.18	0.11 +/- 0.06	0.47 +/- 0.18	0.68 +/- 0.09
In Silico data						
Ames QSAR						
	Outcome	NEGATIVE				
	prob(POS) +/- uncertainty	0.17 +/- 0.14				
Ames Alerts						
	Outcome	No hits				
	prob(POS) +/- uncertainty					
Combining all evidence for T1, A2, A4						
	Outcome	NEGATIVE				
	prob(POS) +/- uncertainty	0.23 +/- 0.21				

In silico Data were generated by ToxGPS prediction tool from MN-AM. Calculation of the overall uncertainty takes model performance and uncertainty in each particular prediction into account.

CONCLUSION

- Analog selection (A2 and A4) was confirmed by the Read-Across approach.
- The Ames mutagenicity of trembotriol-M6 was estimated to be negative.
- The use of QSAR and Alerts increased the reliability of the Read-Across outcome.
  - Using only analogs (A2, A4) without *in silico* data, the Read-Across prediction would be more equivocal.
- A systematic approach from ChemTunes•ToxGPS workflow has been applied.

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