2nd PAN-AMERICAN Conference for Alternative Methods
August 23-24, 2018
Rio de Janeiro

Challenges and opportunities for using AOP-based *in silico* models in regulatory contexts

Carlos E. Matos dos Santos

Altox Ltda

Rio de Janeiro
2018

Overview

- The “AOP-based *in silico* model” concept
- AOP-based *in silico* model – Altox’s Framework
- Examples: Balancing transparency, mechanistic interpretability *and* predictivity
- Challenges and opportunities for using these methods in regulatory contexts

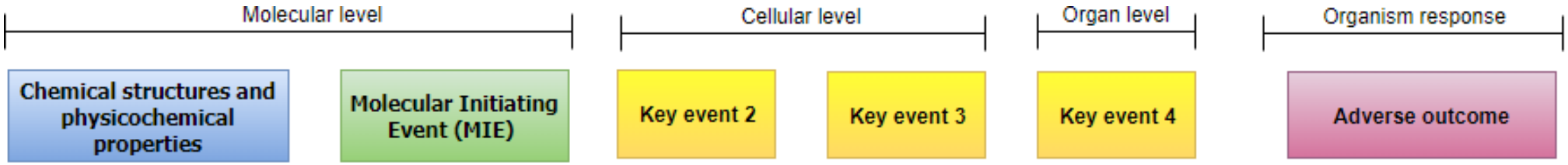
Introduction

Adverse outcome pathways (AOP) framework

- An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect (OECD, 2012)
  - *Regulatory challenge*: to define when there is sufficient ~~confidence~~ predictivity to use one or more alternative models for regulatory purposes

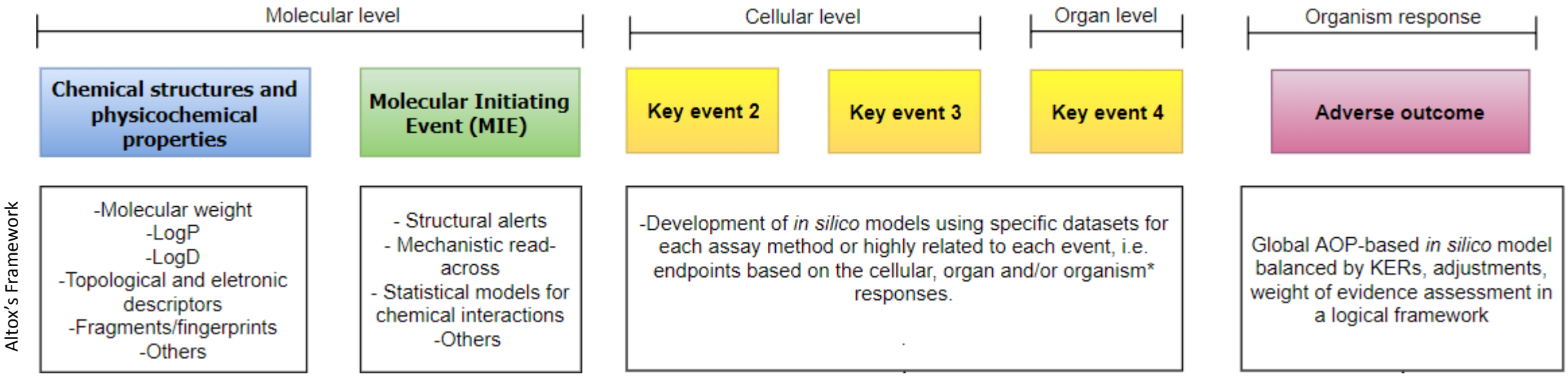
Proposed "AOP-based *in silico* model" Concept

- A framework composed by individual *in silico* models used to identify chemicals that can **activate** the associated modular **AOP components** (MIE/KE) and based in these individual multilevel predictions, balanced by adjustments, relationships and weights, **to predict an adverse outcome.**



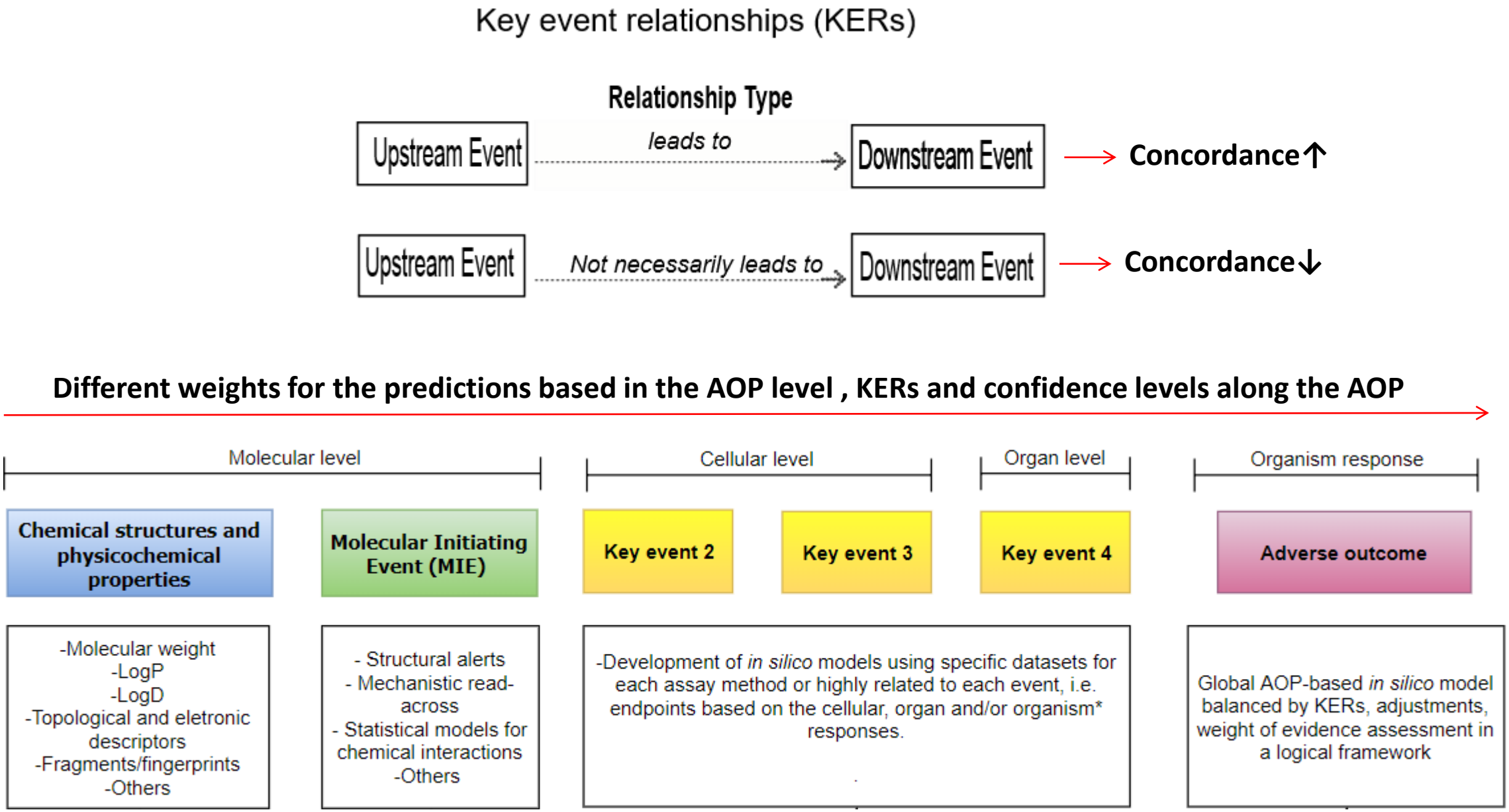
Adapted from: OECD - Organisation for Economic Co-operation and Development. Users' Handbook Supplement to the Guidance Document for Developing and Assessing AOPs. Series on Testing & Assessment, no. 233, Series on Adverse Outcome Pathways, No. 1, 2018.

AOP-based *in silico* model – Altox's Framework



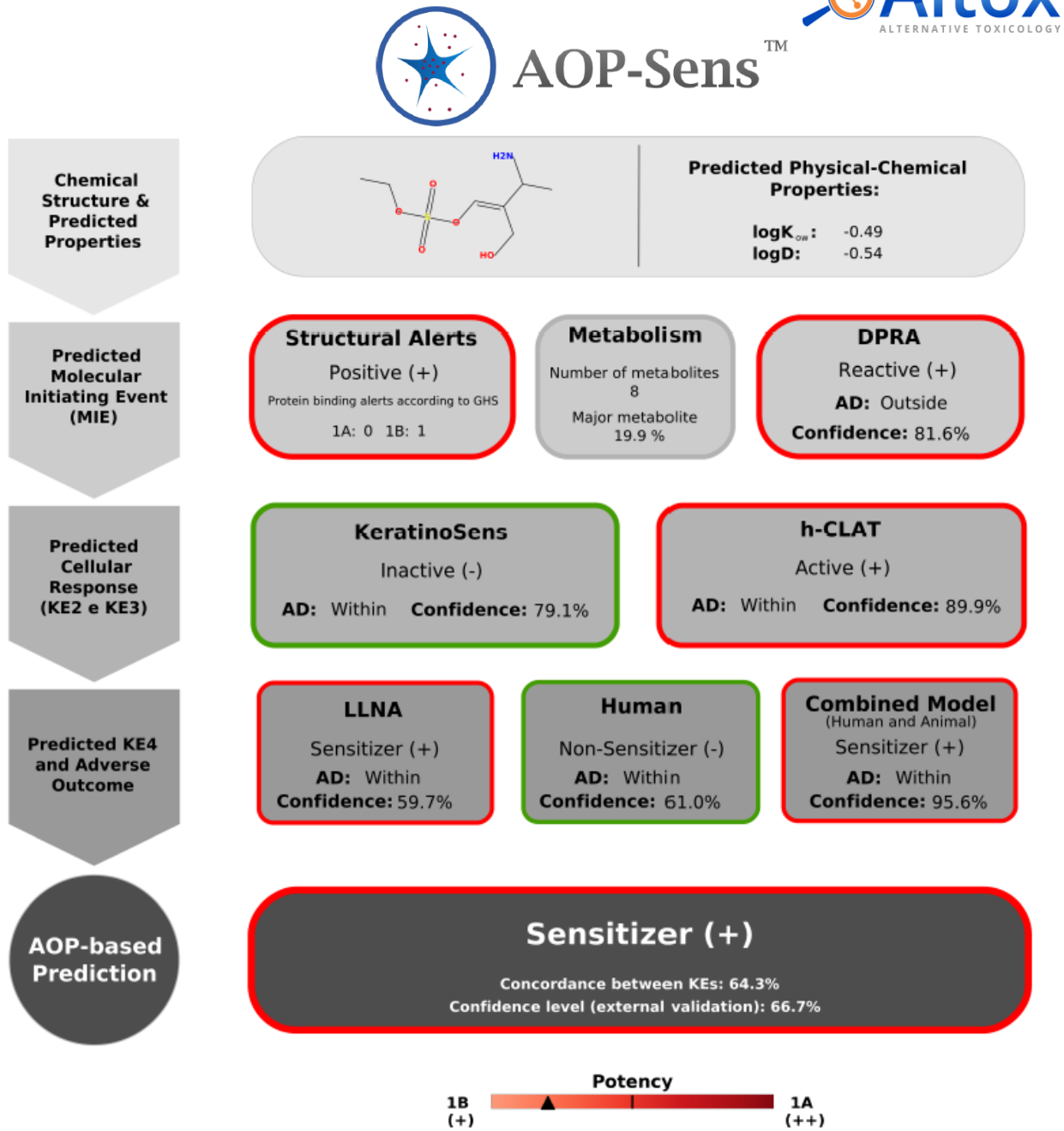
Adapted from: OECD - Organisation for Economic Co-operation and Development. Users' Handbook Supplement to the Guidance Document for Developing and Assessing AOPs. Series on Testing & Assessment, no. 233, Series on Adverse Outcome Pathways, No. 1, 2018.

Key Event Relationships (KERs)



Example – AOP-Sens

- A logical framework balancing transparency, mechanistic interpretability and predictivity;
- Models to predict chemicals that **can activate** the AOP modular components (MIE/KEs);
- **A global model** integrating all multilevel predictions balancing predictivity, key events relationships and WoE adjustments, for predicting the outcome.



Molecular level

AOP-Sens™

Altox ALTERNATIVE TOXICOLOGY

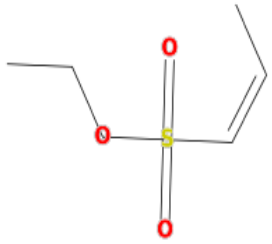
Structures and physicochemical properties

Molecular Query

SMILES: C/C=C\S(=O)(=O)OCC

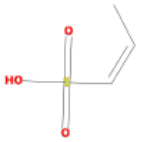
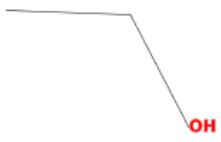
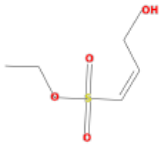
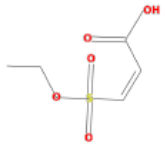
Name: Molecule 2 **logK$_{ow}$:** 0.89

CAS: None **logD:** 1.61



Metabolism prediction and potential for haptenation

To assess both direct and indirect haptens, this module predicts the potential for metabolic activation (pro-hapten formation) by known Phase I reactions, i.e., it can be used to identify potential skin sensitizers which require some type of metabolism to an active metabolite (pro-haptens) before initiation of the key event 1 (KE1) in a skin sensitization AOP (OECD Principle 5).

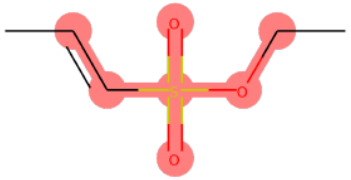
Metabolite (predicted structure)				
SMILES	CC=CS(=O)(=O)O	CCO	CCOS(=O)(=O)C=CCO	CCOS(=O)(=O)C=CC(=O)O
Reaction Rule	Hydrolysis	Hydrolysis	Aliphatic Hydroxylation	Carboxylation
Metabolite Score	17.4 %	17.4 %	4.9 %	1.6 %

Molecular Initiating Event

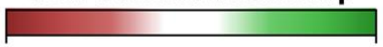
Structural Alert Analysis

Result: (+) Positive

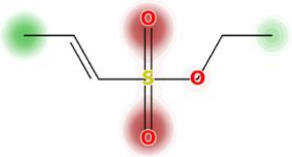
Alerts were found in the molecule. The results are in the table below and a description is provided at the end of the report.

Category	Alert	Alert ID	References
Skin Sensitization Category 1A (Protein binding Alerts, EC3 (LLNA) ≤ 2%; NOEL (HRIPT)≤ 500 µg/cm2)		Polarised Alkenes - sulfonates	Roberts, D., Api, A.M., Safford, R., Lalko, J. Regulatory Toxicology and Pharmacology 72 (2015) 683–693. Aptula A.O. et al., (2006) Chemical Research in Toxicology, 19, 1097.

STR Contribution Map



Reactive / Active (+) Non-Reactive / Inactive (-)

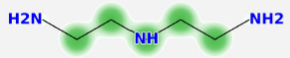
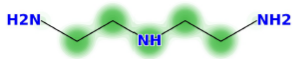
Predicted endpoint/Method	Predicted class (Confidence)	STR Contribution Mapping
Direct Peptide Reactivity Assay (DPRA, OECD 442D) Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, logK$_{ow}$, logD)	Reactive (+) (82.8%)	

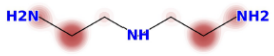
Cellular and organ levels

Key Events

STR Contribution Map

Reactive / Active (+) — Non-Reactive / Inactive (-)

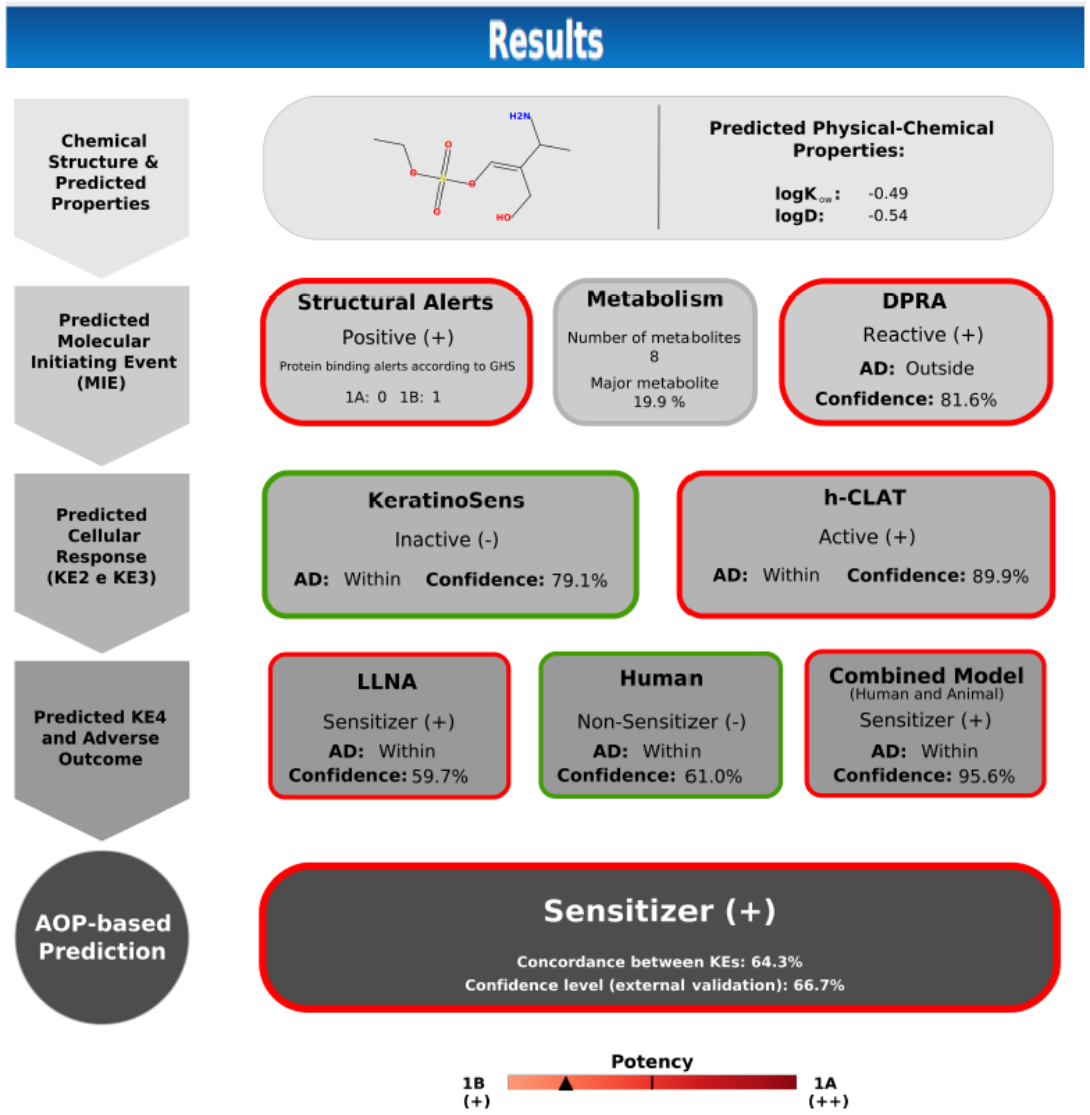
Predicted endpoint/Method	Predicted class (Confidence)	STR Contribution Mapping
KeratinoSens™ (OECD 442D)		
Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Inactive (-) (89.7%)	
Human Cell Line Activation Test (h-CLAT, OECD 442E)		
Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Inactive (-) (76.5%)	

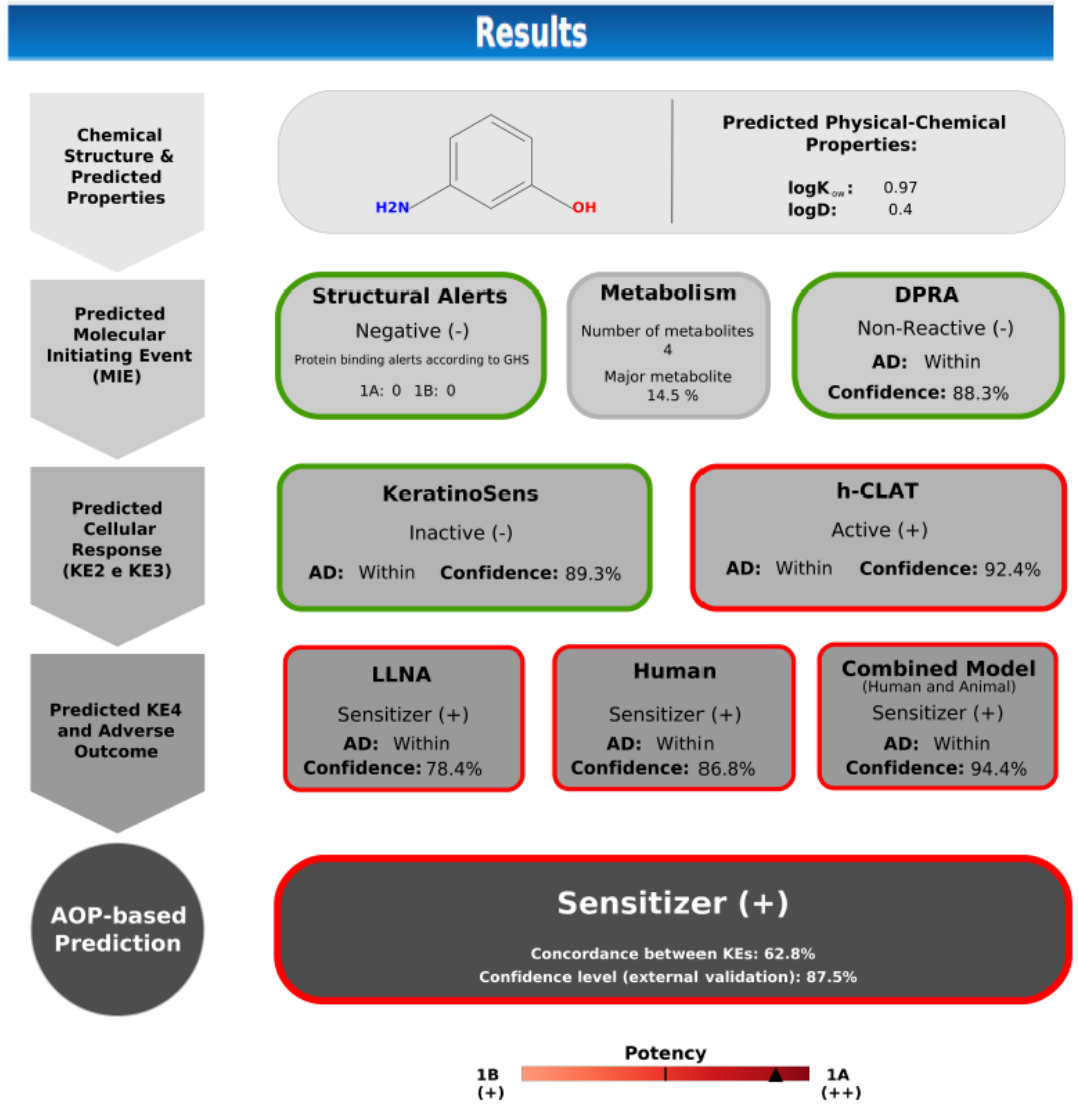
Predicted Outcome/Assay	Predicted class (Confidence)	STR Contribution Mapping
Local Lymph Node Assay (LLNA, OECD 429)		
Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Sensitizer (+) (76.4%)	

Organism response

AOP-based algorithm = $$\sum_{j=1}^{n} p_j \; c_j \; a_j \; w_j$$

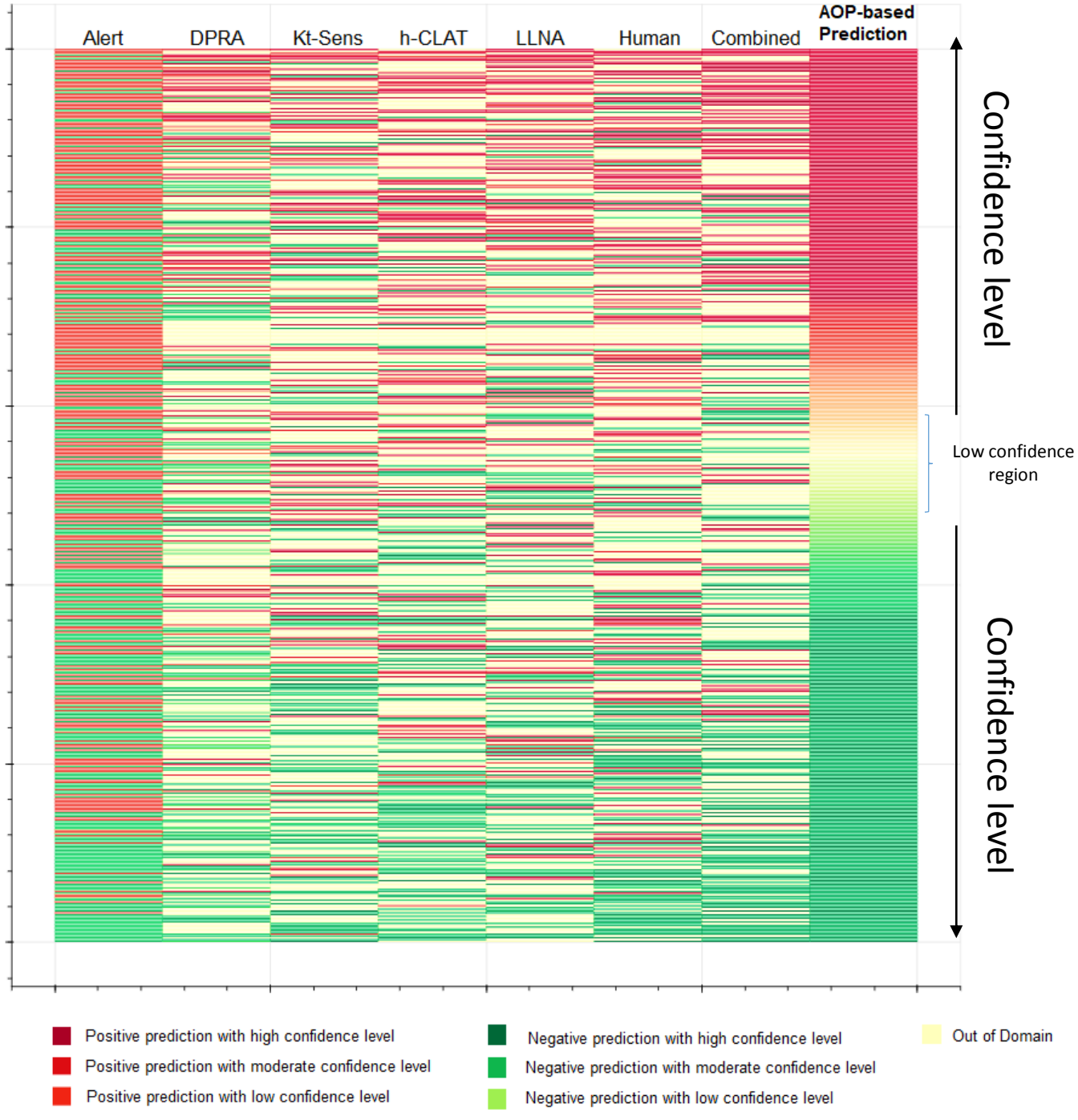
*Where **p** is a prediction result, **c** is the confidence level, **a** is the applicability domain and **w** is a weight (adjusted by KERs)*





Challenges and opportunities

- Different results with variable confidence levels
- Identifying patterns of predictive combinations;
- Identifying patterns for pre and pro-haptens.



Measures of robustness and predictivity

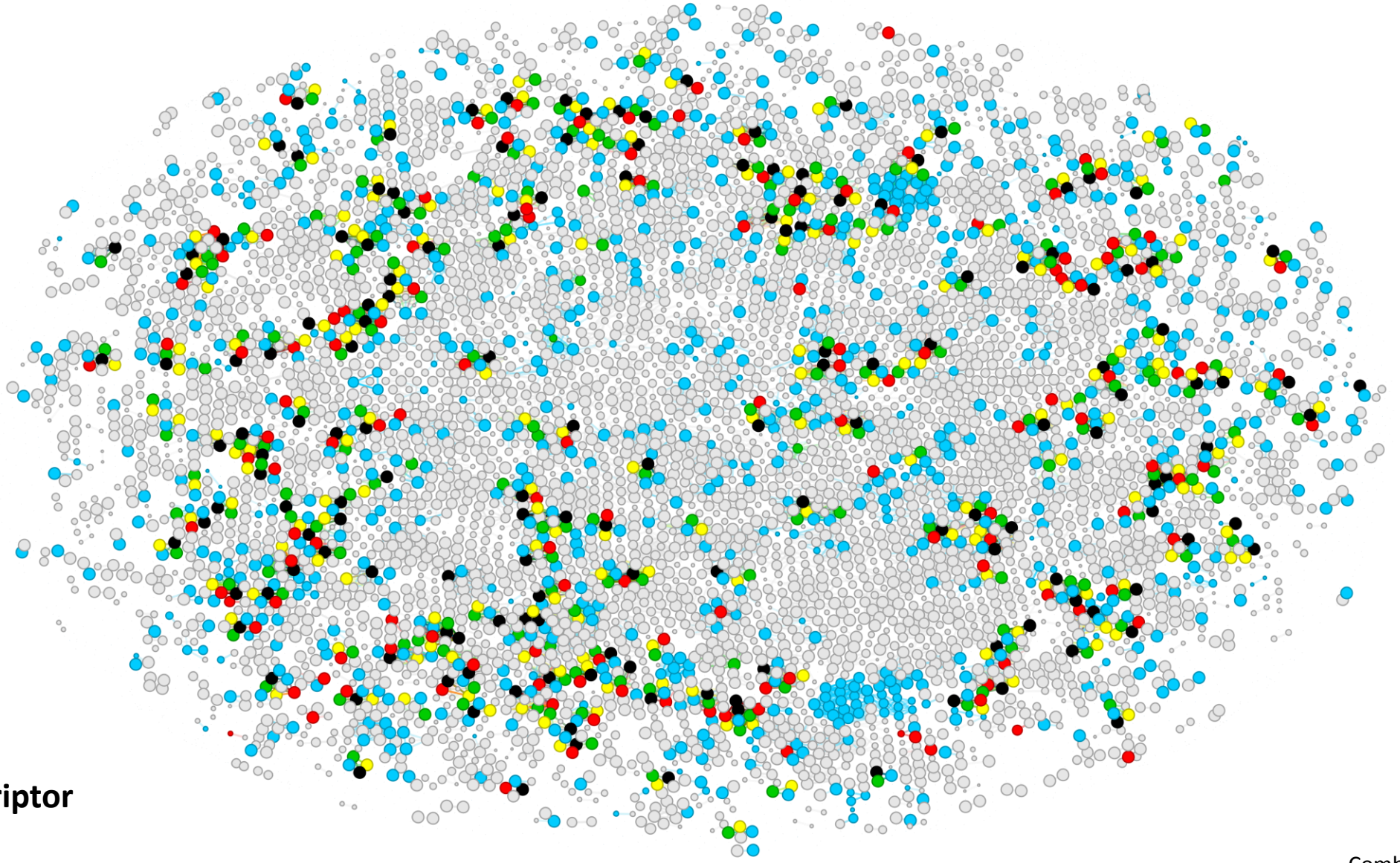
Benchmark - Combined dataset (GMTP, LLNA and human data)

(Q)SAR Models	Model Dataset	Specificity	Sensitivity	Accuracy	*N (external dataset)*
Alert analysis	**197** 128: 1A 69: 1B	0.57	0.59	0.58	6422
DPRA	**195**	0.76	0.32	0.54	6422
KeratinoSens	**190**	0.78	0.27	0.52	4050
H-CLAT	**161**	0.65	0.41	0.53	4178
LLNA	**997**	0.46	0.68	0.57	4932
Human Skin	**389**	0.82	0.35	0.59	4177
Combined dataset (GMTP, LLNA and human data)**	**6971**	**0.75**	**0.92**	**0.84**	**1284**
AOP-based prediction	**-**	**0.76**	**0.71**	**0.74**	**6422**

** Internal validation

Chemical and Toxicological Space for Skin Sensitization

Grouped by Similarity neighbors and Endpoint

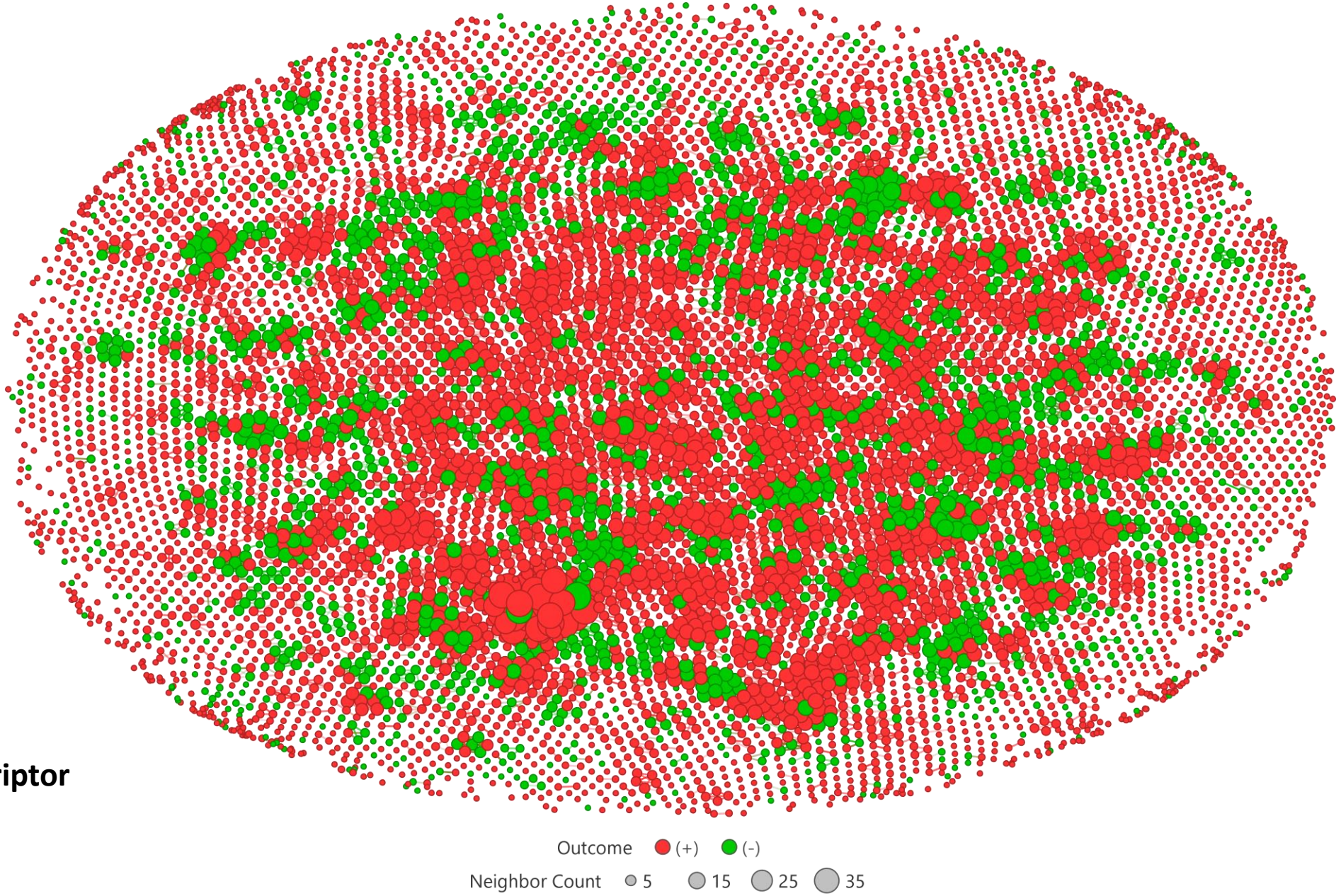


Hybrid descriptor

Combined dataset =
(GMTP, LLNA and human data)

Chemical and Toxicological Space for Skin Sensitization

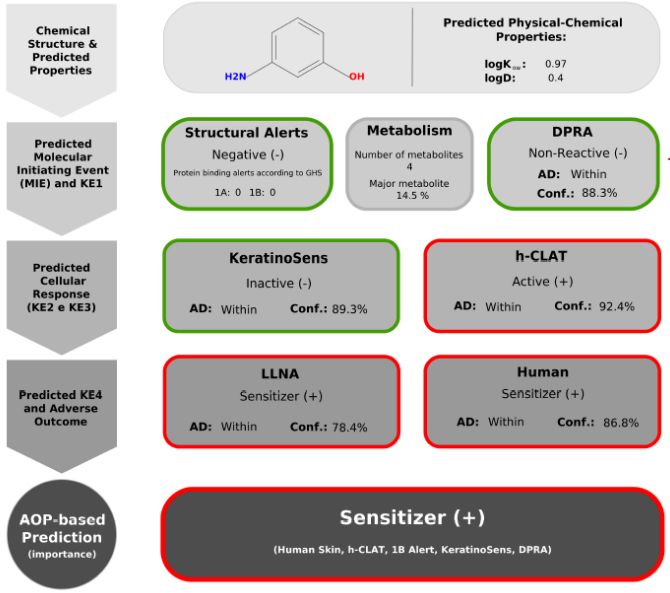
Grouped by Similarity neighbors and all Endpoints Outcome: Positive (+) or Negative (-)



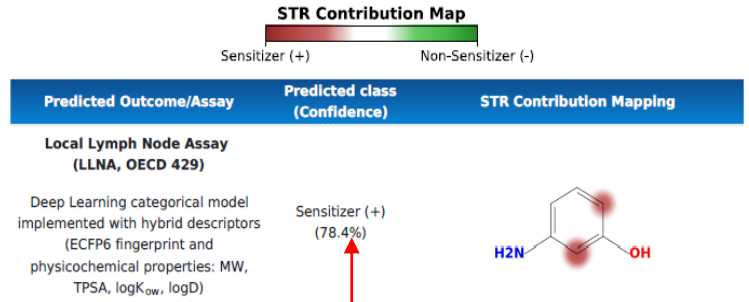
Hybrid descriptor

OECD Principles of (Q)SAR Validation for regulatory purposes

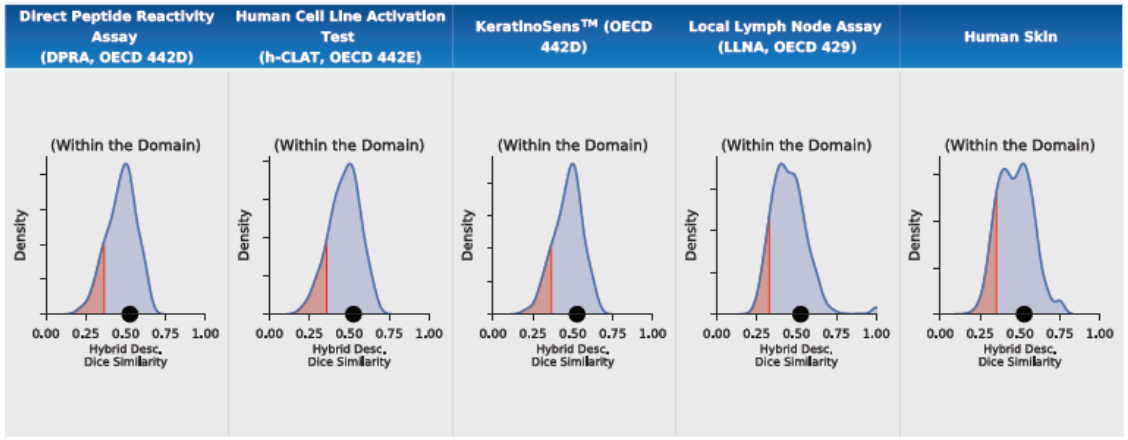
1. A defined endpoint;



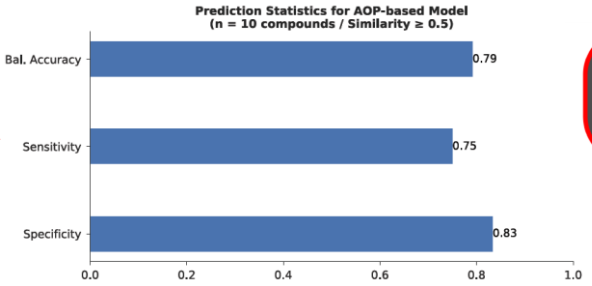
2. An unambiguous algorithm;

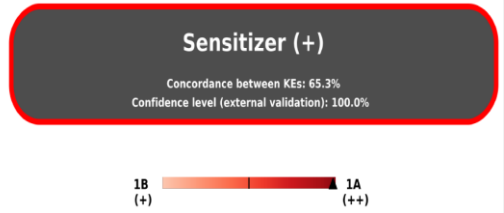


3. A defined domain of applicability;

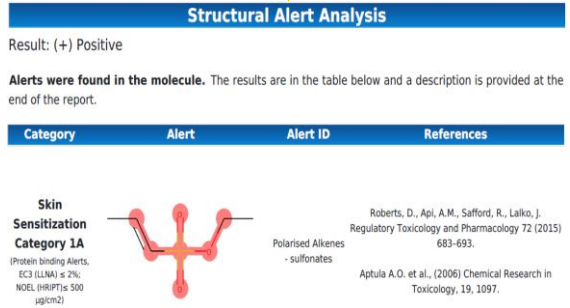


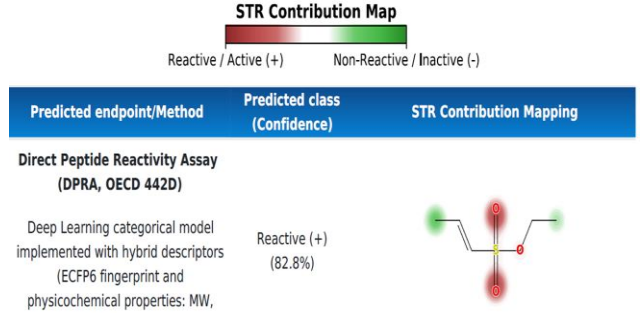
4. Appropriate measures of goodness-of-fit, robustness and predictivity;





5. A mechanistic interpretation, if possible.





Challenges and opportunities

- Balancing predictivity and mechanistic intepretation of the models at different levels (adverse outcome pathways - AOP)
- Looking for patterns with predictivity level similar or higher than biological assays
- Filling gaps in safety assessments with *in silico* predictions
- Making new regulatory criteria based in predictive integrated approaches containing *in silico* models

iS Tox Platform™

Machine-learning models for a new toxicology

Altox
ALTERNATIVE TOXICOLOGY

We are seeking partnerships and collaborations with our tools!

iS-Ocular™

Pred-CYP2D™

Pred-Oral™

DevTox-iS™

Genotox-iS™

Artificial intelligence and advanced machine learning algorithms for mutagenicity assessment

AOP-Sens™

IrriTest™

iS-Liver™

Acute-Tox™

BCF-Test™

Pred-Ecotox™

Research funding agency
- Project sponsored by FAPESP

FAPESP

Thank you for your attention!

carlos@altox.com.br