A Comparison of Mode- and Mechanism-Based *In Silico* Methods to Classify Environmental Toxicants Leading to an Improved Classification Scheme

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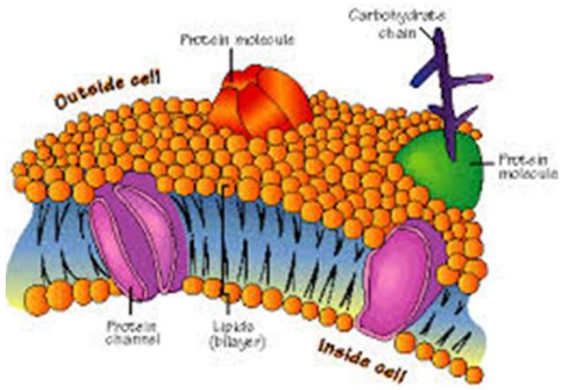
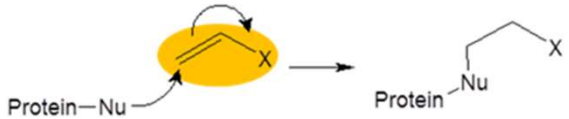
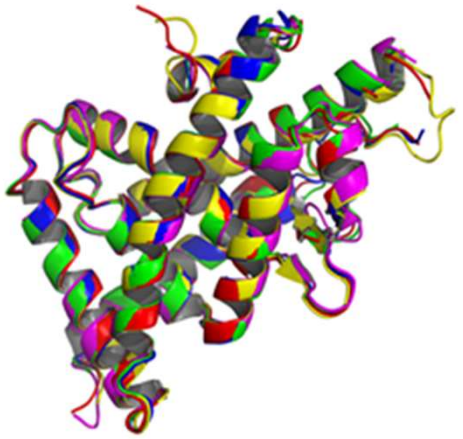
Modes and Mechanisms of Eco-Toxicological Action

Narcosis Reactive Specific Chronic

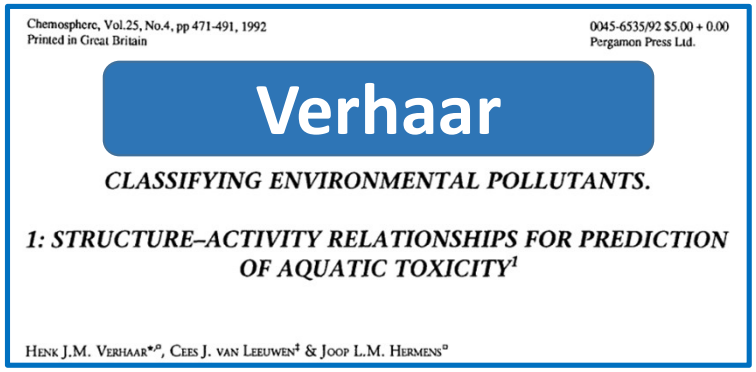


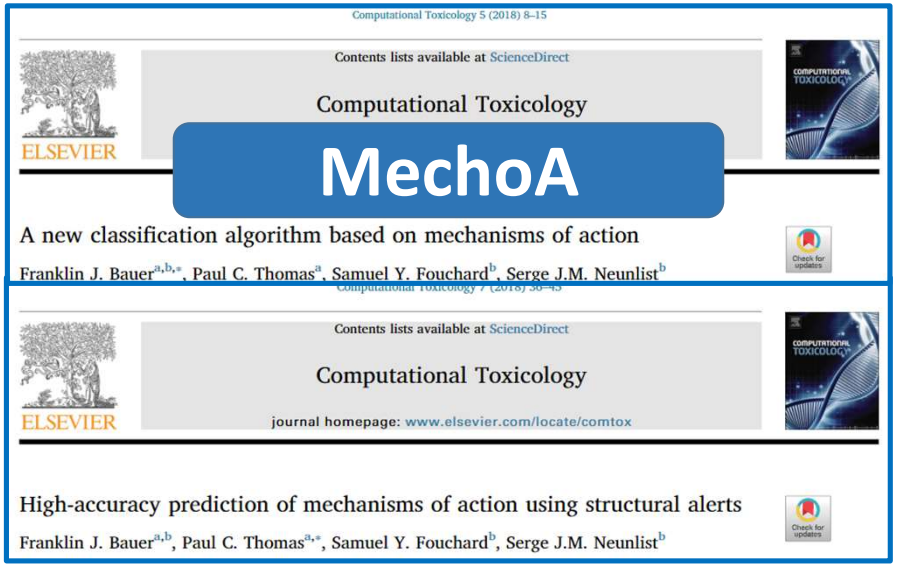


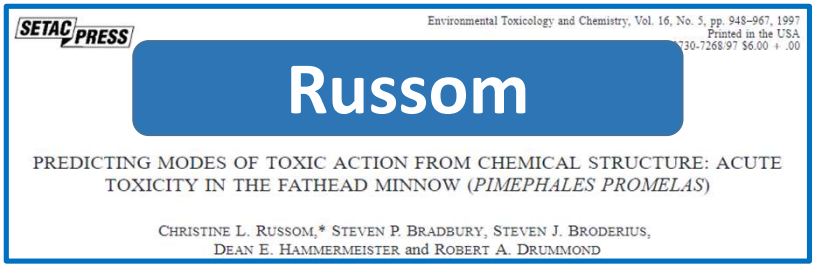


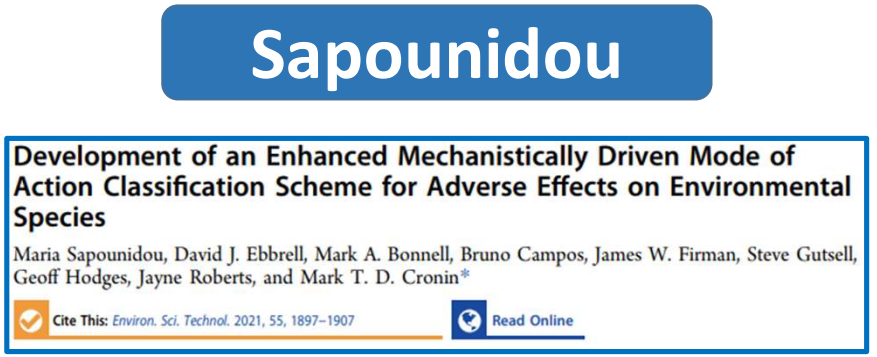
- Assist with assigning Quantitative Structure-Activity Relationships (QSARs), forming categories, read-across etc

Existing Schemes to Assign Chemicals to a Mode or Mechanism of Toxicological Action









Schemes vary according to implementation, coverage, relevance to AOPs / MIEs, species relevance etc

Aim of Investigation

To compare the schemes for classification in terms of:

- Coverage
- Mechanistic relevance
- Applicability to risk assessment scenarios

and to identify opportunities to improve and integrate the schemes

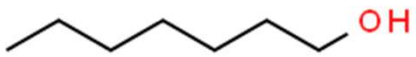
Evaluating the Schemes

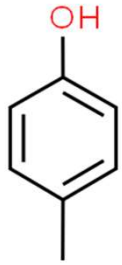
- Classification of 5,500 common industrial chemicals
  - Verhaar: OECD QSAR Toolbox v. 4.4.1
  - Russom: ChemProp 7.1.0
  - MechoA: MechoA (v. 2.2) in iSafeRat© Desktop (v. 2.1.0)
  - Sapounidou: 183 structural alerts coded in KNIME

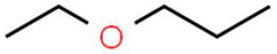
QSAR TOOLBOX Chemprop iSK Open for Innovation KNIME

Mechanistic Organisation: Sapounidou Narcosis Domain

Domain	Mechanistic Group	Mechanistic Sub-Group
1. Narcosis	1.1. Non-polar narcosis	1.1.1. Non-polar
	1.2. Enhanced narcosis	1.2.1. Polar
		1.2.2. Alkyl amine
		1.2.3. Carboxylic acid ester







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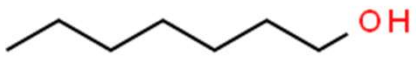

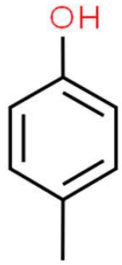

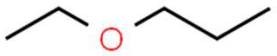

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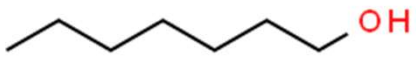

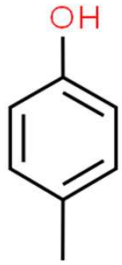

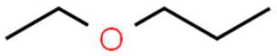

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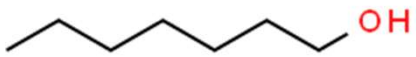
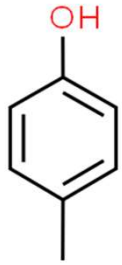
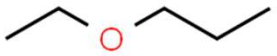
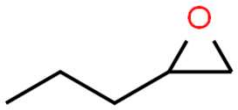


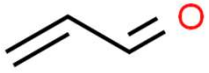


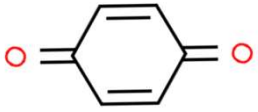


Mechanistic Organisation: Sapounidou Reactive Domain

Domain	Mechanistic Group	Mechanistic Sub-Group
2. Reactive	2.1. Electrophilic	2.1.1. Soft
		2.1.2. Hard
		2.1.3. Pre-reactive (electrophilic)
	2.2. Nucleophilic	2.2.1. Nucleophilic
	2.3. Free radical generation	2.3.1. Radical damage of tissues
		2.3.2. Redox cycling
		2.3.3. Pre-reactive (free rad generation)







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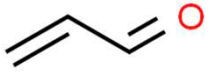

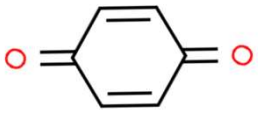

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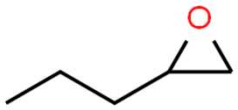
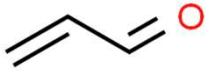
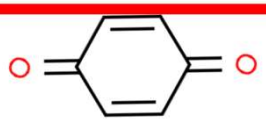
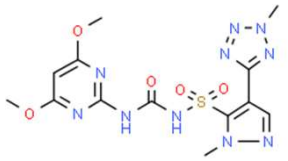
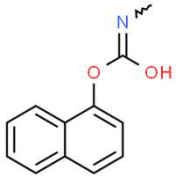




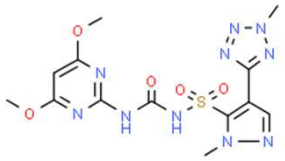




Specific Domain



Domain	Mechanistic Group	Mechanistic Sub-Group
3. Specific	3.1. Enzyme inhibition	3.1.1. Acetylcholinesterase (AChE) inhibition
		3.1.2. Photosynthesis inhibition
	3.2. Ion channel	3.2.1. Modulation of ion channels
	3.3. Cellular function disruption	3.3.1. Amino acid biosynthesis disruption
		3.3.2. Cell structure disruption
		3.3.3. Fatty acid biosynthesis disruption
		3.3.4. Nucleic acid biosynthesis disruption
		3.3.5. Steroid biosynthesis disruption
		3.3.6. Carotenoid biosynthesis disruption
		3.3.7. Protein biosynthesis disruption
		3.3.8. Developmental disruption
	3.4. Mitochondrial disruption	3.4.1. Mitochondrial ETC inhibition (specific)
		3.4.2. Mitochondrial ETC (non-specific)
	3.5. Nuclear receptor	3.5.1. Modulation of nuclear receptors



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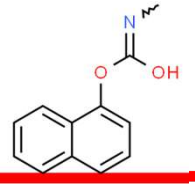

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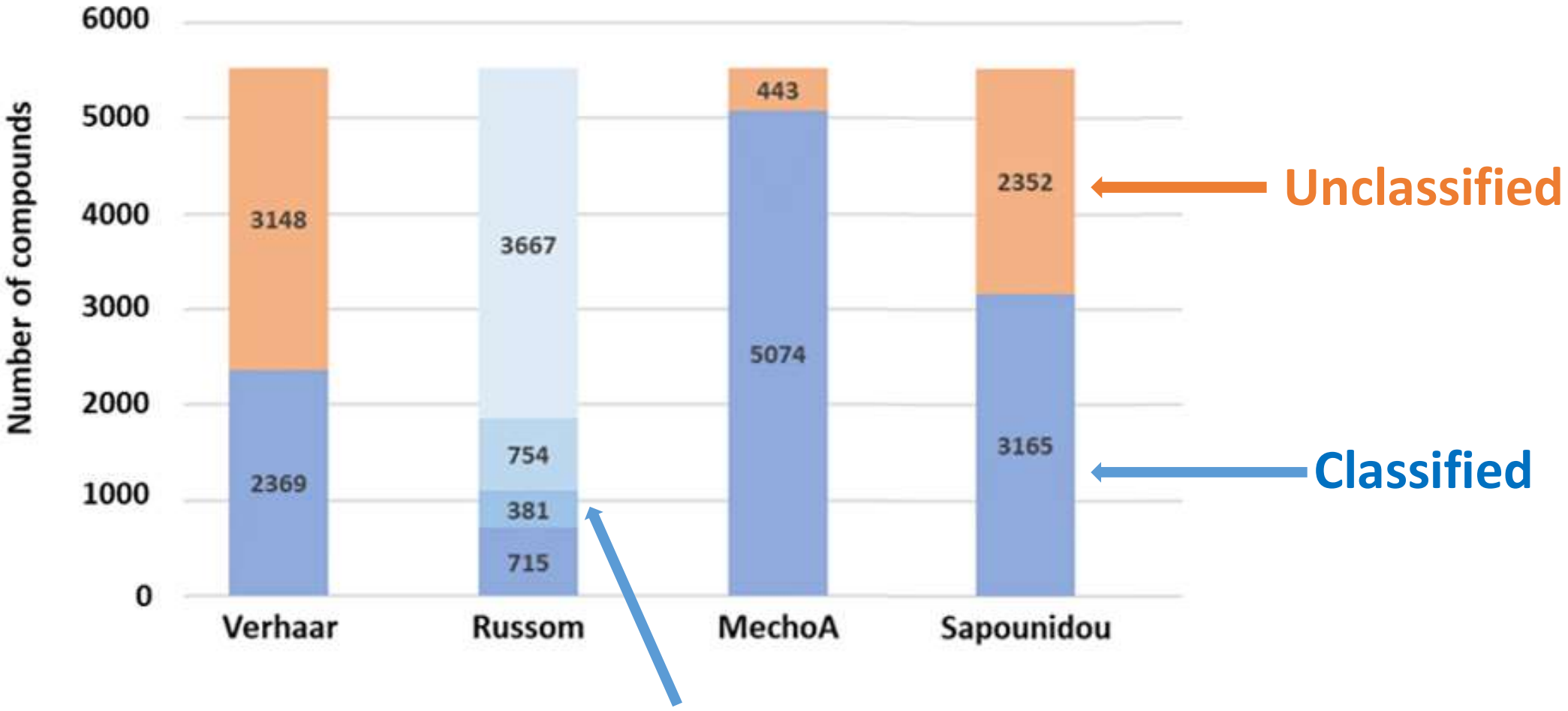
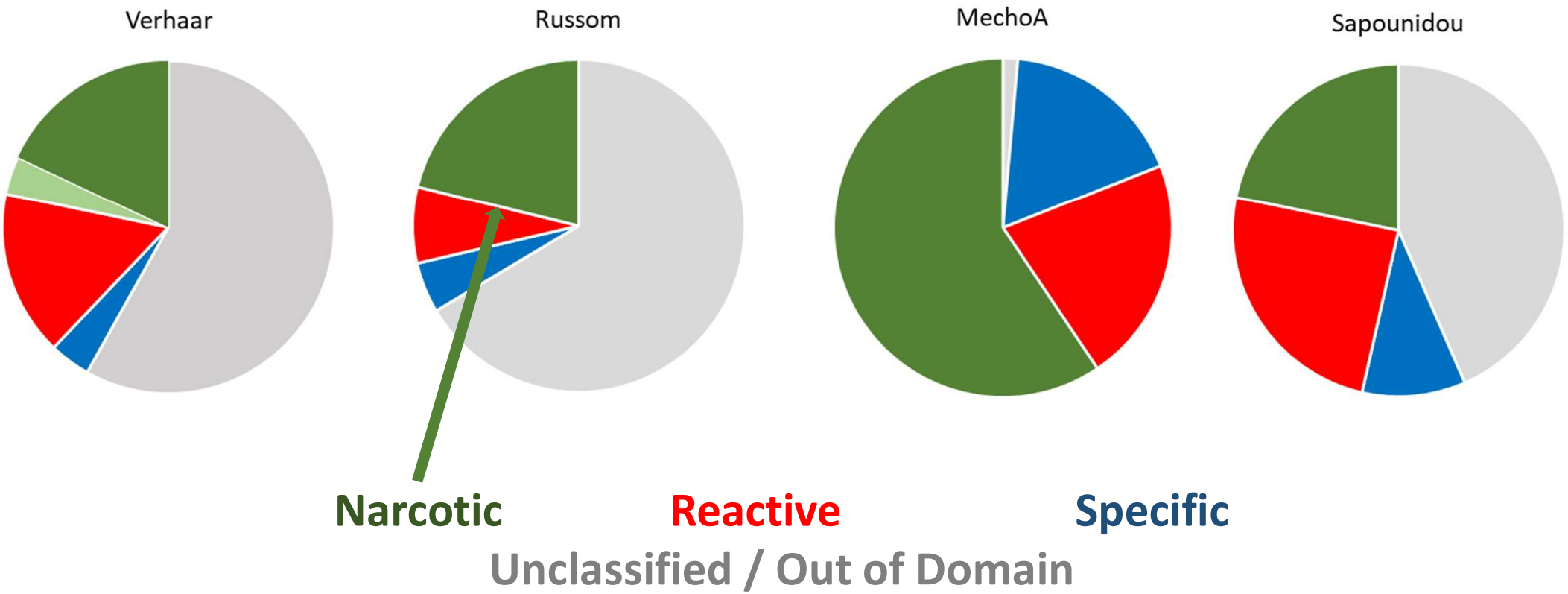
		3.3.5. Steroid biosynthesis disruption
		3.3.6. Carotenoid biosynthesis disruption
		3.3.7. Protein biosynthesis disruption
		3.3.8. Developmental disruption
	3.4. Mitochondrial disruption	3.4.1. Mitochondrial ETC inhibition (specific)
		3.4.2. Mitochondrial ETC (non-specific)
	3.5. Nuclear receptor	3.5.1. Modulation of nuclear receptors

Coverage of the Schemes



Russom: Borderline In / Out of Domain; Out of Domain

Assignments from the Schemes



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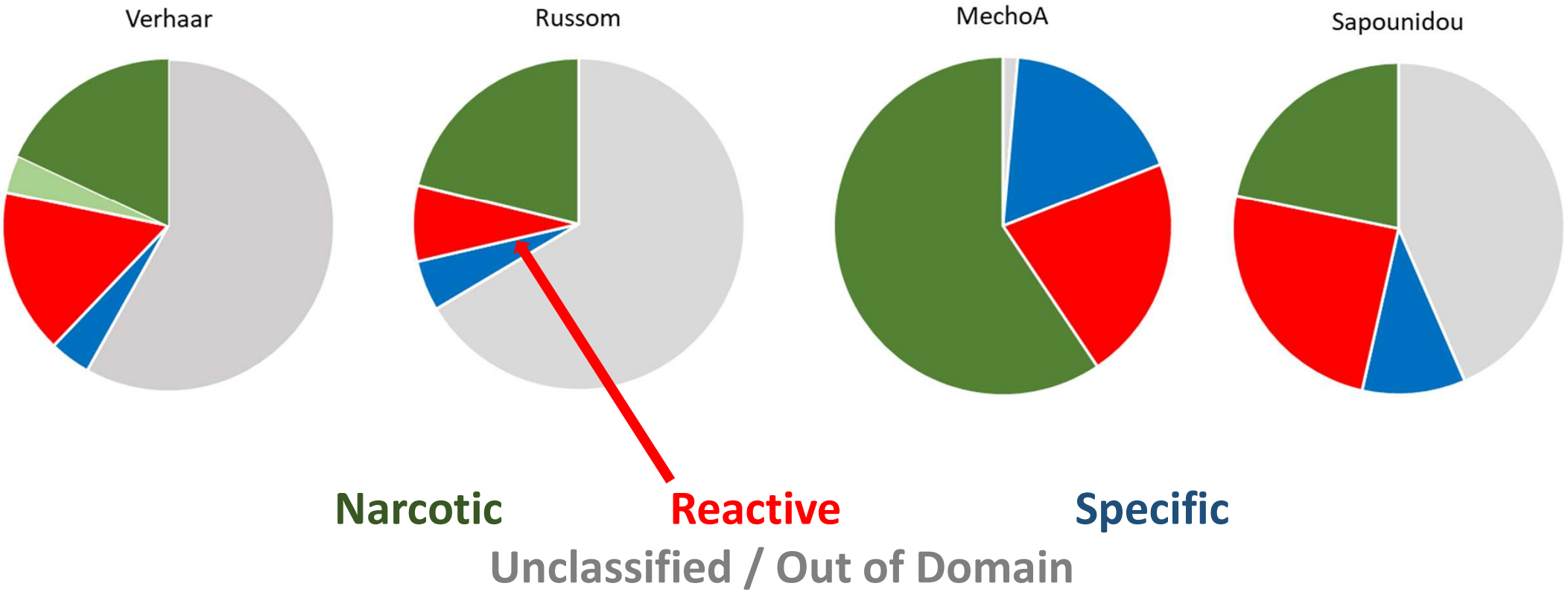

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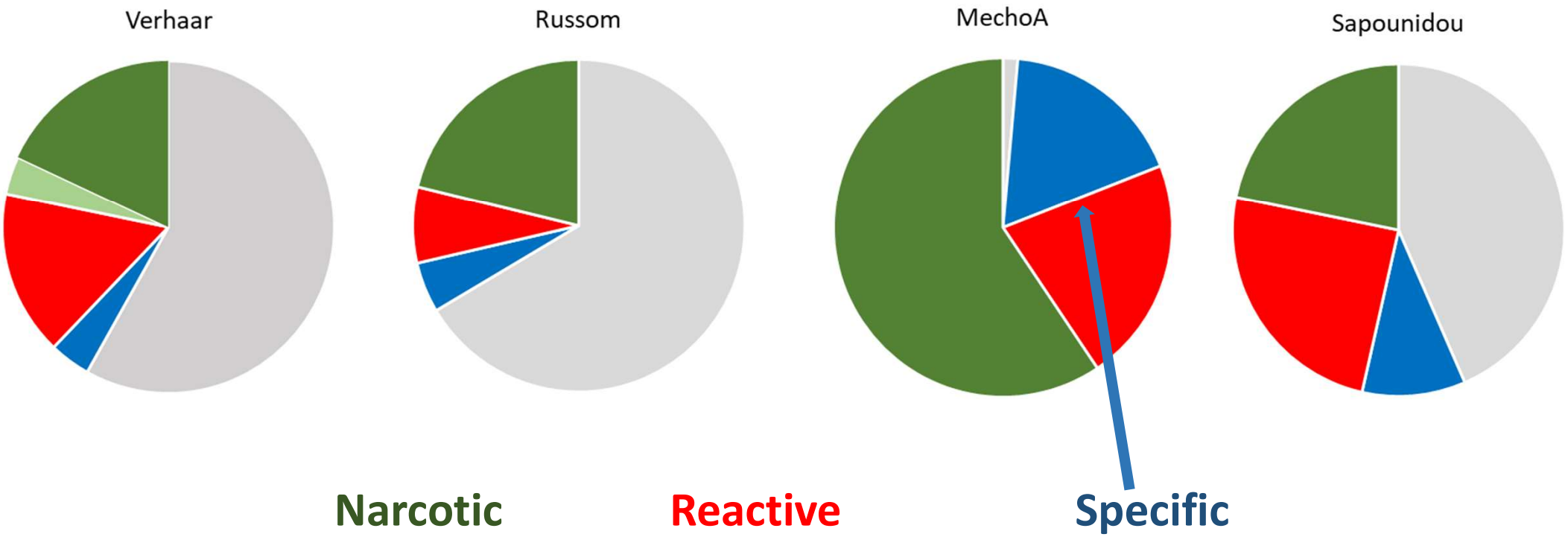

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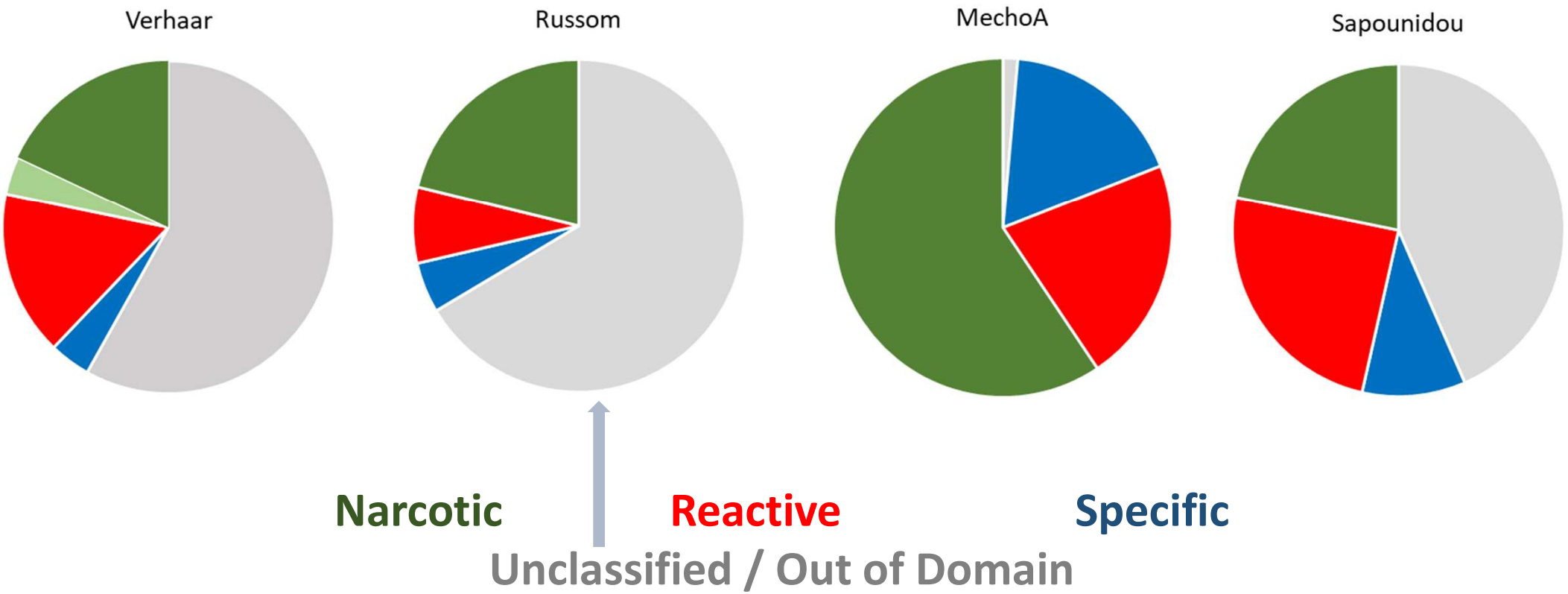

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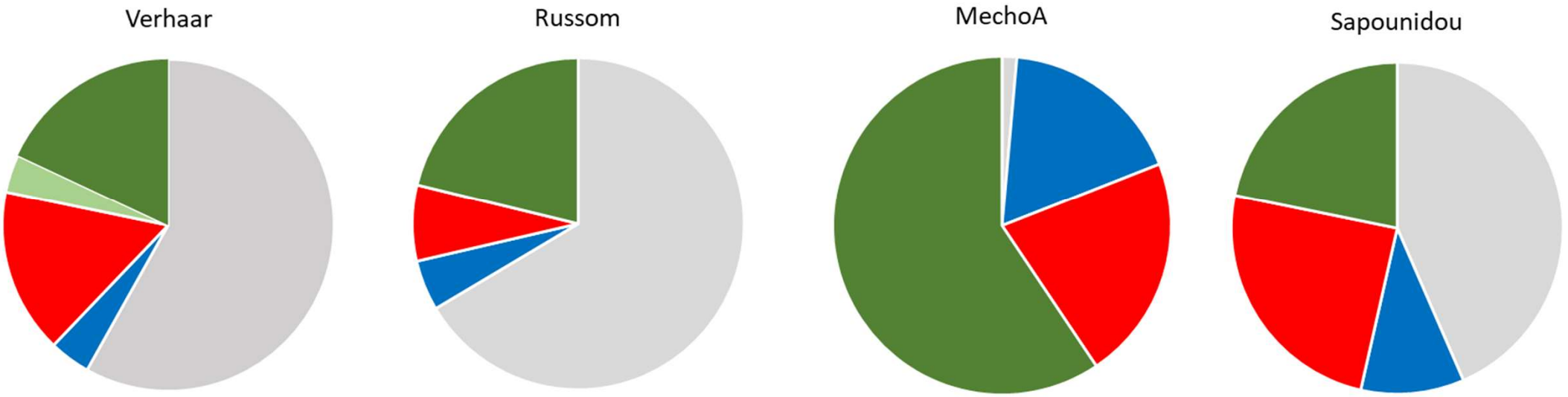

Narcotic **Reactive** **Specific**

Unclassified / Out of Domain

Mechanistic Relevance

- Knowledge of mechanisms helps justify hazard identification and risk assessment decisions
  - Clear linkage to AOPs will assist
- Definitive mechanisms known for very few chemicals
  - Most defined by analogy
- Weight-of-evidence can be established
  - Historical data and information
  - “Similar” chemicals and fitting to QSAR
  - NAMs data

Mechanistic Relevance of Schemes

- MechoA and Sapounidou schemes have increased granularity
- Linkage to AOPs provides information on MIEs enabling development of structural alerts
- Mechanistic relevance allows for species specificity to be built into the schemes

Mechanistic and Species Detail in Sapounidou: Non-Polar Narcosis Domain

Description of Mechanism	Non-specific accumulation within biological membranes – predicted well by baseline toxicity equation
MIE	Accumulation in membrane-based phosopholipids
MIE Target	Cellular membranes
Example Xenobiotics	Aliphatic 2^{o} and 3^{o} amines, ketones, aliphatic alcohols, halogen substituted mono and polycyclic hydrocarbons, ethers, hydrocarbons, aliphatic halides

Mechanistic and Species Detail in Sapounidou: Non-Polar Narcosis Domain

Taxonomical Applicability	Across all taxa and species
No. Structural Alerts	6
References	Ankley et al., 2010; Aruoja et al., 2014; Cronin and Schultz, 1997; Ellison et al., 2008; Klüver et al., 2016; 2018; Könemann, 1981; Nendza et al., 2017; Perkins et al., 2015; Roberts and Costello 2003; Verhaar et al., 1992; Vinken and Blaauboer, 2017; Zhao et al., 1998a; 1998b

Applicability of Updated Schemes to Risk Assessment Scenarios

- Allow coverage and mechanistic detail for MoA classification

- Support risk assessment:
  - Assignment of QSARs
  - More justifiable read-across
  - MIE / AOP informed NAMs

- Greater transparency

Summary and Opportunities

- Mechanistic classification schemes play a vital role in risk assessment
- New schemes (MechoA and Sapounidou) extend our knowledge giving more detail and transparency
- Integration of schemes (by KREATiS) is on-going
- New MechoA classification scheme to be made publicly available
- Scheme designed to be information rich, flexible and updateable