United States Environmental Protection Agency

Revisiting and updating chemical categorization with new approach methodologies: *Lessons learned*

US EPA in collaboration with Health Canada, Environment Climate Change Canada

APCRA
November 11, 2022

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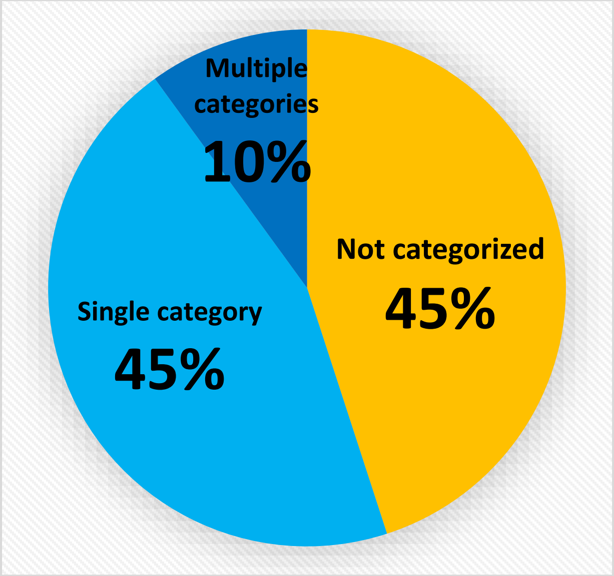
Background: Chemical categorization



- "A chemical category is a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity." – OECD
- Traditional approaches to chemical categorization are based on accumulated data and past decisional precedents.
- Many new chemicals across various regulatory jurisdictions cannot be categorized using existing *in silico* models and methods.
- Almost half of all New Chemical inventories across regulatory jurisdictions cannot be categorized using NCC or ECOSAR.
- Some chemicals fall into multiple categories.

Case study objectives

- ***How do we incorporate new approach methodologies (NAMs) and cheminformatic approaches to assist in identifying new chemical categories?***
- ***Can we use a classified consensus Mode-of-Action (cMOA) dataset (supervised learning) to develop a robust classification model to discriminate between narcotic (N) and specific-acting (S) chemicals for aquatic (fish) toxicity?***

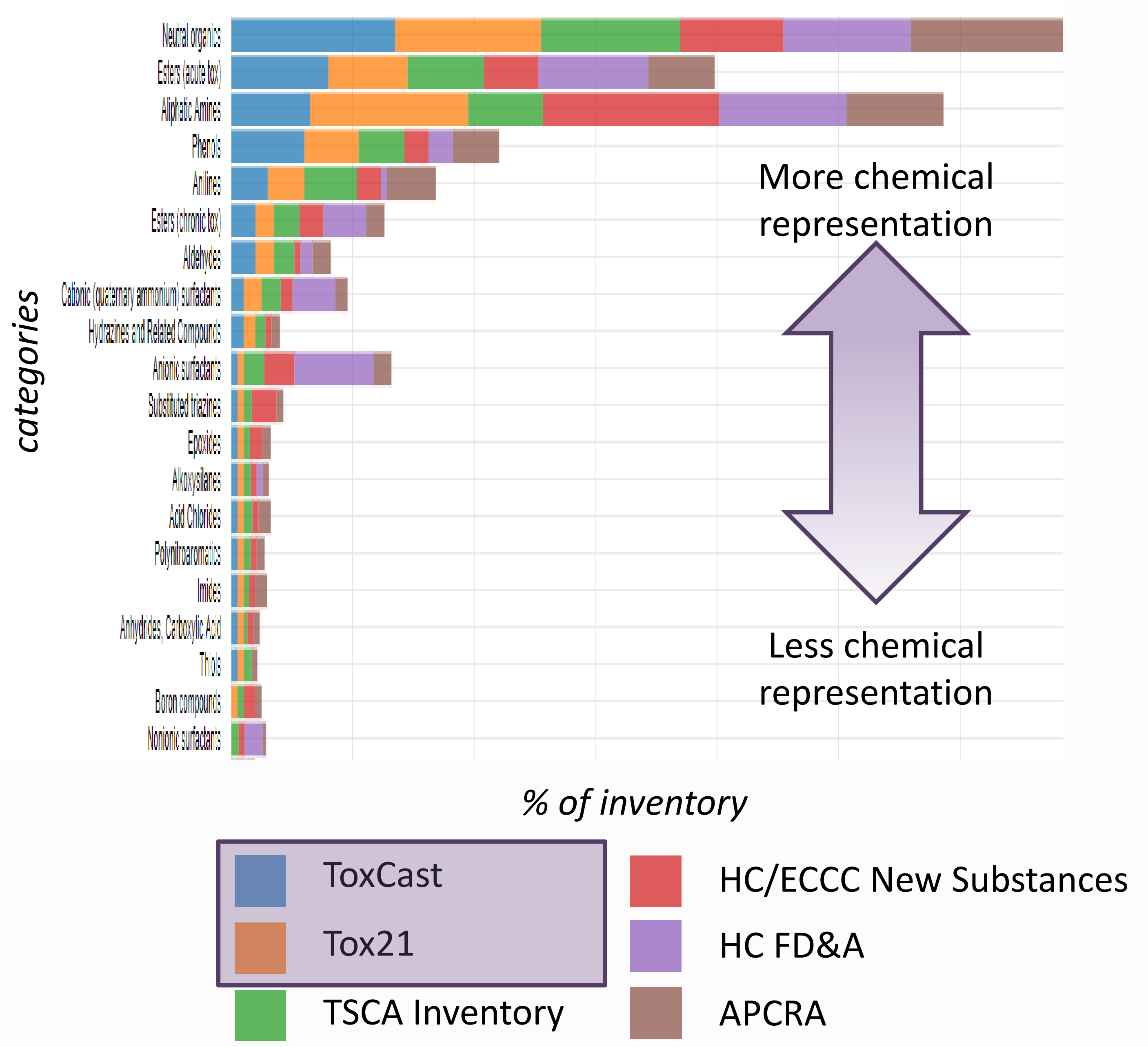
Key case study accomplishments

1. Identified the landscape of regulatory and bioassay chemical inventories based on New Chemical Category (NCC) definitions and ToxPrints (TxP).
2. Developed of a robust N/S classification model for aquatic toxicity.
3. Identified known limitations regarding unclassified cMOA chemicals.
4. Suggested targeted use of NAM information (*i.e.*, use of specific assay data) with Chemotype enrichment workflows.

Office of Research and Development
Center for Computational Toxicology and Exposure

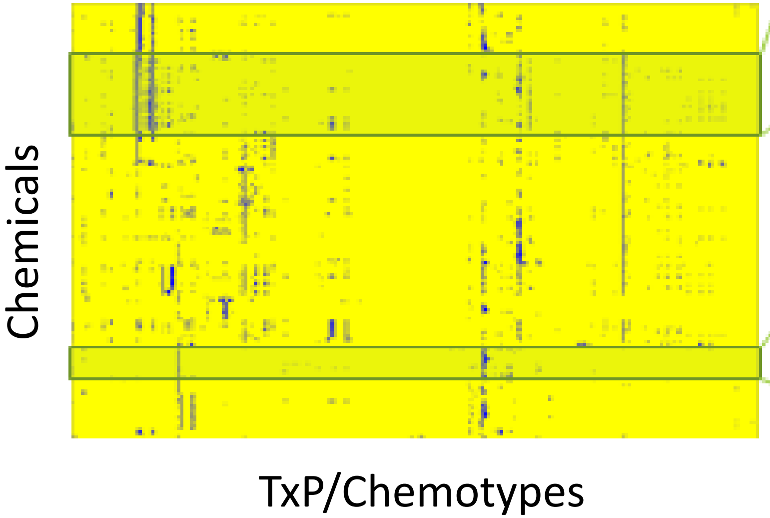
1. Identification of the chemical landscape

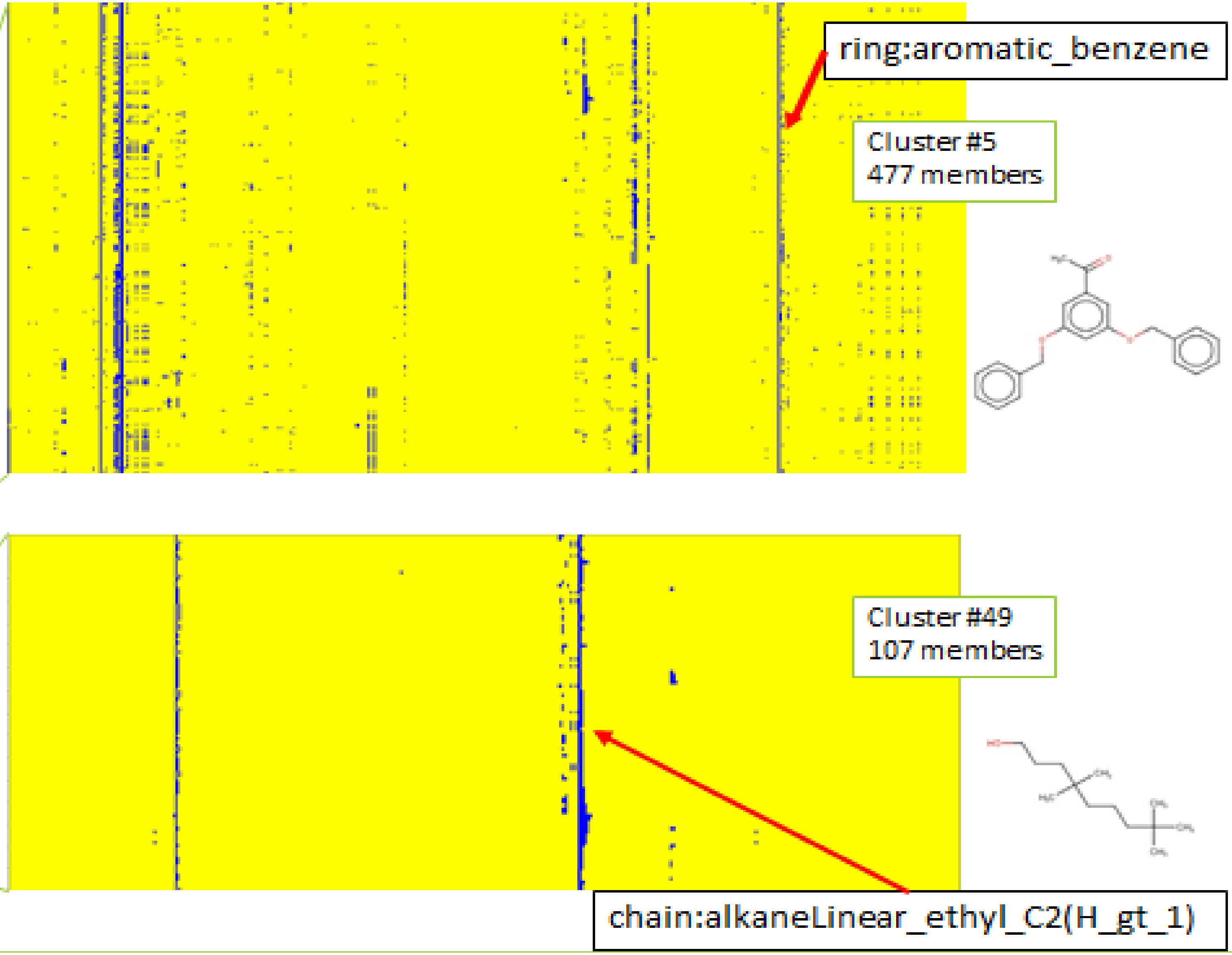
- Can we use existing NAM data rather than traditional *in vivo* data?
- 57 NCC chemical categories are based on structure, phys chem properties and existing *in vivo* data.
- ToxCast and Tox21 HTS data overlaps well with existing regulatory chemical inventories.
- ***For TSCA and APCRA inventories, ToxCast and Tox21 may be appropriate potential surrogates for NAM-based information in larger categories – Neutral Organics, Phenols, etc.***



TSCA Neutral Organics

- TxP analysis with hierarchical clustering
- Identified unique clusters of chemistries/representations
- Potential implications for risks/endpoints





Heatmap of Neutral Organics based on ToxPrint features, hierarchical clustering

2. Classification model for aquatic toxicity

Primary focus of this effort: Identification of narcotic (N) and specific-acting (S) chemicals for aquatic (fish) toxicity using a classified consensus Mode-of-Action (cMOA) dataset.

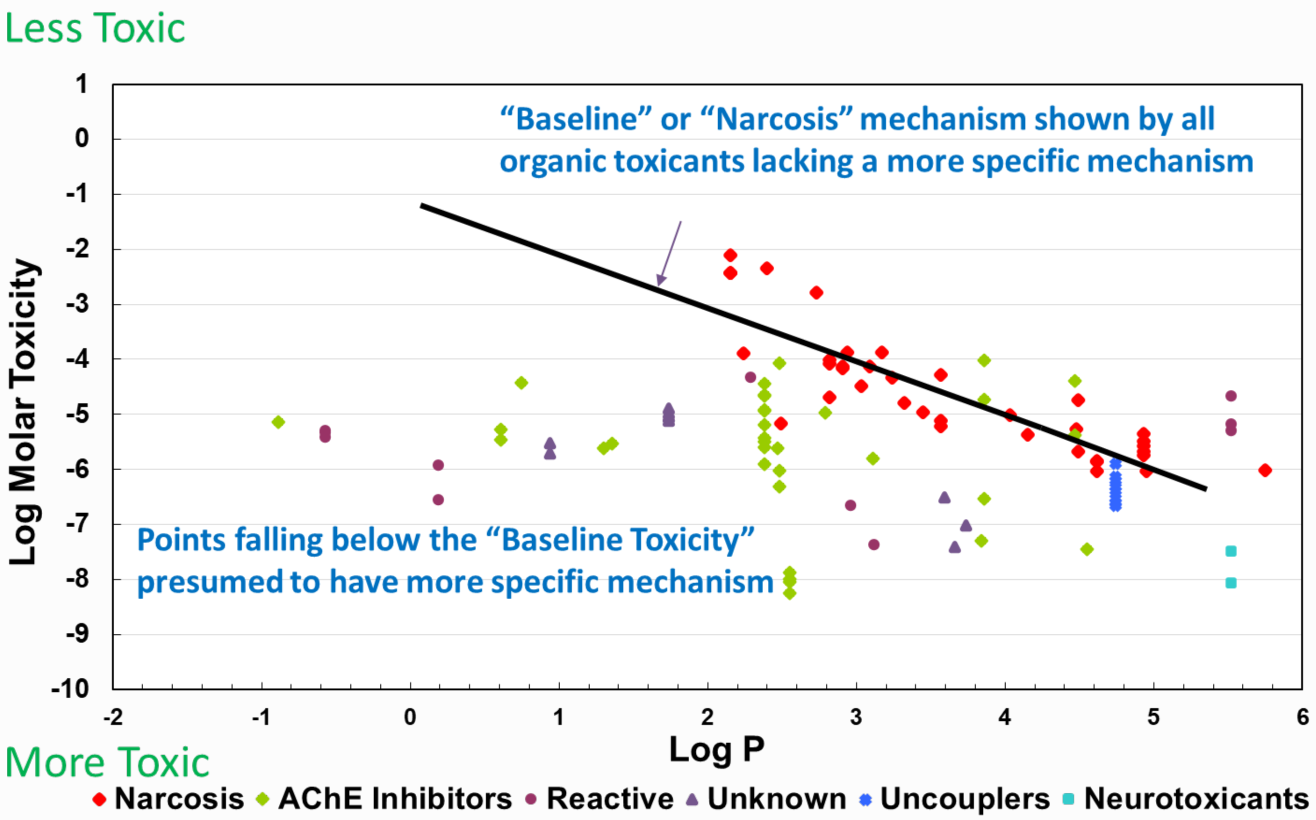
Applications of chemical categorization include first tier assessment efforts and read across from structurally similar analogs – ECOSAR

US EPA ECOSAR chemical classifications

- Class-based SAR to predict aquatic toxicity
- Classification scheme identifies excess toxicity
- Estimates **acute** and **chronic toxicity** based on accumulated data and past decisional precedents

Acute Effects:	Chronic Effects:
Fish 96-hr LC_{50}	Fish ChV
Daphnid 48-hr EC_{50}	Daphnid ChV
Algae 72/96-hr EC_{50}	Algae ChV

- Profiler in OECD QSAR Toolbox



1. ***Regulators consider MOA information to determine the size of assessment factors***
2. ***Can we develop a viable model to use as a tool to discriminate specific-acting & narcotic MOAs for potential category development?***

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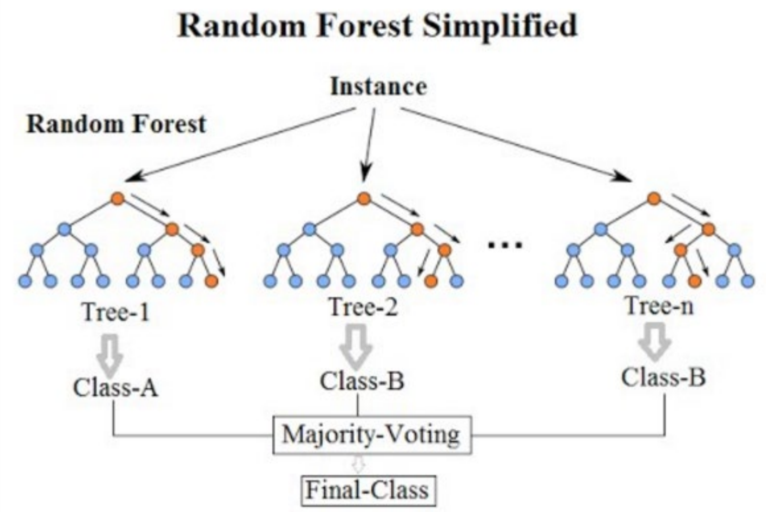
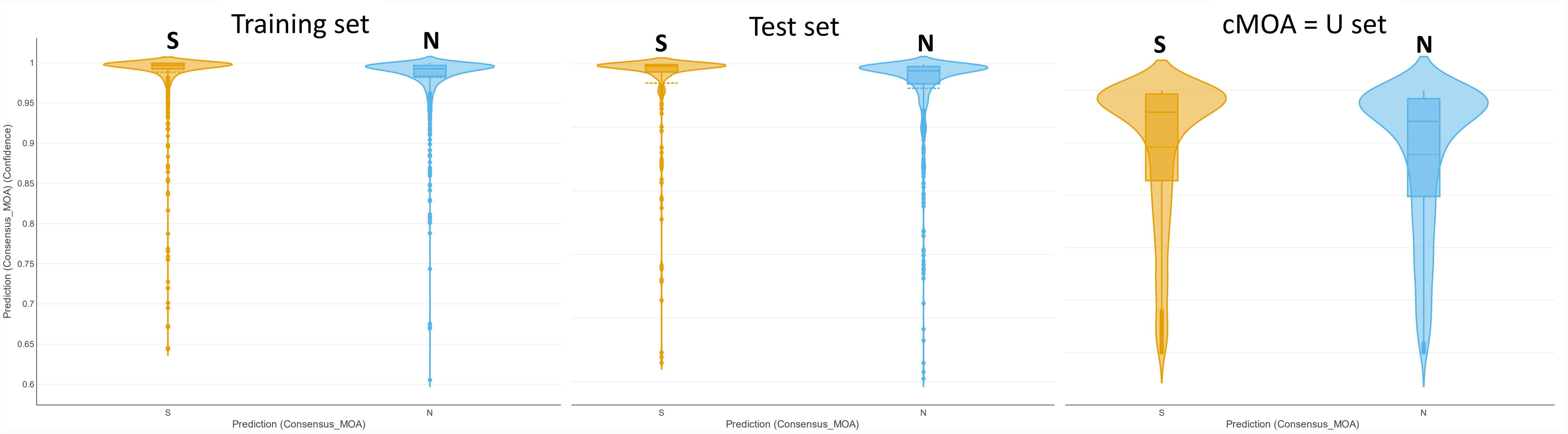
TxP model details

- **Dataset: supervised learning via Consensus MOA (cMOA) dataset**
  - EnviroTox Database: Aquatic toxicity *in vivo* dataset with a consensus call based on 4 structure based models (Health and Environmental Sciences Institute (HESI). 2019. EnviroTox Database & Tools. Version 1.1.0 Available: http://www.envirotoxdatabase.org/)
- **Features: ToxPrints**
  - Richard *et al.*, Chem. Res. Toxicol. 2016, 29(8) 1225 – 1251; Strickland *et al.*, Arch Toxicol. 2018 92(1) 487 – 500; Wang *et al.*, Environment International 2019, 126 377 – 386.
- **Method: Random Forest (Boosted Gradient Method)**
  - Split data into 80% training and 20% hold out (test) sets
  - Hyperparameter tuning with 5-fold cross validation, square-root sampling, etc.
  - Training set: “balanced” down-sampled subset (2104 chemicals w/ a cMOA = N or S)
  - High accuracy in both training and test sets (training = 99.7%; test = 95.8%)
  - Total Accuracy on all N + S data set = 97.6% (4356 cMOA = N or S)
  - Across all N + S chemicals -> 105 chemicals misclassified:
    - 24 F_{pos}{predicted S}
    - 81 F_{neg}{predicted N}



https://medium.com/@williamkoehrsen/random-forest-simple-explanation-377895a60d2d

Distribution of prediction confidence [0,1] by (N,S) class



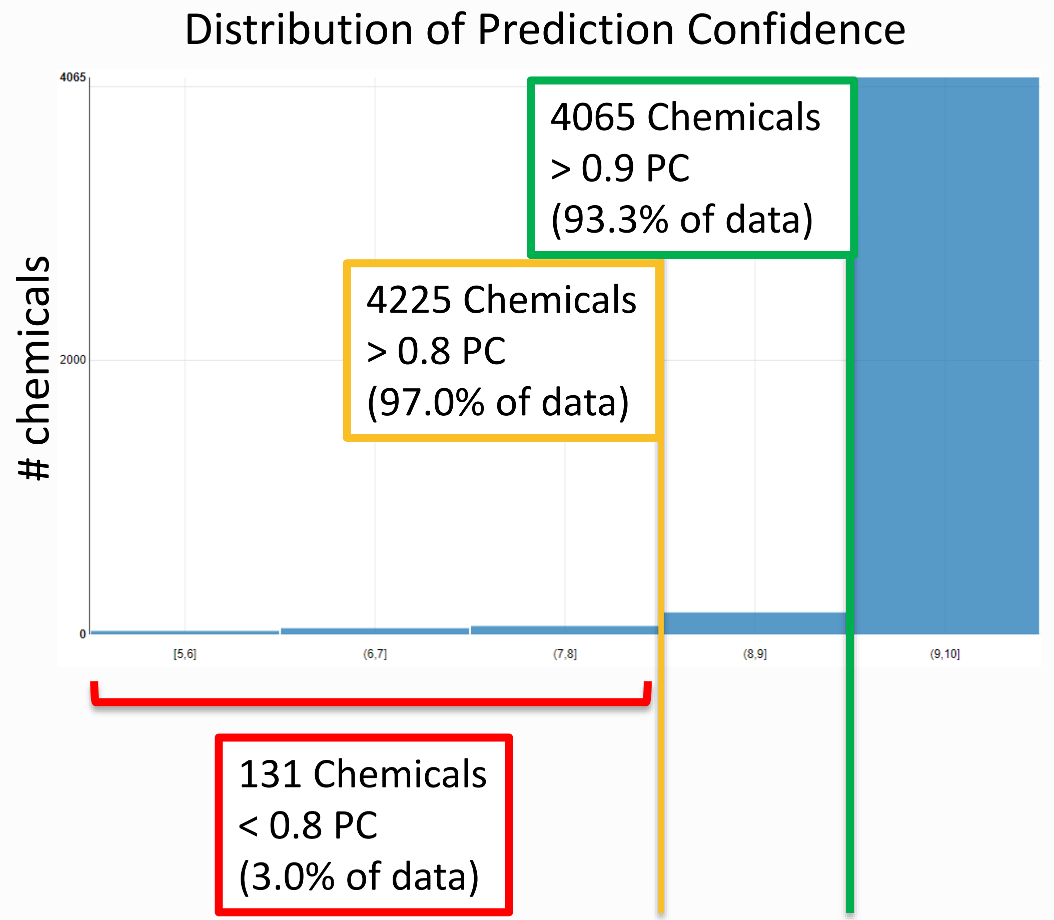
Training Set
Median: 0.999, 0.993
Mean: 0.988, 0.982

Test Set
Median: 0.996, 0.989
Mean: 0.970, 0.962

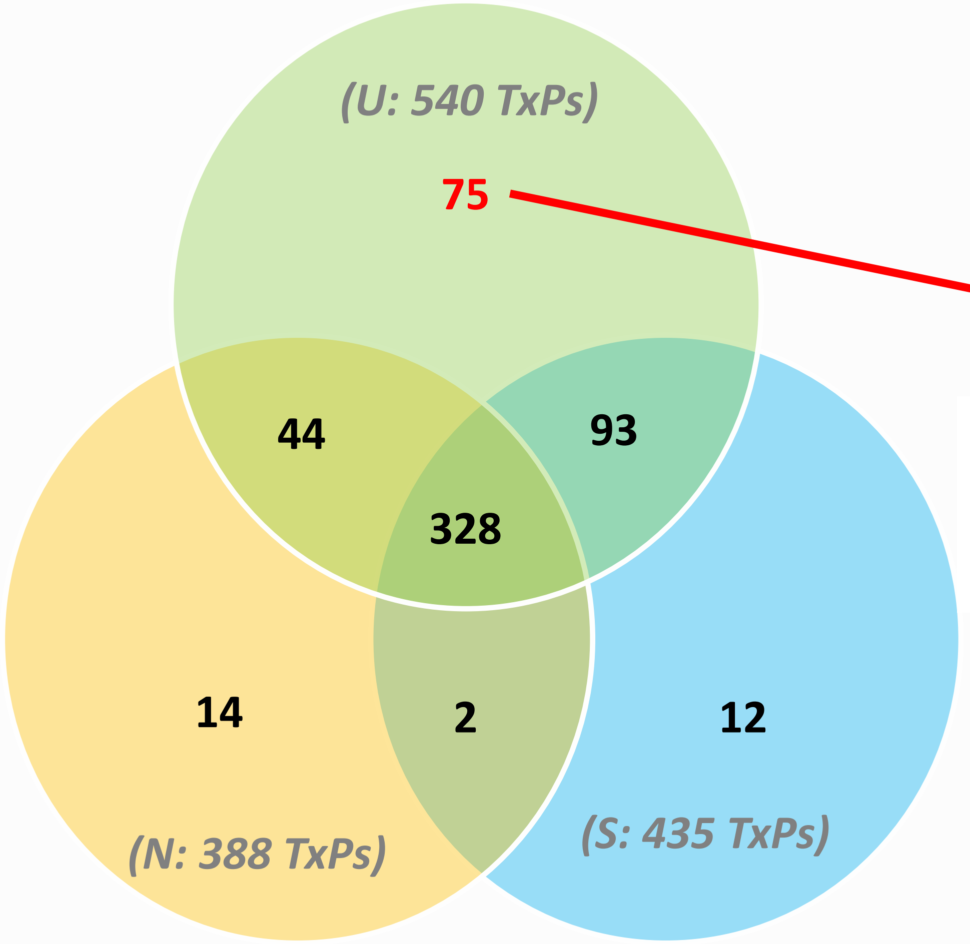
Unclassified Set
Median: 0.958, 0.941
Mean: 0.892, 0.877

Prediction confidence across the cMOA = N or S

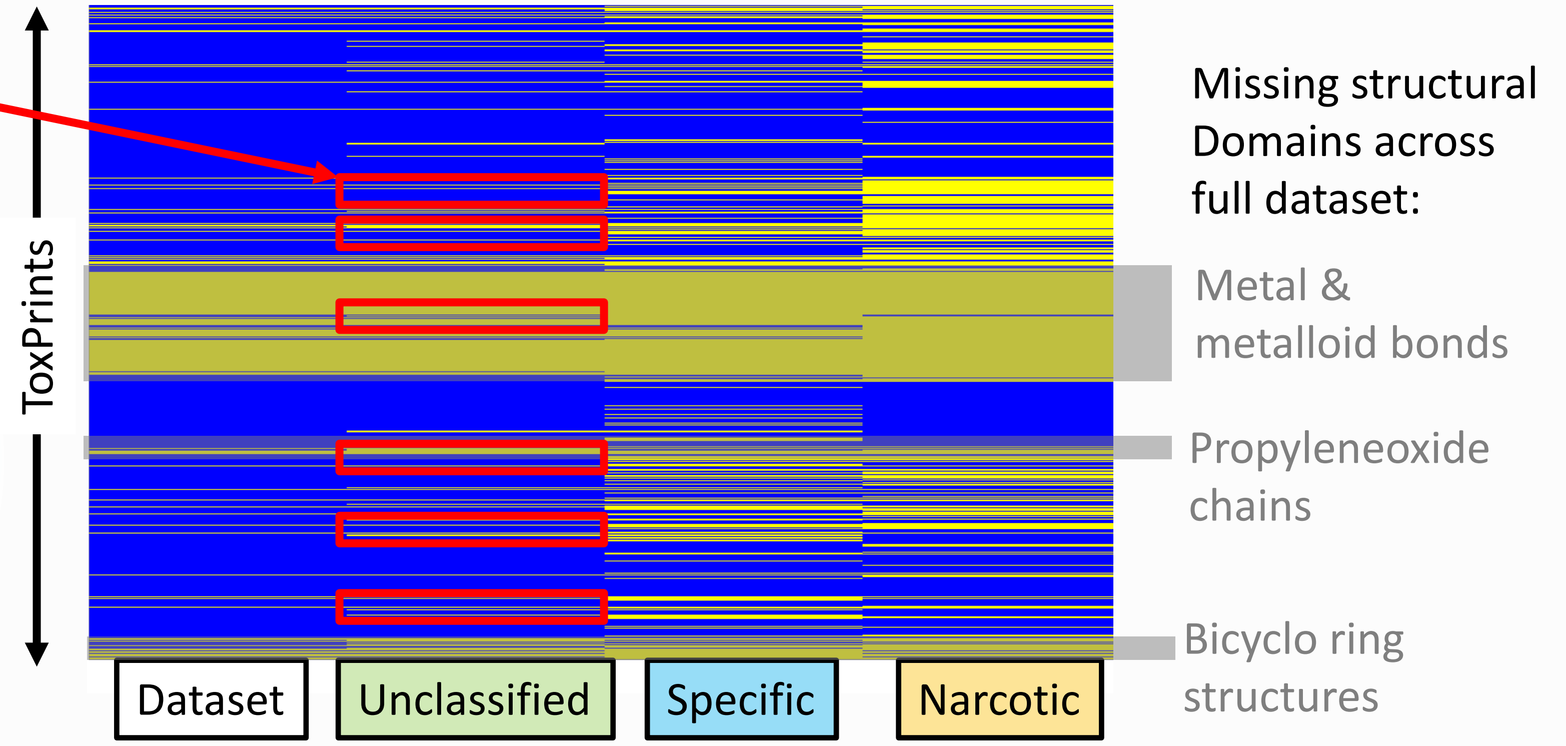
- Distribution of prediction confidence (PC) tends to be > 0.8 for the classified data (cMOA = N or S)
- Model has fewer # misclassifications in S
  - Misclassifications for 93 cMOA confidence = 2, and 12 with 1,3 scores (recall 3>2>1 for confidence)
  - **~46% of the misclassifications can be attributed to the chemicals with PC < 0.8**
  - ~67% of the misclassification can be attributed to chemicals with PC < 0.88



3. Analysis of unclassified consensus MOA chemicals: Characterization of TxP coverage per consensus MOA class



Heatmap representation of ToxPrints

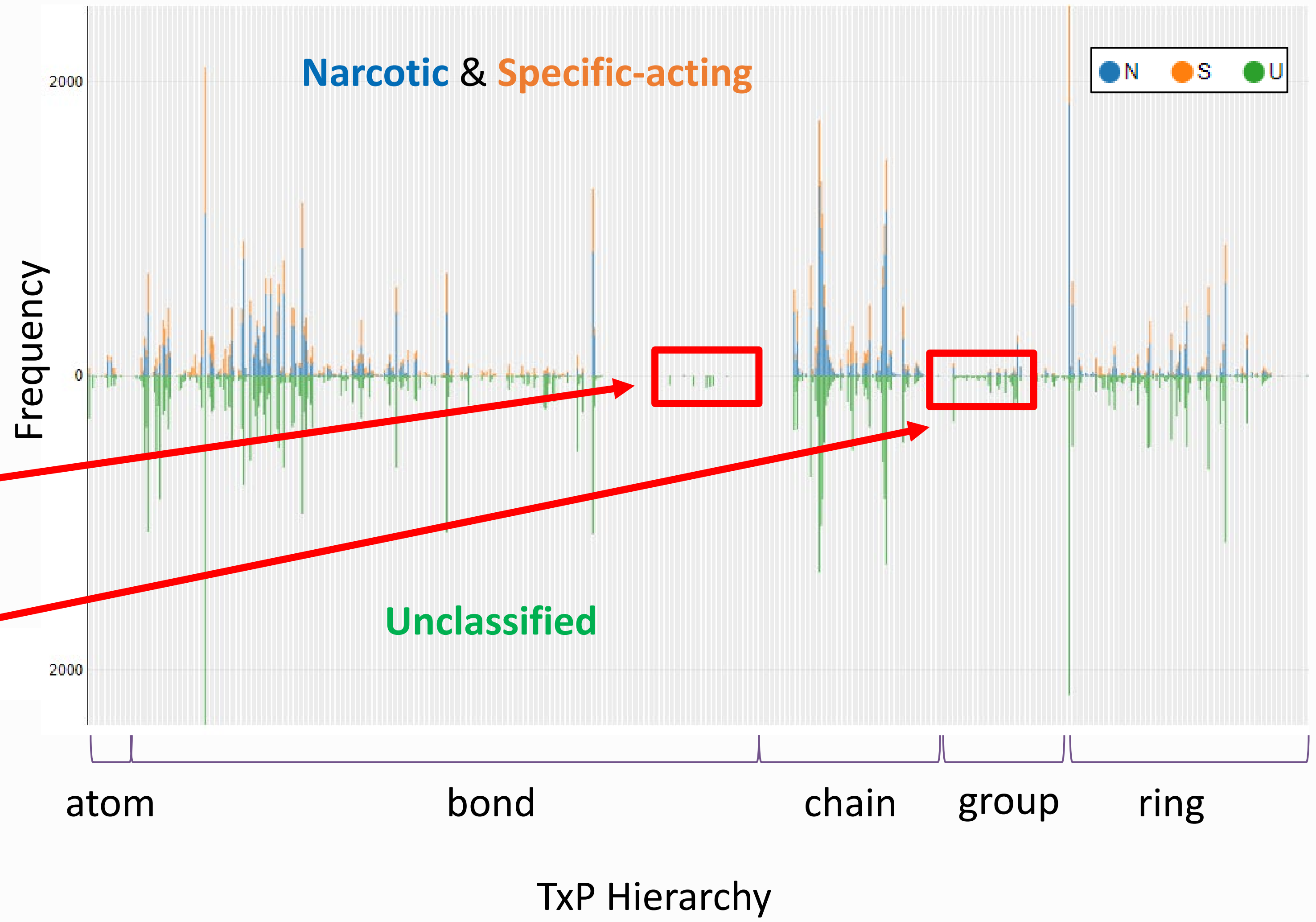


ToxPrints: Dataset > Unclassified > Specific-acting > Narcotic

Unique TxPs in the unclassified set

- ~7x more unique features in U (than in N or S)
- Could explain the lower prediction confidence in N/S classification of the U set
- Potential for additional categories based on structure:
  - 2 atom TxPs (metal group III)
  - 38 bond TxPs (metalloid: silane and siloxanes…)
  - 8 chain TxPs (ethyleneoxide alkanes C10 – C20)
  - 19 group TxPs (amino acids, polydentate ligands)
  - 8 ring TxPs

Frequency of TxPs per consensus MOA class

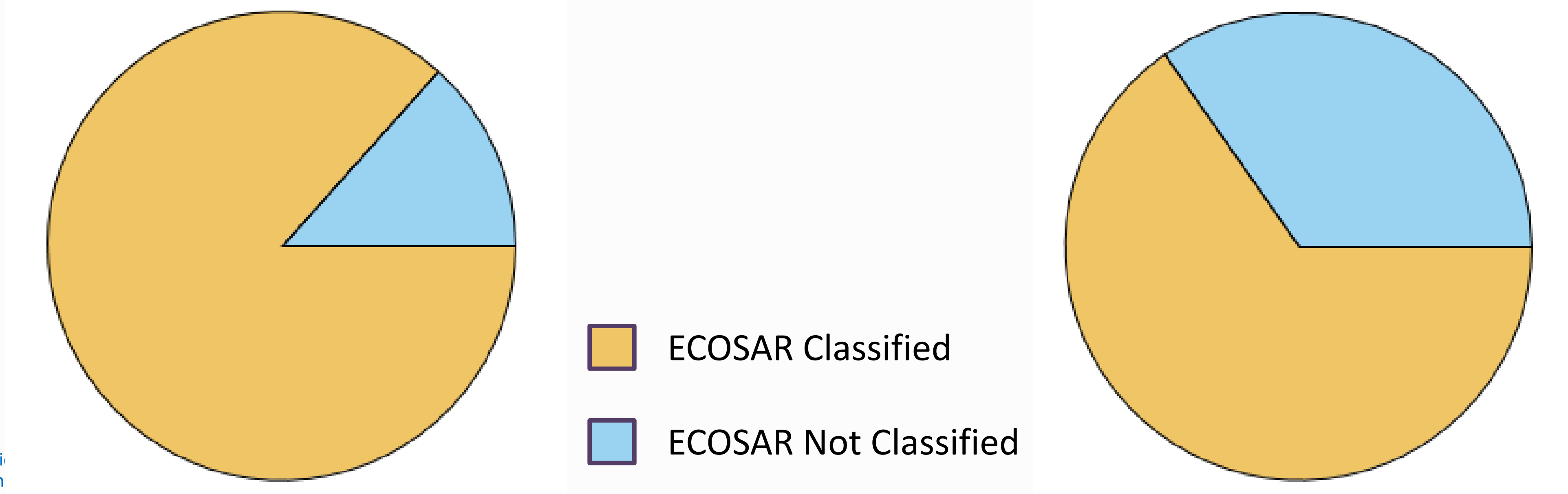


TxP model predicted MOAs of the EnviroTox unclassified set

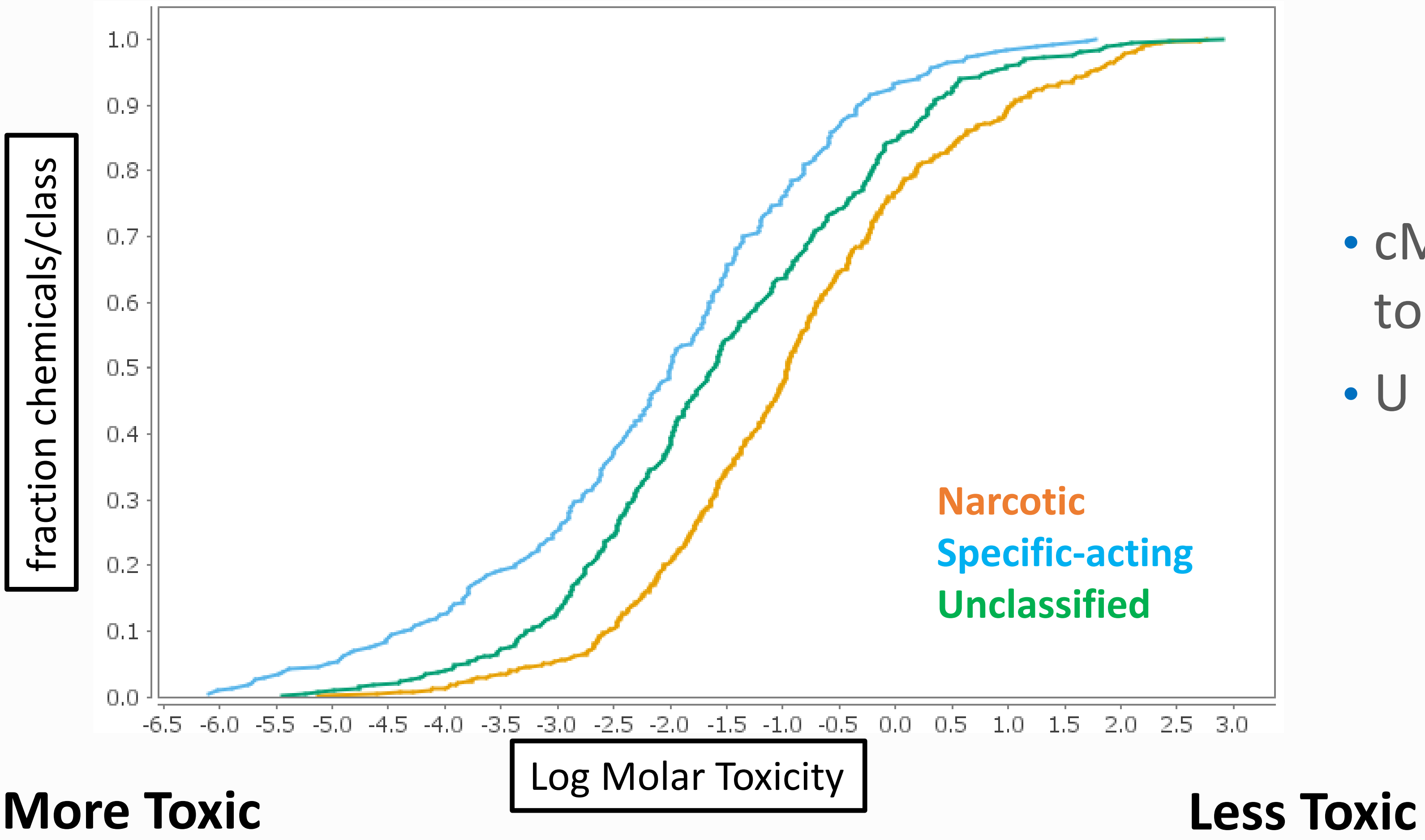
- 674 subset chemicals in the EnviroTox dataset that had low confidence or ambiguous consensus (unclassified)
- Applied TxP model to the unclassified set and compared predictions to ECOSAR classification

361 predicted as Narcotic

313 predicted as Specific-acting



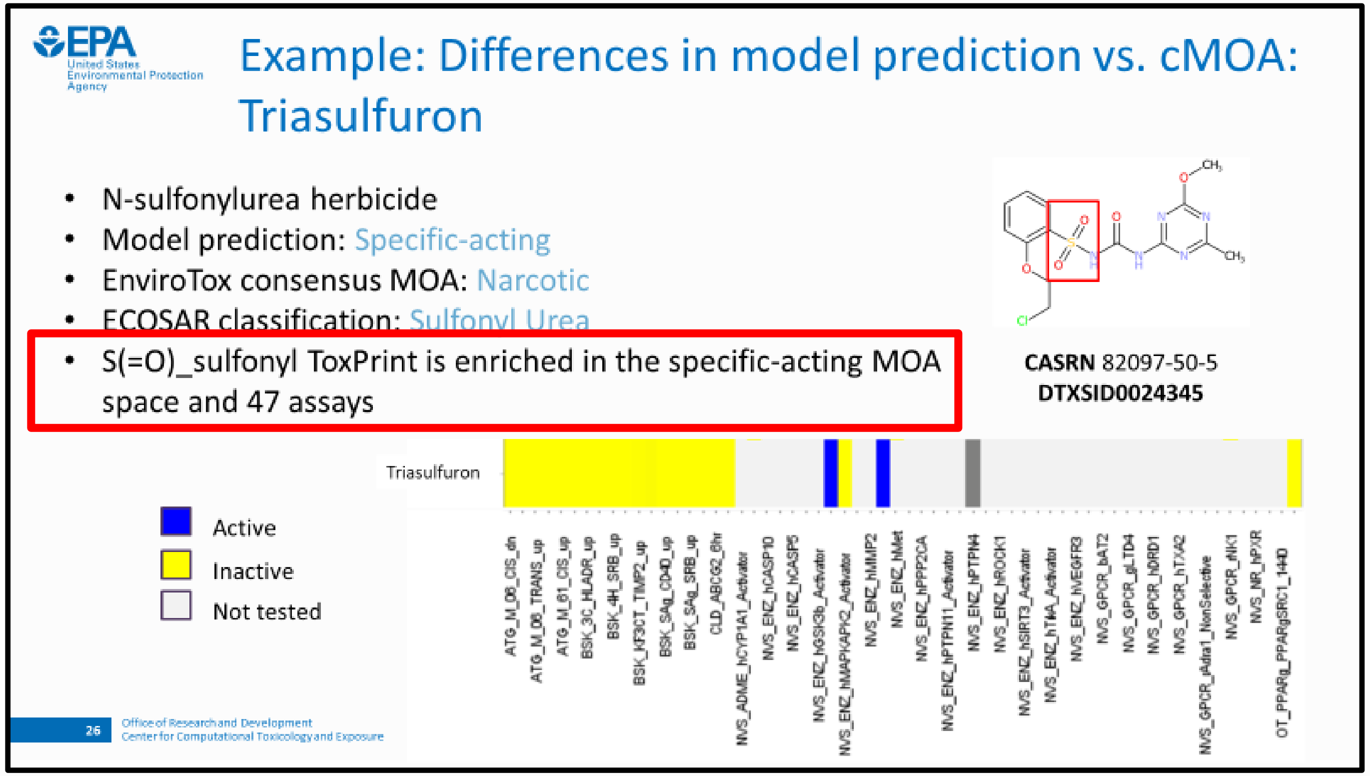
Cumulative distribution function: Log molar toxicity, (LC50, 96h, FISH) for cMOA classes (N,S,U)



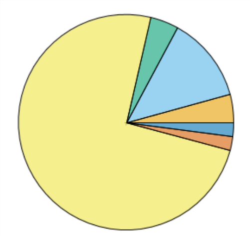
- cMOA classification is sufficient to discriminate N,S
- U presents some challenges

4. Targeted use of NAM information and Txp/Chemotypes

- Use chemotype enrichments to inform potential NAM data streams
- Example: sulfonyl TxP enrichments across NovaScreen (NVS) assay platform
- "…assays measure chemical binding to nuclear receptors, G-protein-coupled receptors (GPCR), transporters, and ion channels, and enzymatic inhibition or activation for a range of proteins including kinases, phosphatases, CYP450s, proteases, and histone deacetylases."
- Identified 47 assays due to sulfonyl TxP enrichment



Assay platform identification:



ATG | BSK | CLD | NVS | OT | TOX21

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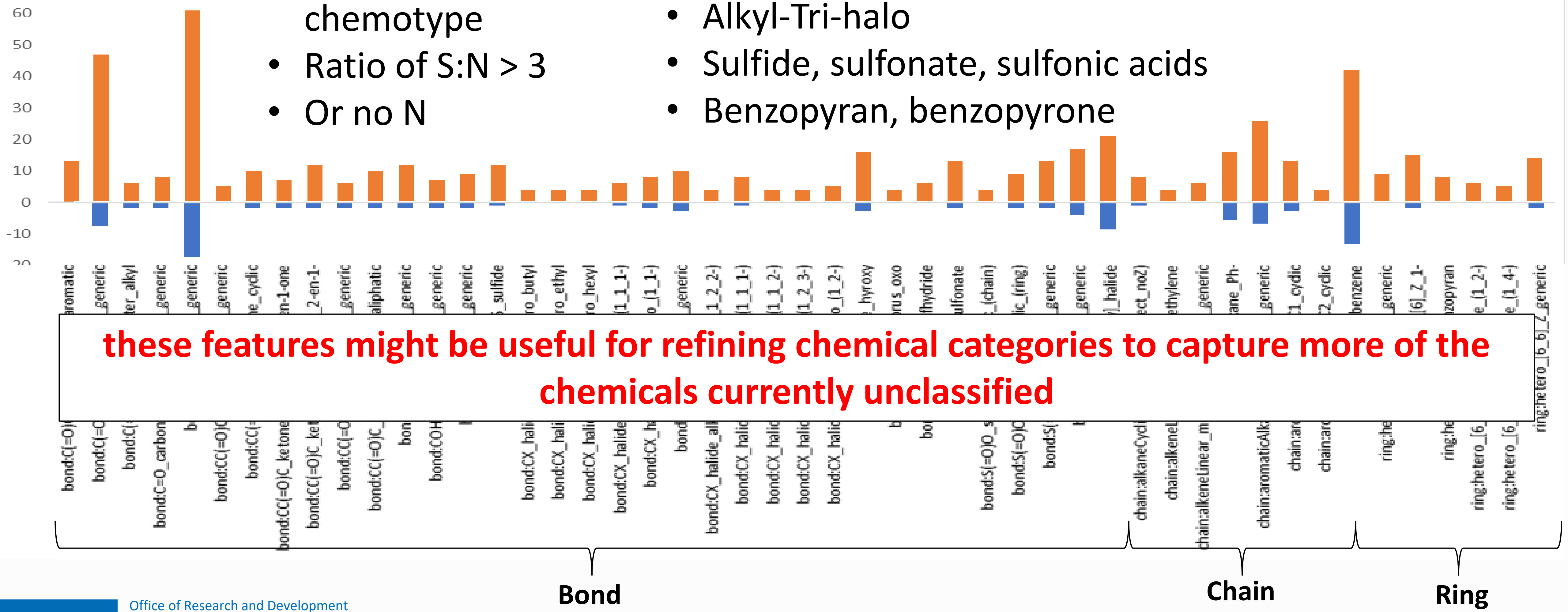
Enriched TxPs: Unclassified chemicals, TxP model predicted specific-acting

Criteria:

- ≥ 3 chemicals per chemotype
- Ratio of S:N > 3
- Or no N

Results:

- Ketones
- Alkyl-Tri-halo
- Sulfide, sulfonate, sulfonic acids
- Benzopyran, benzopyrone



these features might be useful for refining chemical categories to capture more of the chemicals currently unclassified

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Summary and Final Steps

- Developed a robust structural TxP model
  - Good N/S classification
  - Challenges in unclassified chemistries
- Investigated model predictions to inform ECOSAR subset of unclassified chemicals
  - Some unclassified chemicals predicted as potentially specific-acting MOAs
  - Identified primary chemotypes for specific-acting MOAs
- Explored methods to fold in NAM data streams
  - Using chemotype enrichments to identify potential bioassays with bioactivity to provide support of NAM data in category development
- Developing a manuscript on the existing TxP model and analyses

Thank you!