United States Environmental Protection Agency

# Revisiting and updating chemical groupings with new approach methodologies

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

APCRA-5
August 26 – 27, 2020

Office of Research and Development
Center for Computational Toxicology and Exposure

Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. This presentation has not been reviewed for policy and is not for distribution.

# Team members

## Accelerating the Pace of Chemical Risk Assessment (APCRA)

- US EPA
  - Dan Chang
  - Kellie Fay
  - Kristan Markey
  - Martin Phillips
  - Grace Patlewicz
  - Ann Richard
  - Gino Scarano
  - Mahmoud Shobair
  - Ryan Lougee
  - Ellery Saluck (summer intern)
- Environment & Climate Change Canada (ECCC)
  - John Prindiville
  - Cristina Inglis
- Health Canada
  - Mark Lewis
- ILS
  - Kamel Mansouri

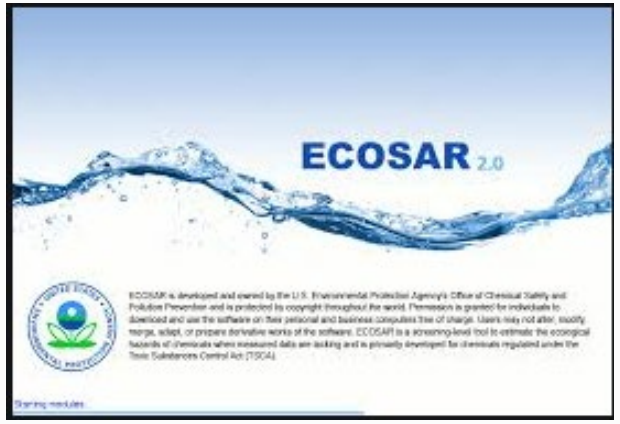
# Overview

**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

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

- Toxic Substances Control Act (TSCA) New Chemical Program Chemical Categories (NCC; US EPA)
- ECOSAR (focus of presented work)

# 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 |

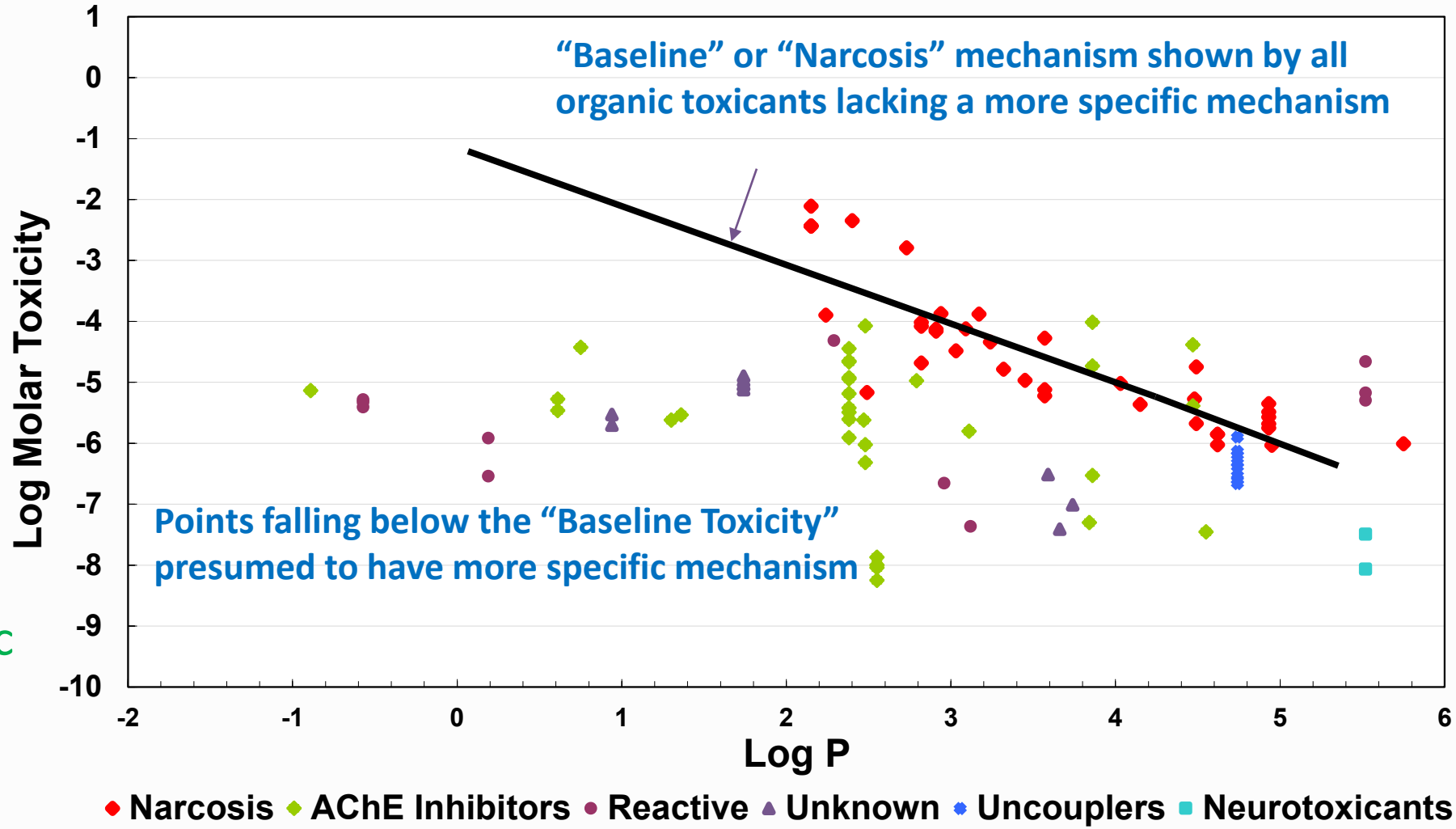
- Profiler in OECD QSAR Toolbox

# Narcosis vs. specific-acting toxicity MOA

Less Toxic

Regulators (ECCC) consider MOA information to determine the size of assessment factors

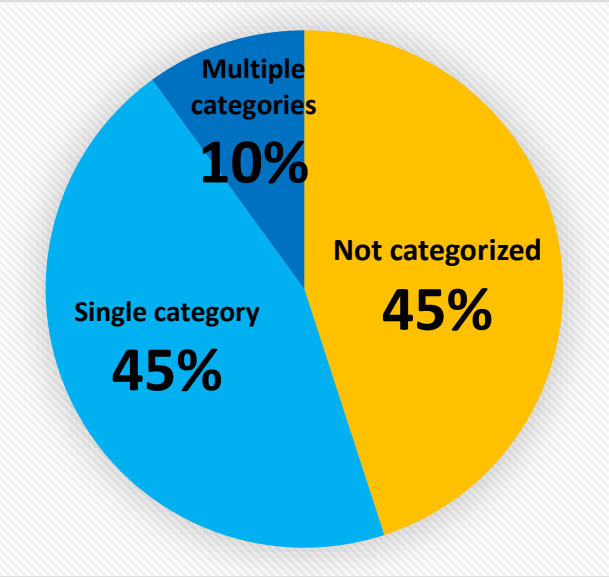
More Toxic





Office of Research and Development
Center for Computational Toxicology and Exposure

# Potential approach for updating chemical categories



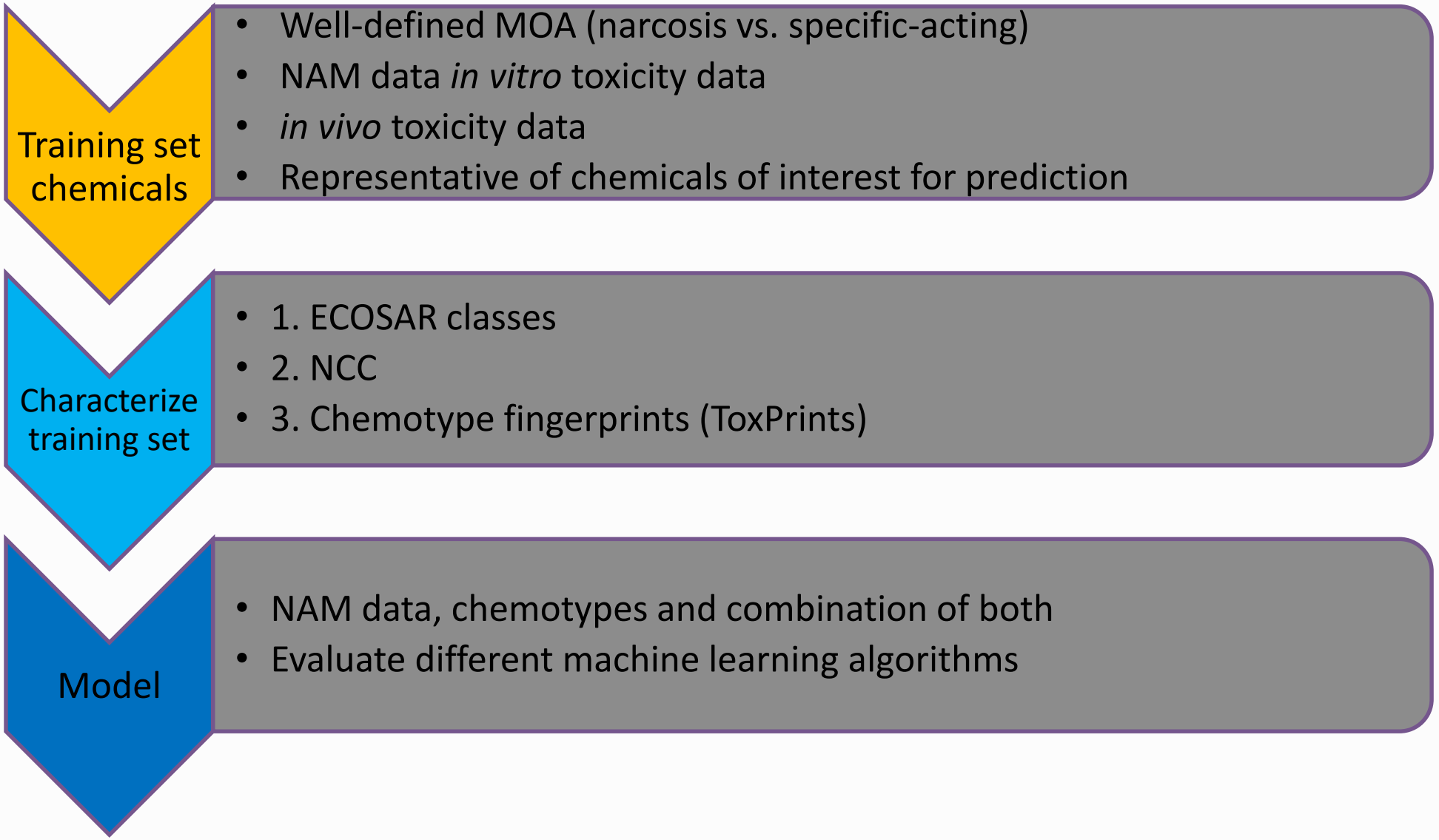

- Almost half of all New Chemical inventories across regulatory jurisdictions cannot be categorized using NCC or ECOSAR
- Some fall into multiple categories

**How do we update?**

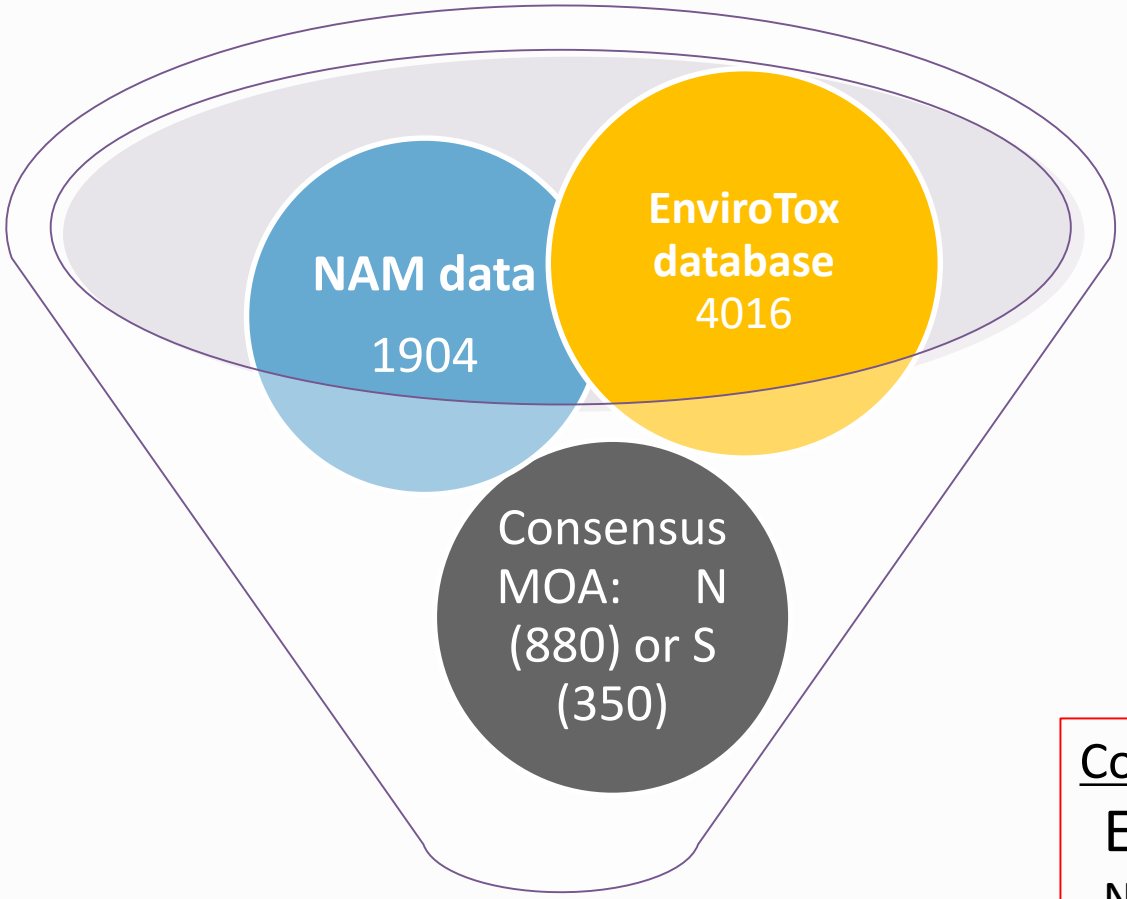
- Incorporate New Approach Methodologies (NAMs) – *i.e.*, ToxCast and Tox21 biological activity information
- Apply cheminformatic approaches

# ToxPrint (TxP) model development

# General approach



# EnviroTox training set chemicals




1. Chemicals with *in vivo* eco-data – from the EnviroTox[1] database – 4016
2. Sub-selection for chemicals with NAM data (ToxCast and Tox21) - 1904
3. MOA predictions based on 4 publicly-available classification models
   - VERHAAR, ASTER, OASIS, TEST
   - Each predicts Narcotic, Specific-Acting or Unclassified

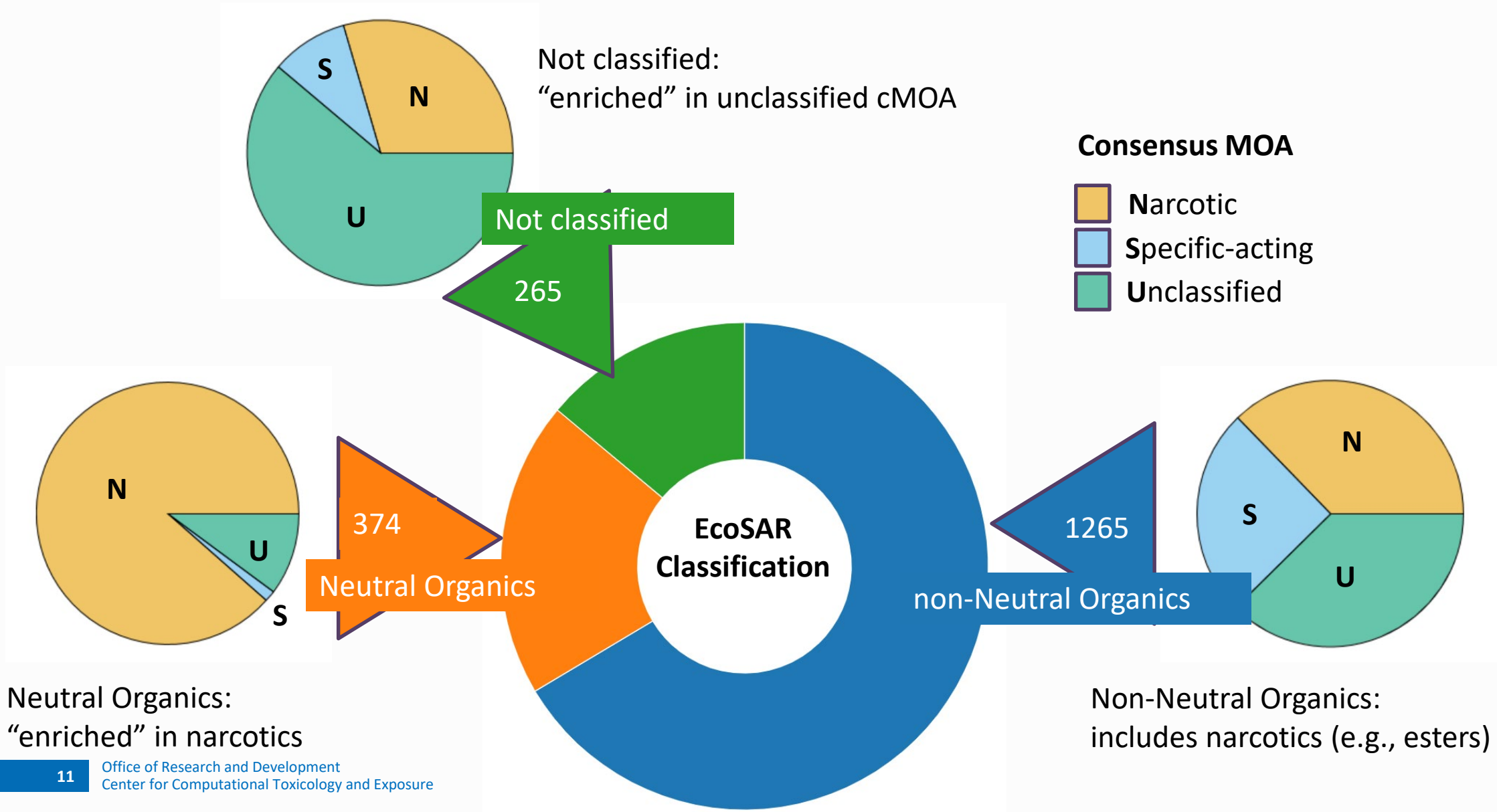
Consensus MOA (cMOA) with confidence scores[2]

| Examples: | Results: |
|---|---|
| NNNN = N, score =3 | 880 Narcotic |
| NNSN = N, score= 2 | 350 Specific-acting |
| SUSS = S, score= 2 | 674 Unclassified |
| NUNS = U, score = 0 | |

[1]Health and Environmental Sciences Institute (HESI). 2019. EnviroTox Database & Tools. Version 1.1.0 Available: http://www.envirotoxdatabase.org/

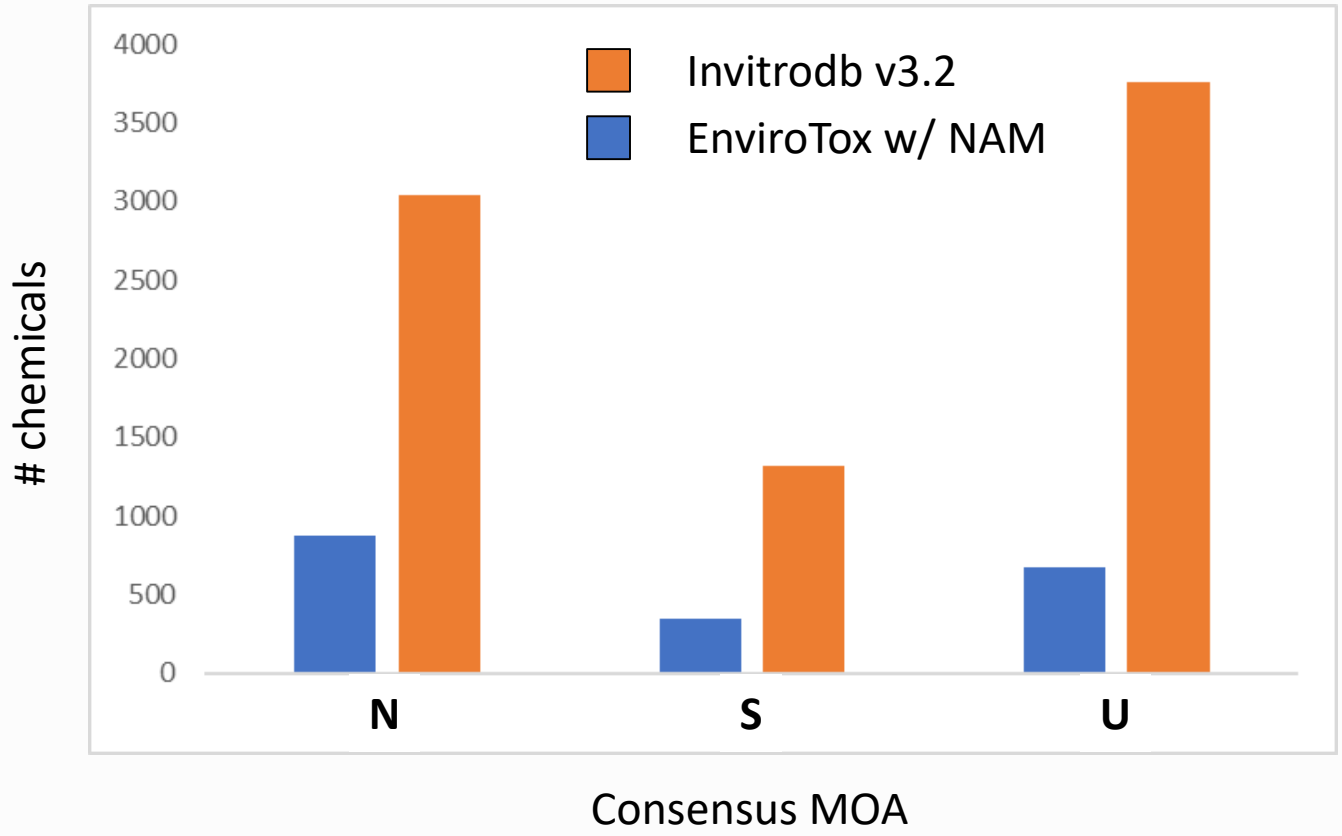
[2] Kienzler et al.. Environ Toxicol and Chem. 2019, 38(10) 2294-2304

# Characterize EnviroTox training set chemicals: ECOSAR classes



# Expanding the Envirotox chemical space

- Additional 6215 chemicals with NAM data (invitrodb v3.2)
- Applied the same consensus MOA methodology



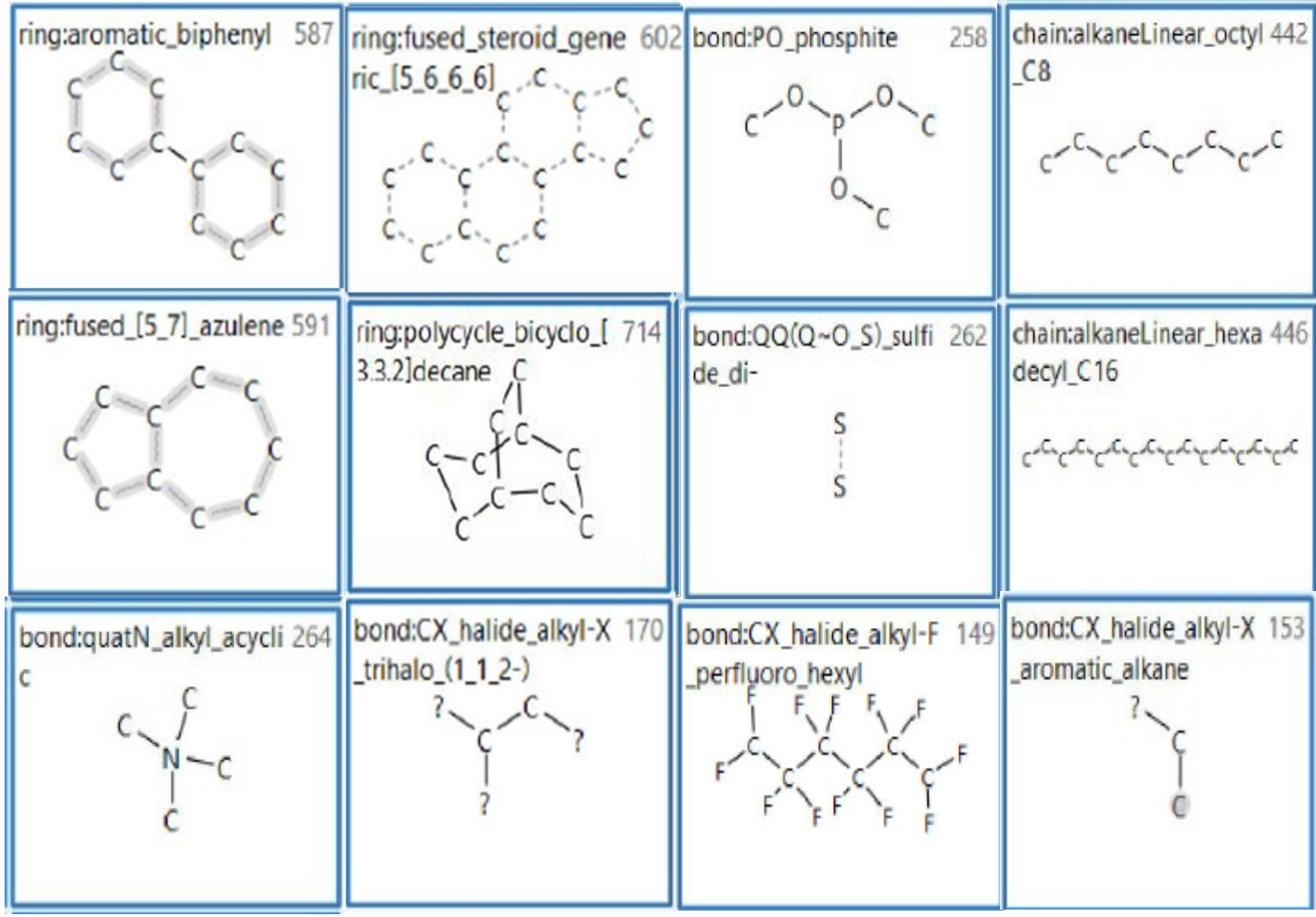

- Increased chemical coverage across all classes, specifically in the unclassified cMOAs relative to N/S classes

# Characterize training set chemicals: ToxPrints

- Pull in chemotype information for our chemicals via ToxPrints (TxPs)
  - Publicly available tool
  - EPA Comptox Chemicals Dashboard

## *ToxPrints:*

- ✓ *729 chemical features*
- ✓ *Chemically interpretable*
- ✓ *Coverage of diverse chemistry*
- ✓ *Hierarchical: Includes scaffolds, functional groups, chains, rings, bonding patterns, atom-types*



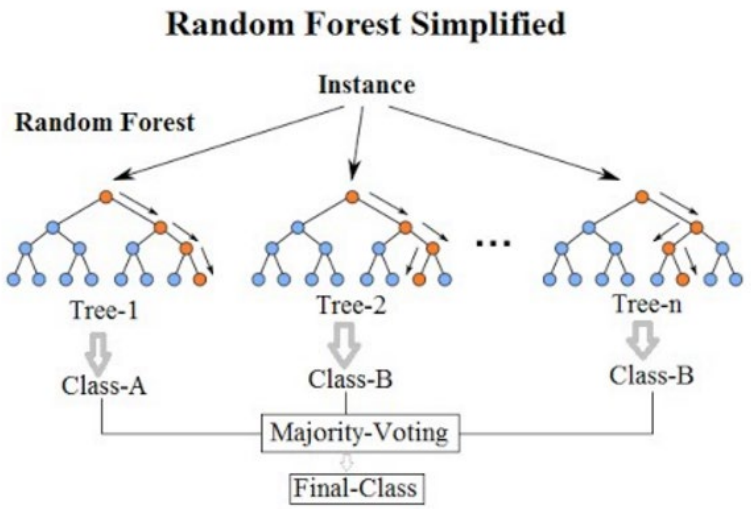

*Yang et al. J. Chem. Inf. Model. 2015. Richard et al., Chem. Res. Toxicol. 2016, 29(8) 1225 – 1251; Strickland et al., Arch Toxicol. 2018 92(1) 487 – 500; Wang et al., Environment International 2019, 126 377 – 386*

# TxP model details

- Random Forest (Boosted Gradient Method) provided the best model results:
  - Split data into 80% training and 20% hold out (test) sets
  - Hyperparameter tuning with 5-fold cross validation, square-root sampling, etc.

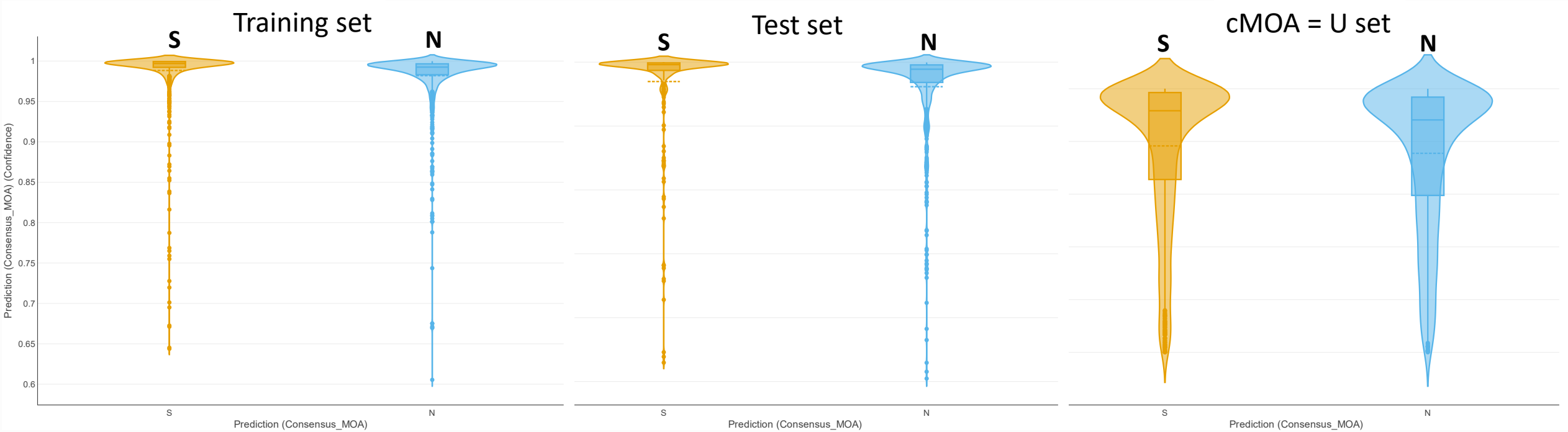
  - Training set: “balanced” down-sampled subset (2104 chemicals w/ a cMOA = N or S)

  - 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



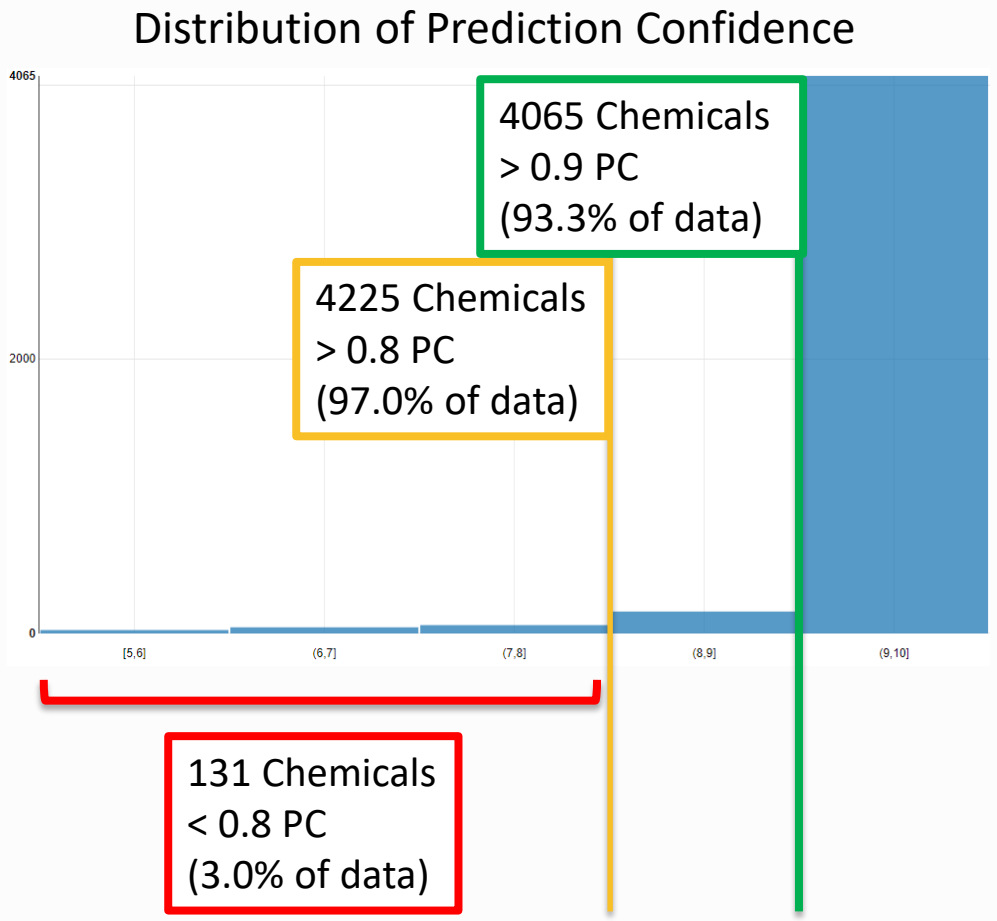

**Training Set**
**Median: 0.999, 0.993**
**Mean: 0.988, 0.982**

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

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

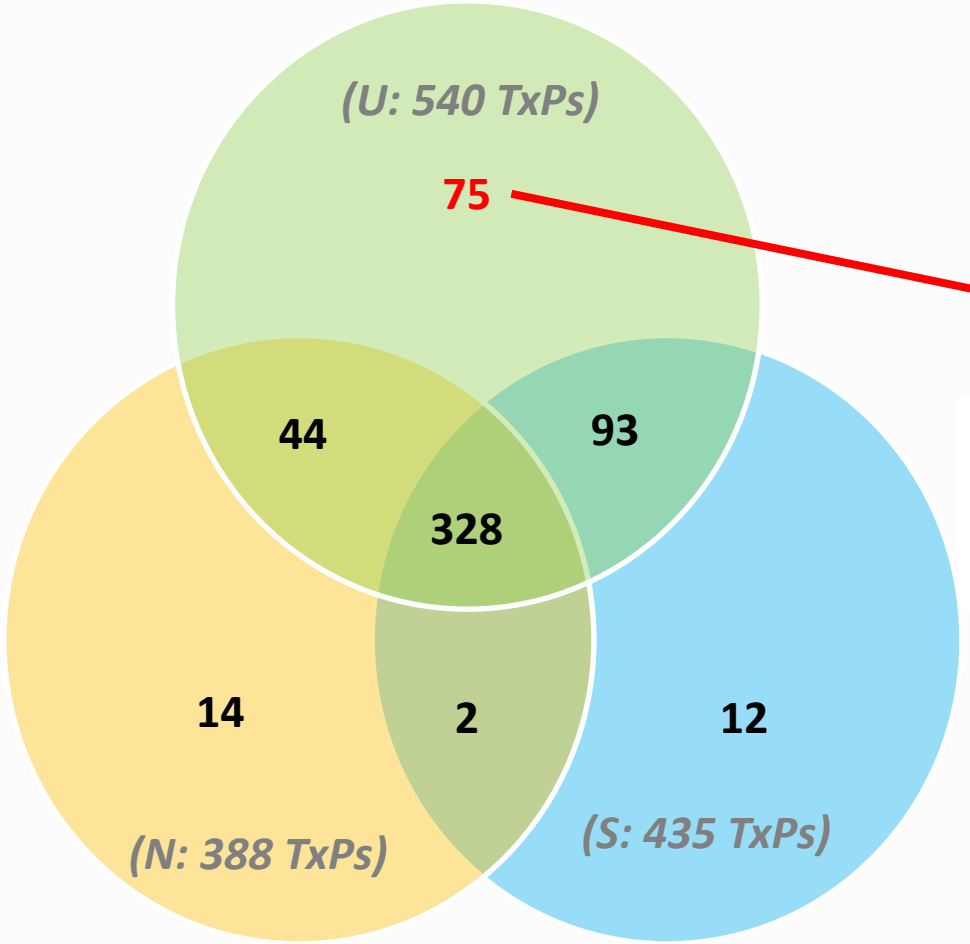
# Prediction confidence across the cMOA = N or S

- 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

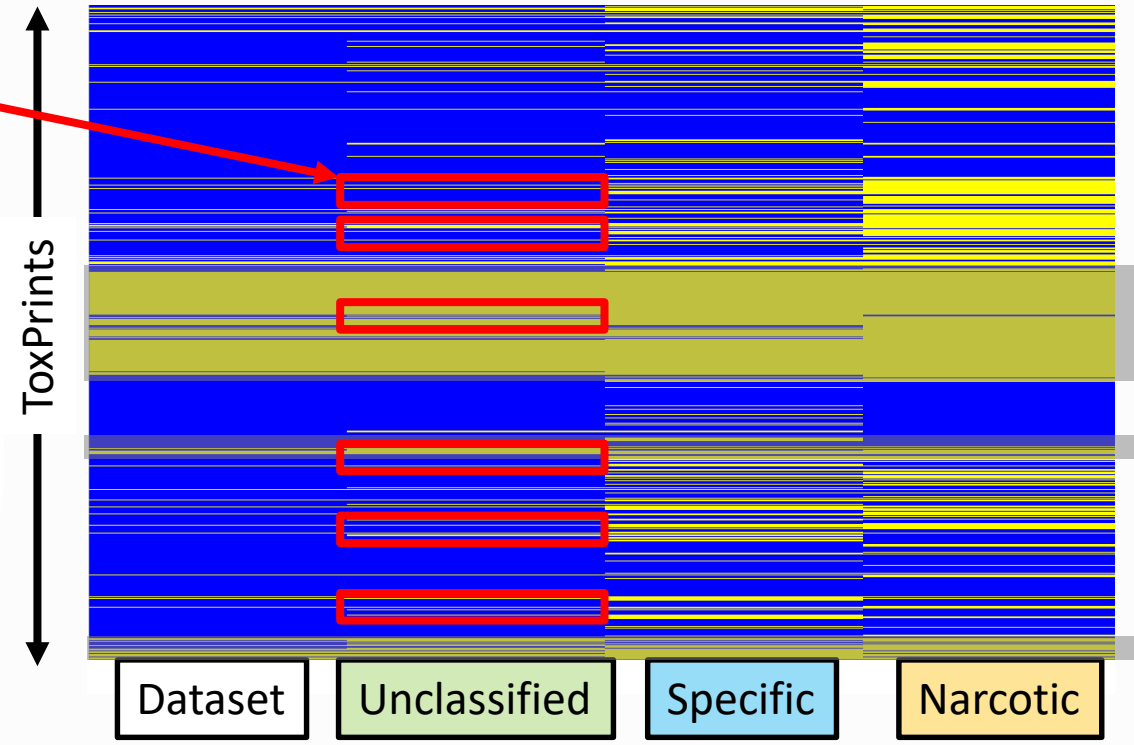


# ToxPrint (TxP) domains

# Characterization of TxP coverage per consensus MOA class




Heatmap representation of ToxPrints



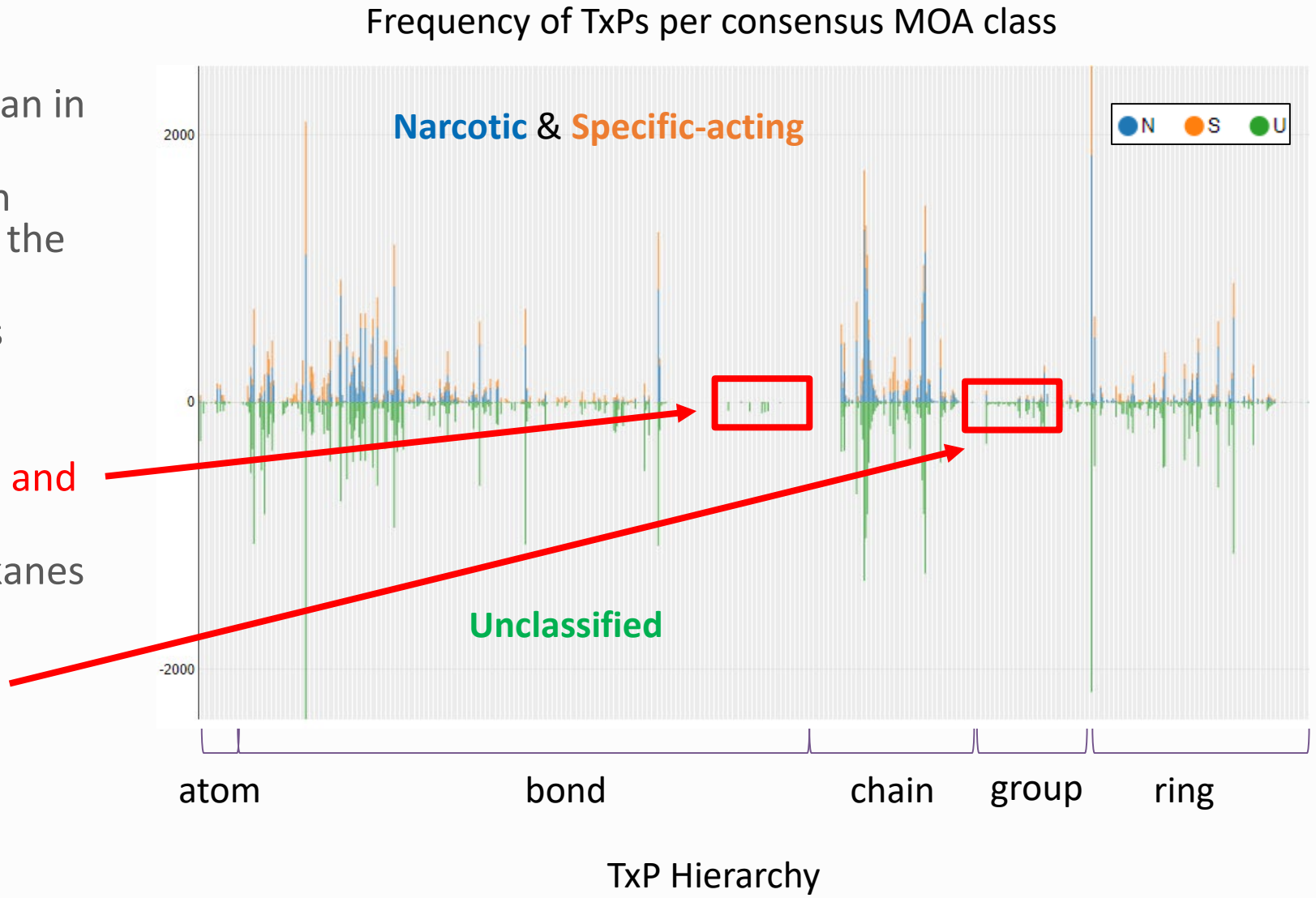

Missing structural Domains across full dataset:

- Metal & metalloid bonds
- Propyleneoxide chains
- Bicyclo ring structures

# 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

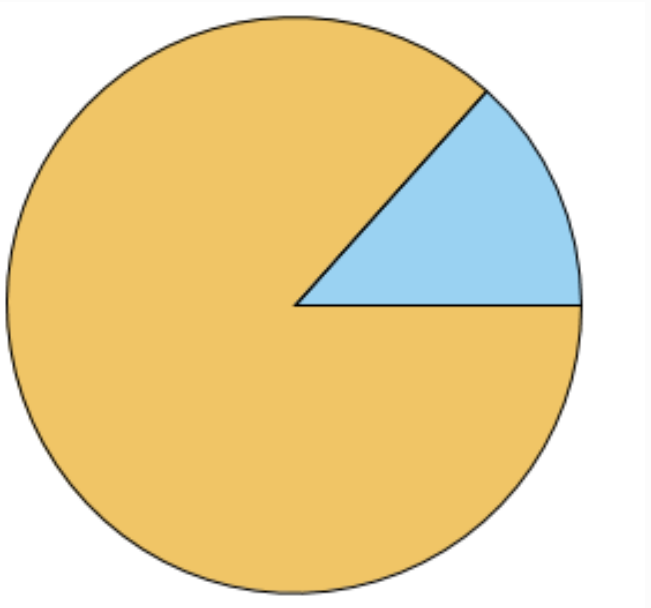


# ToxPrint (TxP) model application to Envirotox dataset

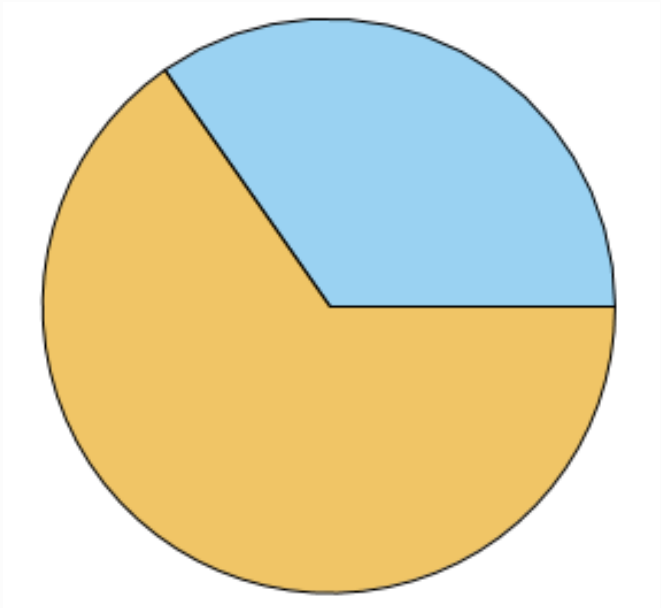
# TxP model predicted MOAs of the EnviroTox unclassified set

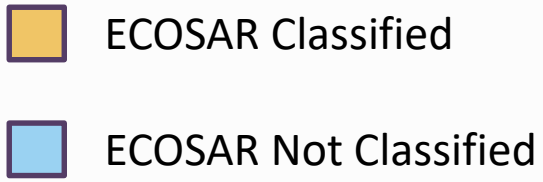
- 674 chemicals in the EnviroTox dataset that had low confidence or ambiguous consensus
- Applied TxP model to the unclassified set and compared predictions to ECOSAR classification
- ***Currently extending this analysis to the additional 3089 unclassified chemicals***

**361 predicted as Narcotic**



**313 predicted as Specific-acting**

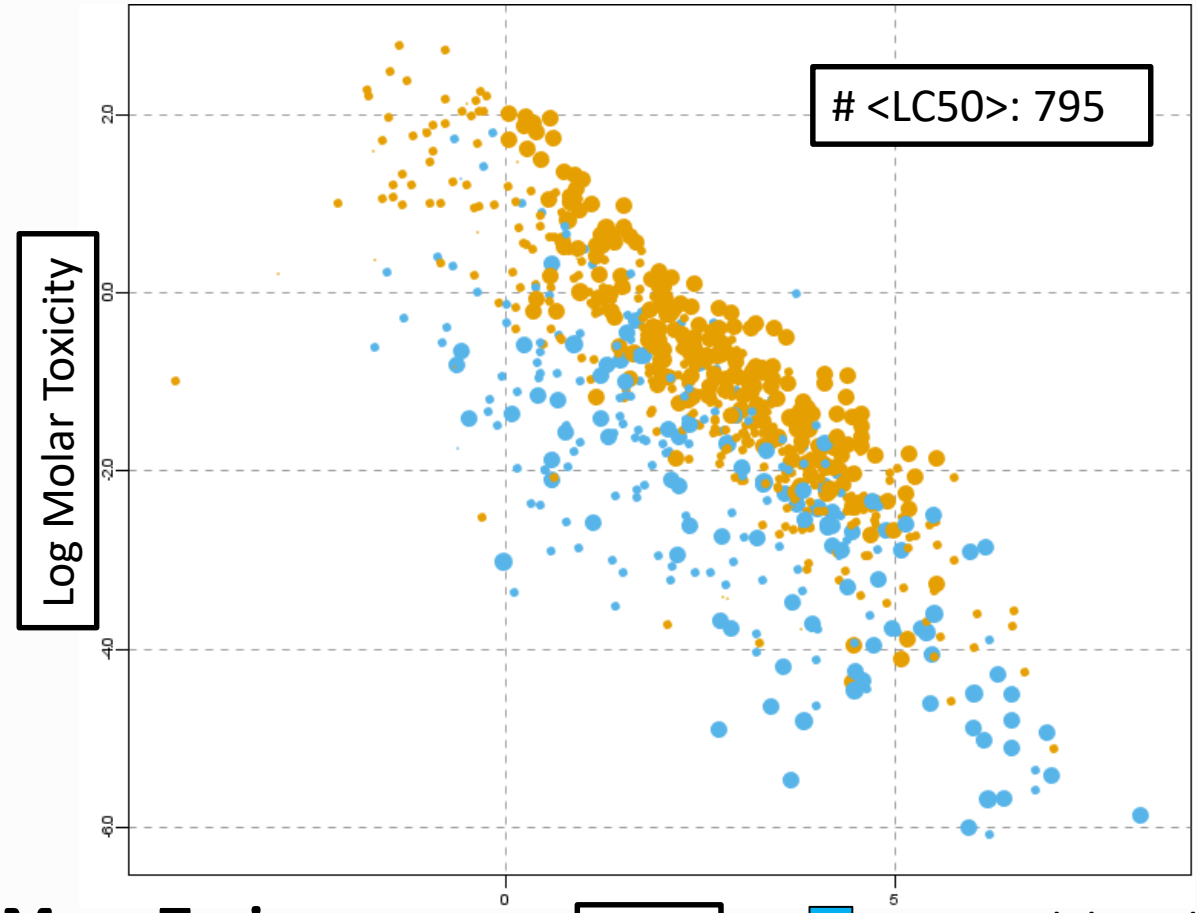




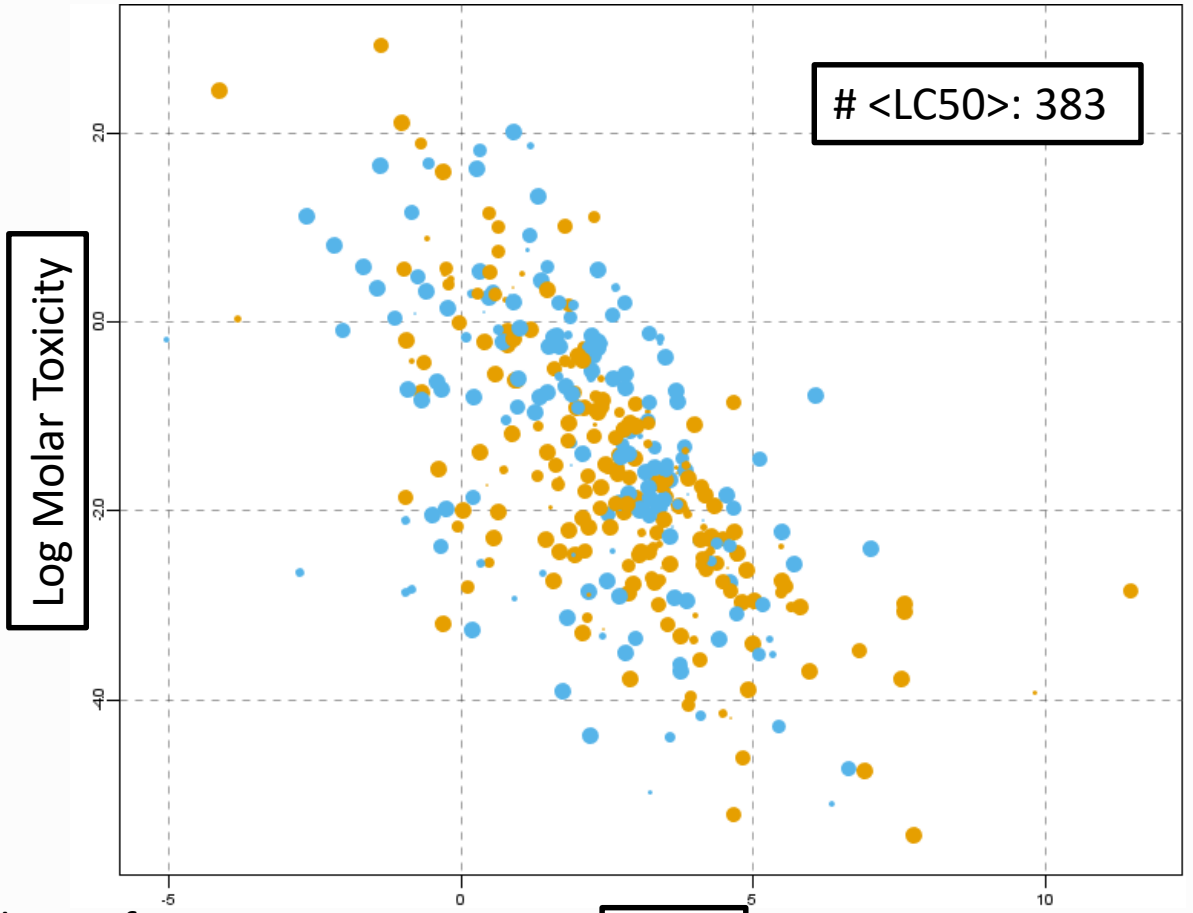
# Log molar toxicity, (LC50, 96h, FISH): TxP model predicted MOA (N,S) for cMOA (N,S,U) data

**Less Toxic**

cMOA classified data (N,S)




cMOA unclassified data (U)



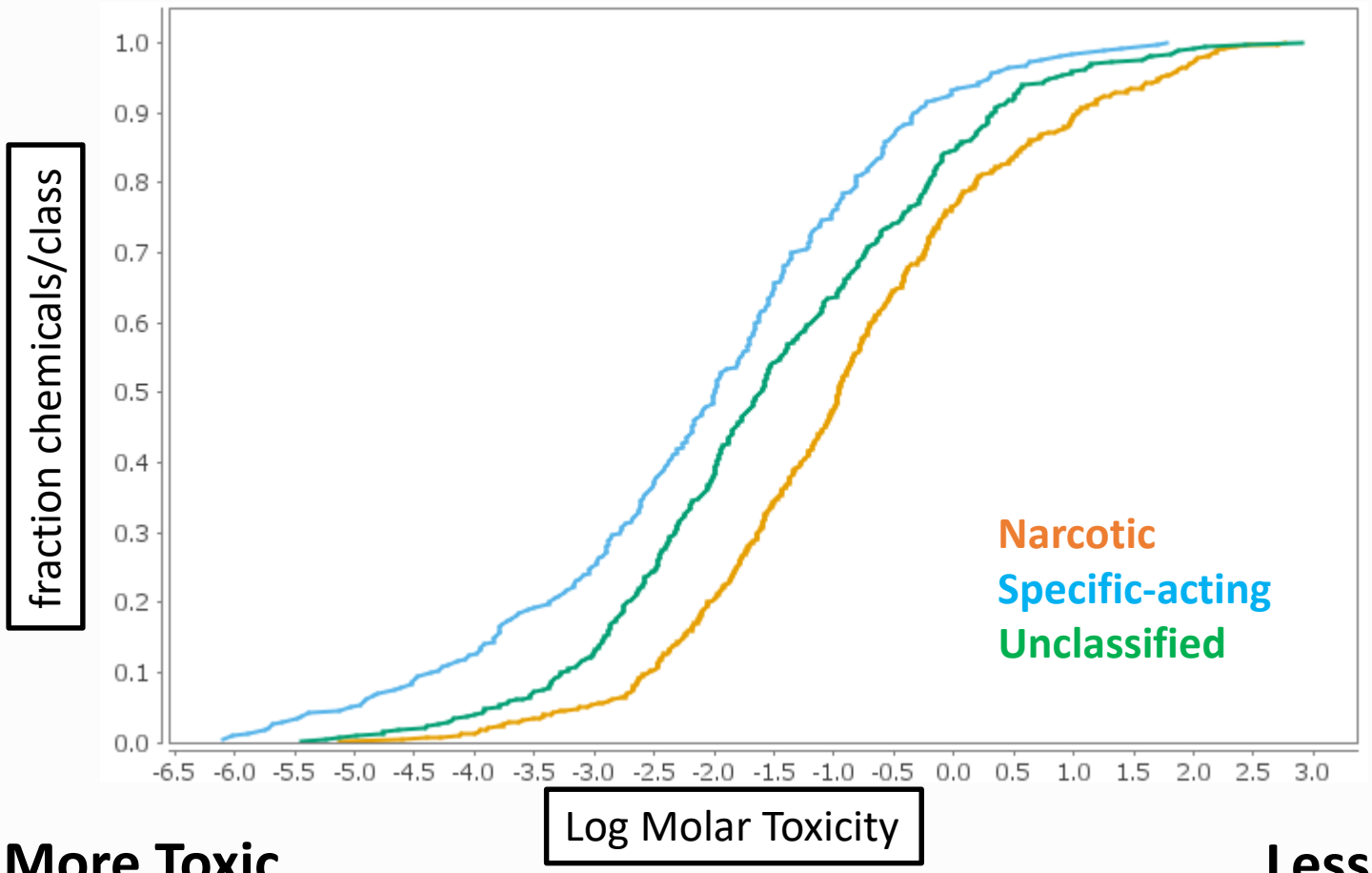

**More Toxic**

TxP model predicted specific-acting

TxP model predicted narcotic

Size proportional to cMOA confidence score = [0,1,2,3]

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



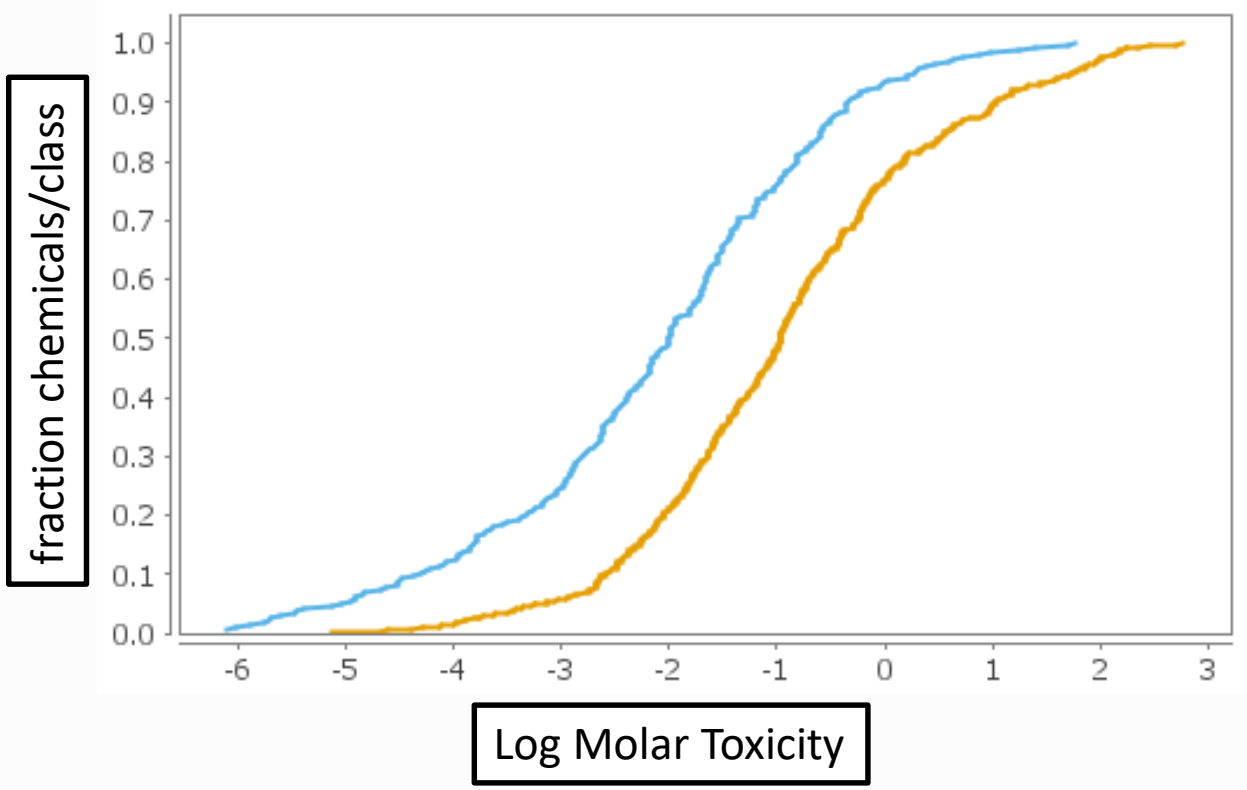

**More Toxic**

**Less Toxic**

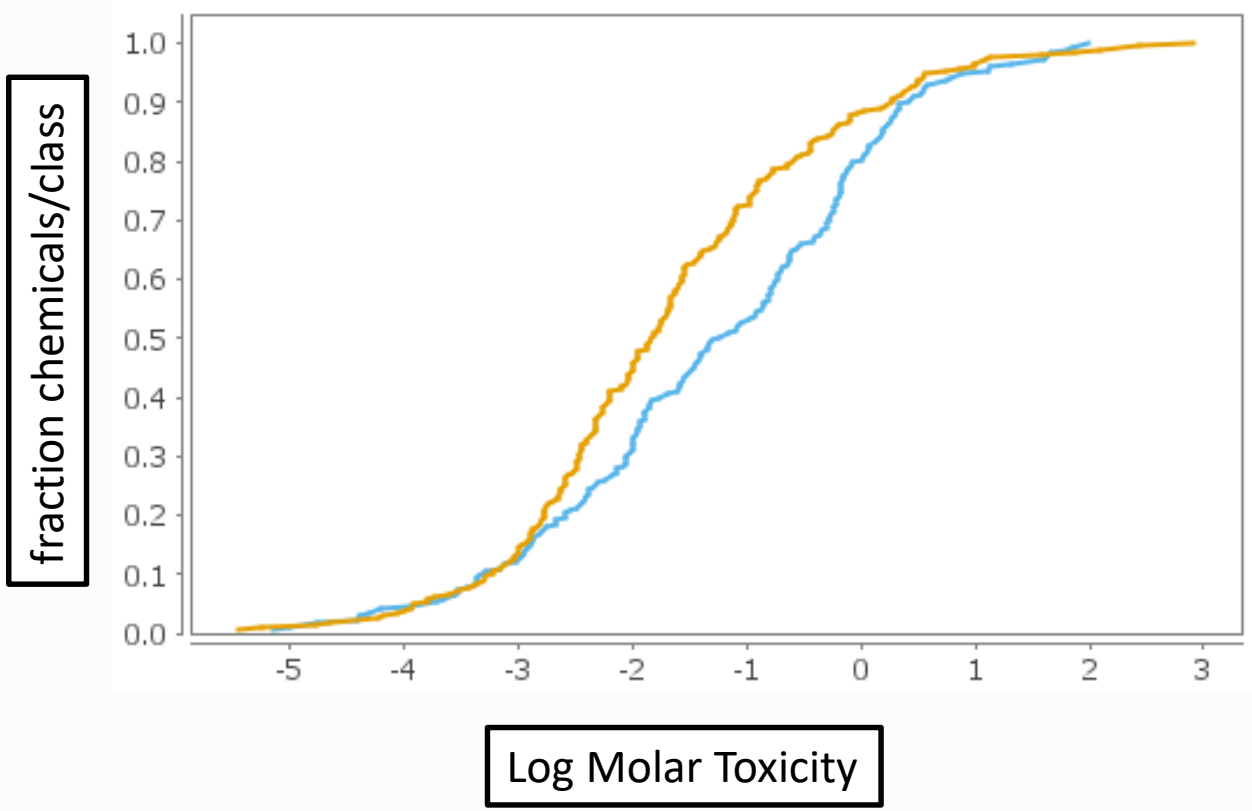
- cMOA classification is sufficient to discriminate N,S
- U presents some challenges

# Cumulative Distribution Function:
# Log molar toxicity, (LC50, 96h, FISH) for TxP model predicted classes (N,S)

cMOA classified data (N,S)




cMOA unclassified data (U)




TxP model predicted specific-acting

TxP model predicted narcotic

# Identifying relevant NAM data

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

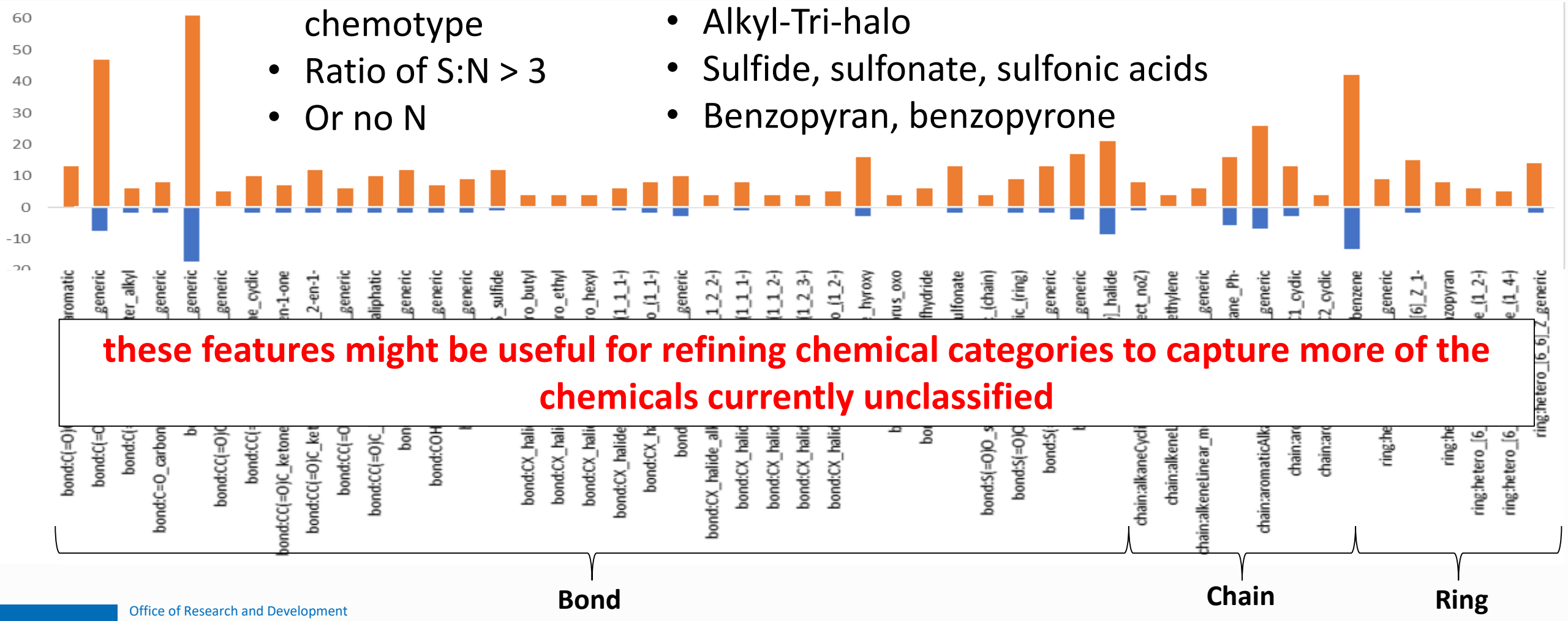
United States Environmental Protection Agency

**Criteria:**

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

**Results:**

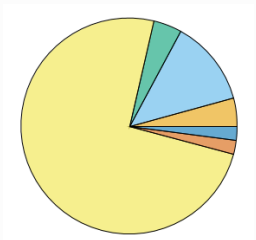
- Ketones
- Alkyl-Tri-halo
- Sulfide, sulfonate, sulfonic acids
- Benzopyran, benzopyrone



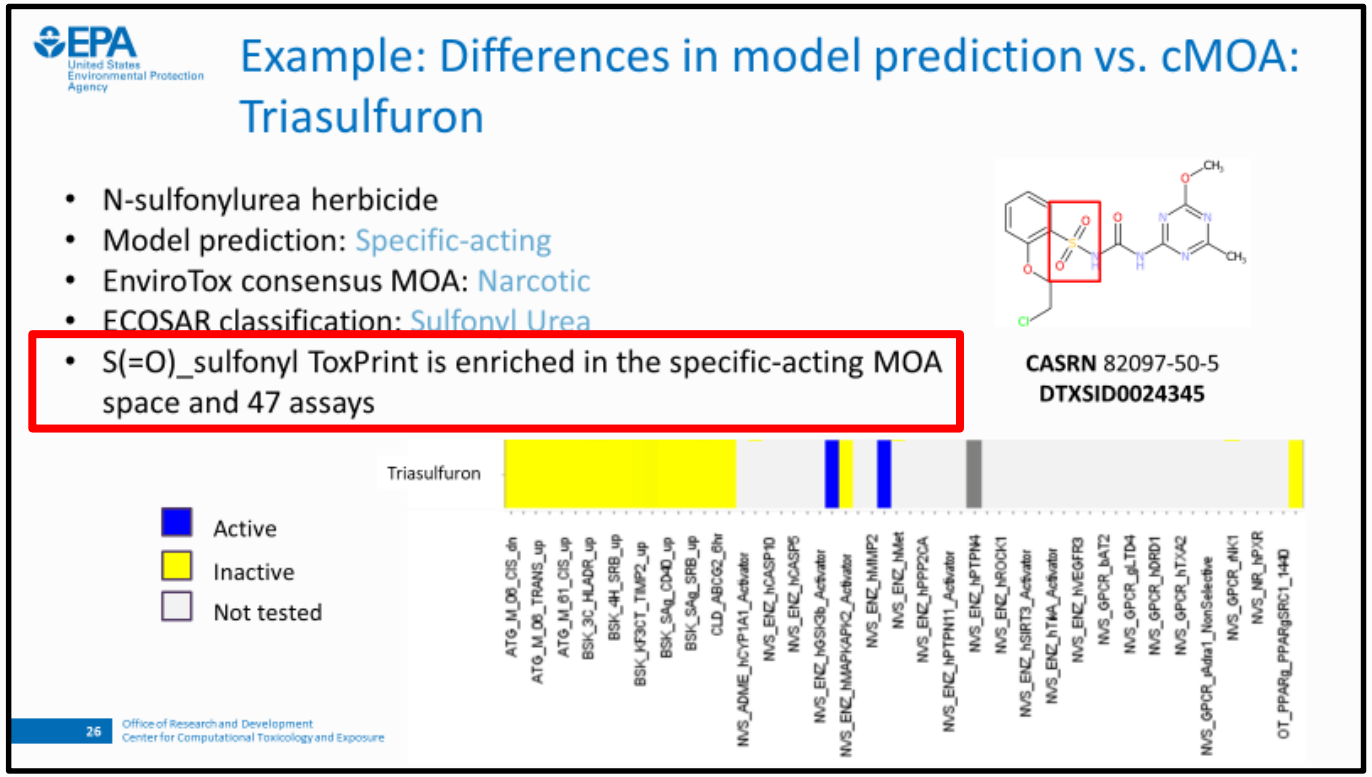
# Exploring assay platforms across TxP model predicted classes

- Use chemotype enrichments to inform potential NAM data streams
- Example: sulfonyl TxP enrichments across NovaScreen (NVS) assay platform
- Identified 47 assays due to sulfonyl TxP enrichment

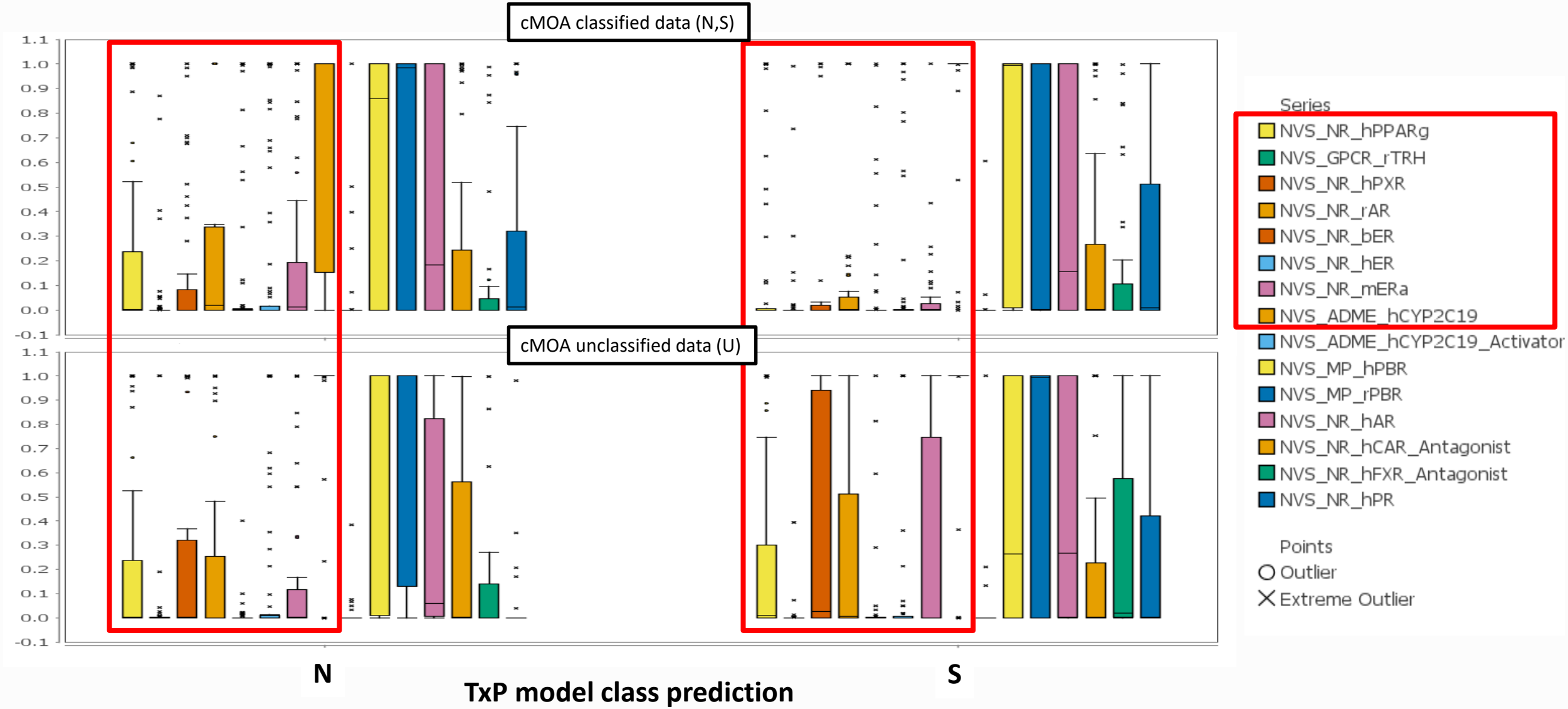
Assay platform identification:



ATG BSK CLD NVS OT TOX21



# NVS Platform: TxP model class predictions



# Summary

- Increased the available chemical space of EnviroTox w/ cMOA classifications
- Developed a robust structural TxP model
  - Robust N/S classification
  - Challenges in unclassified chemistries
- Investigated model predictions to inform ECOSAR preliminary set of unclassified chemicals
  - Majority of unclassified chemicals predicted to have a specific acting MOA
  - Identified primary chemotypes for specific-acting MOAs
- Exploring 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

# Thank you!