

## Background and Purpose

The Threshold of Toxicological Concern (TTC) is widely applied when adequate compound-specific toxicological data are unavailable (e.g., ICH M7, food contact materials, cosmetics, extractables and leachables).

### Current frameworks (e.g., Cramer Decision Tree<sup>1</sup>):

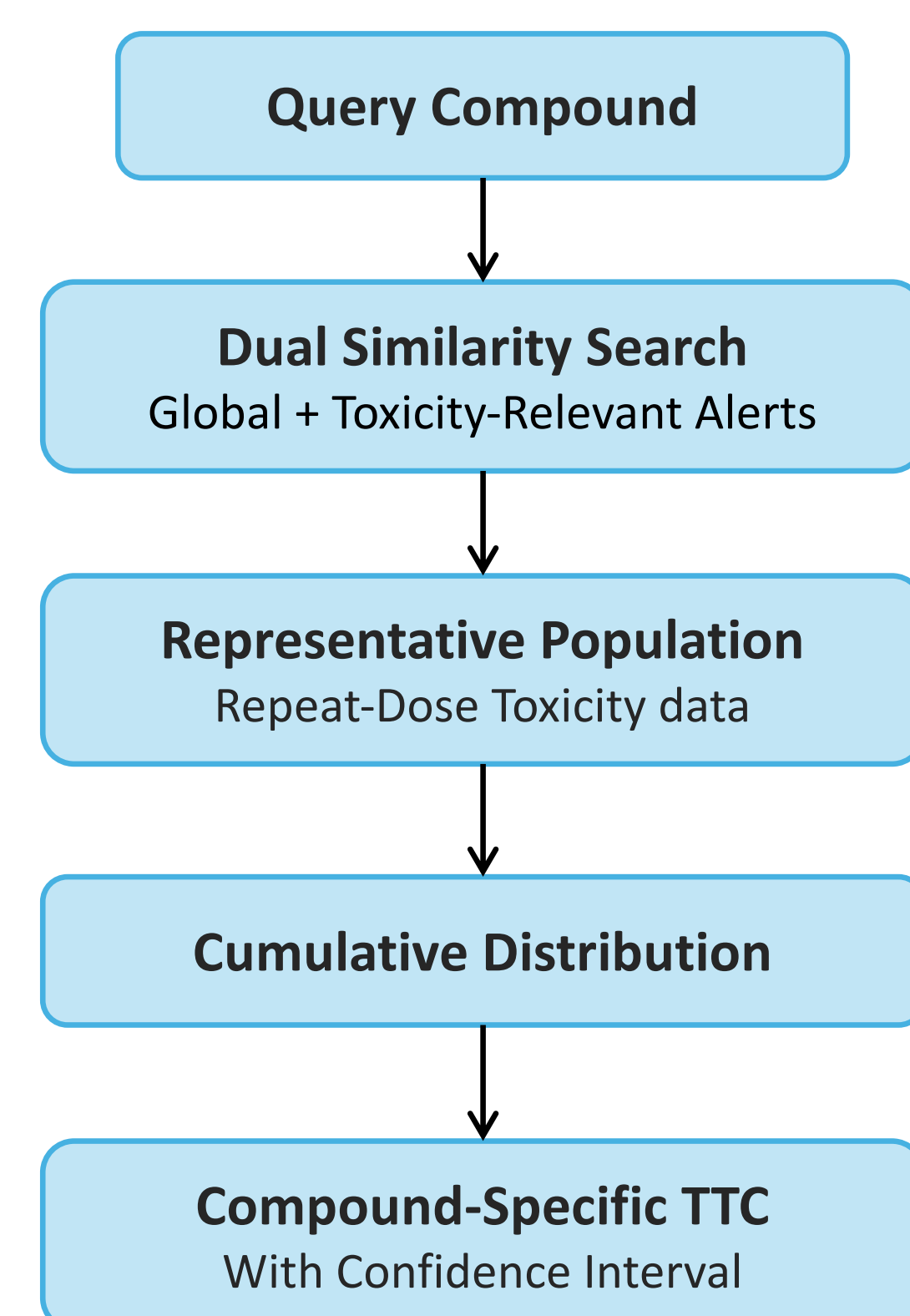
Structure → Decision Tree → Toxicity Class → Fixed TTC

These approaches provide conservative and practical screening thresholds. However, *advances in computational approaches now allow complementary quantitative refinement strategies.*

### Hypothesis

Toxicity thresholds can be derived directly from experimentally observed *in vivo* toxicity data by identifying a structurally and mechanistically relevant neighborhood of compounds.

## Current Approach



### Data Used

- **Reference dataset:** Curated and standardized<sup>2</sup>, 2,047 structurally diverse compounds with *repeat-dose subchronic and chronic in vivo* oral toxicity studies, predominantly in *rats*, with additional studies in dogs, mice, rabbits, and other species.
- **Endpoint:** Duration adjusted No Observed Effect Levels (NOEL/NOAEL; NEL).

### Objectives

A data-driven framework that:

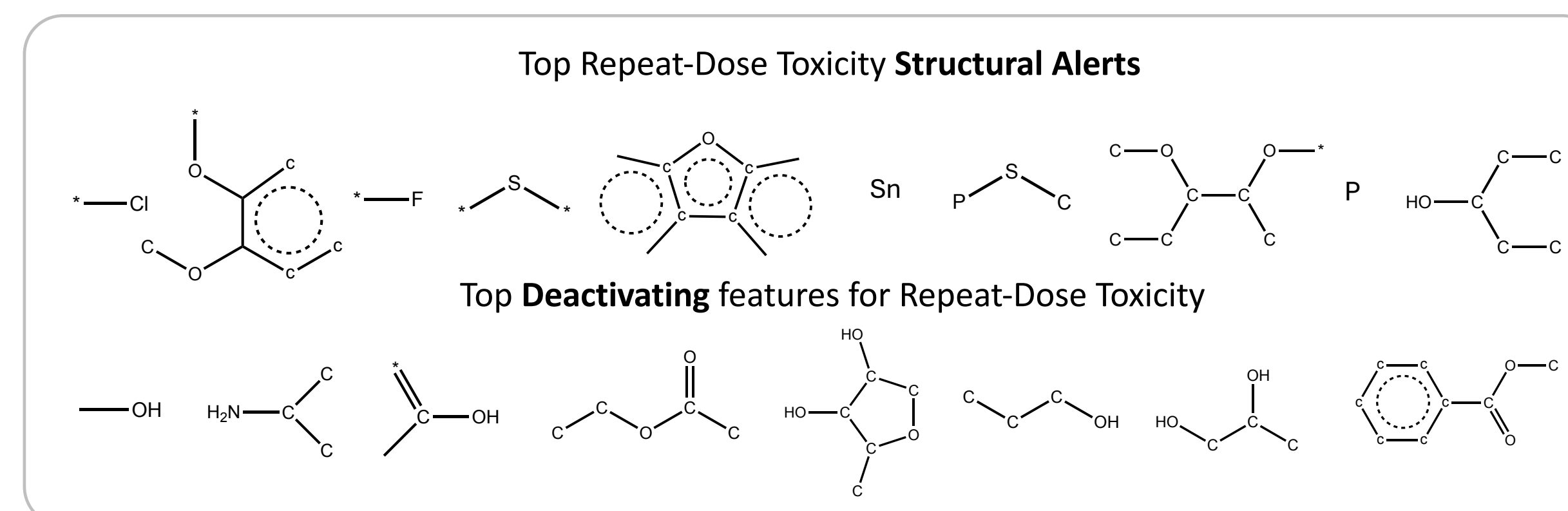
- Uses experimentally observed NEL/NOAEL values.
- Mines and employs toxicity alerts along with their quantitative contributions.
- Identifies structurally and mechanistically relevant analogs.
- Derives a percentile-based TTC.
- Provides compound-specific uncertainty and applicability domain metrics.
- Provides scalability.

### Construction of A Compound Neighborhood to Represent the Query

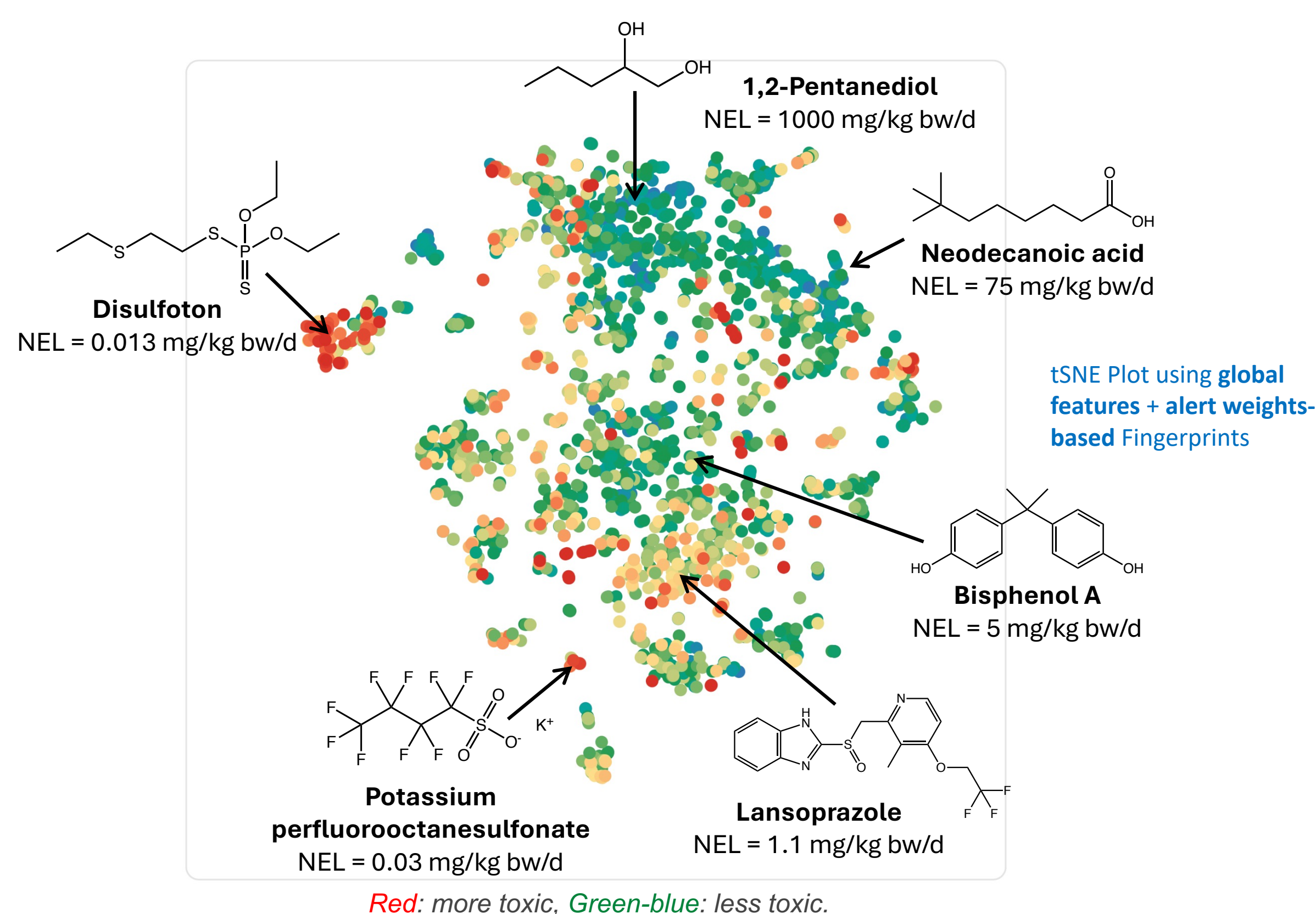
Query → Identify Alerts → Find Matching Analog in the Reference Dataset → Remove Analog with Confounding Alerts → Build Cumulative Curve → Derive TTC

### Alert Mining

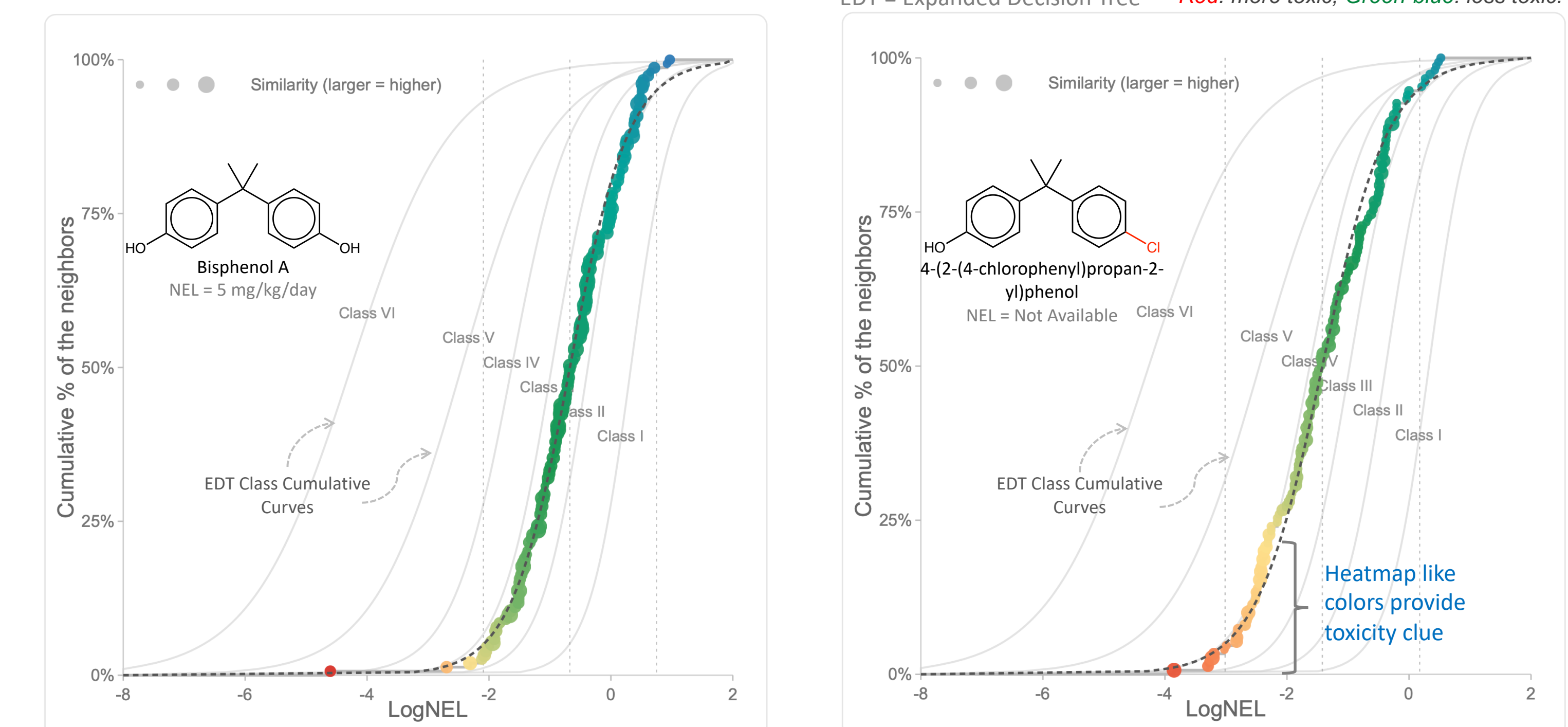
- Positive and deactivating features derived from repeat-dose experimental data (using statistical machine learning methods).
- Continuous toxicity weights (reproducible, derived using statistically validated models<sup>3</sup>).



### Separation of Repeat-Dose Toxicity Levels Using Dual Structural Similarity



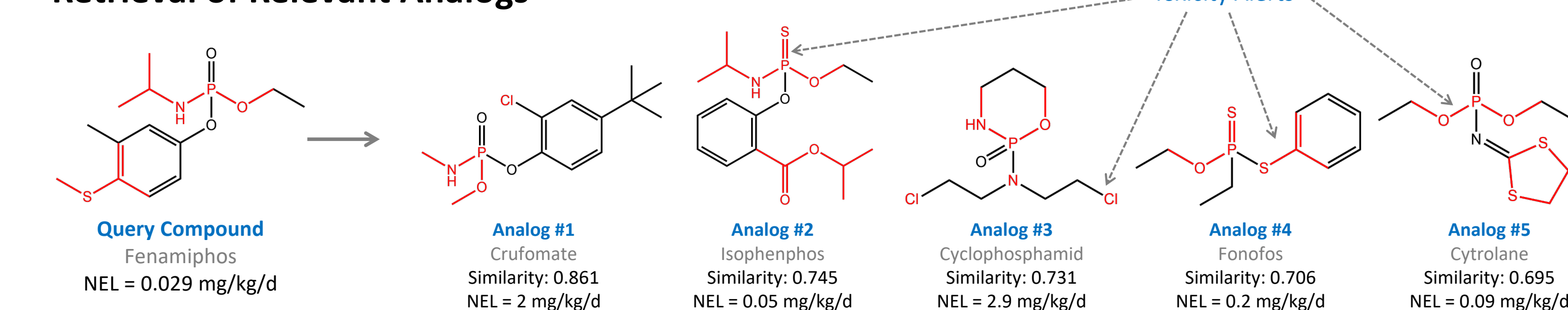
### Impact of Minor Structural Changes on Calculated TTC



**TTC (Current Method):** 18.46 (17.41 – 50.00)  $\mu\text{g}/\text{kg}$  bw/d  
Cramer TTC: 1.5  $\mu\text{g}/\text{kg}$  bw/d; Cramer Class: III  
EDT TTC: 45  $\mu\text{g}/\text{kg}$  bw/d; EDT Class: II

**TTC (Current Method):** 2.40 (1.47 – 5.73)  $\mu\text{g}/\text{kg}$  bw/d  
Cramer TTC: 1.5  $\mu\text{g}/\text{kg}$  bw/d; Cramer Class: III  
EDT TTC: Not determined

### Retrieval of Relevant Analogs



## Conclusions

- A data-driven framework was developed to derive percentile-based TTC values directly from experimentally observed repeat-dose NEL/NOAEL data.
- The approach integrates global structural similarity and toxicity-relevant alerts to identify mechanistically relevant analogs within a curated reference dataset.
- Query-specific cumulative NEL distributions enable estimation of TTC values along with uncertainty ranges and applicability domain diagnostics.
- Results indicate that this framework can provide a complementary quantitative layer to traditional TTC approaches, supporting expert risk assessment when compound-specific data are limited.
- The method is designed to be adaptable as additional high-quality repeat-dose toxicity data become available.
- The current implementation focuses on non-genotoxic systemic repeat-dose toxicity.

### References

1. Cramer, et al., Estimation of Toxic Hazard—A Decision Tree Approach. Food and Cosmetics Toxicology 16, no. 3 (1976): 255–76.
2. <https://www.fda.gov/food/food-chemical-safety/expanded-decision-tree-fdas-food-chemical-toxicity-screening-tool>.
3. Chakravarti et al., QSAR in Safety Evaluation and Risk Assessment, editor: Hong, Huixiao. Academic Press, 2023.