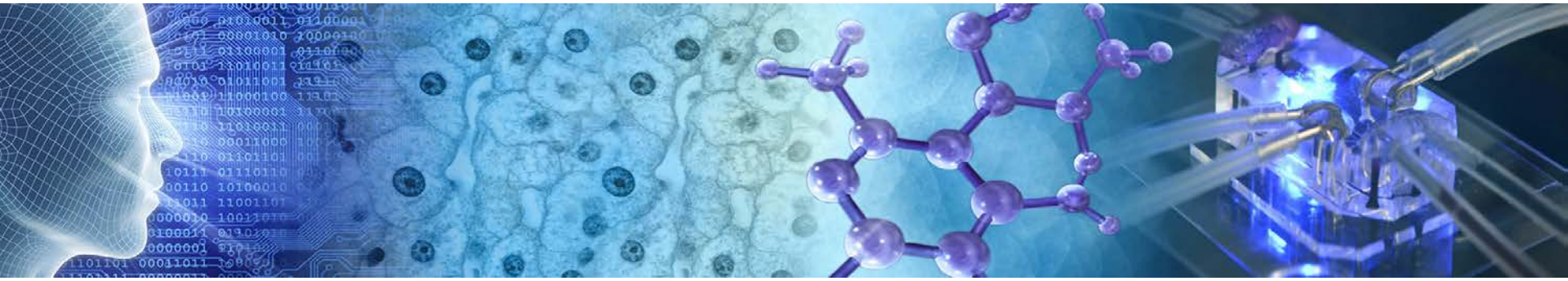
NTP
National Toxicology Program



# Rat Oral Acute Toxicity Database and Evaluation of Variability

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Predictive Models for Acute Oral Systemic Toxicity Workshop
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*Disclaimer: ILS staff provide technical support for NICEATM, but do not represent NIEHS, NTP, or the official positions of any federal agency.*

# OBJECTIVE

**Develop a large inventory of acute oral toxicity data to facilitate an international collaboration for predictive modeling**

1. Establish a dataset of rate acute oral toxicity study LD50 data

2. Characterize the dataset to identify considerations for modeling

3. Evaluate variability of acute oral toxicity LD50 data and identify sources of this variability

# Rat Oral Acute Toxicity LD50 Database

## Data sources and inventory

| Database Resource | Rows of Data (number of LD50 values) | Unique CAS |
|---|---|---|
| ECHA (ChemProp) | 5,533 | 2,136 |
| JRC AcutoxBase | 637 | 138 |
| NLM HSDB | 3,981 | 2,205 |
| OECD (eChemPortal) | 10,119 | 2,290 |
| PAI (NICEATM) | 364 | 293 |
| TEST (NLM ChemIDplus) | 13,069 | 12,974 |

Rat oral LD50s:
16,297 chemicals total
34,508 LD50 values

Require unique LD50 values with mg/kg units

**15,688 chemicals total**
21,200 LD50 values

# Rat Oral LD50 per Chemical Across Sources

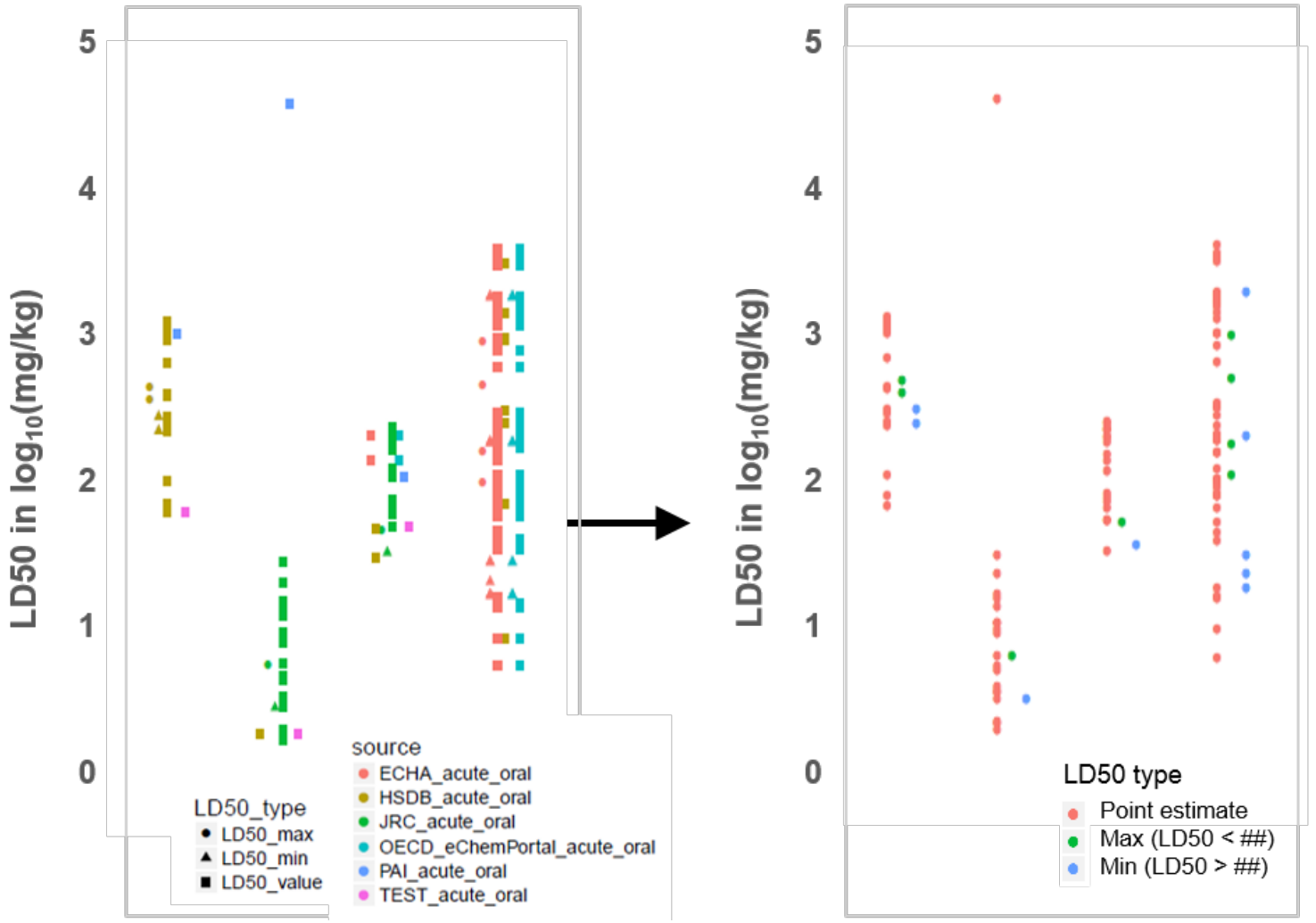
# Data Processing

- Identify unique LD50 values per chemical
  - Remove values that may be replicated across sources
- Include limit test and point estimate LD50 values
- Identify representative LD50 values where necessary

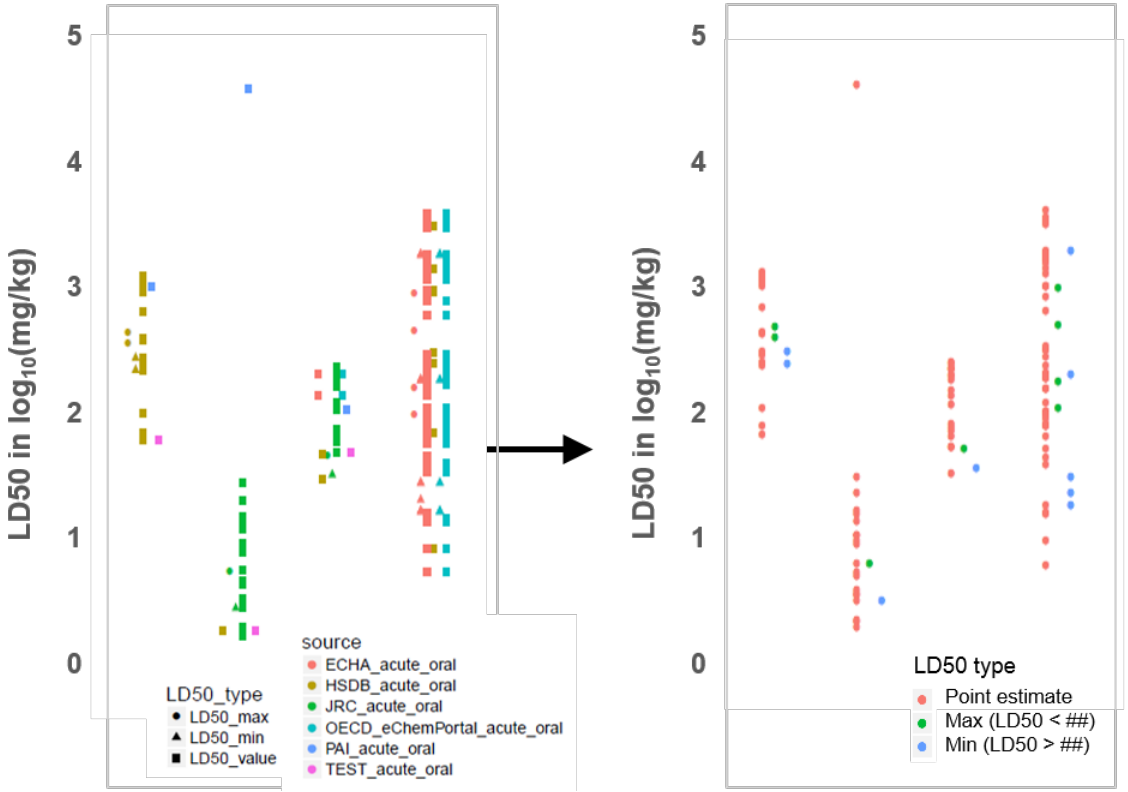
- Evaluate variability and impacts on hazard categorization and modeling
- Quantify variability and estimate reproducibility of acute oral toxicity bioassay

# Evaluating the LD50 Inventory

## Remove duplicate values



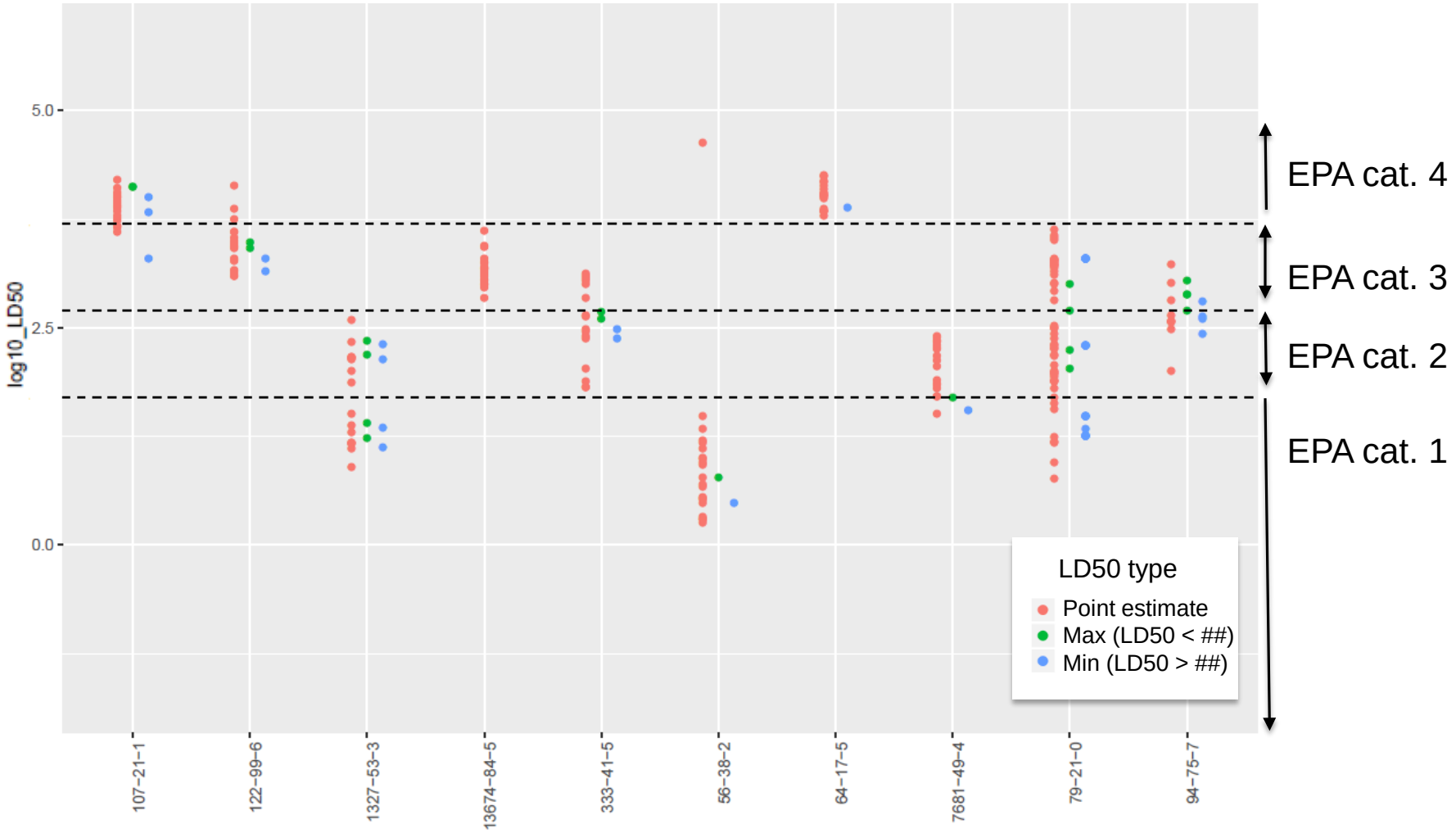
# Impact on Hazard Categorization




| | LD50 (mg/kg) | |
|---|---|---|
| **EPA IV** *Caution (optional)* | 5,000 | **Not classified** |
| **EPA III** *Caution* | 2,000 | **GHS 5** *Warning* |
| | 500 | **GHS 4** *Warning* |
| **EPA II** *Warning* | 200 | **GHS 3** *Danger* |
| | 50 | |
| **EPA I** *Danger - Poison* | 5 | **GHS 2** *Danger* |
| | | **GHS 1** *Danger* |

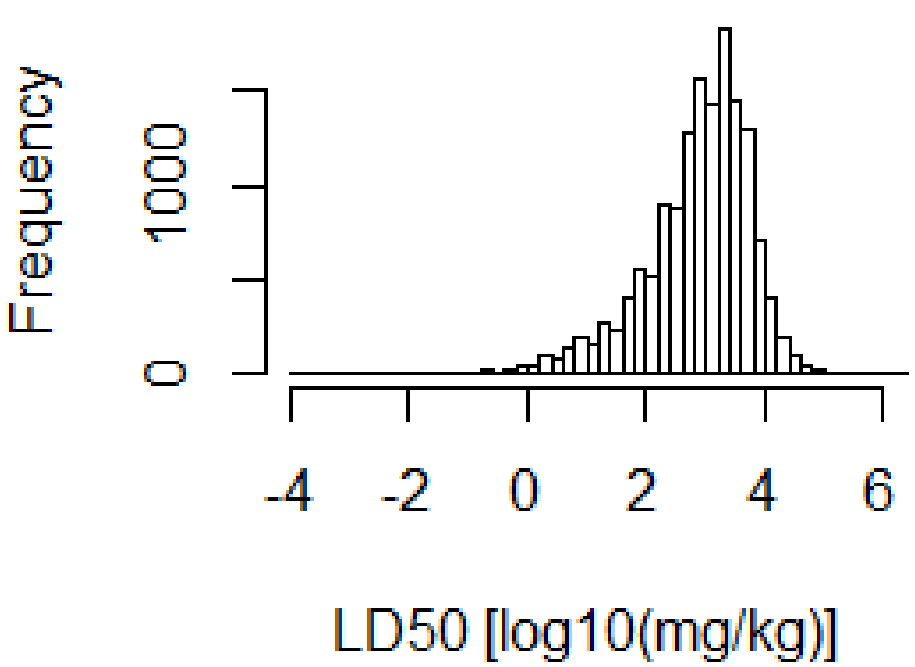
# Impact on Hazard Categorization

## Example: EPA Classification

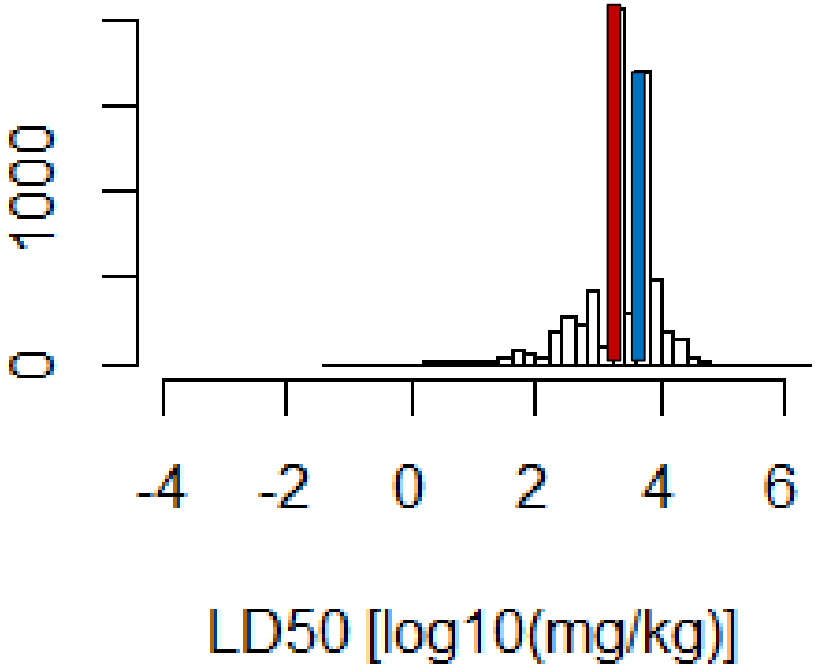


# Evaluating the LD50 Inventory

**14,745 point estimate LD50 values**




**6,455 limit test LD50 values**



# Dataset Inventory

## EPA Hazard Categorization

| EPA Category | LD50 Range | # of chemicals* |
|---|---|---|
| **Category I** | ≤ 50 mg/kg | 1,094 |
| **Category II** | > 50 ≤ 500 mg/kg | 3,037 |
| **Category III** | > 500 ≤ 5000 mg/kg | 7,492 |
| **Category IV** | > 5000 mg/kg | 3,418 |

*Number of chemicals from entire dataset (15,688 chemicals)

**2,349 chemicals have ≥2 LD50 values:**

| Number of categories | Number of chemicals |
|---|---|
| 1 | 1,949 (83%) |
| 2 | 391 (17%) |
| 3 | 9 (<1%) |

| Categories | Number of chemicals |
|---|---|
| I & II | 11 |
| I & III | 1 |
| I & IV | 2 |
| **II & III** | **146** |
| II & IV | 1 |
| **III & IV** | **230** |
| I & II & III | 3 |
| II & III & IV | 6 |

# Dataset Inventory

## GHS Hazard Categorization

| GHS Category | LD50 Range | # of chemicals* |
|---|---|---|
| **Category 1** | ≤ 5 mg/kg | 228 |
| **Category 2** | > 5 ≤ 50 mg/kg | 869 |
| **Category 3** | > 50 ≤ 300 mg/kg | 1,831 |
| **Category 4** | > 300 ≤ 2000 mg/kg | 4,725 |
| **Category 5** | > 2000 mg/kg | 7,158 |

*Number of chemicals from entire dataset (15,688 chemicals)

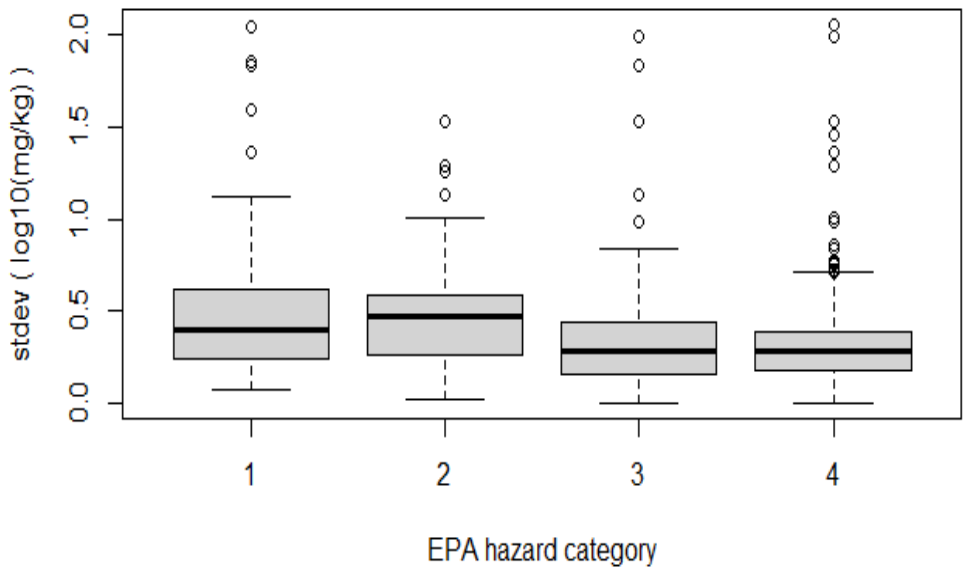
**2,349 chemicals have ≥2 LD50 values:**

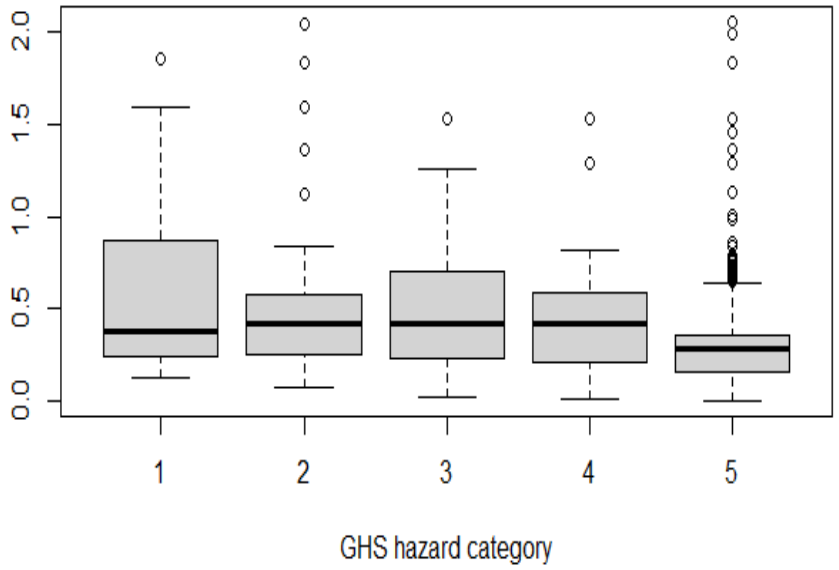
| Number of categories | Number of chemicals |
|---|---|
| 1 | 2180 (93%) |
| 2 | 160 (7%) |
| 3 | 8 (<1%) |
| 4 | 1 (<1%) |

| Categories | Number of chemicals |
|---|---|
| 1 & 2 | 1 |
| 1 & 3 | 2 |
| 1 & 4 | 9 |
| **1 & 5** | **72** |
| 2 & 3 | 3 |
| 3 & 4 | 10 |
| **4 & 5** | **63** |
| 1 & 4 & 5 | 5 |
| 3 & 4 & 5 | 3 |
| 1 & 3 & 4 & 5 | 1 |

# Variability vs. Hazard Categories

- Standard deviations of the 2,349 chemicals with ≥2 LD50 values were plotted per hazard category, revealing **no association between potency and variability**



# Acute Oral LD50 Dataset Replicate Inventory

## Breakdown from the 15,688 chemical inventory

- 13,339 chemicals with one LD50 value
- 2,349 chemicals with ≥2 LD50 values
- 1,120 chemicals with ≥3 LD50 values
- 609 chemicals with ≥4 LD50 values
- 347 chemicals with ≥5 LD50 values

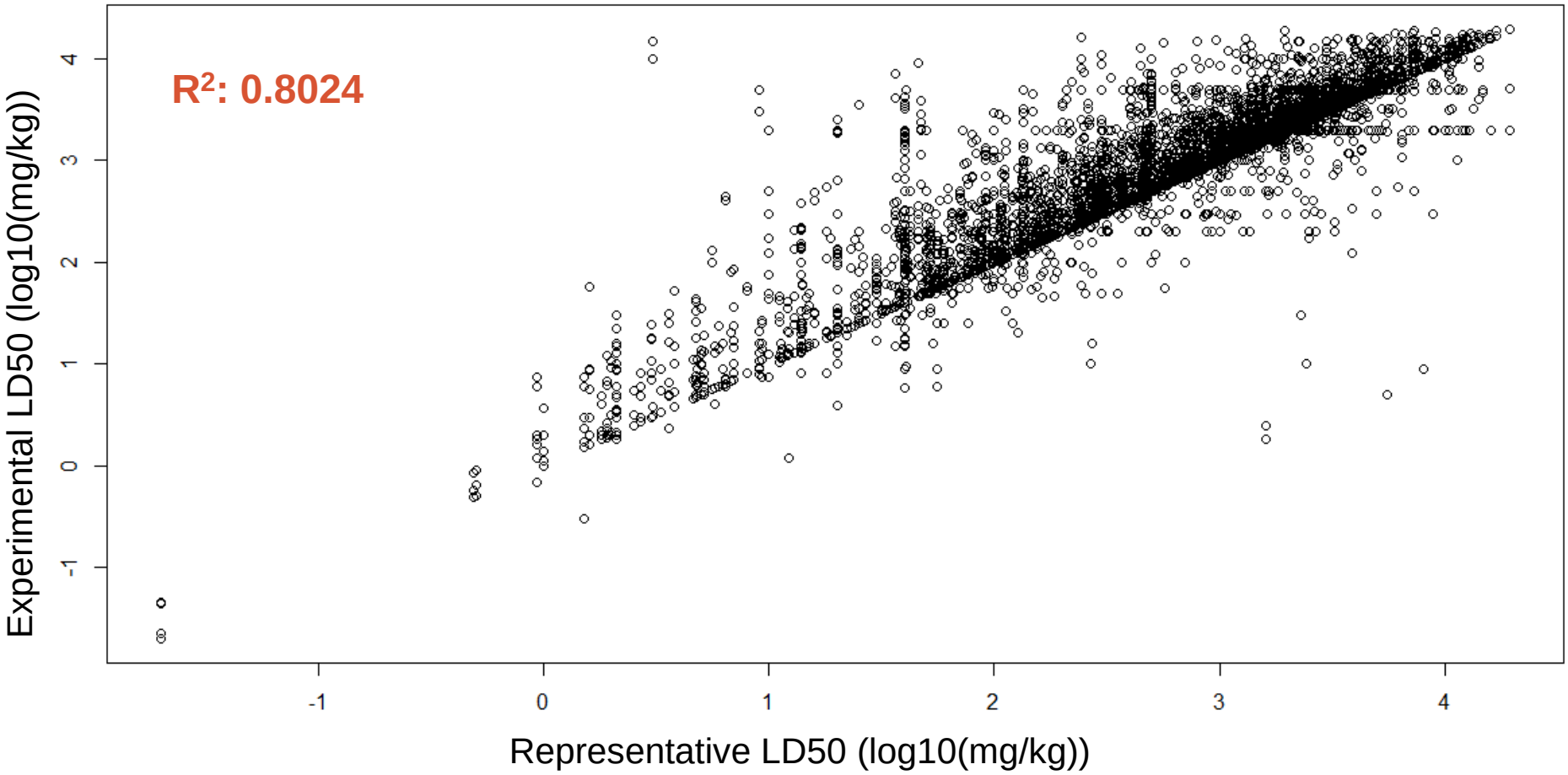
| Orders of magnitude for LD50s | Number of chemicals |
|---|---|
| 0 | 546 (49%) |
| 1 | 519 (46%) |
| 2 | 39 (3%) |
| 3 | 8 (0.7%) |
| 4 | 8 (0.7%) |

# Evaluating "Performance" of the Animal Assay

1. Utilize subset of chemicals with ≥3 values (1,120 of 15,688)
   - These 1,120 chemicals represent 5,270 LD50 values
2. Identify a representative LD50 value for every chemical
   - Member of ATWG and ICCVAM agency feedback was solicited
   - Calculated as the median of the lower quantile
   - Derive hazard categories associated with the representative LD50 value
3. Using the representative LD50 value as "truth", assess every one of the experimental values with summary statistics:
   - Accuracy with 95% confidence interval
   - Sensitivity, specificity, and balanced accuracy

# Evaluating “Performance” of the Animal Assay

## Representative LD50 vs. Experimental Values




RMSE of 0.42 was also computed for this dataset based on the LD50 values

# Evaluating "Performance" of the Animal Assay

## EPA Hazard Categorization

- The accuracy of the experimental data when compared to the representative LD50-derived hazard category was 78%

95% confidence interval: 76% – 79%

| | Representative EPA Hazard Category | | | |
|---|---|---|---|---|
| **Experimental** | **1** | **2** | **3** | **4** |
| **1** | **384** | 27 | 4 | 2 |
| **2** | 177 | **1006** | 59 | 4 |
| **3** | 42 | 459 | **2180** | 100 |
| **4** | 10 | 23 | 253 | **433** |

# Evaluating "Performance" of the Animal Assay

## GHS Hazard Categorization

- The accuracy of the experimental data when compared to the representative LD50-derived hazard category was 74%

  95% confidence interval: 73% – 75%

| | Representative GHS Hazard Category | | | | |
|---|---|---|---|---|---|
| **Experimental** | **1** | **2** | **3** | **4** | **5** |
| **1** | **75** | 3 | 0 | 2 | 0 |
| **2** | 59 | **247** | 25 | 2 | 4 |
| **3** | 2 | 152 | **598** | 30 | 8 |
| **4** | 0 | 46 | 311 | **1395** | 48 |
| **5** | 4 | 25 | 42 | 586 | **1561** |

# Hazard Categorization "Performance" Summary

By integrating replicate acute oral toxicity studies, representative LD50 values were derived and used as "truth" to assess the performance of the animal assay for identifying EPA and GHS hazard categories.

The accuracy for predicting EPA and GHS categories was 78% and 74%, respectively, and balanced accuracy ranged from 0.75-0.89 across the hazard categories.

| | EPA 1 | EPA 2 | EPA 3 | EPA 4 |
|---|---|---|---|---|
| **Sensitivity** | 0.63 | 0.66 | 0.87 | 0.80 |
| **Specificity** | 0.99 | 0.93 | 0.77 | 0.94 |
| **Balanced Accuracy** | **0.81** | **0.80** | **0.82** | **0.87** |

| | GHS 1 | GHS 2 | GHS 3 | GHS 4 | GHS 5 |
|---|---|---|---|---|---|
| **Sensitivity** | 0.54 | 0.52 | 0.61 | 0.69 | 0.96 |
| **Specificity** | 0.99 | 0.98 | 0.95 | 0.87 | 0.82 |
| **Balanced Accuracy** | **0.77** | **0.75** | **0.78** | **0.78** | **0.89** |

# Additional “Performance” Evaluation

## “Non-Toxic” and “Very Toxic” Endpoints

- Other endpoints of interest to ICCVAM agencies:
  - non-toxic (≤50 mg/kg)
  - very toxic (≥2000 mg/kg)
- The animal experimental data, and representative values were also used to evaluate performance for these endpoints:

| | Representative | |
|---|---|---|
| **Experimental** | **Non-toxic** | **false** |
| **false** | **2953** | 60 |
| **Non-toxic** | 658 | **1561** |

**Non-toxic Endpoint Performance**
Accuracy 86%
95% Confidence Interval: 85.3% - 87.2%
Sensitivity 96%, Specificity 82%
Balanced Accuracy 89%

| | Representative | |
|---|---|---|
| **Experimental** | **Very toxic** | **false** |
| **false** | **4624** | 229 |
| **Very toxic** | 33 | **384** |

**Very Toxic Endpoint Performance**
Accuracy 95%
95% Confidence Interval: 94.4% - 95.6%
Sensitivity 63%, Specificity 99%
Balanced Accuracy 81%

# Summary

## Hazard Categorization & “Performance” Evaluation

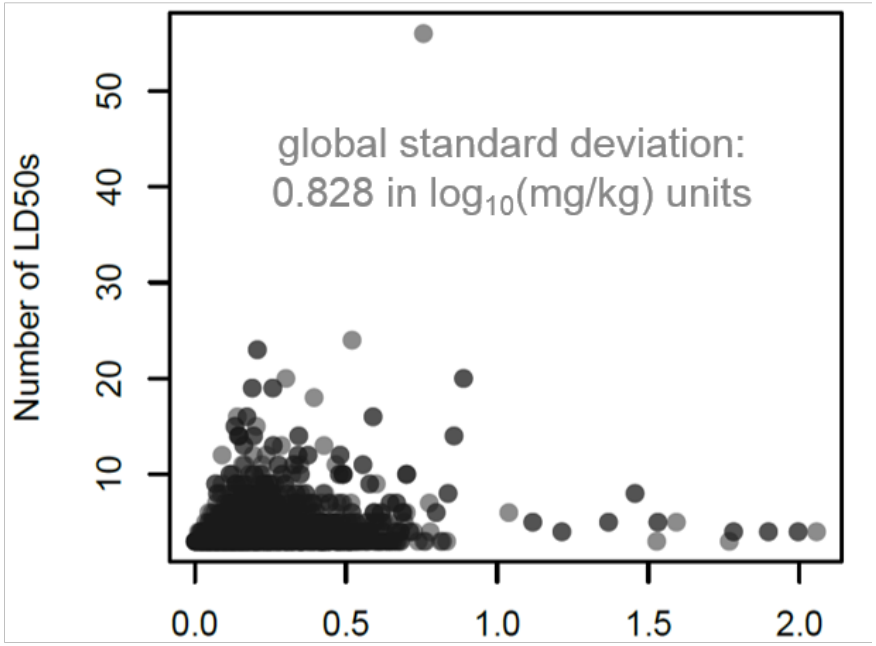
LD50 variability can result in implications for Hazard Categorization

**Follow-up question:**
**What are the sources of the variability?**

# Evaluation of Variability

- Standard deviation does not increase as a function of how many LD50 values there are per chemical

Standard deviations computed for 1,120 chemicals with ≥3 LD50 values (5,270 LD50 values)

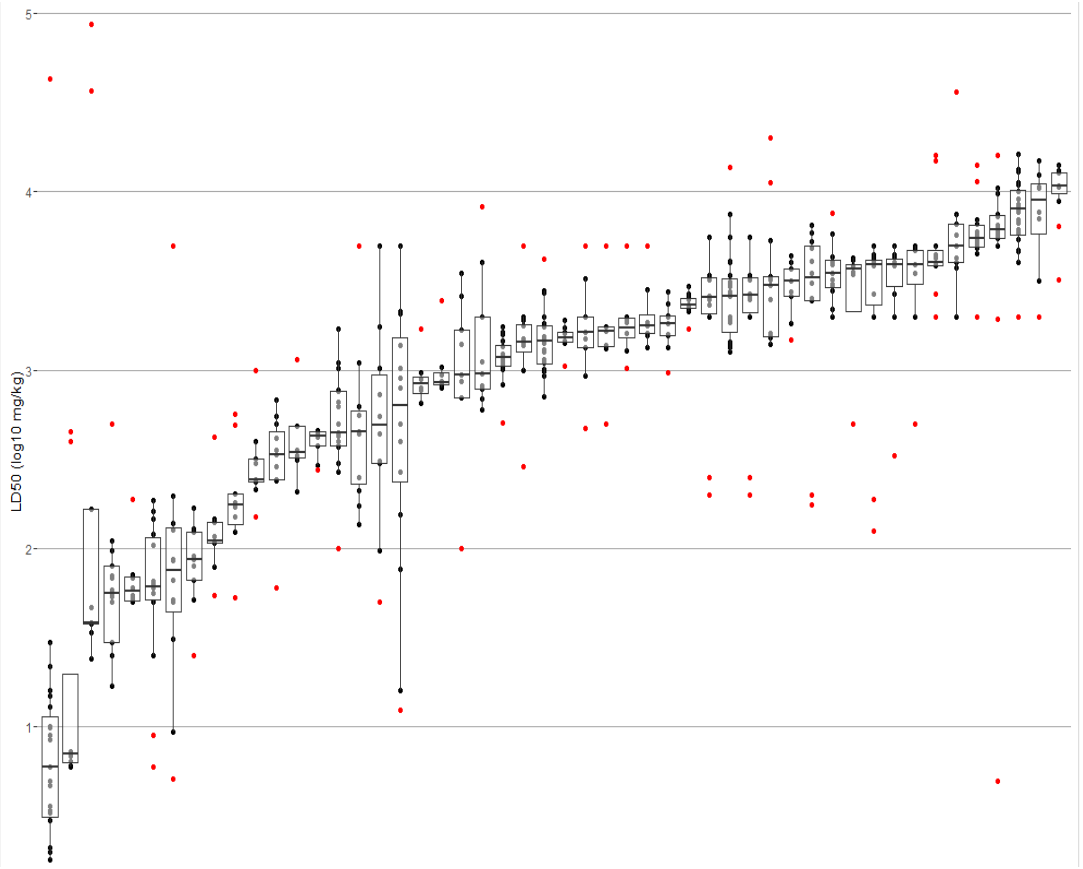


# Evaluation of Variability

## Identifying “Extreme” Values

- Tukey Fences (>1.5x interquartile range) applied to identify “extreme” values for all chemicals with ≥3 LD50 values (1,120 chemicals)

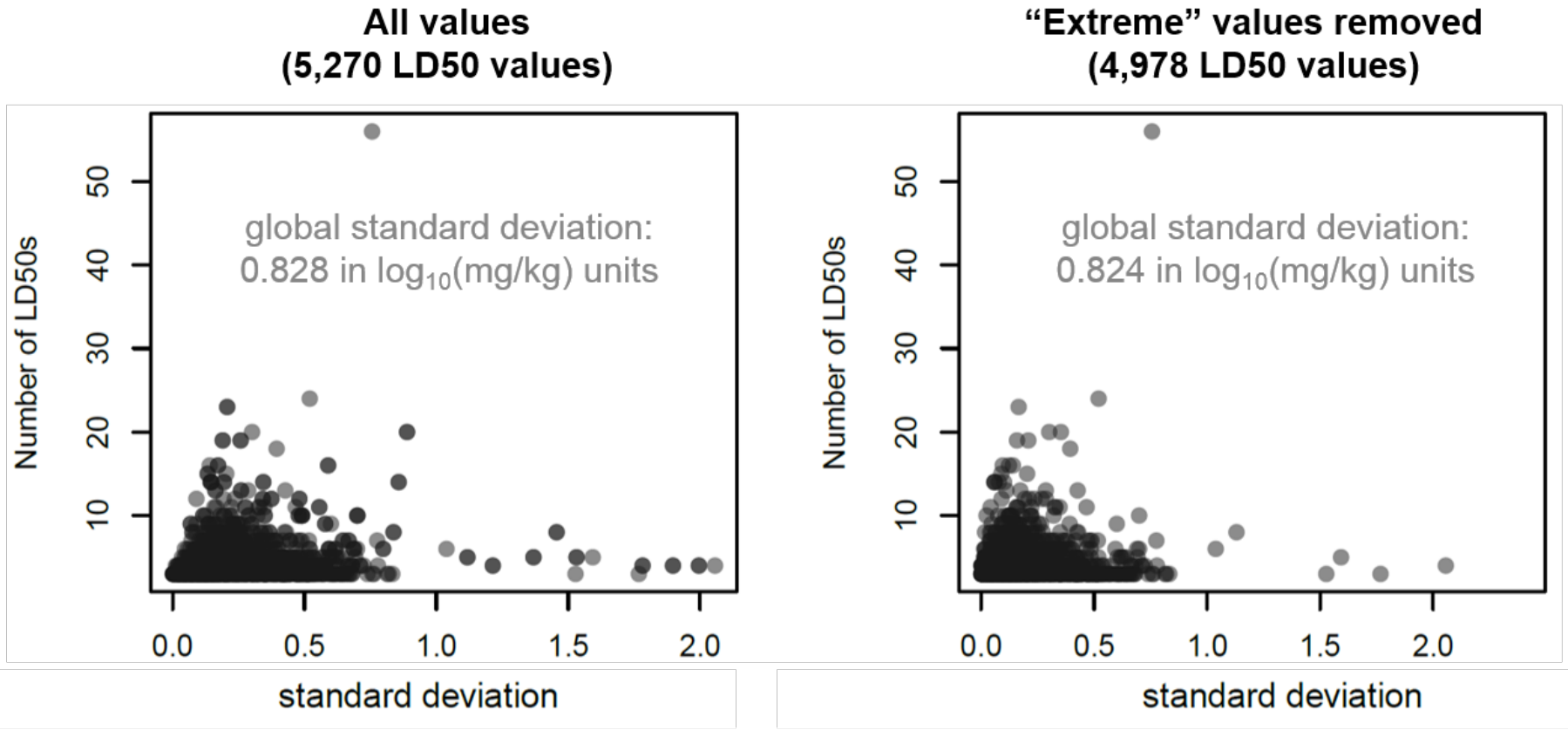
- 253 chemicals (23% of the 1,120 chemicals in the analysis) had at least one “extreme” value.
  - 292 values were identified



# Evaluation of Variability

## Impact of "Extreme" Values on Standard Deviation

- Standard deviation does not increase as a function of how many LD50 values there are per chemical



# Evaluation of Variability

## Association with Chemical Use

- CPCat includes 6,127 chemicals from the oral acute dataset
- 1,108 of the 1,120 chemicals with ≥3 LD50 values had use information
  - There were 181 unique use terms associated with these chemicals
  - Chemicals were associated with anywhere from 1 to 737 use terms
    - 1,815 chemicals had only one use term
    - 1,115 chemicals had >10 use terms

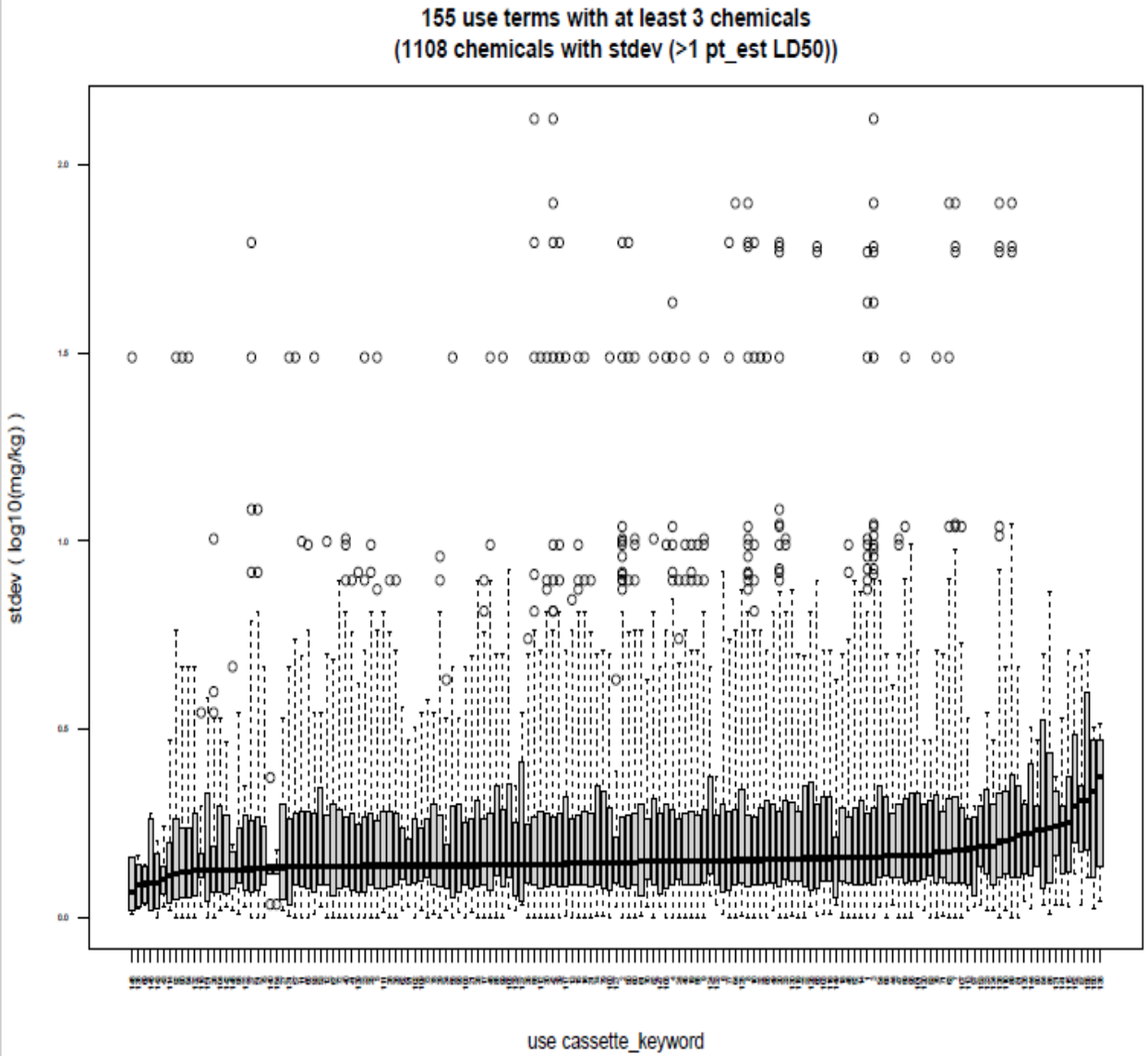
Four use terms with more than 500 chemicals associated:

- Manufacturing
- Consumer use
- Pesticide
- Industrial manufacturing

# Evaluation of Variability

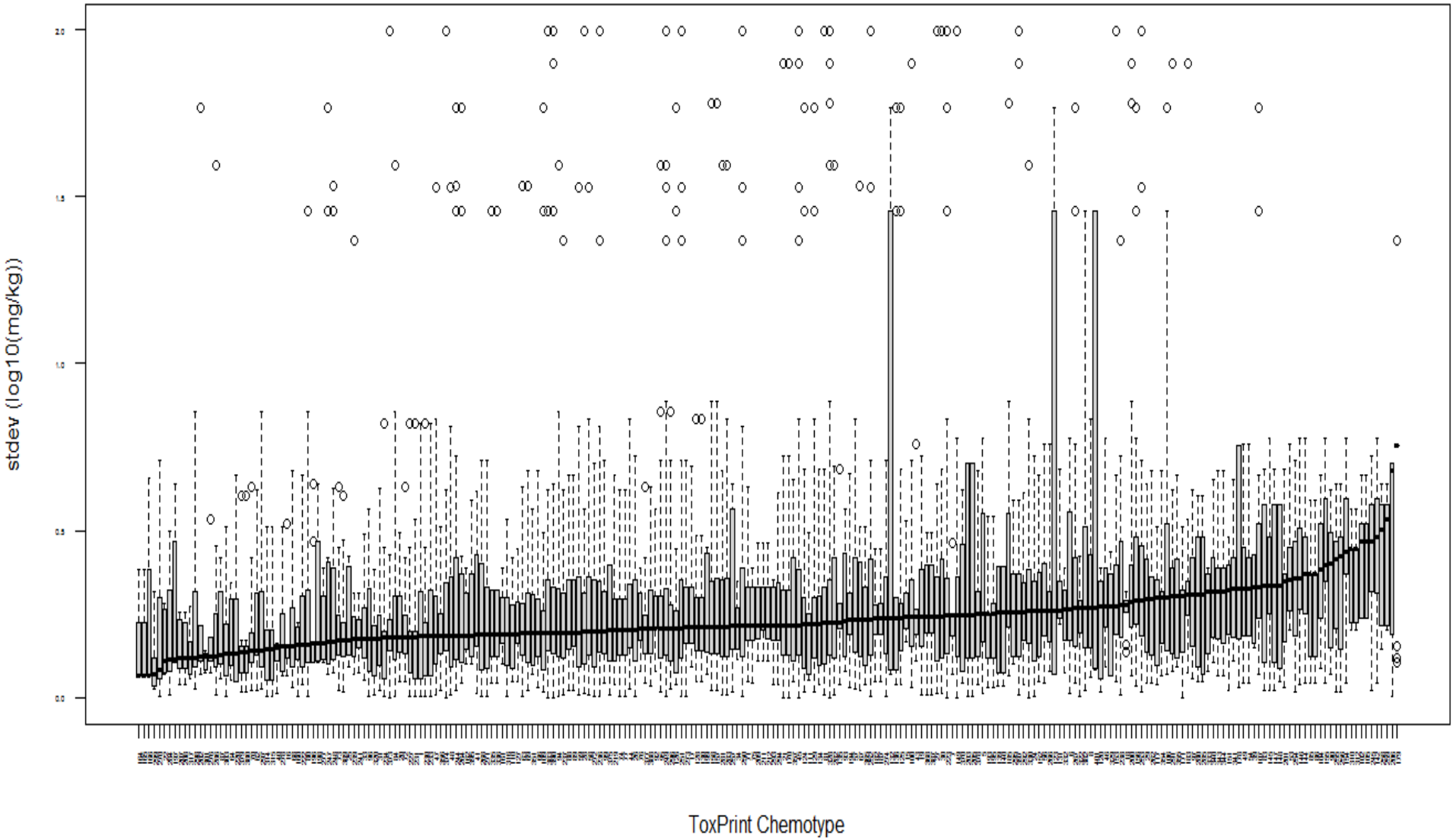
## Variability per Use Term

- 155 use terms had at least three chemicals for which standard deviation was available
- There was no significant enrichment of any use term being associated with higher variability
- Use terms with highest mean standard deviation:
  - Antiwrinkle (N = 5; SD = 0.37)
  - Glass (N = 5; SD = 0.33)
  - Polymer (N = 5; SD = 0.31)
  - Power generation (N = 11; SD = 0.31)
  - Antishell (N = 4; SD = 0.3)



# Evaluation of Variability

## Variability by ToxPrint Chemotype



# Evaluation of Variability

## Manual Curation from Primary Literature

- Some “extreme” values were evaluated manually by retrieving the primary literature source of the data.
- While most studies were guideline studies, some old sources had different sex and strain usage that may contribute to some variability.
- Study design differences may account for some of the variability.
  - To investigate this further, a more detailed data extraction from primary literature would be required.

# Summary

## Evaluation of Sources of Variability

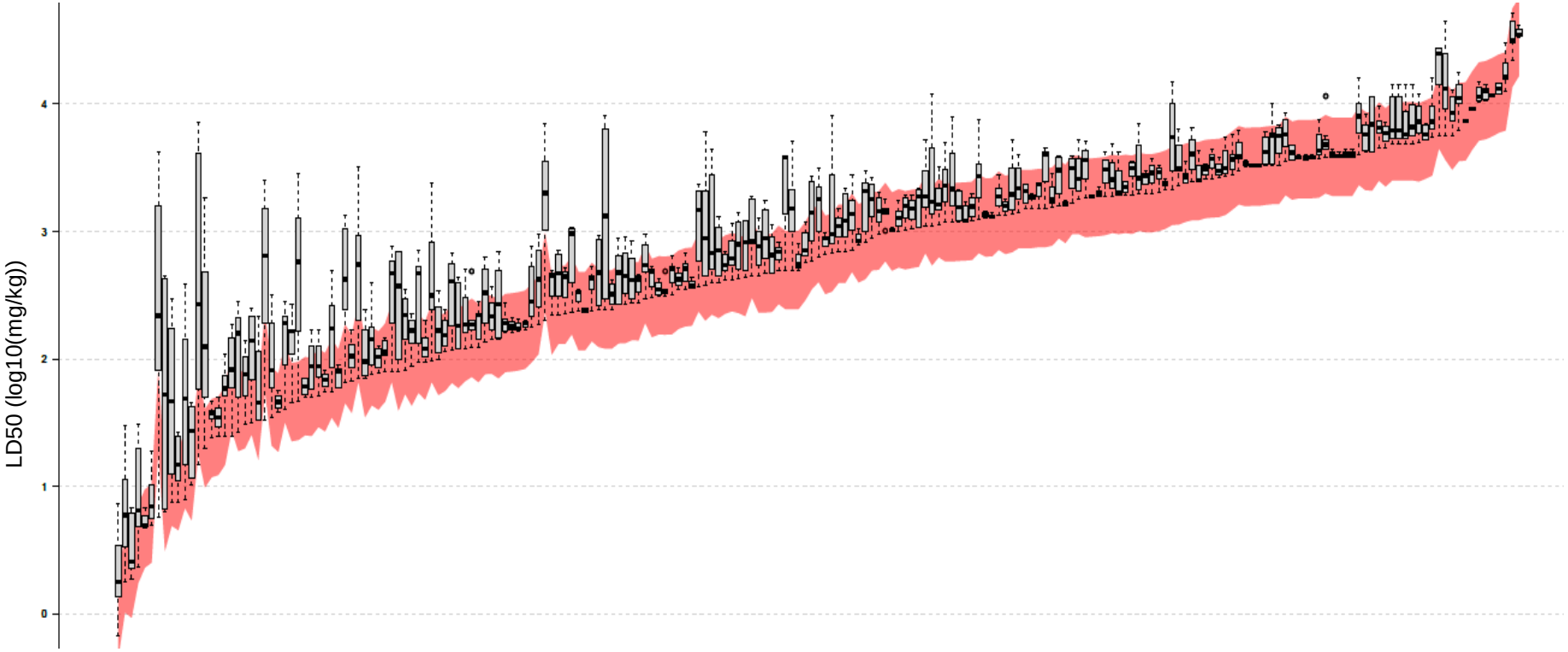
Neither study replication, LD50 potency, chemical use, nor structure were significantly correlated with increased variability.

Variation in study design may underlie some, but not all, of the variability.

**Final consideration:**
**Can we determine a confidence range for acute oral toxicity LD50 values?**

# Defining a Confidence Range

- Bootstrapping of the standard deviations identified a 95% confidence interval for acute oral toxicity LD50 values of to $\pm 0.31$ $\log_{10}$(mg/kg)



# Summary

- A large database of acute oral systemic toxicity LD50 values was compiled from numerous resources
- Chemicals with multiple LD50 values were used to evaluate the performance of this *in vivo* assay as well as characterize variability
  - Highly variability results in multiple hazard categories per chemical
  - Accuracy of the *in vivo* assay for hazard categorization was 74-78%
- Sources of variability were investigated:
  - Number of experiments, potency, chemical use category, or physchem properties do not correlate with increased variability
  - Some of the chemicals with variable data had slight variations in study design
- The 95% confidence interval identified for Acute Oral Systemic Toxicity LD50 values was $\pm 0.31$ $\log_{10}$(mg/kg)

# Acknowledgements

## THANK YOU!


- ICCVAM Acute Toxicity Working Group
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**Feedback welcome:**
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