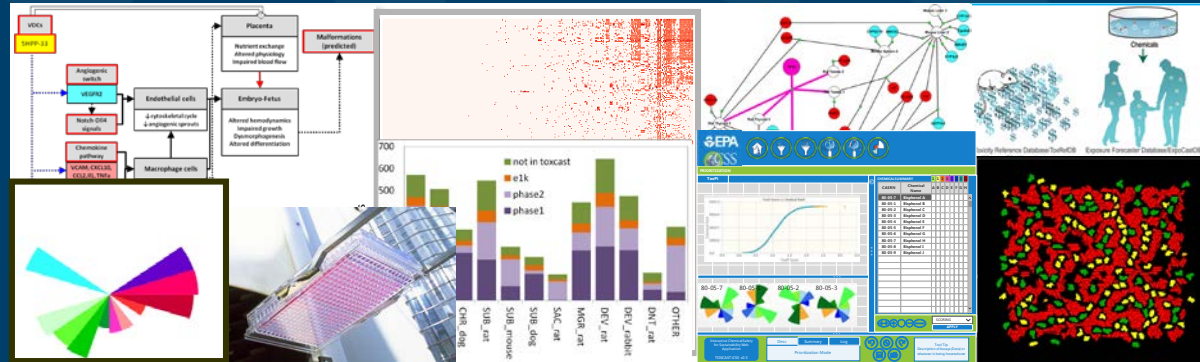


# Implementation of Non-Animal Approaches for Acute Systemic Toxicity



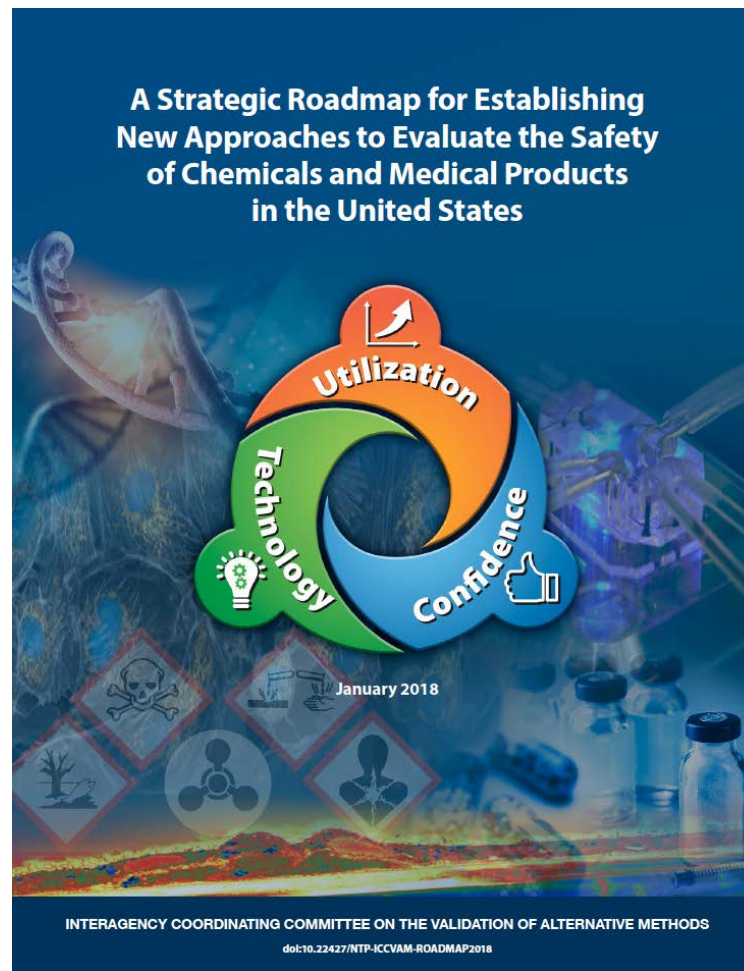
Grace Patlewicz

National Center for Computational Toxicology (NCCT), US EPA

Presenting as co-chair & member of the ICCVAM Acute Toxicity Work Group (ATWG)

# Acknowledgements

- **NICEATM**
- Nicole Kleinstreuer
- **ILS**
- Agnes Karmaus
- Kamel Mansouri
- Dave Allen
- **EPA-NCCT**
- Jeremy Fitzpatrick
- Prachi Pradeep



<https://ntp.niehs.nih.gov/go/natl-strategy>

# Acute Toxicity Implementation Plan

- Coordinate activities via ICCVAM Workgroups
- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for acute toxicity data
- Coordinate efforts with stakeholders
- Identify, acquire, and curate high quality data from reference test methods
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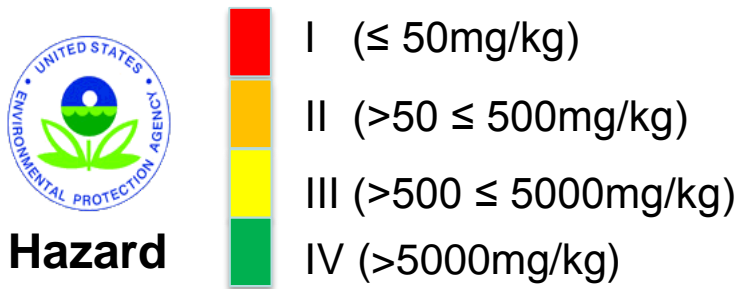
# Acute Toxicity Workgroup

- \*Grace Patlewicz (EPA)
  - \*Donald Cronce (DOD)
  - Kent Carlson (CPSC)
  - Xinrong Chen (CPSC)
  - John Gordon (CPSC)
  - Joanna Matheson (CPSC)
  - Lyle Burgoon (DOD)
  - Natalia Vinas (DOD)
  - Jeffery Gearhart (DOD)
  - David Mattie (DOD)
  - Ronald Meris (DOD)
  - Heather Pangburn (DOD)
  - Michael Phillips (DOD)
  - Emily N. Reinke (DOD)
  - Mark Williams (DOD)
  - Aiguo Wu (DOD)
  - Ryan Vierling (DOT)
  - Anna Lowit (EPA)
  - Thao (Tina) Pham (EPA)
  - Christopher Schlosser (EPA)
  - Warren Casey (NIEHS)
  - Nicole Kleinstreuer (NIEHS)
  - Elizabeth Maul (NIEHS)
  - George Fonger (NLM)
  - Pertti (Bert) Hakkinen (NLM)
  - Surender Ahir (OSHA)
  - Deana Holmes (OSHA)
- ICATM Liaison Members
- Pilar Prieto Peraita (EURL ECVAM)
  - Seung-Tae Chung (KoCVAM)
- NICEATM Support Staff (ILS)
- Judy Strickland
  - Agnes Karmaus
  - David Allen

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# Agencies that Use Acute Oral Toxicity Data



**Hazard**

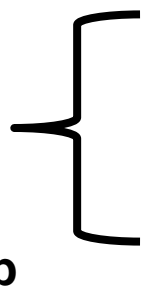


Highly toxic ( $\leq 50\text{mg/kg}$ )

Toxic ( $>50-5000\text{mg/kg}$ )



**Packing Group**



- |  |                                     |
|--|-------------------------------------|
|  | I ( $\leq 5\text{mg/kg}$ )          |
|  | II ( $>5 \leq 50\text{mg/kg}$ )     |
|  | III ( $>50 \leq 300\text{mg/kg}$ )  |
|  | IV ( $>300 \leq 2000\text{mg/kg}$ ) |



**Hazard**

**GHS**

See Presentations by E Reinke, L Scarano

# Acute Systemic Toxicity: U.S. Statutes and Regulations

Statute/Regulations	Agency
Federal Hazardous Substances Act (FHSA) (1964): 16 CFR 1500.3: <b>Consumer Products</b>	CPSC
Poison Prevention Packaging Act (1970): 16 CFR 1700: <b>Hazardous Household Substances</b>	CPSC
Federal Hazardous Material Transportation Act (1975): 49 CFR 173.132: <b>Transported Substances</b>	DOT
Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6): 40 CFR 156, 40 CFR 158.500, 40 CFR 158.2140, 40 CFR 158.2230: <b>Pesticides</b>	EPA
Toxic Substances Control Act (TSCA; 1976): 40 CFR 700-799: <b>New or Imported Chemicals</b>	EPA
Occupational Safety and Health Act (1970): 29 CFR 1910.1200: <b>Workplace Chemicals</b>	OSHA



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- > 60 participants from industry, academia, and ICCVAM agencies
- Recommendations:
  - Clear understanding of agency requirements
    - Strickland et al., Reg Tox Pharm, 2018*
  - Emphasise training and education
    - NICEATM and PISC outreach/reviewer training
  - International harmonisation of existing approaches
    - ICATM and OECD coordination, NC3Rs satellite
  - Use of existing data (curation and sharing efforts) for development of new *in vitro* and *in silico* approaches
    - ICE, CLA stakeholder discussions, inhalation tox workgroups

*Hamm et al., Tox In Vitro, 2017*




### Status of acute systemic toxicity testing requirements and data uses by U.S. regulatory agencies

Judy Strickland<sup>a,\*</sup>, Amy J. Clippinger<sup>b</sup>, Jeffrey Brown<sup>c</sup>, David Allen<sup>d</sup>, Abigail Jacobs<sup>e-1</sup>, Joanna Matheson<sup>d</sup>, Anna Lowit<sup>f</sup>, Emily N. Reinke<sup>g</sup>, Mark S. Johnson<sup>h</sup>, Michael J. Quinn Jr.<sup>i</sup>, David Mattie<sup>j</sup>, Suzanne C. Fitzpatrick<sup>k</sup>, Surender Ahir<sup>l</sup>, Nicole Kleinstreuer<sup>l</sup>, Warren Casey<sup>m</sup>

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<sup>b</sup> PETA International Science Consortium Ltd., Society Building, 8 All Saints Street, London, UK  
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Acute systemic toxicity

### ABSTRACT

Acute systemic toxicity data are used by a number of U.S. federal agencies, most commonly for hazard classification and labeling and/or risk assessment for acute chemical exposures. To identify opportunities for the implementation of non-animal approaches to produce these data, the regulatory needs and uses for acute systemic toxicity information must first be clarified. Thus, we reviewed acute systemic toxicity testing requirements for six U.S. agencies (Consumer Product Safety Commission, Department of Defense, Department of Transportation, Environmental Protection Agency, Food and Drug Administration, Occupational Safety and Health Administration) and noted whether there is flexibility in satisfying data needs with methods that replace or reduce animal use. Understanding the current regulatory use and acceptance of non-animal data is a necessary starting point for future method development, optimization, and validation efforts. The current review will inform the development of a national strategy and roadmap for implementing non-animal approaches to assess potential hazards associated with acute exposures to industrial chemicals and medical products. The Acute Toxicity Workgroup of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), U.S. agencies, non-governmental organizations, and other stakeholders will work to execute this strategy.



### Review

### Alternative approaches for identifying acute systemic toxicity: Moving from research to regulatory testing

Jon Hamm<sup>a,\*</sup>, Kristie Sullivan<sup>b</sup>, Amy J. Clippinger<sup>c</sup>, Judy Strickland<sup>d</sup>, Shannon Bell<sup>e</sup>, Barun Bhattarai<sup>d</sup>, Bas Blaauboer<sup>f</sup>, Warren Casey<sup>g</sup>, David Dorman<sup>h</sup>, Anna Forsby<sup>h</sup>, Natàlia Garcia-Reyero<sup>i</sup>, Sean Gehen<sup>j</sup>, Rabea Graepel<sup>k</sup>, Jon Hotchkiss<sup>l</sup>, Anna Lowit<sup>l</sup>, Joanna Matheson<sup>m</sup>, Elissa Reaves<sup>n</sup>, Louis Scarnano<sup>o</sup>, Catherine Sprankle<sup>a</sup>, Jay Tunkel<sup>o</sup>, Dan Wilson<sup>o</sup>, Menghang Xia<sup>p</sup>, Hao Zhu<sup>q</sup>, David Allen<sup>r</sup>

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<sup>h</sup> Stockholm University and Swedish Toxicology Sciences Research Center (Swetox), Söderstråle, Sweden  
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<sup>l</sup> U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC, USA  
<sup>m</sup> U.S. Consumer Product Safety Commission, Washington, DC, USA  
<sup>n</sup> U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, USA  
<sup>o</sup> SRC, Inc., Syracuse, NY, USA  
<sup>p</sup> National Center for Advancing Translational Sciences, Rockville, MD, USA  
<sup>q</sup> Department of Chemistry Rutgers University-Camden, Camden, NJ, USA  
<sup>r</sup> U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, USA

# Workshop on Acute Toxicity Inhalation Testing (2016)

- 2016 webinar series & workshop
- > 50 participants from industry, NGOs, academia, and ICCVAM agencies
  - Developing a database of existing acute systemic toxicity data
  - Preparing a state-of-the-science review on mechanisms and non-animal approaches for acute inhalation toxicity (final draft under review & internal clearance)
  - Summarising global regulatory and non regulatory data requirements (workshop report)
- ***Clippinger et al., Tox in Vitro, 2018***
- Developing an in silico decision tree
- Designing and conducting an in vitro proof-of-concept



Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report

Amy J. Clippinger<sup>a,b</sup>, David Allen<sup>b</sup>, Annie M. Jarabek<sup>c</sup>, Marco Corvaro<sup>d</sup>, Marianna Gaça<sup>e</sup>, Sean Gehen<sup>f</sup>, Jon A. Hotchkiss<sup>g</sup>, Grace Patlewicz<sup>h</sup>, Jodie Melbourne<sup>i</sup>, Paul Hinderliter<sup>j</sup>, Miyoung Yoon<sup>k</sup>, Dongeun Huh<sup>l</sup>, Anna Lowit<sup>m</sup>, Barbara Buckley<sup>n</sup>, Michael Bartels<sup>o</sup>, Kelly BéruBé<sup>o</sup>, Daniel M. Wilson<sup>p</sup>, Ian Indans<sup>q</sup>, Mathieu Vincken<sup>r</sup>

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<sup>f</sup> Dow AgroSciences, Indianapolis, IN, United States

<sup>g</sup> The Dow Chemical Company, Midland, MI, United States

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<sup>i</sup> Syngenta, Greensboro, NC, United States

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<sup>l</sup> U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Washington, DC, United States

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<sup>n</sup> Cardiff University, School of Biosciences, Cardiff, Wales, UK

<sup>o</sup> Health and Safety Executive, London, UK

<sup>p</sup> Free University of Brussels Belgium, Brussels, Belgium



~50 international participants

ICATM Regional Updates:

- Europe, Japan, Korea, Brazil

U.S. National Strategy and Roadmap

Industry Perspectives:

- Current regulatory climate
- GHS additivity calculations

International Harmonisation:

- OECD coordination
- ECVAM perspectives on credibility and validation
- Cosmetics Europe skin sensitisation collaboration

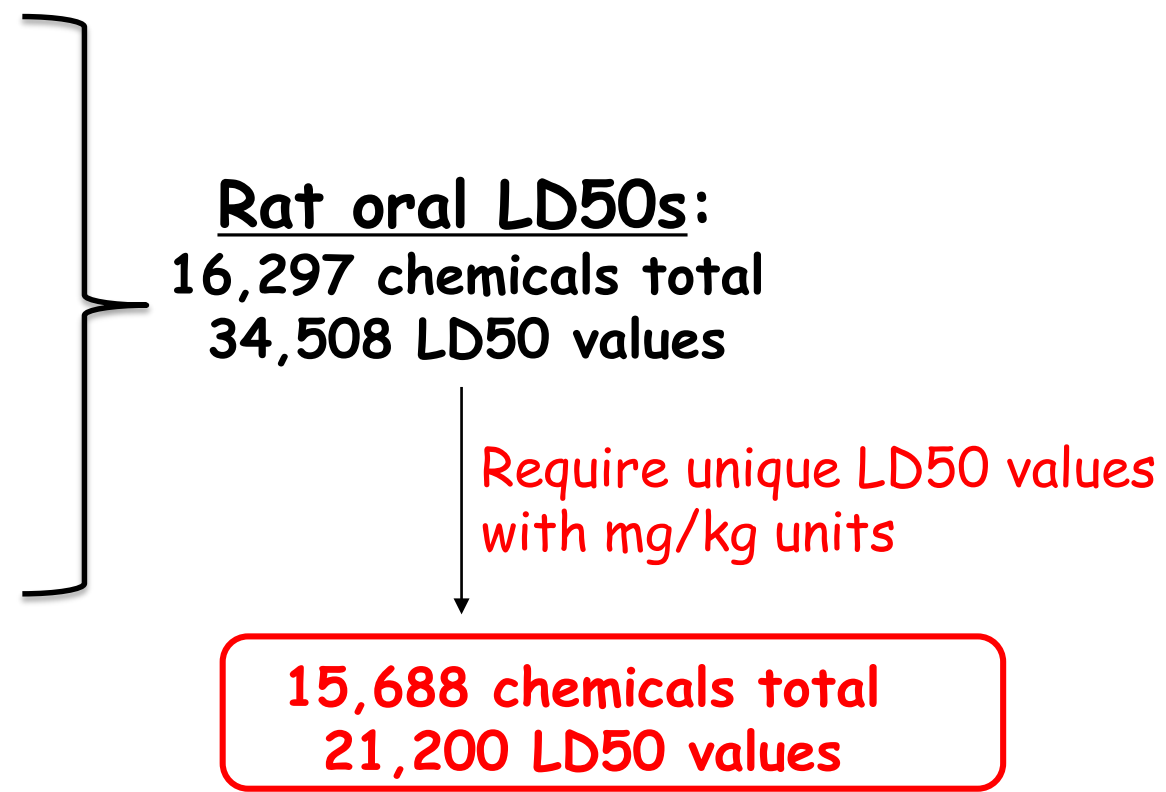
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# Establishing a dataset of acute oral toxicity data

See Agnes Karmaus's presentation

Database Resource	Rows of Data (number of LD50 values)	Unique CAS
ECHA (ChemProp)	5533	2136
JRC AcutoxBase	637	138
NLM HSDB	4082	2238
OECD (eChemPortal)	10206	2314
PAI (NICEATM)	364	293
TEST (NLM ChemIDplus)	13689	13545



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# Identify and evaluate non-animal alternative approaches to acute toxicity testing

- Establish a dataset of rat oral acute toxicity study LD50 data 😊
- Evaluate the variability of the experimental data collected 😊
  - to inform data curation efforts
  - to inform considerations for evaluating performance and coverage of existing models
  - to inform considerations for new model development
- Identify endpoints to be modeled based on ICCVAM agency needs 😊
- Evaluate existing models for acute toxicity
- Investigate the feasibility of developing new models for acute toxicity
- Initiate a project to leverage the expertise of the international modelling 😊 community to develop predictive models of acute oral toxicity
- Evaluate the applicability of the existing and new models for chemistries of interest to ICCVAM agencies



# Identify and evaluate non-animal alternative approaches to acute toxicity testing

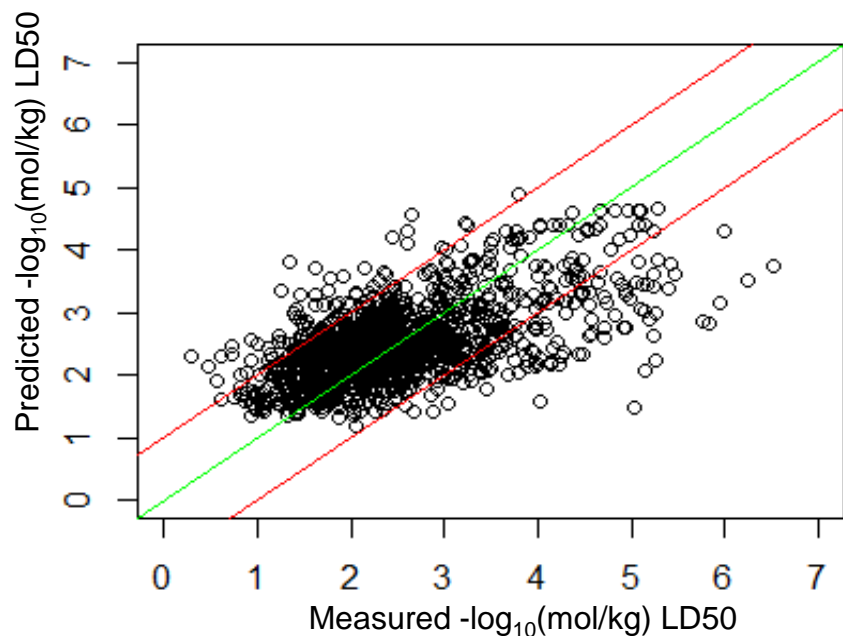
- Evaluating existing *in silico* models

Model	Number of substances in dataset	Number of substances that could be predicted	Accuracy for substances with one Value	Accuracy for substances with multiple values	Overall Accuracy
TIMES Model	1787	315 (17.6%)	85 of 93 (91%)	206 of 222 (93%)	291 of 315 (92%)
TEST-Acute Oral Consensus Model	1787	1673 (93.6%)	433 of 490 (88%)	1092 of 1183 (92%)	1525 of 1673 (91%)

Fitzpatrick et al., Presented at ASCCT 2017; SOT 2018, manuscript in preparation  
EPA NCCT - NICEATM

# Identify and evaluate non-animal alternative approaches to acute toxicity testing

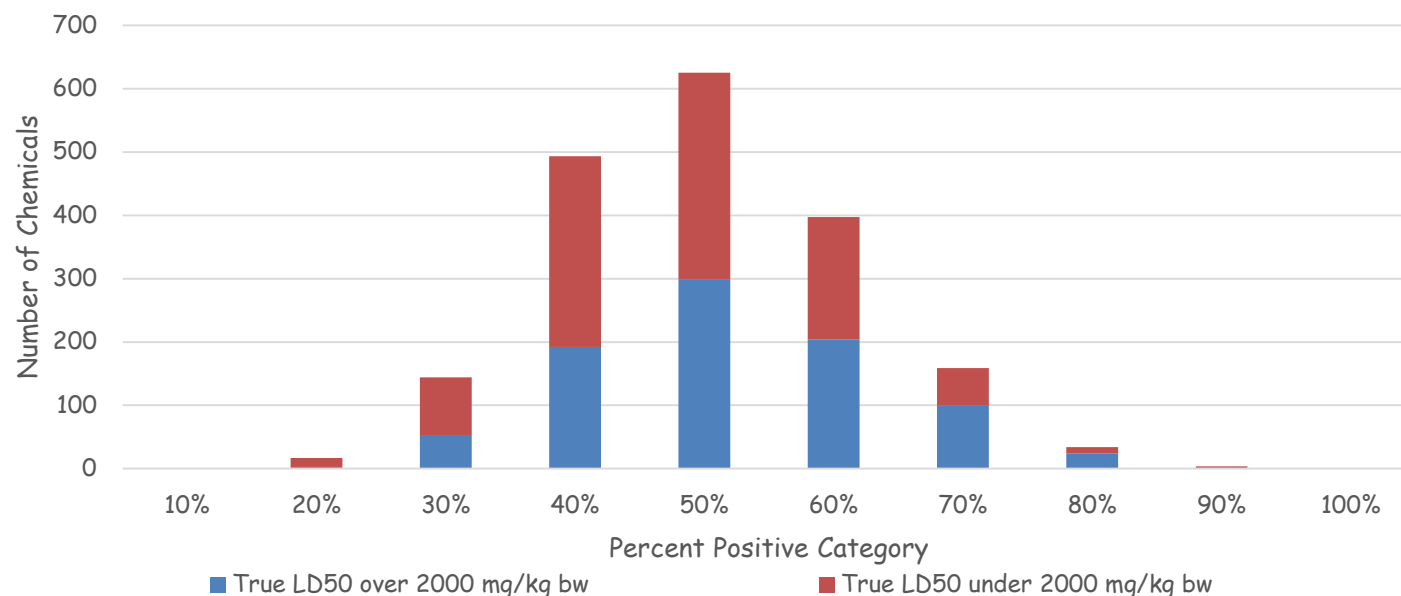
- Developing new models:
- Global Regression Model



- ◆ Global ridge regression model used both experimental and predicted ToxCast™ and Tox21 assay outcomes as descriptors.
- ◆ Training set (4164), Test set (1387)
- ◆ 85% of the substances were found to be within one log unit of their predicted LD50 value.

- Global Random Forest Model

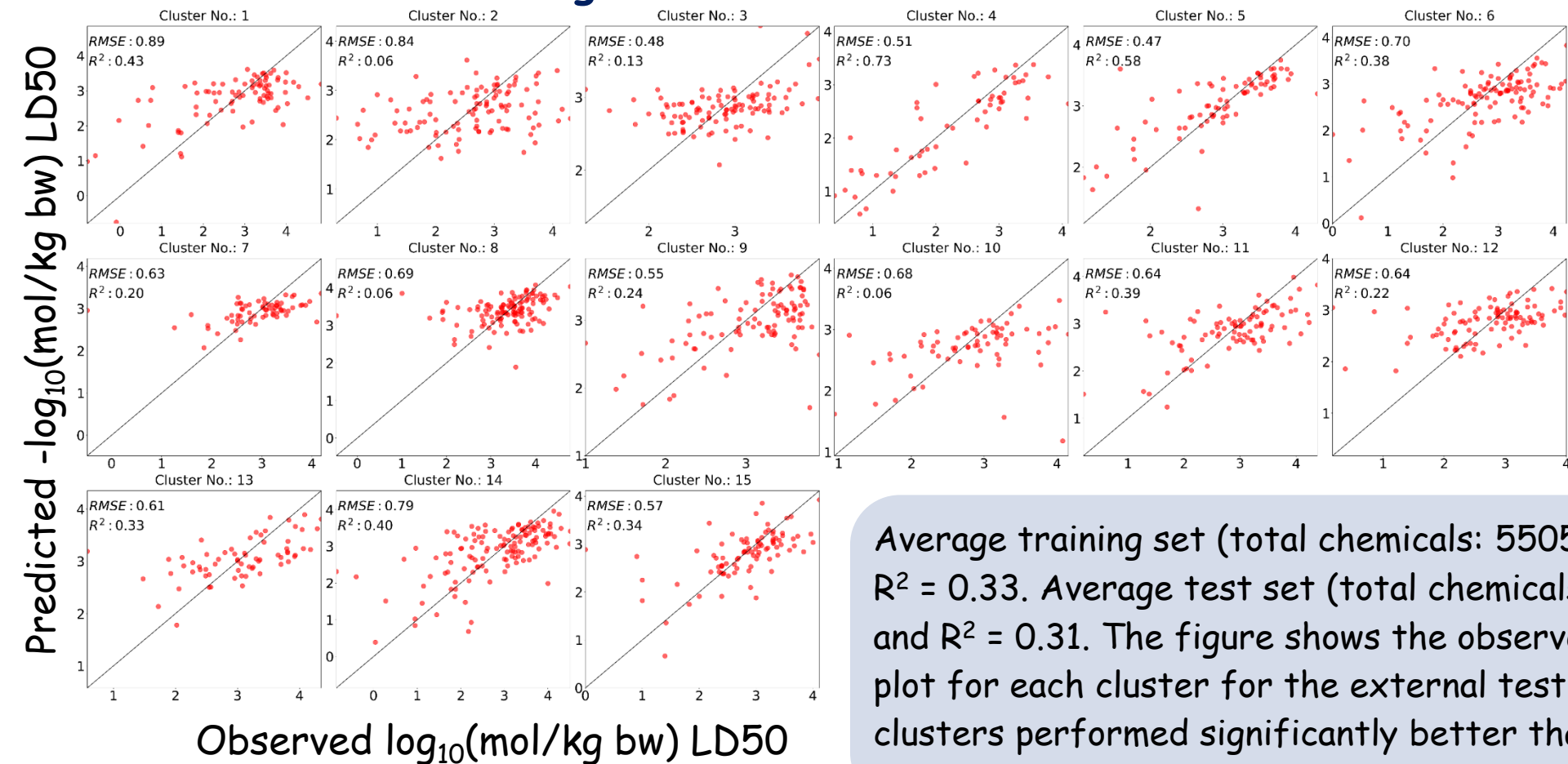
Over/Under Model For Acute Toxicity



- ◆ Model for predicting compounds over and under a LD50 of 2000 mg/kg bw had an accuracy of 57%, a balanced accuracy of 56%, a sensitivity of 57%, and a specificity of 56%.

# Identify and evaluate non-animal alternative approaches to acute toxicity testing

- Developing new models:
- Local Cluster-based Regression Model



Average training set (total chemicals: 5505) RMSE = 0.65 and R<sup>2</sup> = 0.33. Average test set (total chemicals: 1377) RMSE = 0.65 and R<sup>2</sup> = 0.31. The figure shows the observed versus predicted plot for each cluster for the external test dataset. Some clusters performed significantly better than others with R<sup>2</sup> > 0.4.

# Identify and evaluate non-animal alternative approaches to acute toxicity testing

- Establish a dataset of rat oral acute toxicity study LD50 data ☺
- Evaluate the variability of the experimental data collected ☺
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# Identify and evaluate non-animal alternative approaches to acute toxicity testing

See Kamel Mansouri's presentation

- Initiate a project to leverage the expertise of the international modelling community to develop predictive models of acute oral toxicity
- 32 groups from the US, Europe, and Asia responded with 135 models for LD50, EPA and GHS categories, and binary nontoxic vs all others and very toxic vs all others.

- **Outlined ATWG charges**
- **Substantial progress has been made in outlining the decision contexts, needs and gathering the acute data to inform the array of in silico modelling efforts**
- **This workshop is critical to practically actualising the ATWG implementation plan**