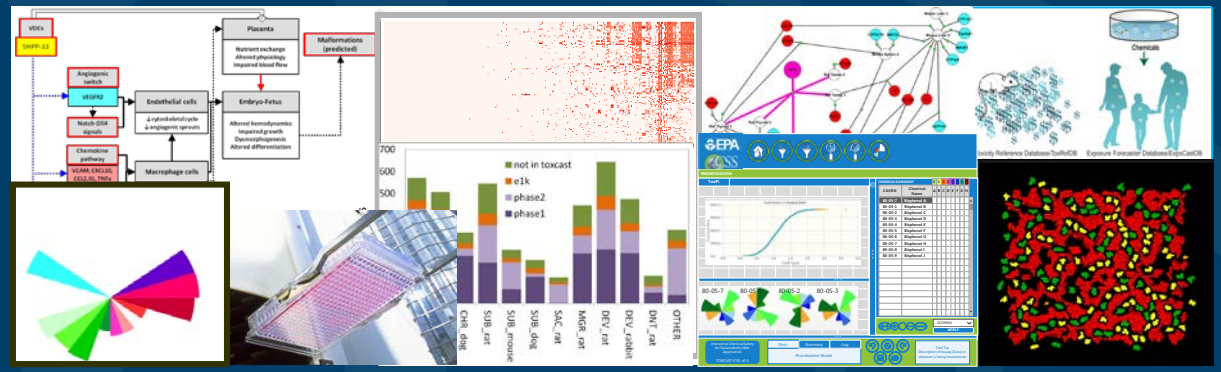
EPA United States Environmental Protection Agency

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
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- Agnes Karmaus
- Kamel Mansouri
- Dave Allen
- **EPA-NCCT**
- Jeremy Fitzpatrick
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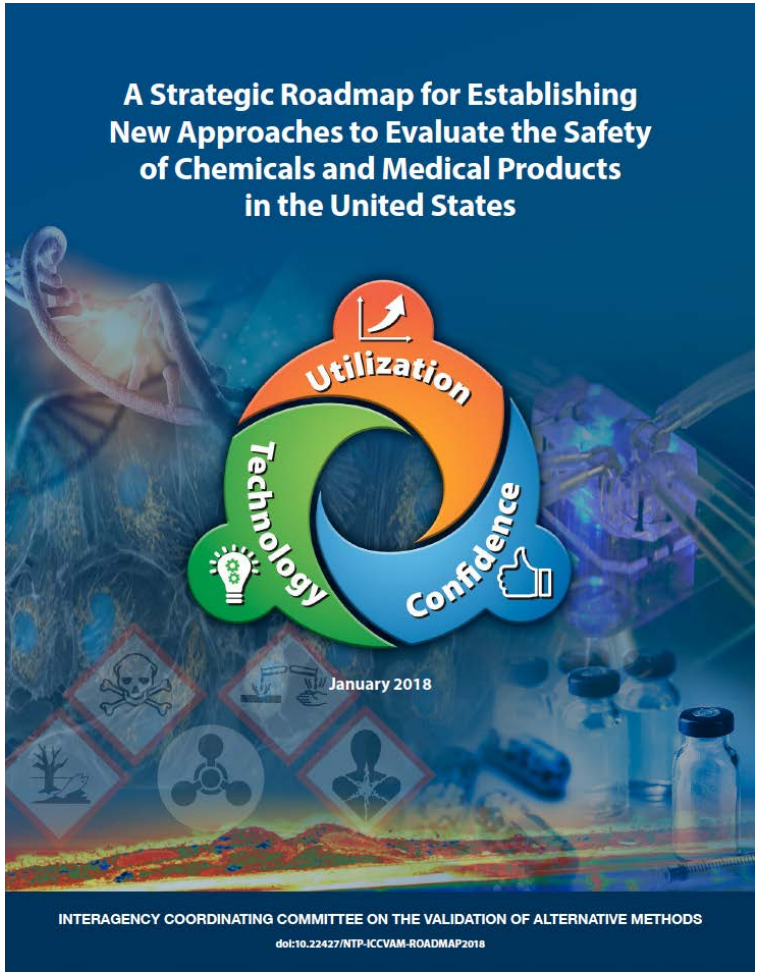

https://ntp.niehs.nih.gov/go/natl-strategy

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
- Identify and evaluate non-animal alternative approaches to acute toxicity testing
- Gain regulatory acceptance and facilitate use of non-animal approaches

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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)
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NICEATM Support Staff (ILS)

- Judy Strickland
- Agnes Karmaus
- David Allen

*co-chairs

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Agencies that Use Acute Oral Toxicity Data

Hazard

- I (≤ 50mg/kg)
- II (>50 ≤ 500mg/kg)
- III (>500 ≤ 5000mg/kg)
- IV (>5000mg/kg)

Hazard

- Highly toxic (≤50mg/kg)
- Toxic (>50-5000mg/kg)

Packing Group

- I (≤ 5mg/kg)
- II (>5 ≤ 50mg/kg)
- III (>50 ≤ 300mg/kg)
- IV (>300 ≤ 2000mg/kg)

OSHA®

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: **Consumer Products**	CPSC
Poison Prevention Packaging Act (1970): 16 CFR 1700: **Hazardous Household Substances**	CPSC
Federal Hazardous Material Transportation Act (1975): 49 CFR 173.132: **Transported Substances**	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: **Pesticides**	EPA
Toxic Substances Control Act (TSCA; 1976): 40 CFR 700-799: **New or Imported Chemicals**	EPA
Occupational Safety and Health Act (1970): 29 CFR 1910.1200: **Workplace Chemicals**	OSHA

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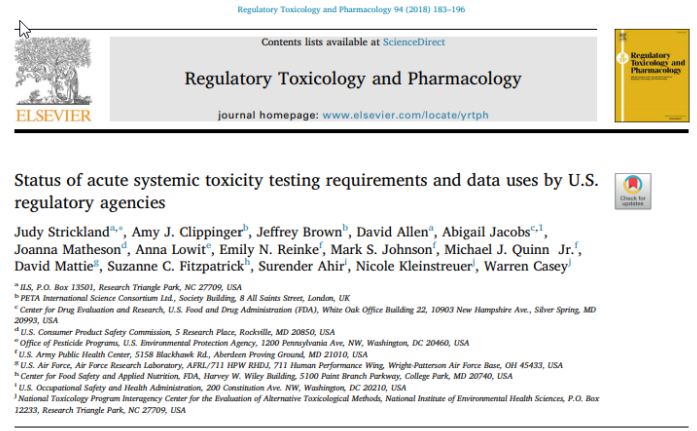
Workshop on Acute Toxicity Testing (2015)

- > 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


Regulatory Toxicology and Pharmacology 94 (2018) 183–196

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

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

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

ARTICLE INFO

ABSTRACT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Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Review

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

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

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


Toxicology in Vitro 48 (2018) 53–70

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

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

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

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[n] Cardiff University, School of Biosciences, Cardiff, Wales, UK
[o] Health and Safety Executive, London, UK
[p] Free University of Brussels-Belgium, Brussels, Belgium

Workshop on Acute Toxicity Testing (2017)

~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

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

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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

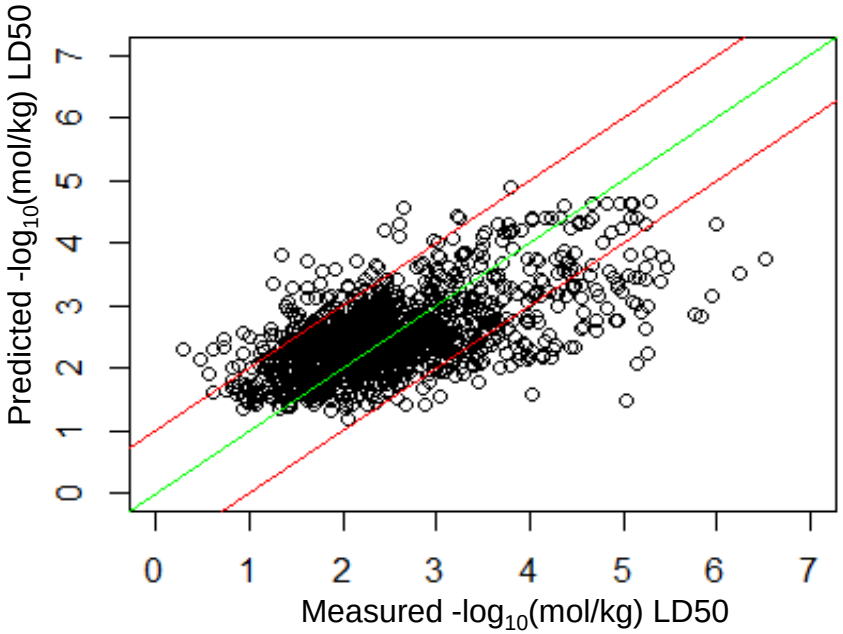
- Evaluating existing *in silico* models

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%)

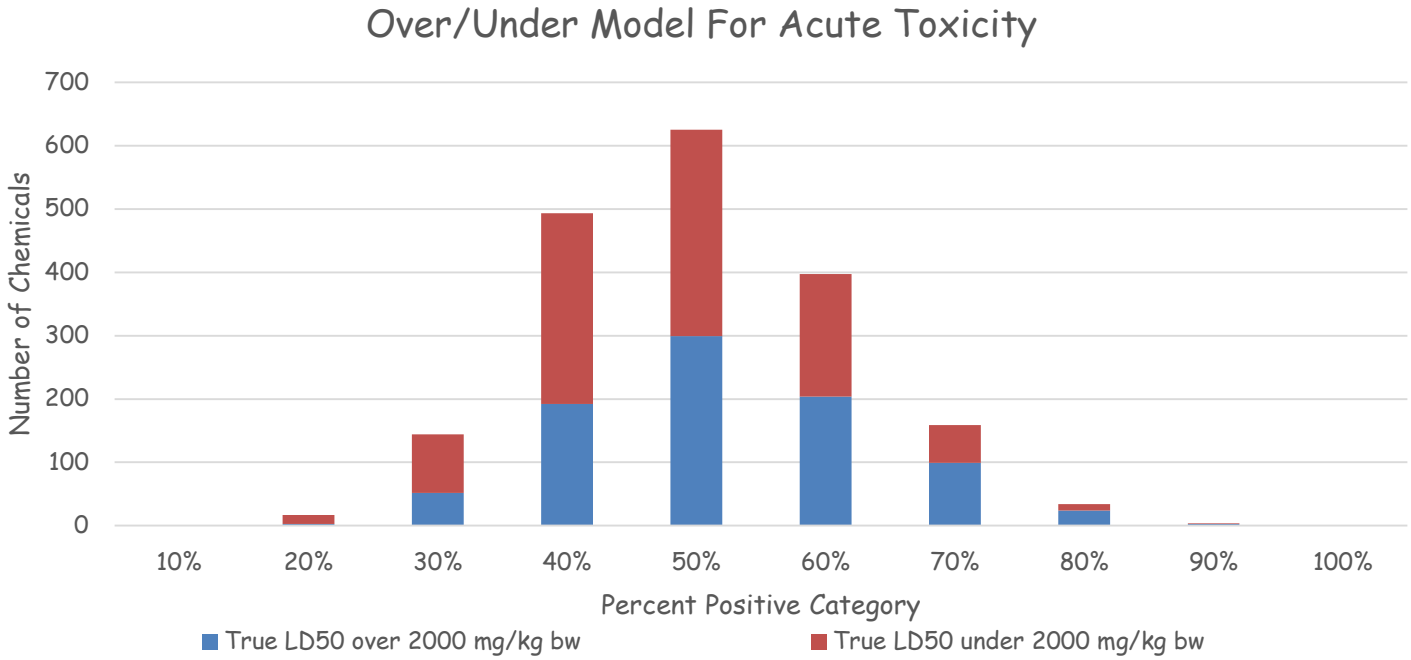
Fitzpatrick et al., Presented at ASCCT 2017; SOT 2018, manuscript in preparation

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

- Developing new models:
- Global Regression Model



- Global Random Forest Model



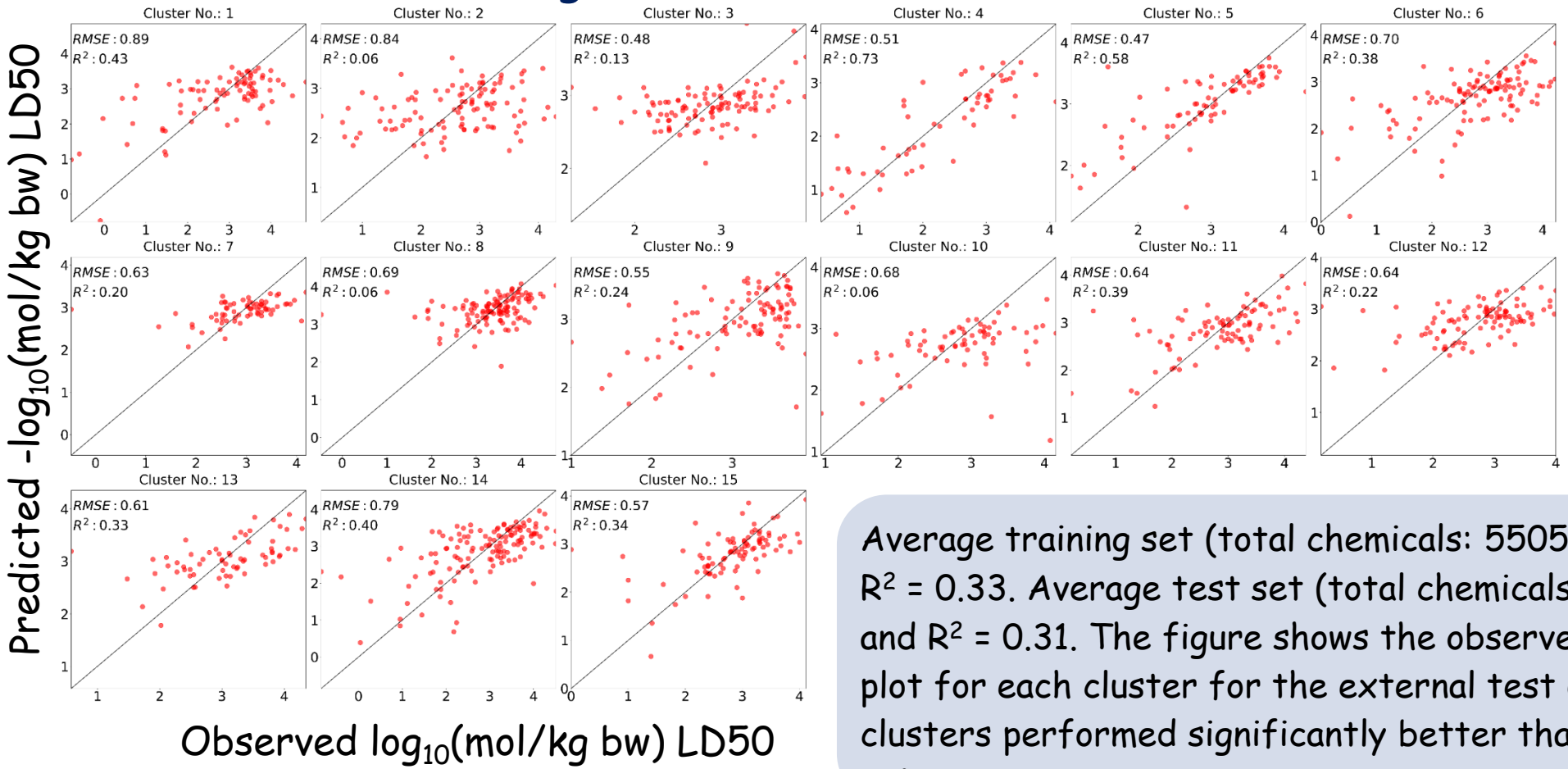
- Global ridge regression model used both experimental and predicted ToxCast™ and Tox21 assay outcomes as descriptors.
- Training set (4164), Test set (1387)
- 85% of the substances were found to be within one log unit of their predicted LD50 value.

- 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%.

Fitzpatrick et al., Presented at ASCCT 2017; SOT 2018, manuscript in preparation

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^2 = 0.33. Average test set (total chemicals: 1377) RMSE = 0.65 and R^2 = 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^2 > 0.4.

Fitzpatrick et al., Presented at SOT 2018, manuscript in preparation

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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.

Summary remarks

- 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