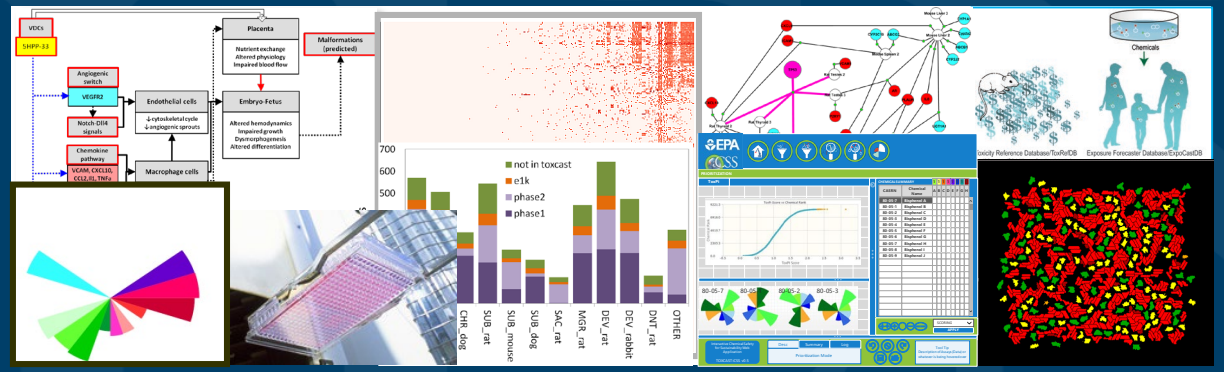
Variability of NAMs and traditional methods in the context of predicting acute toxicity

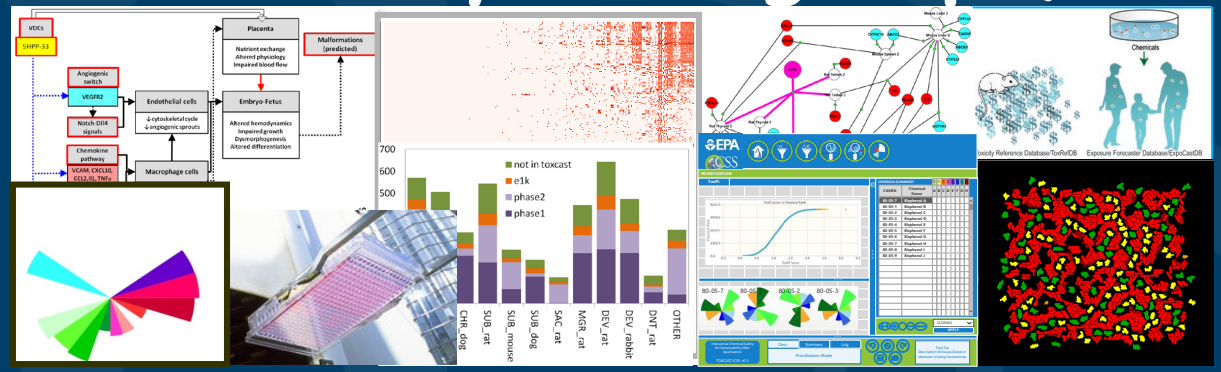


Grace Patlewicz
National Center for Computational Toxicology (NCCT), US EPA

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

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

Challenges in 'benchmarking' in silico models against traditional toxicity methods – insights gained from the ICCVAM Acute Toxicity Workgroup (ATWG) efforts



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- Jeremy Fitzpatrick**
- Prachi Pradeep**
- George Helman**
- Imran Shah

**Also provided all slide materials for this presentation

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

Outline

- ICCVAM Workgroup on Acute Toxicity – Charges & Scope
- Progress and challenges in developing new and evaluating existing non-animal alternative approaches to acute toxicity testing
- Summary remarks

ATWG 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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- Gain regulatory acceptance and facilitate use of non-animal approaches

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Identify U.S. agency requirements, needs, and decision contexts for acute 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)

Hazard

GHS

Regulatory Toxicology and Pharmacology 94 (2018) 183–196

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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

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.

ATWG Acute Toxicity Implementation Plan

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

Rat oral acute toxicity LD50 Database

- Mined and merged multiple existing resources containing rat oral acute toxicity LD50 data (collaboration between NICEATM & NCCT)
- Identify transcription errors (e.g. 20005000 mg/kg)
- Manual curation of highly variable chemicals; identify source data
- Often (typically) meta data not available for vast majority of the substances collected
- Explore the variability of the data - representative LD50, variability across hazard categories

Data source	Number of LD50 values	Number of unique chemicals
ECHA ChemProp	5,533	2,136
NLM HSDB	3,981	2,205
JRC AcutoxBase	637	138
NLM ChemIDplus	13,072	12,977
NICEATM PAI	364	293
OECD eChemPortal	10,119	2,290

Total:
34,511 LD50 values
16,307 chemicals

↓ Identify unique data in mg/kg

21,210 LD50 values
15,698 chemicals

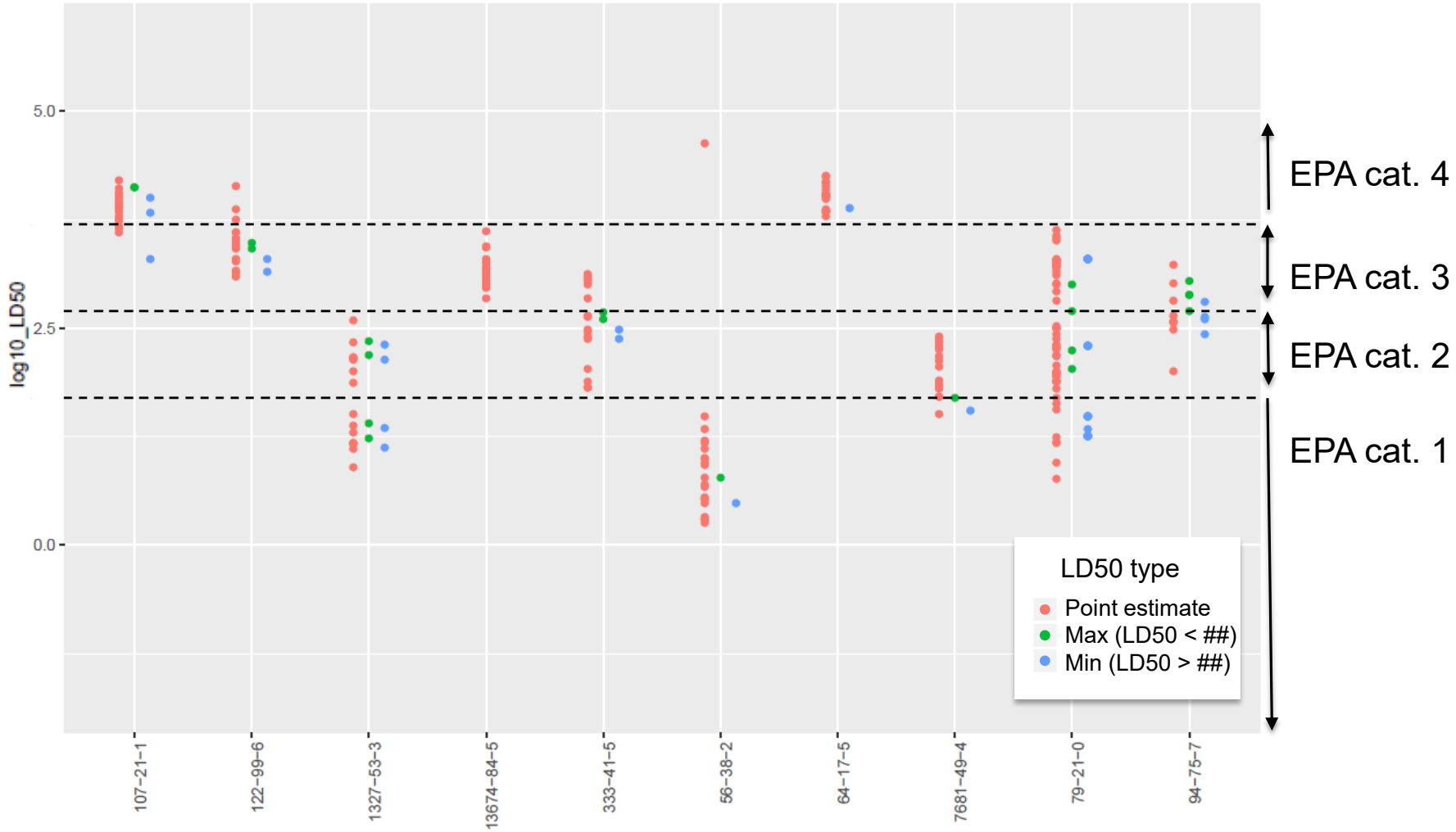
Acute Oral LD50 Dataset Replicate Inventory

- 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%)

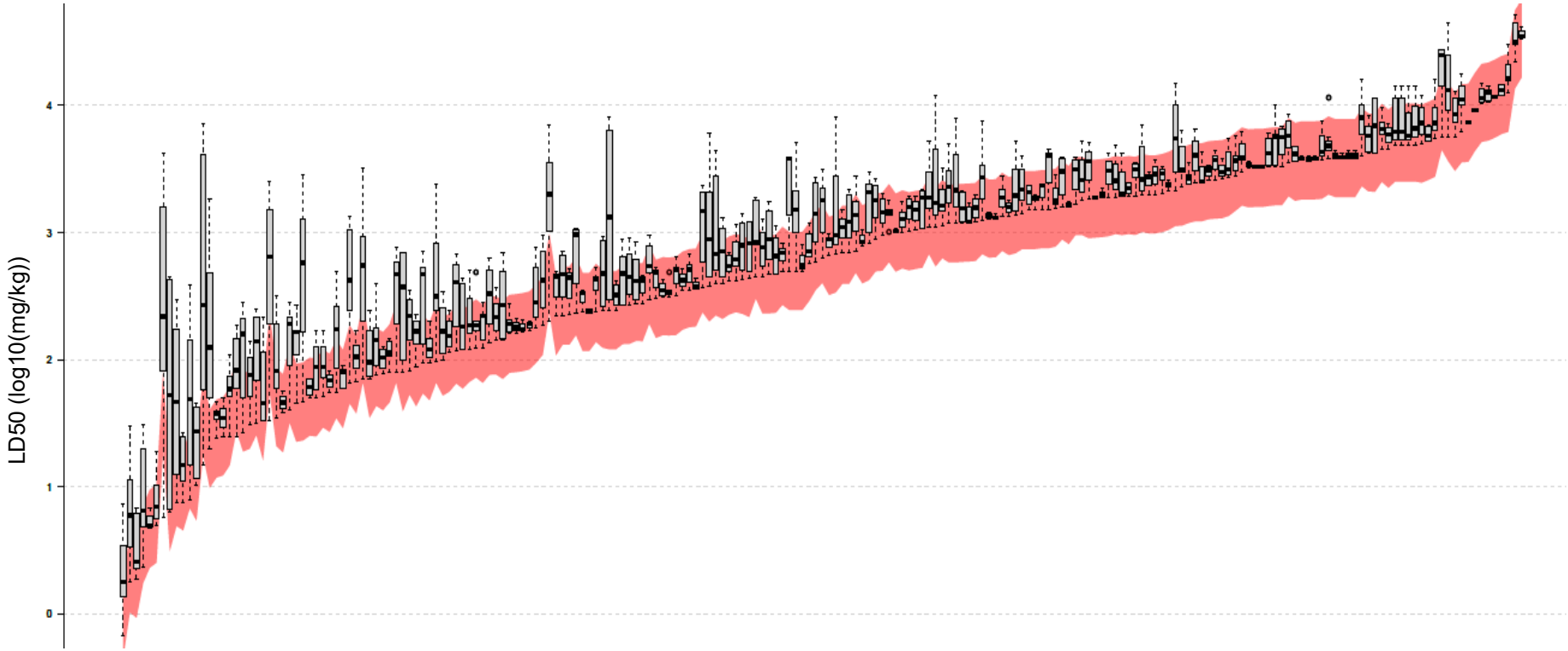
Impact on Hazard Categorization

Example: EPA Classification



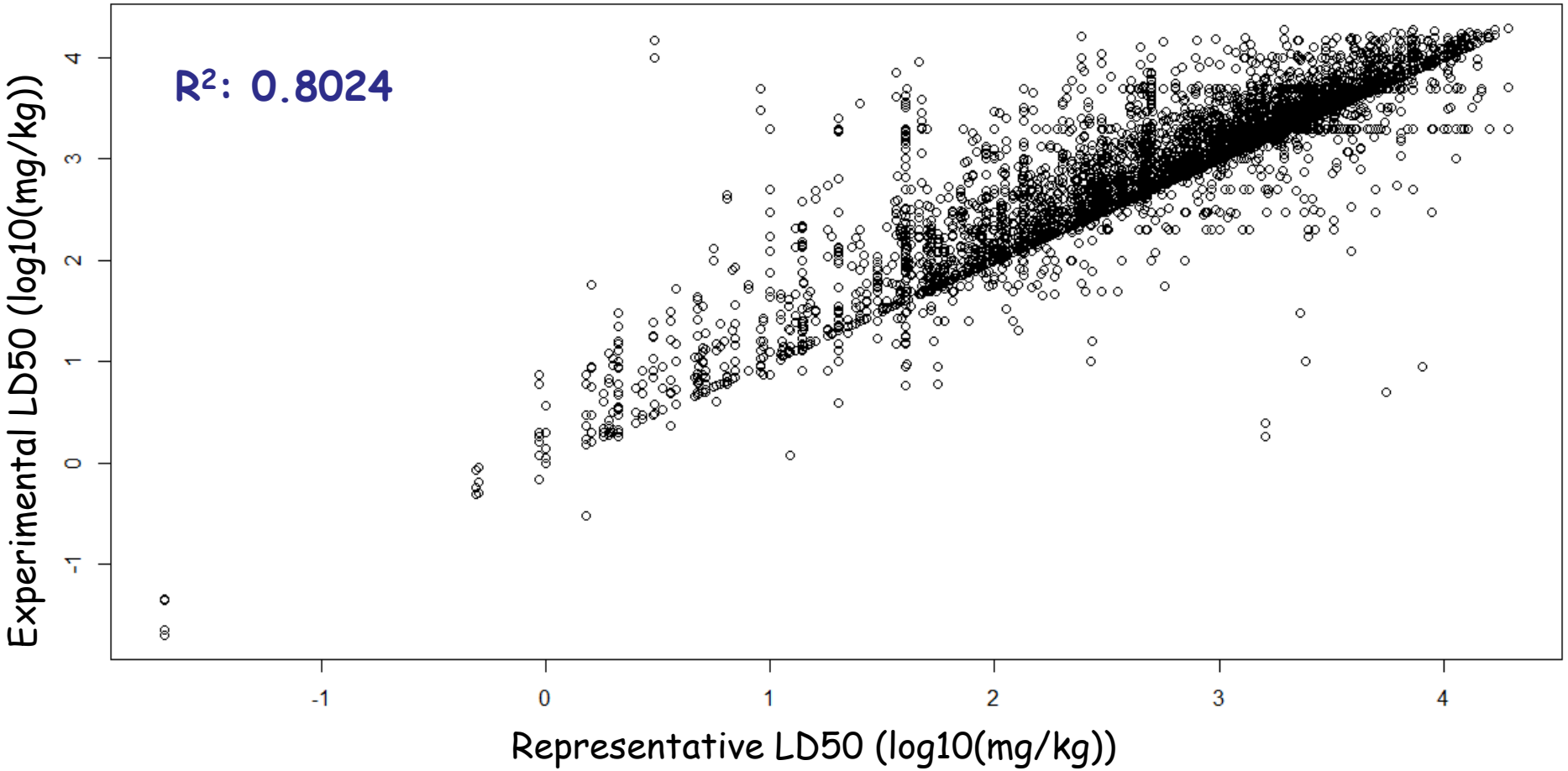
Defining a Confidence Range

Bootstrapping of the standard deviations for repeat test chemicals (~1120 with >3 replicates) identified a 95% confidence interval for LD50 values of ± 0.31 $\log_{10}$(mg/kg)



Assessing "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

Acute Toxicity Implementation Plan

- Coordinate activities via ICCVAM Workgroups
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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 US 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 US agencies

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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

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

Informed by the Conceptual Framework outlined in the NRC 2015 report prepared for DOD

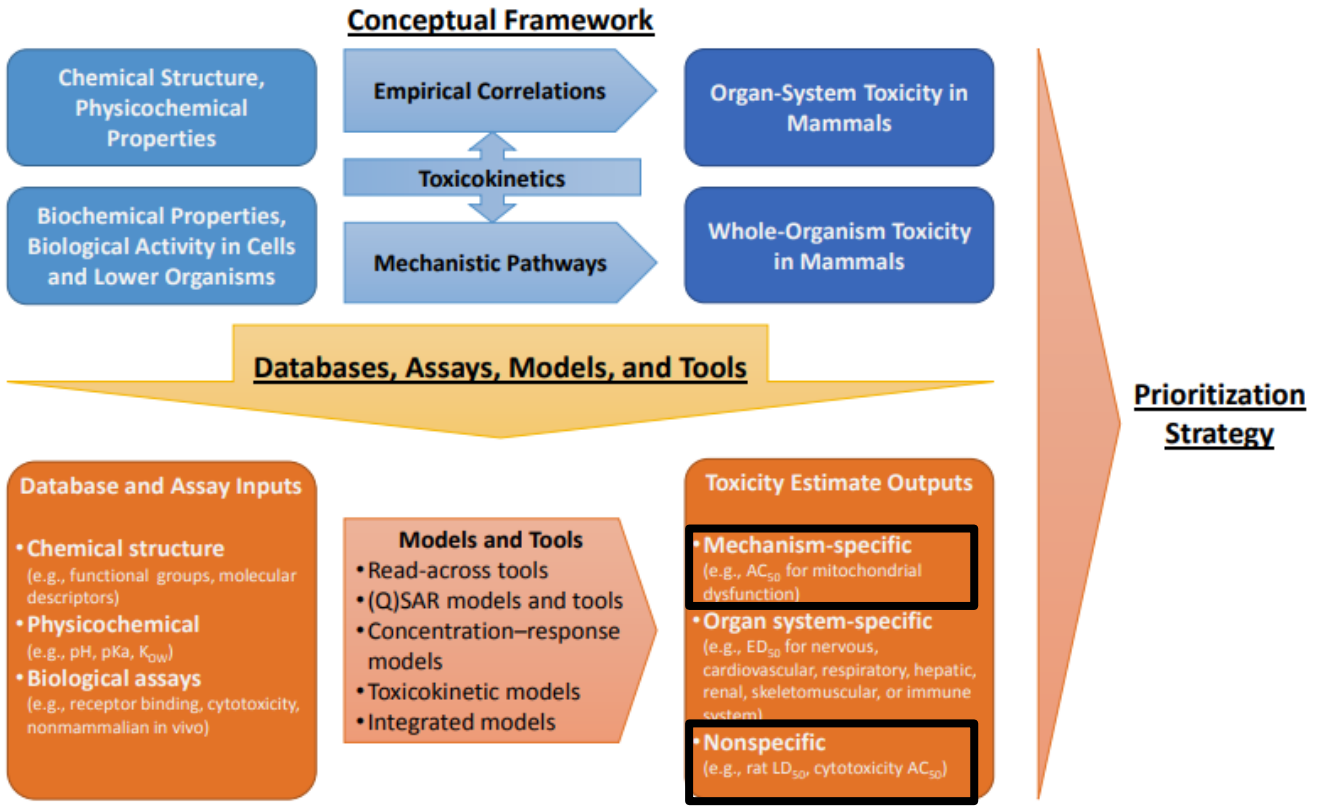
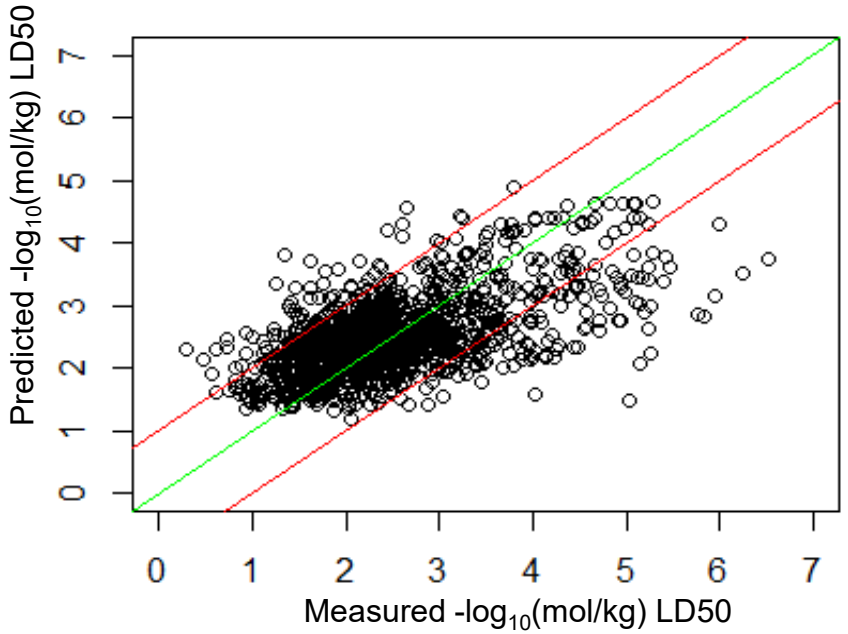


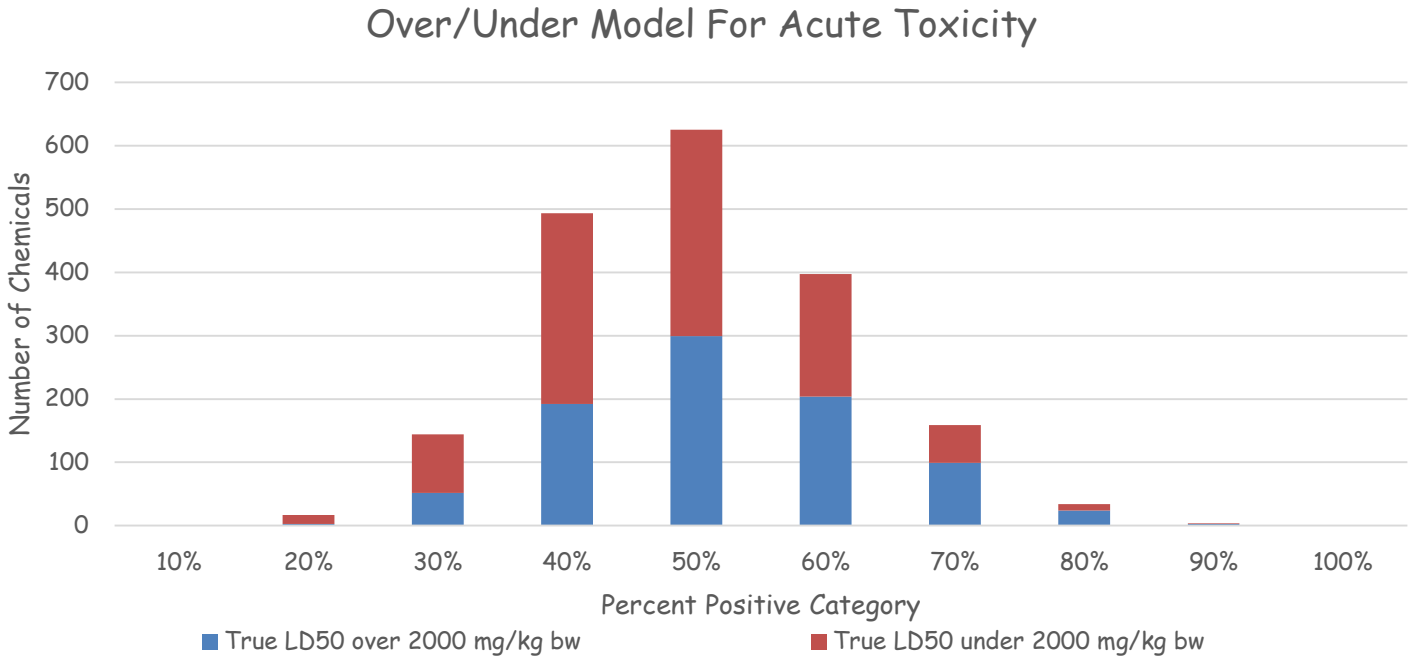
FIGURE S-1 Conceptual framework and examples of databases, assays, models, and tools for predicting acute chemical toxicity.

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

- Developing new Global 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

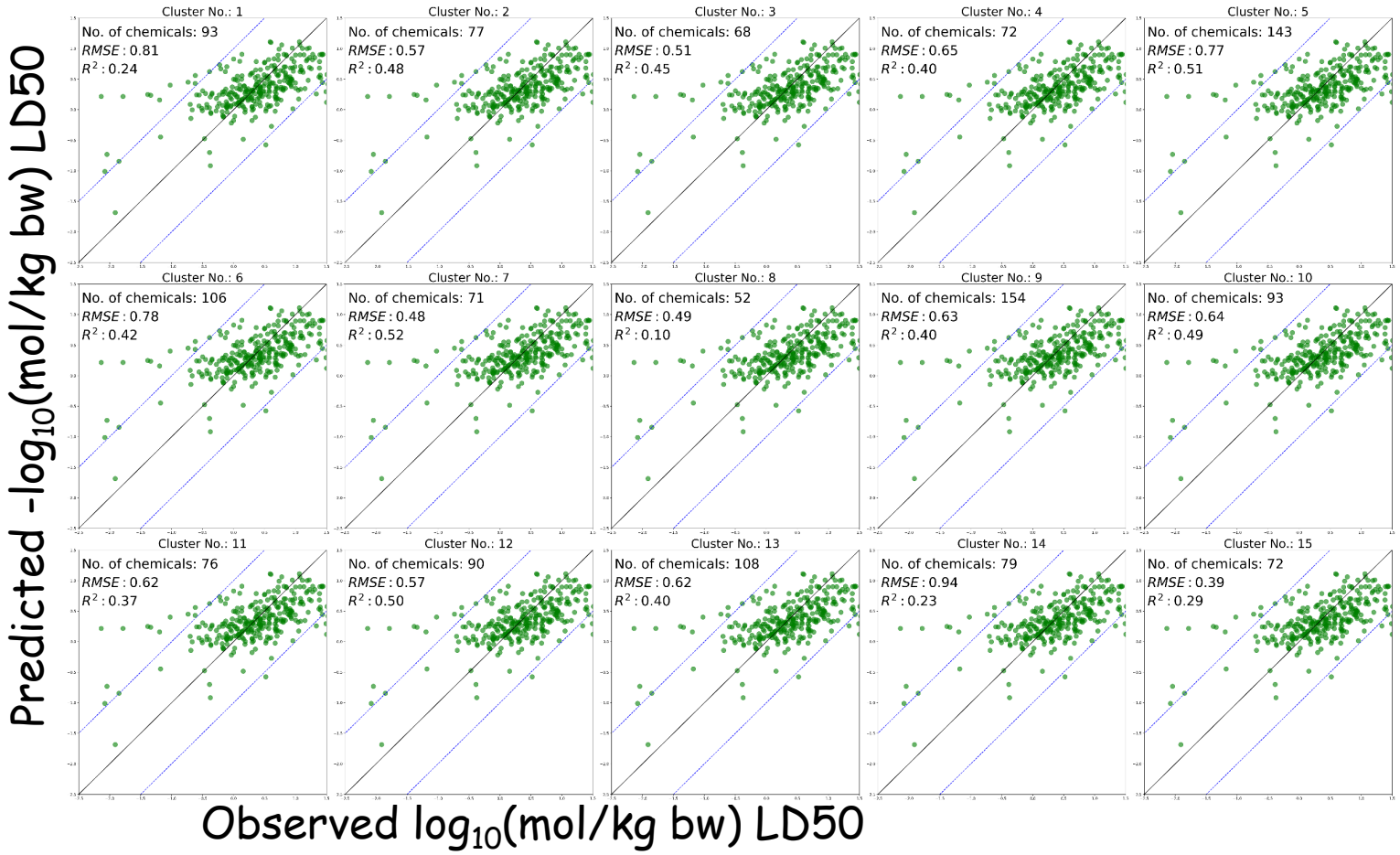
- **Developing new Local models:**
- **Local Cluster-based Regression Models – chemical, biological, hybrid and MOA-chemical**

1. Chemical Clusters-Chemical QSAR	2. Chemical Clusters-Biological QSAR	3. Biological Clusters-Chemical QSAR	4. Chemical-Biological Clusters, QSAR	5. MOA Clusters-Chemical QSAR
• **Chemical Descriptors** – ToxPrints – PaDEL Descriptors – CDK Descriptors	• **Chemical Descriptors** – ToxPrints • **Biological Descriptors** – ToxCast Group B assays	• **Biological Descriptors** – ToxCast Group B assays • **Chemical Descriptors** – ToxPrints – PaDEL Descriptors – CDK Descriptors	• **Chemical Descriptors** – ToxPrints • **Biological Descriptors** – ToxCast Group B assays	• Replace each assay by MOA • Final MOA outcome: = 1, if chemical active in any assay for the given MOA threshold = 0, otherwise • **Chemical Descriptors** • ToxPrints • PaDEL Descriptors • CDK Descriptors

Pradeep et al., in preparation

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

- Developing new Local models:
- Local Cluster-based Regression Model – chemical, biological, hybrid and MOA-chemical



$R^2 > 0.40$
10/15 clusters

Pradeep et al., in preparation

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

- Developing new Local models:
- Local Cluster-based Regression Model – chemical, biological, hybrid and MOA-chemical

TABLE 2-1 Biological Processes and Cellular Targets Associated with Acute Toxicity in Humans or Laboratory Animals[a, b]

Biological Process or Cellular Target	Example	Chemical or Biological Agent	Example Target Organ System	Examples of in vitro Assay Approaches[c]
Change in neurotransmitter function				
Altered axonal transport	Disruption of microtubule function	Vinca alkaloids β, β'-iminodipropionitrile	Nervous	Tubulin polymerization assessed with flow
Altered impulse conduction by axonal membrane	Blocking of Na^+ ion channel	Tetrodotoxin	Nervous	
Reduced precursor availability or neurotransmitter synthesis and storage	Inhibition of acetylcholine uptake into synaptic vesicle	Vesamicol Reserpine (dopamine)	Nervous	
Altered neurotransmitter release	Blocking of release of acetylcholine at neuromuscular junction Presynaptic release of acetylcholine and other neurotransmitters	Botulinum toxin α-latrotoxin	Nervous	
Altered neurotransmitter binding at receptor sites	Neurotransmitter agonists Neurotransmitter antagonists	Opioids, benzodiazepines, nicotine, anatoxin-a, kainic acid Curare, α-bungarotoxin, **3-quinuclidinyl benzilate**	Nervous	
Impaired neurotransmitter inactivation mechanisms	Acetylcholinesterase inhibition Altered dopamine transporter Altered serotonin reuptake Altered dopamine reuptake	**Nerve gas agents** Cocaine Fluoxetine Amphetamine	Nervous	
Altered ion flow				
Altered electrical conduction of heart or cardiomyocyte contractility	Sodium–potassium ATPase blockers	Digoxin	Cardiovascular	
Altered ion pump (Na^+, Ca^{++}, K^+) activity	Inhibit K^+ channel function Inhibit Na^+ channel function	Dendrodotoxin, 4-aminopyridine Tetrodotoxin, **saxitoxin**	Cardiovascular	

Pathways annotated in NRC report

	source	moa	assay_component_endpoint_name
2	NRC	Immune-mediated effects	BSK cell surface markers may inform l
3	NRC	Increased permeability of cellular membranes	mito mem potential/depolarization a
4	NRC	Altered bioenergetics	NA
5	NRC	Altered oxygen transport	NA
6	Hamm	Sodium-potassium ATPase inhibition	NA
7	Hamm	Protein synthesis inhibition	NA
8	Hamm	GSH depletion (followed by covalent binding of reactive metabol	NA
9	Hamm	Michael acceptor reaction	NA
10			
11	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_IC_hKhERGCh
12	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_IC_rCaBTZCHL
13	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_IC_rCaChN
14	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_IC_rCaDHPRCh_L
15	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_IC_rKAR
16	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_IC_rKATPCh
17	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_IC_rKCaCh
18	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_IC_rNaCh_site2
19	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_bGABAR_Agonist
20	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_bGABARa1
21	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_bGABARa5
22	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_h5HT3
23	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_hNNR_NBungSens
24	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_rAMPA
25	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_rGABAR_NonSelective
26	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_rGABARa6
27	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_rGluNMDA_Agonist
28	NRC; Hamm	altered ion flow; (voltage-gated) sodium channel inhibition	NVS_LGIC_rGluNMDA_MK801_Agonist

Alignments with existing ToxCast assays

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

- Developing new Local models:
- Local Cluster-based Regression Model – chemical, biological, hybrid and MOA-chemical

4 unique MOAs based on ToxCast assay data

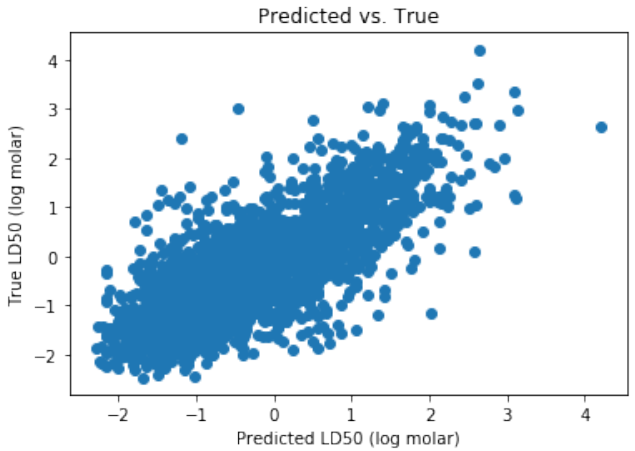
1. Cytotox
2. Oxidative Stress or ROS formation; Cell stress relevant
3. Mitochondrial inhibition
4. Anticoagulation

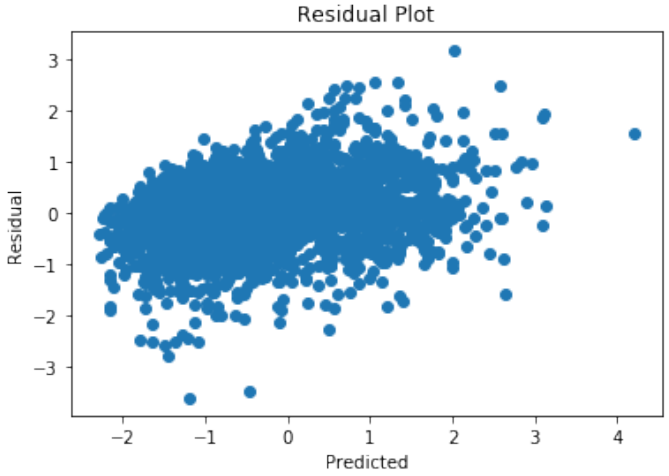
MOA threshold	Number of Clusters	Best R^2	Number of chemicals in the cluster
1	16	0.27	2779
2	5	0.27	3062
3	4	0.30	3179
4	4	0.31	3263

Pradeep et al., in preparation

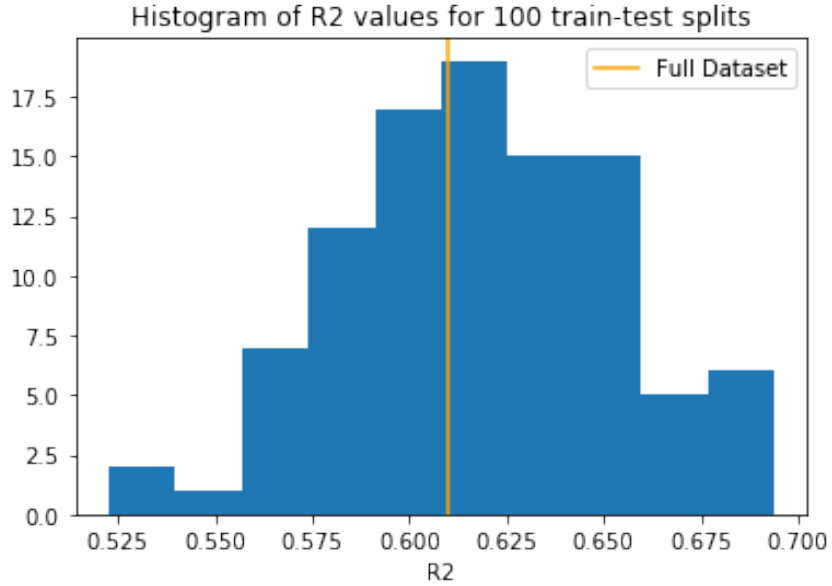
Identify and evaluate non-animal alternative approaches to acute toxicity testing

- Developing read-across models: using GenRA





- R^2 = 0.61
- RMSE = 0.58
- A few outliers, but not too extreme
- Residuals clustered around zero with no obvious patterns



- 75-25 train-test splits
- R^2 values range from 0.52 to 0.69

Helman et al., in preparation

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

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

- Use large database of rat oral LD50 values to train (and test) QSAR models to predict acute oral systemic 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.
- Models were qualitatively and quantitatively assessed and combined into consensus models.
- Consensus model performance compared with animal test reproducibility for binary, categorical, and quantitative models

https://ntp.niehs.nih.gov/go/tox-models

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

Predictive models for acute oral systemic toxicity: A workshop to bridge the gap from research to regulation

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Read-across
Acute oral toxicity
ICCVAM
Workshop

ABSTRACT

In early 2018, the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) published the "Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States" [1]. Cross-agency federal workgroups have been established to implement this roadmap for various toxicological testing endpoints, with an initial focus on acute toxicity testing. The ICCVAM acute toxicity workgroup (ATWG) helped organize a global collaboration to build predictive *in silico* models for acute oral systemic toxicity, based on a large dataset of rodent studies and targeted towards regulatory needs identified across federal agencies. Thirty-two international groups across government, industry, and academia participated in the project, culminating in a workshop in April 2018 held at the National Institutes of Health (NIH). At the workshop, computational modelers and regulatory decision makers met to discuss the feasibility of using predictive model outputs for regulatory use in lieu of acute oral systemic toxicity testing. The models were combined to yield consensus predictions which demonstrated excellent performance when compared to the animal data, and workshop outcomes and follow-up activities to make these tools available and put them into practice are discussed here.

Predictive Models for Acute Toxicity: Performance vs Animal Data

	Rat Oral LD50: Reproducibility			Consensus Model Performance (Tr/Ts Avg)		
	Sensitivity	Specificity	BA	Sensitivity	Specificity	BA
VT	63%	99%	81%	77%	95%	86%
NT	96%	82%	89%	82%	92%	87%
EPA	74%	91%	82%	62%	94%	78%
GHS	66%	92%	79%	54%	92%	73%

	R2	RMSE	R2	RMSE
LD50	0.8	0.42	0.74	0.42

Summary remarks

- Outlined ATWG charges
- Substantial progress has been made in outlining the decision contexts, needs and gathering the acute toxicity data to inform the array of in silico modelling efforts
- Evaluating the variability of the acute toxicity data is a key consideration both in terms of the impact this has in current hazard assessments but also in managing expectations of the performance of new models