UNITED STATES ICCVAM
Advancing Alternatives to Animal Testing

Interagency Coordinating Committee on the Validation of Alternative Methods

Implementation: Alternatives to Acute Systemic Toxicity Testing and Other Areas

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SACATM Meeting
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Agency for Toxic Substances and Disease Registry • Consumer Product Safety Commission • Department of Agriculture
Department of Defense • Department of Energy • Department of the Interior • Department of Transportation
Environmental Protection Agency • Food and Drug Administration • National Institute for Occupational Safety and Health
National Institutes of Health • National Cancer Institute • National Institute of Environmental Health Sciences Institute • National Institute of Standards and Technology • Occupational Safety and Health Administration

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2017

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

Acute Toxicity Workgroup

- *Alison Myska (DOD)
- *Grace Patlewicz (EPA)
- Kent Carlson (CPSC)
- Xinrong Chen (CPSC)
- John Gordon (CPSC)
- Joanna Matheson (CPSC)
- Lyle Burgoon (DOD)
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- Judy Strickland
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*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

GHS

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

Hazard

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

Label Review Manual

Chapter 10: Worker Protection Label

~300 formulation submissions/year

~10 active ingredient submissions/year

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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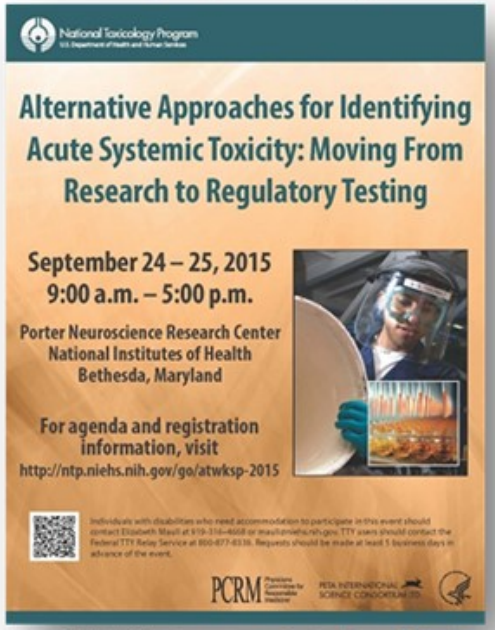
Workshop on Acute Toxicity Testing (2015)

- > 60 participants from industry, academia, and ICCVAM agencies
- Recommendations:
  - Clear understanding of agency requirements
    - *Strickland et al., in preparation*
  - Emphasize training and education
    - NICEATM and PISC outreach/reviewer training
  - International harmonization 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 https://ntp.niehs.nih.gov/go/atwksp-2015

Interagency Coordinating Committee on the Validation of Alternative Methods

Workshop on Acute Toxicity Testing (2017)

NC 3Rs

~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 Harmonization:

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

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Rat oral acute toxicity LD50 Database

- Multiple existing resources containing rat oral acute toxicity LD50 data were mined and merged

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

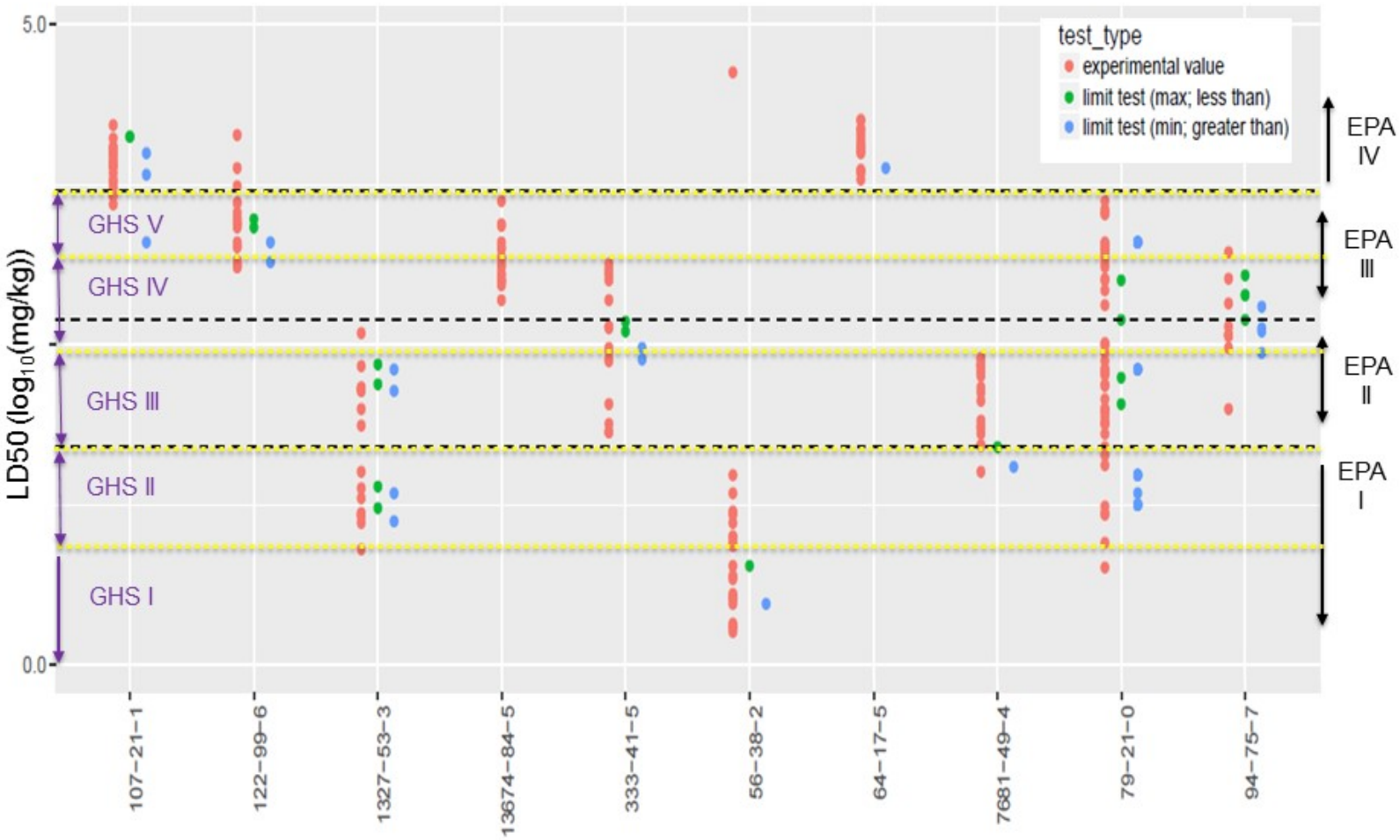
Total:
34,511 LD50 values
16,307 chemicals

Identify unique data in mg/kg

21,210 LD50 values
15,698 chemicals

- LD50 data comprised point estimates as well as limit tests

Impact of Variability on Hazard Classification



EPA: Data Extraction from Pesticide Formulations

816	Product Names
437	Products with 1 a.i.
227	Products with 2 a.i.
152	Products with ≥3 a.i.

- NICEATM CBI-cleared to extract data from FIFRA DERs
- Data from all “6-pack” endpoints have been extracted for 816 products
- Final database entry (ICE): October 2017

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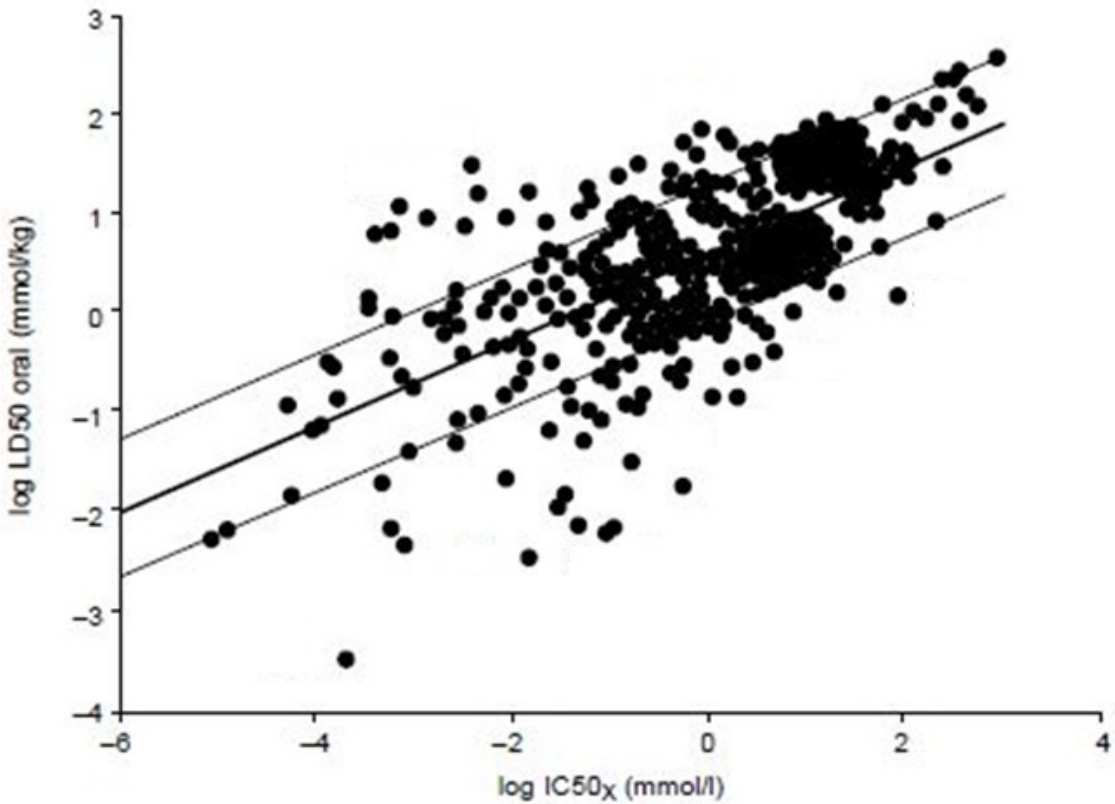
Using Cytotoxicity to Predict Acute Toxicity: A Historical Perspective



Registry of Cyotoxicity (Halle. 2003. ATLA 31:89-198)

Using HTS Assays to Predict Acute Oral Toxicity

- No single in vitro Tox21/ToxCast assay predicted in vivo lethality
- Combining assay results using machine learning marginally improved LD50 prediction
  - The best performance was obtained using random forest for a binary prediction of toxic vs. nontoxic
  - Accuracy of 62-71% is inadequate for animal replacement
- Structure-based models outperform in vitro models (80-90%)
- Next steps to improve predictions
  - Develop large curated database to train hybrid QSAR models
  - Design strategies that integrate chemical structure, physicochemical properties, and mechanistic information
  - Build local models based on chemical characteristics/use cases

Development of In Silico Models for Acute Oral Toxicity

- Interested QSAR modelers will be tasked with building models to predict acute oral systemic toxicity
- Agency input on model output has been solicited
  - Various quantitative and categorical endpoints requested
- Training and test data will be derived from the dataset used to analyze LD50 variability
  - 15,698 chemicals with 21,210 LD50 values
  - Coordinated with ICCVAM ATWG

EPA Guidance on Waiving Dermal Toxicity

EPA United States Environmental Protection Agency

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New EPA Guidance for Testing Pesticides Will Reduce Animal Testing

For Release: November 29, 2016

EPA is issuing guidance for requesting waivers of acute dermal toxicity testing requirements for pesticide formulations, which will lead to fewer animal tests for acute dermal toxicity for pesticides. Last March, EPA released a "Draft Retrospective Analysis for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations," which included guidance for pesticide manufacturers to request waivers of acute dermal toxicity studies for formulations.

EPA is now finalizing its Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations. This guidance is in line with the Office of Pesticide Programs' Strategic Vision for implementing the 2007 National Research Council's report on Toxicity Testing in the 21st Century. EPA's Pesticide Program receives about 200-300 dermal formulation toxicity tests annually, each of which generally use 10 animals per test. We expect this waiver guidance to save 2,500 or more laboratory animals every year.

3.0 Waiver Guidance.

The agency believes this retrospective analysis fully supports the conclusion that waivers may be granted for acute dermal toxicity studies for formulated pesticide products. Applicants should submit formal waiver requests as part of their registration application through existing processes[7]. Waiver requests should contain all relevant information to support the waiver (e.g., acute oral LD_{50} and dermal irritation study data) and cite this guidance.

https://www.epa.gov/pesticides/new-epa-guidance-testing-pesticides-will-reduce-animal-testing

Waiving Dermal Toxicity Testing: International Status

> 2000mg/kg via the oral route (2015)

OECD Guidance Document 237: > 2000 mg/kg via the oral route (2016)

Any category, pesticide formulations only (2016)

Pesticide products and active ingredients (2017)

Challenges

- Animal methods currently provide the reference data for evaluating alternatives
  - Results are variable
  - Need to identify appropriate summary metrics & characterize uncertainty
- Data requirements vary across U.S. and global regulatory authorities and are often ambiguous
- Overcoming regulatory and institutional inertia
  - Education and training, communication with method/model developers

Other Areas

- Three new workgroups have recently been formed:
  - Developmental and Reproductive Toxicology
    - Sponsoring Agency: FDA
  - In Vitro to In Vivo Extrapolation
    - Sponsoring Agencies: ATSDR, EPA
  - Read Across
    - Sponsoring Agencies: EPA, FDA

END

BACK POCKET – if asked

Acute Inhalation Toxicity Testing

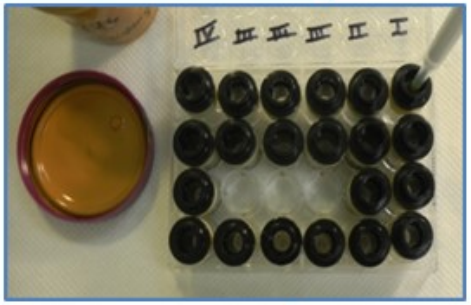
- 2016 webinar series and workshop outcomes - stakeholder workgroups tasked with:
  - Developing a database of existing acute systemic toxicity data
  - Preparing a state-of-the-science review on mechanisms and non-animal approaches for acute inhalation toxicity (*final draft under review*)
  - Developing an *in silico* decision tree
  - Designing and conducting an in vitro proof-of-concept
- *Workshop report to be submitted this Fall*

Eye Irritation: Private-Public Partnership

- Crop Life America-EPA-NICEATM
  - BASF, Dow, Bayer, Syngenta, Dupont
- Paired data for approximately 200 pesticides
- Rabbit eye test data + in vitro data in one or more assays:
  - Bovine corneal opacity and permeability (BCOP, OECD TG 437)
  - Isolated chicken eye (ICE, OECD TG 438)
  - EpiOcular (EO, OECD TG 492 and ET40 protocol)
  - Neutral red release (NRR)
  - Chorioallantoic membrane vascular assay (CAMVA)

Evaluating Alternative Approaches for Eye Irritation: OptiSafe Method

- Manufactured kit for ocular irritant/non-irritant classification
- Irritation prediction based on measured molecular damage
- 2-Phase Validation Study
  - Bottom-up approach (non-irritants vs all irritant classes)
  - Phase I: Initial qualification of naïve labs and protocol refinement
  - Phase II: Testing of 30 chemicals by all 3 labs, additional 60 tested by main lab
- ICCVAM Ocular/Dermal Irritation Workgroup members make up the VMT

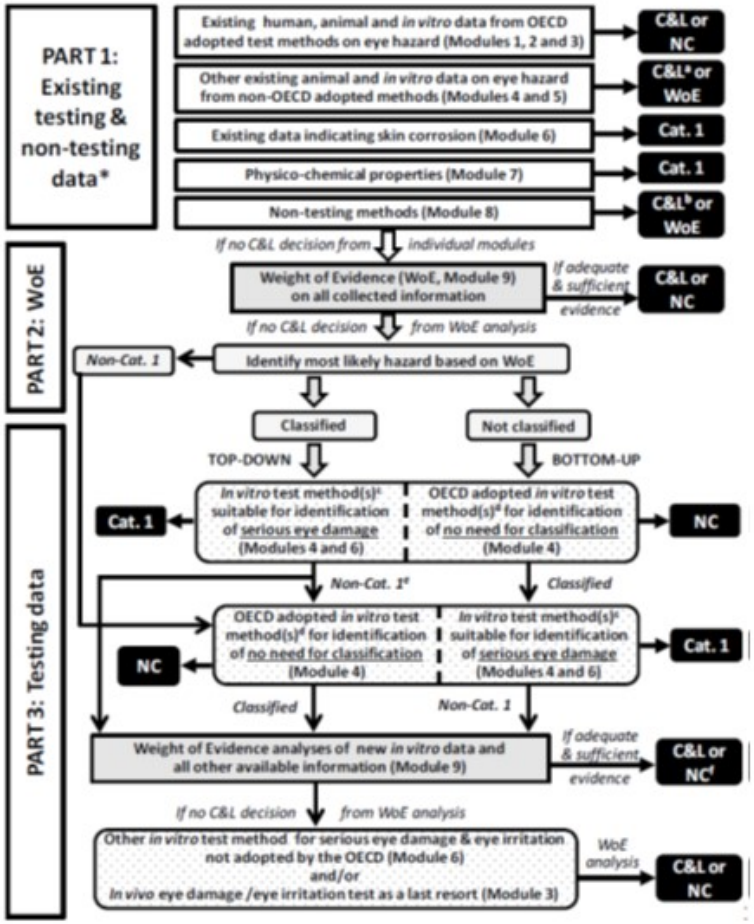


Ongoing Eye Irritation Data Collection/Curation

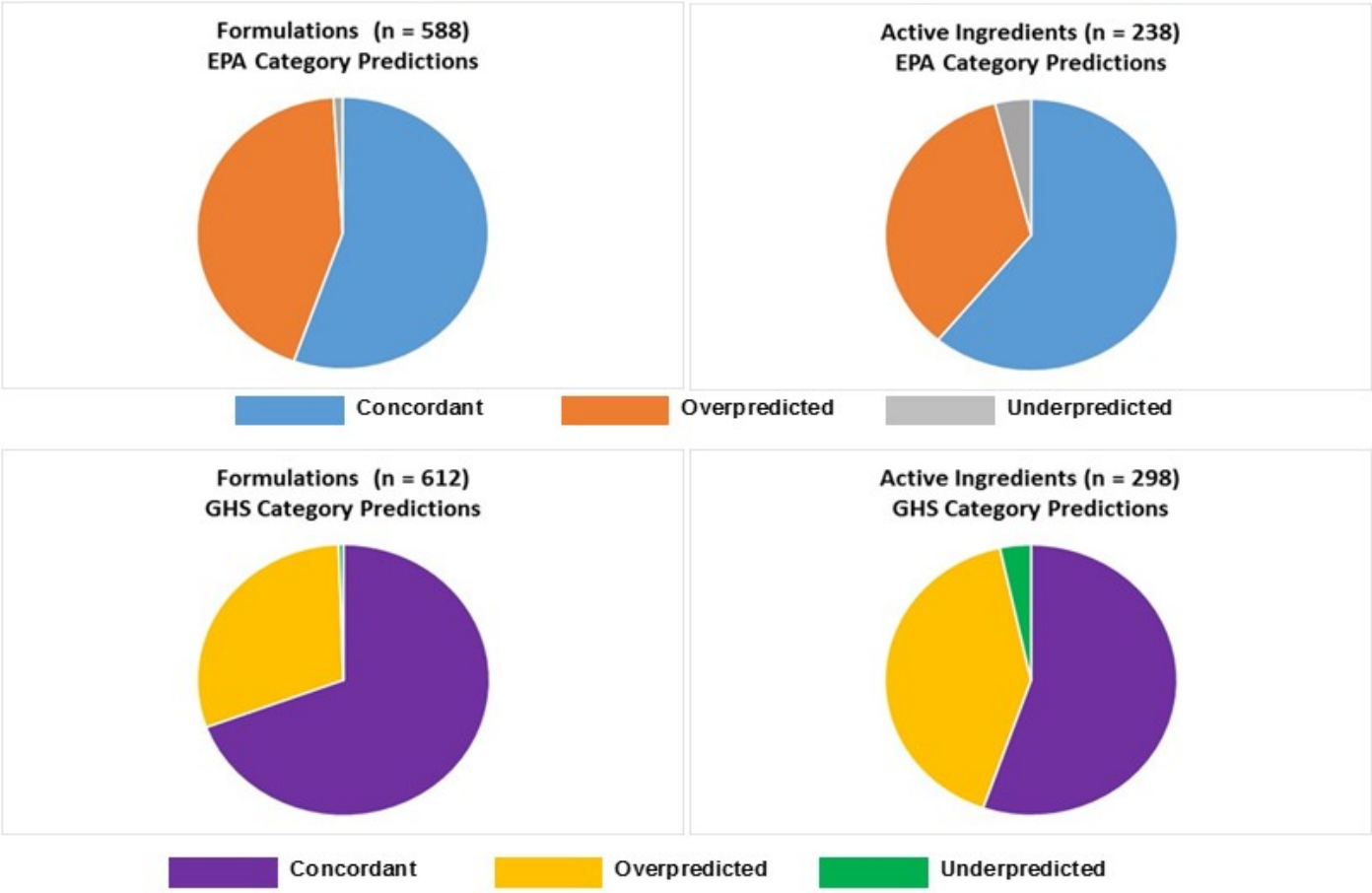
- EPA FIFRA
- CropLife America (paired in vivo and in vitro ocular)
- Other stakeholders (EPA-led stakeholder discussions)
- Obtaining physicochemical properties for individual chemicals
- Generating QSAR predictions where feasible to use in integrated approaches

International Harmonization: IATA for Eye Irritation

- OECD Guidance Document 263 (US and EU co-led project)
- Three parts:
  1. Existing and available information (physchem properties QSAR, read across, bridging)
  2. Weight of evidence
  3. New testing (in vitro and/or in vivo)

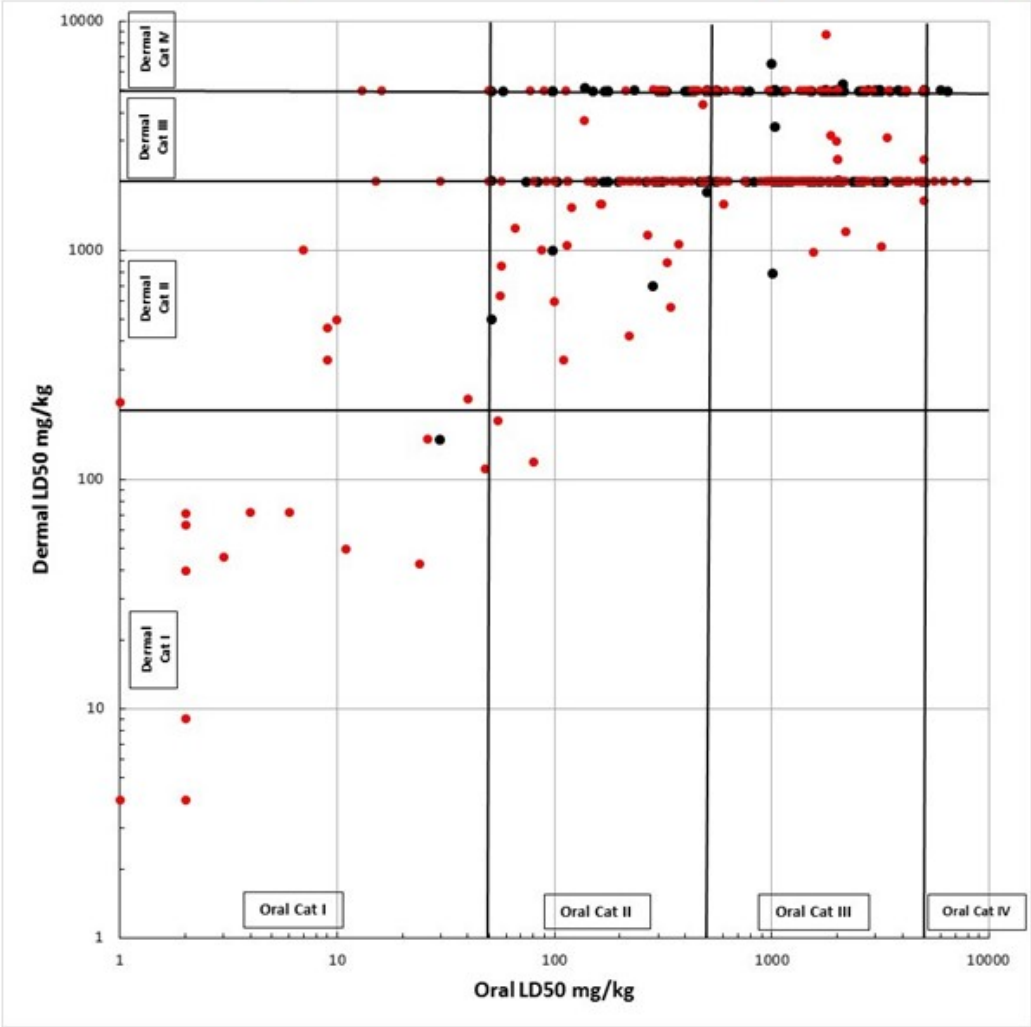


Waiving Dermal Toxicity Testing



- Comparison of Concordance, Overprediction, and Underprediction of Formulations and Active Ingredients using EPA or GHS Classification Systems

Waiving Dermal Toxicity Testing



● pesticide formulations

● pesticide active ingredients