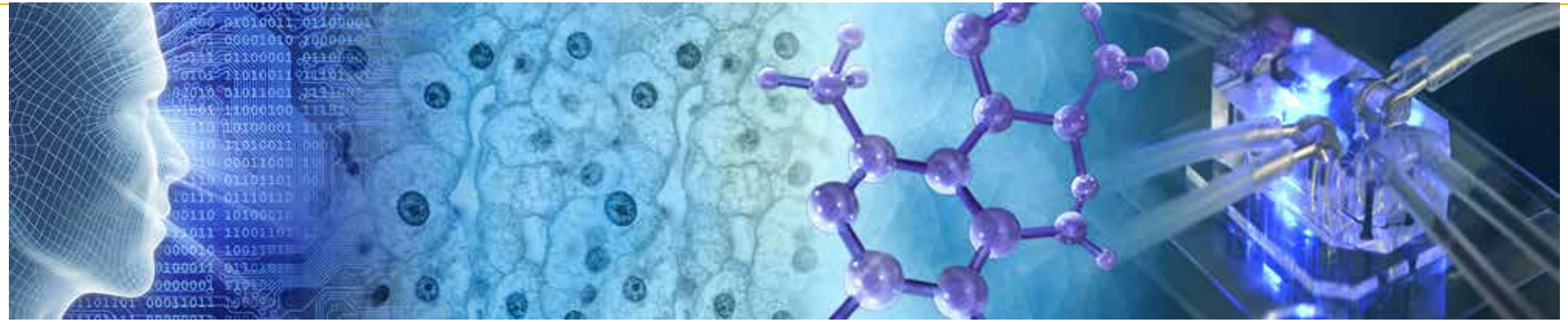
NTP
National Toxicology Program



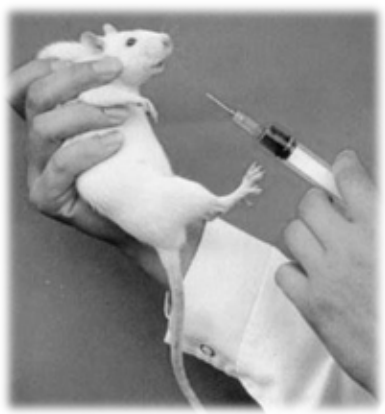
Regulatory needs: Can existing data be used to derive acute lethality estimates without animal tests?

Warren Casey, PhD, DABT
Director, NICEATM

October 30-31, 2019

LD50

- Introduced in 1928, acute oral lethality (Rat LD50), is the most commonly conducted toxicity test worldwide.

1928	2019
	
	
	

U.S. Statutes and Regulations

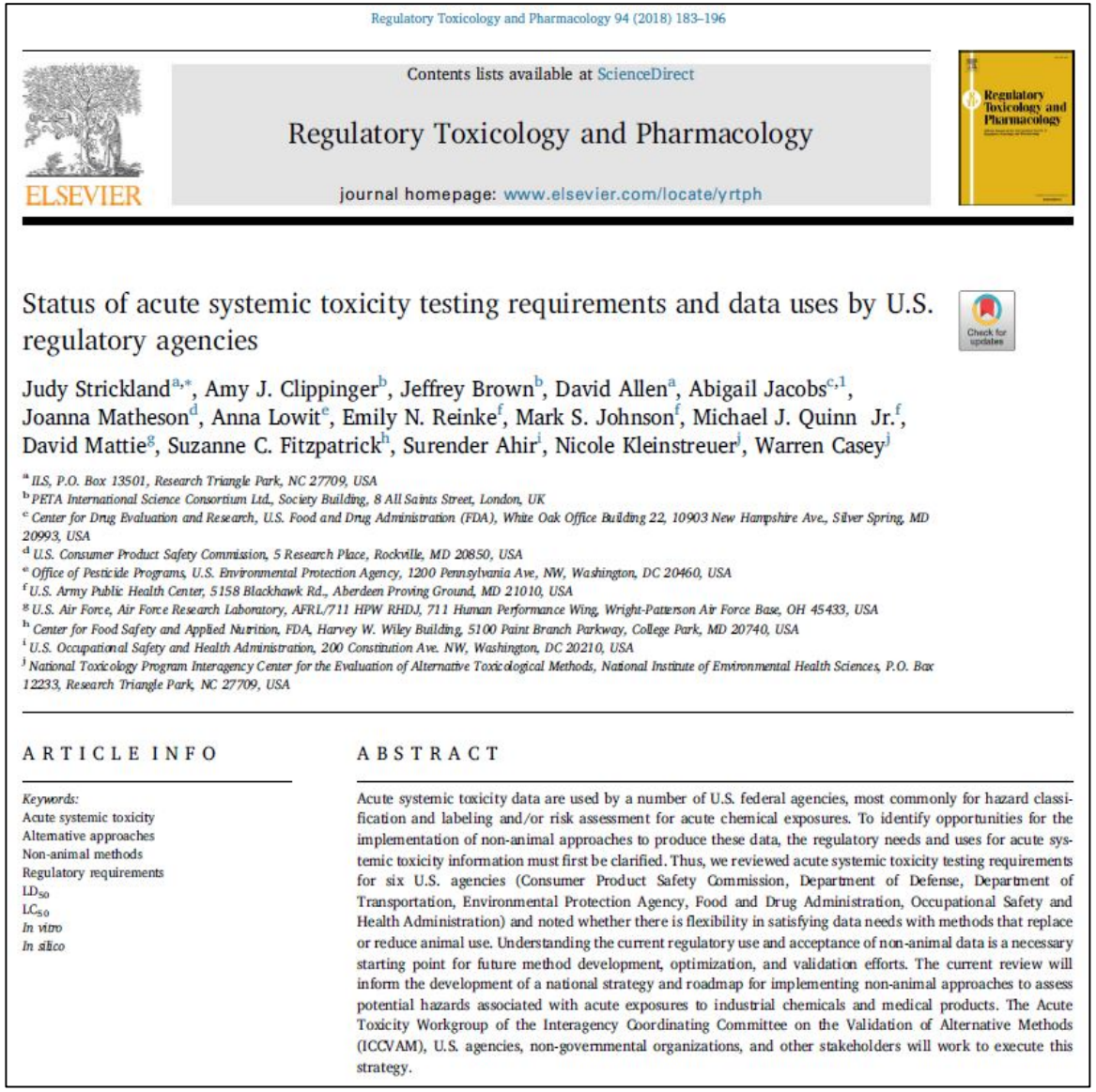
US Statute/Regulations	Agency
Federal Hazardous Substances Act (FHSA) (1964): 16 CFR 1500.3: **Consumer Products**	CPSC
Poison Prevention Packaging Act (1970): 16 CFR 1700: **Hazardous Household Substances**	CPSC
Hazardous Materials Transportation Act (1970); 49 CFR 173.132: **Transported Hazardous Substances**	DOT
Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6): 40 CFR 156; 40 CFR 158.500**: Pesticides**; CFR 158.2230: **Antimicrobials**	EPA
Toxic Substances Control Act (TSCA; 1976, amended 2016): 40 CFR 720.50: **Industrial Chemicals**	EPA
Federal Food, Drug, and Cosmetic Act (1938): **Biologicals**	FDA
Federal Food, Drug, and Cosmetic Act (1938): **Food Ingredients**	FDA
Occupational Safety and Health Act (1970): 29 CFR 1910.1200: **Workplace Chemicals**	OSHA

+ DoD

Scoping Regulatory Needs

ICCVAM Acute Toxicity Workgroup

- Identifies federal agency requirements, needs, and decision contexts for using acute systemic toxicity data



Regulatory Toxicology and Pharmacology 94 (2018) 183–196

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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

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ARTICLE INFO

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Acute systemic toxicity
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Non-animal methods
Regulatory requirements
LD_{50}
LC_{50}
In vitro
In silico

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.

LD50

- Single chemicals
- Formulations and mixtures

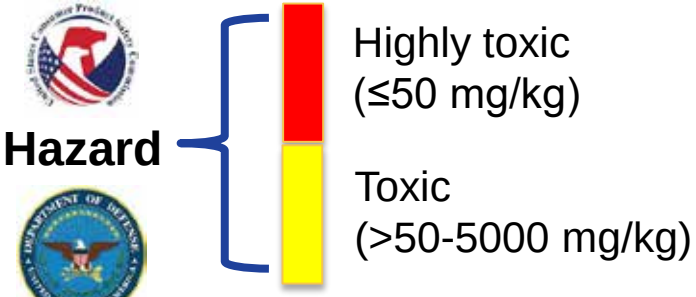
LD50

- Quantitative Risk Assessment for Human Health and Eco Tox
- Classification and Labelling

Agency Data Needs

Binary Models

Hazard



+ Nontoxic (>2000 mg/kg)

Continuous Model

Point estimates of LD50 values

Categorical Models

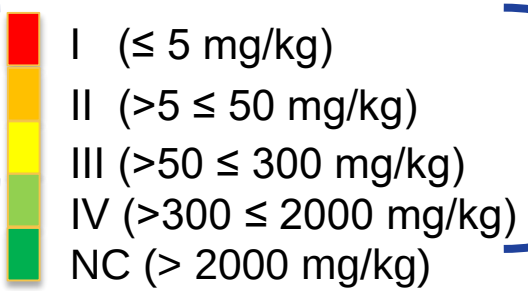
Hazard

EPA Categories

Hazard

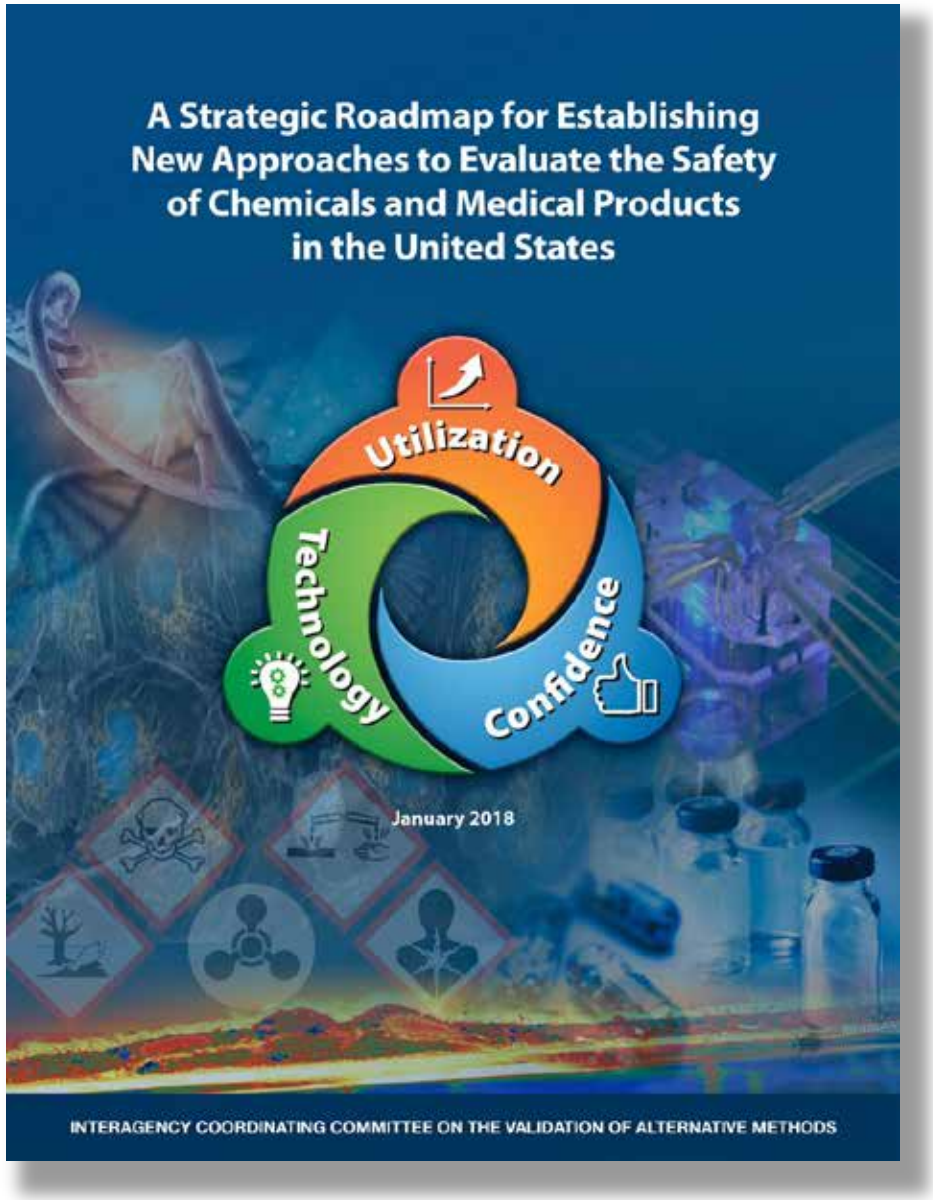
GHS Categories

Packing Group



OSHA

Hazard



Integrate Processes That:

- **Connect end users with the developers of alternative methods**
- **Establish new validation approaches that are more flexible and efficient**
- **Ensure adoption and use of new methods by both regulators and industry**

The way forward?

In Silico (+) predictions for individual chemicals

+

Model(s) used to combine data for individual chemicals

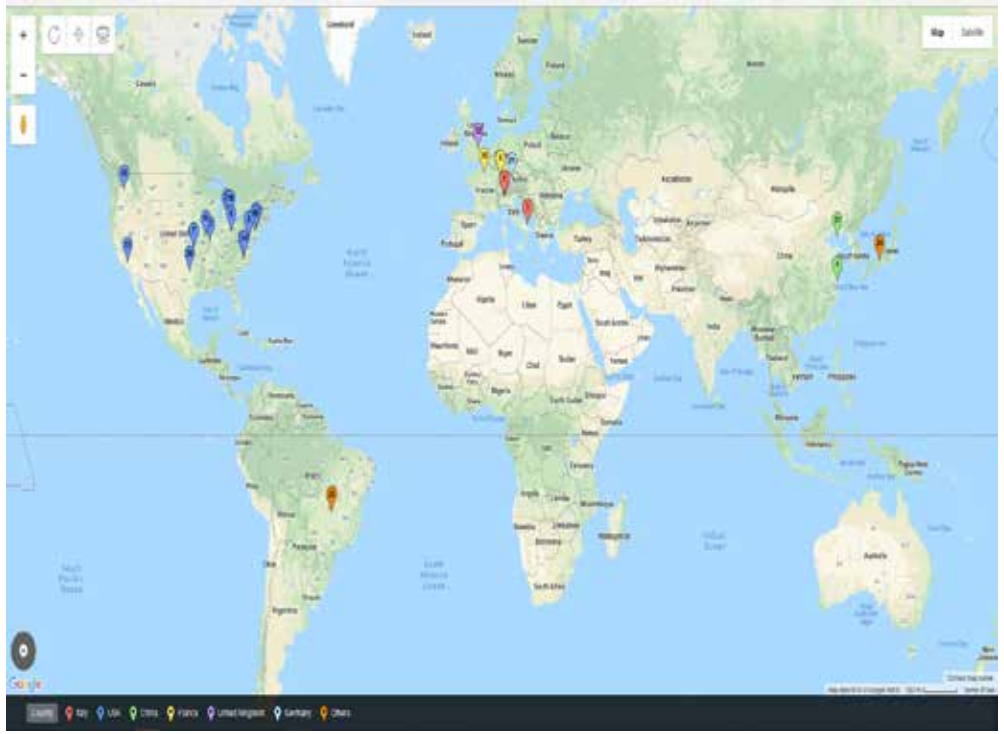
ICCVAM Workshop: Predictive Models for Acute Oral Systemic Toxicity, April 11-12, NIH, Bethesda

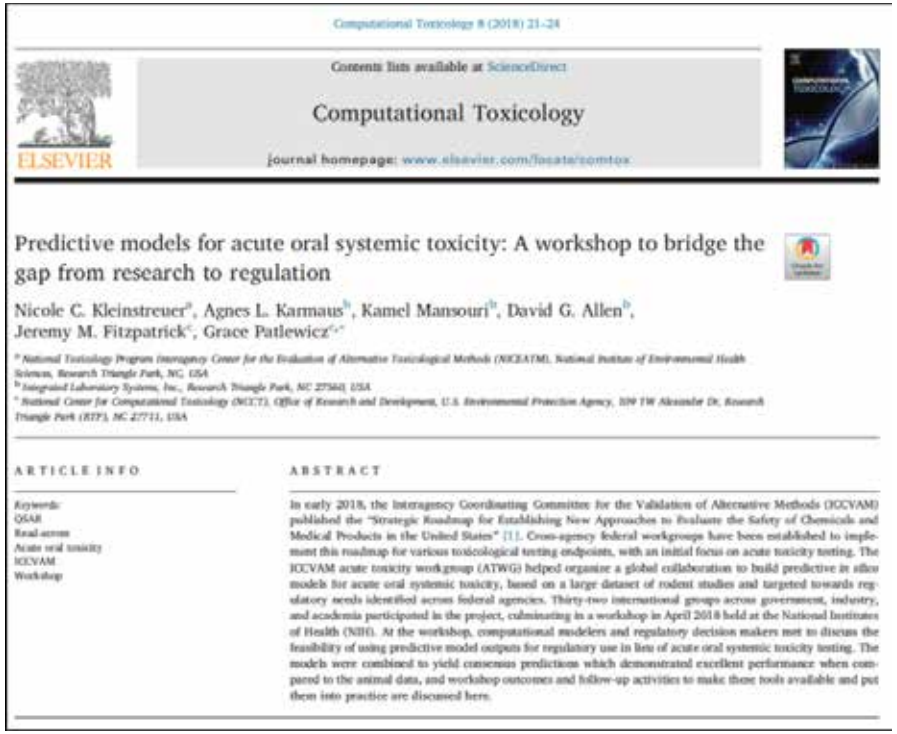
- Scientists were invited to submit in silico models that use chemical structure information to predict LD50 values and hazard categories
  - Largest set of curated LD50 data ever assembled: ~21,000 LD50 values for ~15,000 chemicals (available on NICEATM web site)
  - 130 Models, 32 Groups (20 Academic, 8 Industry, 4 Fed), 8 Countries
  - Attendance: 90 in-person, 170 Webcast
  - Results are promising and continue to be evaluated!

International Collaboration

Consortium:

- **35 Participants/Groups** from around the globe representing academia, industry, and government contributed **139 models**





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ELSEVIER

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.

CATMoS consensus modeling

Steps of combining the single models into consensus

Initial models & predictions

- VT (32 models)
- NT (33 models)
- GHS (23 models)
- EPA (26 models)
- LD50 (25 models)

Combining models

Step 1

Weighted average /majority rule

Independent consensus models/predictions

- VT
- NT
- GHS
- EPA
- LD50

A consensus model per endpoint (~20-~30 models)

Weight of Evidence approach (WoE)

Step 2

Majority rule

Consistent consensus models/predictions

- VT
- NT
- GHS
- EPA
- LD50

Consensus representing all ~140 models

Predicting Acute Toxicity of Mixtures

- GHS additivity formulas for classifying formulations and mixtures for the acute toxicity

The acute toxicity estimate (ATE) of ingredients should be considered as follows:

- Include ingredients present at 1% or greater with a known acute toxicity, which fall into any of the GHS acute toxicity categories.
- Ignore ingredients that are presumed not acutely toxic (e.g., water, sugar).
- Ignore ingredients if the oral limit test does not show acute toxicity at 2,000 mg/kg/body weight.

The ATE of the mixture is determined by calculation from the ATE values for all relevant ingredients according to the following formula below for Oral, Dermal or Inhalation Toxicity:

$$\frac{100}{ATE_{mix}} = \sum_{\eta} \frac{C_i}{ATE_i}$$

where:
C_i= concentration of ingredient i
n ingredients and i is running from 1 to n
ATE_i = Acute Toxicity Estimate of ingredient I

Leveraging Existing Data for Formulations Using Additivity

Regulatory Toxicology and Pharmacology 82 (2016) 99–110

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

GHS additivity formula: A true replacement method for acute systemic toxicity testing of agrochemical formulations

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GHS
Theory of additivity
Replacement
Global policy change
3Rs

ABSTRACT

Acute systemic (oral, dermal, inhalation) toxicity testing of agrochemical formulations (end-use products) is mainly needed for Classification and Labelling (C&L) and definition of personal protection equipment (PPE). A retrospective analysis of 225 formulations with available *in vivo* data showed that: A) LD_{50}/LC_{50} values were above limit doses in <20.2% via oral route but only in <1% and <2.4% of cases via dermal and inhalation route, respectively; B) for each formulation the acute oral toxicity is always equal or greater than the Acute Toxicity Estimate (ATE) via the other two routes; C) the GHS (Global Harmonised System) computational method based on ATE, currently of limited acceptance, has very high accuracy and specificity for prediction of agrochemical mixture toxicity according to the internationally established classification thresholds.

By integrating this evidence, an exposure- and data-based waiving strategy is proposed to determine classification and adequate PPE and to ensure only triggered animal testing is used. Safety characterisation above 2000 mg/kg body weight or 1.0 mg/L air should not be recommended, based on the agrochemical exposure scenarios. The global implementation of these tools would allow a remarkable reduction (up to 95%) in *in vivo* testing, often inducing lethality and/or severe toxicity, for agrochemical formulations.

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GHS additivity formula: can it predict the acute systemic toxicity of agrochemical formulations that contain acutely toxic ingredients?

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ARTICLE INFO

Keywords:
GHS additivity formula
Mixtures
Formulations
Agrochemicals
Theory of additivity
Acute toxicity
Selection bias

ABSTRACT

In vivo acute systemic testing is a regulatory requirement for agrochemical formulations. GHS specifies an alternative computational approach (GHS additivity formula) for calculating the acute toxicity of mixtures. We collected acute systemic toxicity data from formulations that contained one of several acutely-toxic active ingredients. The resulting acute data set includes 210 formulations tested for oral toxicity, 128 formulations tested for inhalation toxicity and 31 formulations tested for dermal toxicity. The GHS additivity formula was applied to each of these formulations and compared with the experimental in vivo result. In the acute oral assay, the GHS additivity formula misclassified 110 formulations using the GHS classification criteria (48% accuracy) and 119 formulations using the USEPA classification criteria (43% accuracy). With acute inhalation, the GHS additivity formula misclassified 50 formulations using the GHS classification criteria (61% accuracy) and 34 formulations using the USEPA classification criteria (73% accuracy). For acute dermal toxicity, the GHS additivity formula misclassified 16 formulations using the GHS classification criteria (48% accuracy) and 20 formulations using the USEPA classification criteria (36% accuracy). This data indicates the acute systemic toxicity of many formulations is not the sum of the ingredients' toxicity (additivity); but rather, ingredients in a formulation can interact to result in lower or higher toxicity than predicted by the GHS additivity formula.

Additivity Calculation: GHS Classification

Van Cott et al. 2018 - Additivity

In vivo	1	2	3	4	5	NC	Total
1	0	0	0	0	0	0	0
2	0	0	1	0	0	0	1
3	0	0	5	16	2	7	30
4	0	0	2	60	18	34	114
5	0	0	2	5	6	4	17
NC	0	0	0	11	8	29	48
Total	0	0	10	92	34	74	210

Correct classification: 48% (100/210)
Over classification: 39% (82/210)
Under classification: 13% (28/210)

Corvaro et al. 2016 - Additivity

In vivo	1	2	3	4	5	NC	Total
1	0	0	0	0	0	0	0
2	0	1	0	0	0	0	1
3	0	0	3	4	0	0	7
4	0	0	2	22	10	3	37
5	0	0	0	9	16	27	52
NC	0	0	0	1	9	92	102
Total	0	1	5	36	35	122	199

Correct classification: 67% (134/199)
Over classification: 11% (21/199)
Under classification: 22% (44/65)

1 (≤ 5 mg/kg)
2 (>50 ≤ 50 mg/kg)
3 (>50 ≤ 300 mg/kg)
4 (>300 ≤ 2000 mg/kg)
5 (> 2000 ≤ 5000 mg/kg)
NC (> 5000 mg/kg)

Additivity Calculation: EPA Classification

Van Cott et al. 2018 - Additivity

In vivo	I	II	III	IV	Total
I	0	1	0	0	1
II	0	12	42	19	73
III	0	7	69	45	121
IV	0	0	5	10	15
Total	0	20	116	74	210

Correct classification: 43% (91/210)
Over classification: 6% (12/210)
Under classification: 51% (107/210)

Corvaro et al. 2016 - Additivity

In vivo	I	II	III	IV	Total
I	0	0	0	0	0
II	0	6	9	0	15
III	0	1	51	30	82
IV	0	1	9	92	102
Total	0	8	69	122	199

Correct classification: 75% (149/199)
Over classification: 6% (11/199)
Under classification: 19% (39/199)

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

Overall Assessment of the Additivity Calculation

- Datasets are skewed towards less toxic substances (e.g., Covaro et al. 184/199 are EPA Category III or IV)
- For most in vivo Category IV substances that are identified as “false positive” based on the additivity equation, the calculated value is 2000 mg/kg < LD50 < 5000 mg/kg
- For most in vivo Category III substances that are identified as “false negative” based on the additivity equation, the in vivo LD50 is 2000 mg/kg < LD50 < 5000 mg/kg
- EPA pilot program: GHS Mixtures Equation Pilot
  - OPP has been accepting submissions of oral and inhalation toxicity data paired with calculations done in accordance with the GHS to support evaluations of pesticide product formulations
  - NICEATM data analyses ongoing and will compare to the trends seen above

Other considerations

- Variability
- ADMET; bioaccumulation, protein binding, metabolism/clearance (species specific)
- Combined approach: Global + Local + Read Across + In Vitro (mechanistic)

Priorities?

- Refine QSAR(+) for individual chemicals (need engaged stakeholders with historical data)
- Obtain necessary data for further evaluation (optimization?) of additivity formula
  - Difficult/impossible to optimize further without details of the mixture components
  - EPA pilot will expand the available data and includes conventional pesticides and antimicrobial cleaning product

Building Models to Predict Toxicity of Mixtures