

Highlights

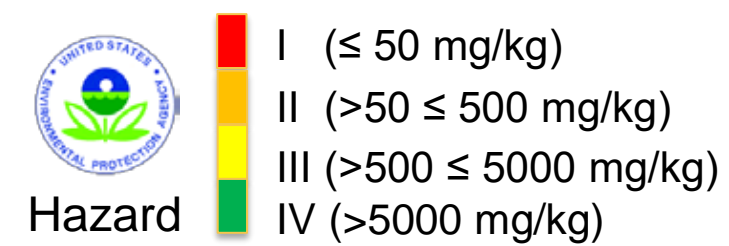
- We evaluated the concordance of *in vivo* acute toxicity results and LD₅₀ values calculated using the GHS mixtures equation.
- Overall concordance was between 55% to 82% depending on the ranges of LD50 values used.
- Most mispredictions occurred between the two least toxic categories.

Introduction

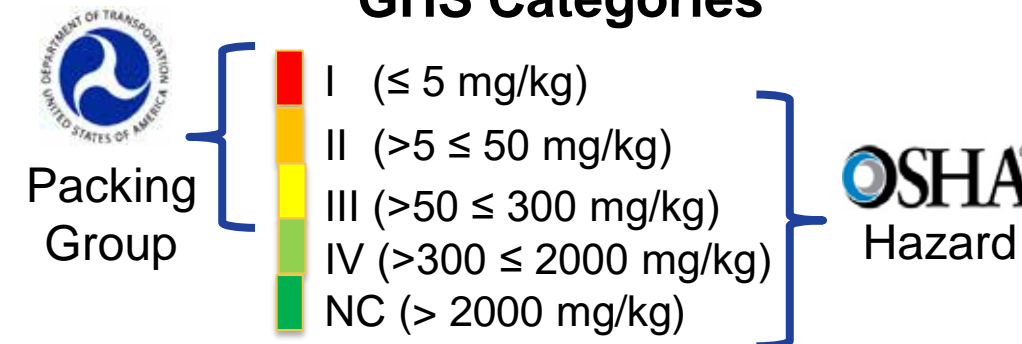
- The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) is used internationally for hazard classification.
- The GHS Mixtures Equation provides a mathematical approach to calculating toxicity of mixtures, considering the toxicity of each mixture component in a weighted manner.
- To evaluate the utility of this approach, we compared LD₅₀s predicted for formulations based on the GHS Mixtures Equation to those determined from *in vivo* results with the complete formulation. Comparisons were made using both the EPA and GHS classification systems.
- LD₅₀s based on *in vivo* results and calculated using the Mixtures Equation for the same substances were collected by the U.S. Environmental Protection Agency (EPA) from studies submitted for pesticide registration and provided to the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).
- We calculated concordance by determining the percentage of formulations for which classifications derived from *in vivo* data agreed with classifications derived from GHS Mixtures Equation calculations.

Classification and Precautionary Labeling

EPA Categories



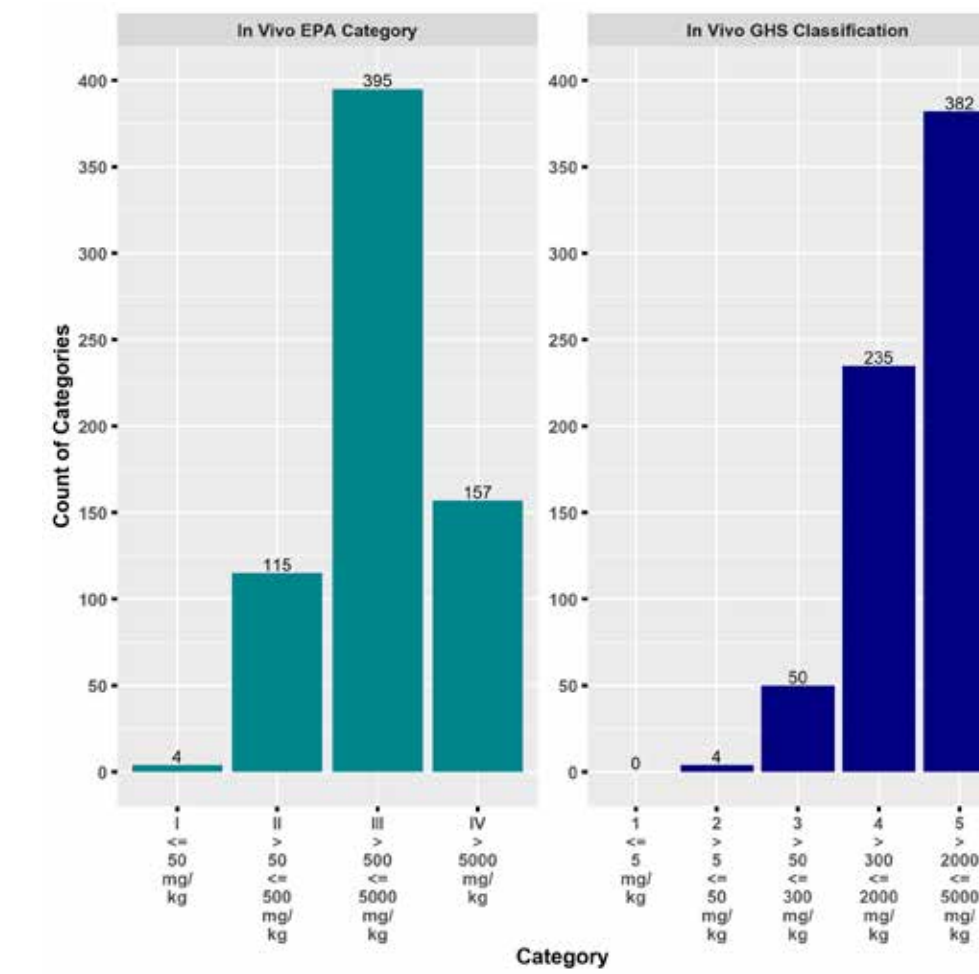
GHS Categories



EPA Category	Signal Word	Statement
I (LD ₅₀ ≤ 50 mg/kg)	Danger/Poison	Fatal if swallowed.
II (>50 < LD ₅₀ ≤ 500 mg/kg)	Warning	May be fatal if swallowed.
III (500 < LD ₅₀ ≤ 5000 mg/kg)	Caution	Harmful if swallowed.
IV (LD ₅₀ > 5000 mg/kg)	Caution (optional)	No statement is required. May use Category III statement

Dataset Description

- The data set consisted of 671 formulations produced by eight companies:
 - 51 antimicrobial cleaning products (AMCPs)
 - 620 agrochemical formulations
- The bargraph shows the distribution of the dataset substances according to their classifications in the EPA and GHS hazard classification systems.
- We used conservative classifications for *in vivo* LD₅₀s expressed as ranges (e.g., would use 300 mg/kg for 300 to 2000 mg/kg) and limit doses (e.g., would use 2000 for > 2000 mg/kg).



Primary Analysis

- Concordance analysis was determined according to EPA and GHS classification systems.

<i>In vivo</i> Classification	EPA Additivity Classification				Within-class Concordance
	I	II	III	IV	
I	3	1	0	0	75%
II	4	30	61	20	26%
III	1	34	197	163	50%
IV	0	1	19	137	87%
Total	8	66	277	320	55%

<i>In vivo</i> Classification	GHS Additivity Classification					Within-class Concordance
	1	2	3	4	5/NC	
1	0	0	0	0	0	NA
2	0	3	1	0	0	75%
3	0	4	10	26	10	20%
4	0	0	17	134	85	57%
5/NC	0	1	4	39	337	88%
Total	0	8	32	199	432	72%

- 79% (128/163) of "discordant" substances (EPA Cat III predicted as Cat IV, yellow highlight) had *in vivo* LD₅₀ values measured between 2000 and 5000 mg/kg or a limit test LD₅₀ > 2000 mg/kg.

Supplementary Analysis

- Precautionary labeling for substances, which also impacts packaging and required personal protective equipment (PPE), is based on the LD₅₀.
- The precautionary statements and associated PPE are much more stringent with LD₅₀ < 500 mg/kg.
- We performed a supplementary analysis that combined all substances with LD₅₀ > 500 mg/kg together.

<i>In vivo</i> LD ₅₀	Additivity LD ₅₀ Prediction (mg/kg)			Within-class Concordance
	≤50	>50 to ≤500	>500	
≤50	3	1	0	75%
>50 to ≤500	4	30	81	26%
>500	1	35	514	93%
Total	8	66	595	82%

Concordance Analysis Summary

All Substances	Primary Approach			Supplementary Analysis		
	Full	AMCP	Agrochem	Full	AMCP	Agrochem
EPA	55% (367/671)	84% (43/51)	52% (324/620)	82% (547/669)	100% (51/51)	80% (496/618)
GHS	72% (484/671)	98% (50/51)	70% (434/620)	NA	NA	NA

Less Toxic Substances	Primary Approach (Cat IV or 5/NC)			Supplementary Analysis (>500 mg/kg)		
	Full	AMCP	Agrochem	Full	AMCP	Agrochem
EPA	87% (138/157)	95% (38/40)	85% (99/117)	93% (514/550)	100% (51/51)	93% (463/496)
GHS	88% (337/381)	100% (49/49)	87% (288/332)	NA	NA	NA

Conclusions and Future Directions

- Most "discordant" substances had *in vivo* LD₅₀s values measured between 2000 and 5000 mg/kg or a limit test LD₅₀ > 2000 mg/kg.
- When considering formulations with LD₅₀ > 500 mg/kg together, overall concordance increased from 55% to 82%.
- Within-class concordance for less toxic substances was consistently over 85% regardless of classification system.
- Animal tests are inherently variable. Similar underclassification could also be observed following a repetition of the animal test.
- Our results suggest the mixtures equation is promising for identifying substances that would not be expected to induce toxicity.
- However, the lack of more toxic formulations in the dataset preclude us from reaching definitive conclusions across the spectrum of hazard categories.

More Information

Subscribe to the NICEATM News email list:
<https://list.nih.gov/cgi-bin/wa.exe?SUBED1=niceatm-I&A=1>

This project was funded in whole or in part with federal funds from the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN273201500010C.

The views expressed above do not necessarily represent the official positions of any federal agency. Since the poster was written as part of the official duties of the authors, it can be freely copied.