

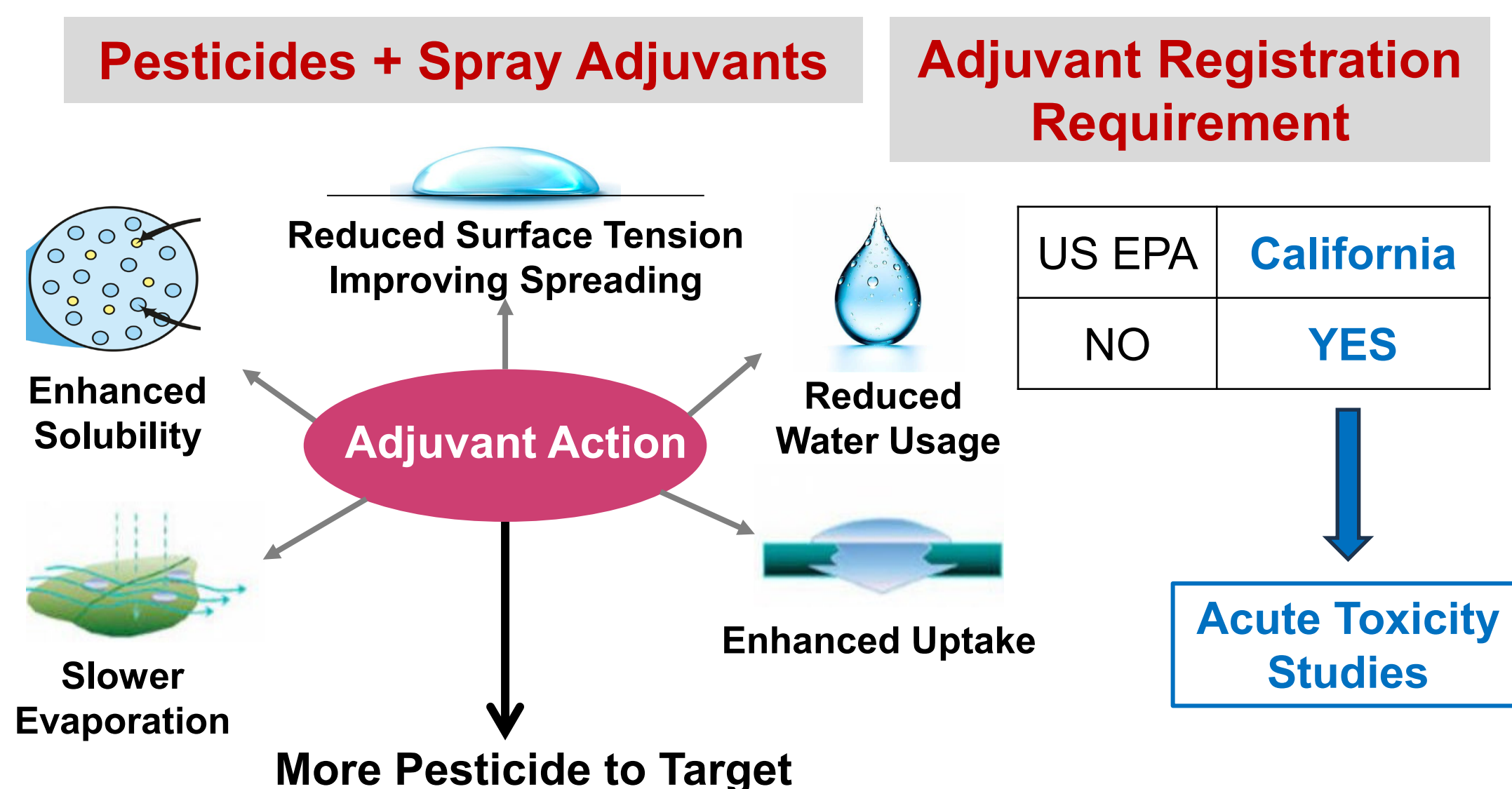
A study evaluating performance of the GHS Mixtures Equation for predicting the acute oral toxicity (LD₅₀) of spray adjuvants

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SPRAY ADJUVANTS AND THEIR REGULATION



OBJECTIVE

Acute Oral Toxicity Study

Animal Test
(Traditional Method)

New Approach Methodologies
(NAMs)

QUESTION: Could the United Nations' Globally Harmonized System (GHS) Mixtures Equation serve as an alternative method?

METHODS

SAMPLE SIZE : 51 Spray Adjuvants registered with the California Department of Pesticide Regulation

In vivo LD₅₀ : Acute Oral Toxicity Studies in Rats of 51 Spray Adjuvants

Single Chemical LD₅₀ : Integrated Chemical Environment (ICE)
(<https://ice.ntp.niehs.nih.gov/Search>)

Equation 1

$$\frac{100}{ATE_{mix}} = \sum \frac{C_i}{ATE_i}$$

Equation 2

$$\frac{100 - (\sum C_{unknown} \text{ if } > 10\%)}{ATE_{mix}} = \sum \frac{C_i}{ATE_i} \quad n$$

ATE_{mix} = *In silico* LD₅₀ estimate of the mixture

ATE_i = Acute Toxicity Estimate (LD₅₀) of ingredient i

C_i = Concentration of ingredient i in the mixture

n = number of ingredients in the mixture where i is running from 1 to n

Fig 1. GHS Mixtures Equation for calculating the LD₅₀ of a mixture

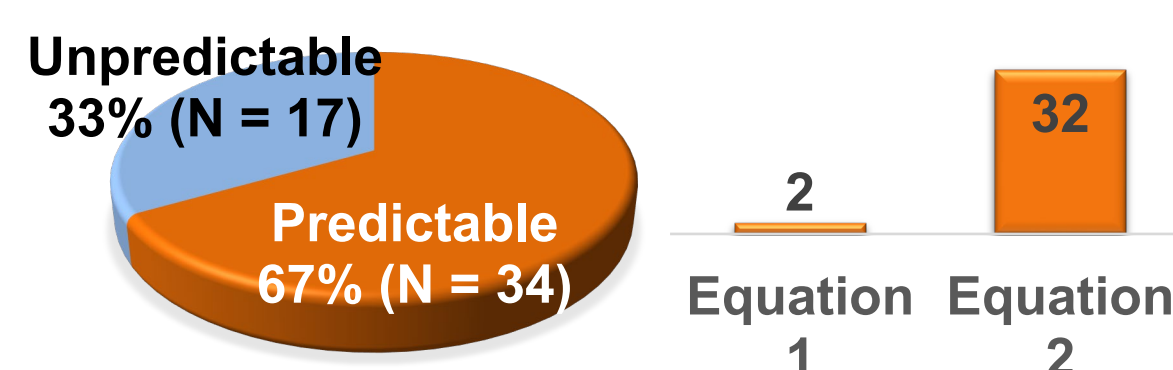
Table 1. Acute Oral Toxicity Categories and Classification Criteria

| US EPA* | Toxicity Category | I | II | III | IV | |
|--------------|--------------------------------|------|---------------|-----------------|-----------------|----------------|
| | LD ₅₀ range (mg/kg) | ≤ 50 | > 50 ≤ 500 | > 500 ≤ 5000 | > 5000 | |
| GHS (OSHA**) | Toxicity Category | 1 | 2 | 3 | 4 | Not Classified |
| | LD ₅₀ range (mg/kg) | ≤ 5 | > 5 ≤ 50 | > 50 ≤ 300 | > 300 ≤ 2000 | > 2000 |

* : <https://www.epa.gov/sites/production/files/2018-04/documents/chap-07-mar-2018.pdf>

** OSHA: United States Occupational Safety and Health Administration

RESULTS



The availability of LD₅₀ values for the relevant ingredients in the mixture limited the equation's predictive power.

Fig 2. Predictive Power of the GHS Mixtures Equation for 51 Spray Adjuvant Mixtures

Table 2. Concordance using the US EPA Classification System

| US EPA Classification | | GHS Mixtures Equation Category Prediction | | | | |
|------------------------|----------|-------------------------------------------|---------|----------|---------|-------|
| | | Cat. I | Cat. II | Cat. III | Cat. IV | Total |
| In vivo Classification | Cat. I | 0 | 0 | 0 | 0 | 0 |
| | Cat. II | 0 | 0 | 0 | 1 | 1 |
| | Cat. III | 0 | 0 | 8 | 7 | 15 |
| | Cat. IV | 0 | 0 | 2 | 16 | 18 |
| | Total | 0 | 0 | 10 | 24 | 34 |
| Consistency: | | 71% | | | | 24/34 |
| Overprediction: | | 6% | | | | 2/34 |
| Underprediction: | | 23% | | | | 8/34 |

- The predictive power of the GHS Mixtures Equation for oral hazards was 77% using the US EPA classification system.
- 5 of 8 under-classified adjuvants had 20% or more of the components with unknown LD₅₀s and the *in vivo* LD₅₀ > 2000 mg/kg (limit test).

Table 3. Concordance using the GHS (OSHA) Classification System

| GHS (OSHA) Classification | | GHS Mixtures Equation Category Prediction | | | | |
|---------------------------|----------------|-------------------------------------------|-------|-------|-------|----------------|
| | | Cat.1 | Cat.2 | Cat.3 | Cat.4 | Not Classified |
| In vivo Classification | Cat.1 | 0 | 0 | 0 | 0 | 0 |
| | Cat.2 | 0 | 0 | 0 | 0 | 0 |
| | Cat.3 | 0 | 0 | 0 | 0 | 0 |
| | Cat.4 | 0 | 0 | 0 | 0 | 1 |
| | Not Classified | 0 | 0 | 0 | 5 | 28 |
| | Total | 0 | 0 | 0 | 5 | 29 |
| Consistency: | | 82% | | | | 28/34 |
| Overprediction: | | 15% | | | | 5/34 |
| Underprediction: | | 3% | | | | 1/34 |

- The predictive power of the GHS Mixtures Equation for oral hazards was 97% using the GHS (OSHA) classification system.

CONCLUSION

- Although this study had a relatively small sample size, the concordance between the toxicity categories assigned using *in vivo* studies and those determined by the GHS Mixture Equation was consistent for the US EPA and GHS (OSHA) Classification Systems.
- The study indicates that the GHS Mixtures Equation can serve as an alternative method for predicting the acute oral toxicity category for spray adjuvants using the GHS (OSHA) Classification System.
- The GHS Mixtures Equation can reliably predict the acute oral toxicity category for low-toxicity mixtures, provided that the LD₅₀s for all active ingredients are available, and fewer than 20% of the other components in the formulation have unknown LD₅₀s.

CONFLICT OF INTEREST: The authors declare no conflicts of interest.

REFERENCES:

- Hamm *et al.* (2021), *Performance of the GHS Mixtures Equation for Predicting Acute Oral Toxicity*, Regulatory Toxicology and Pharmacology **125**.