

UTILITY OF THE GHS MIXTURES EQUATION FOR ACUTE ORAL TOXICITY CLASSIFICATION FOR PESTICIDE FORMULATIONS



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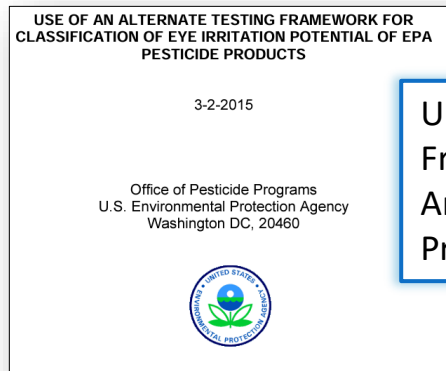
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OVERVIEW

- Background on OPP Initiatives to Reduce Reliance on Animal Testing
- Pesticide Registration Requirements
- GHS Mixtures Pilot Program
- Analysis of GHS Mixtures Predictions
- Use of GHS Mixtures Prediction for Pesticide Formulations

ANIMAL REDUCTION MILESTONES



Updated Alternative Testing Framework for Antimicrobial Cleaning Products

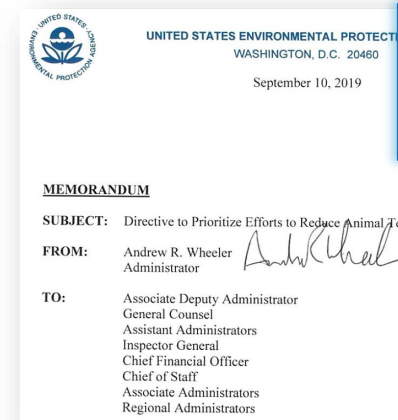
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2016

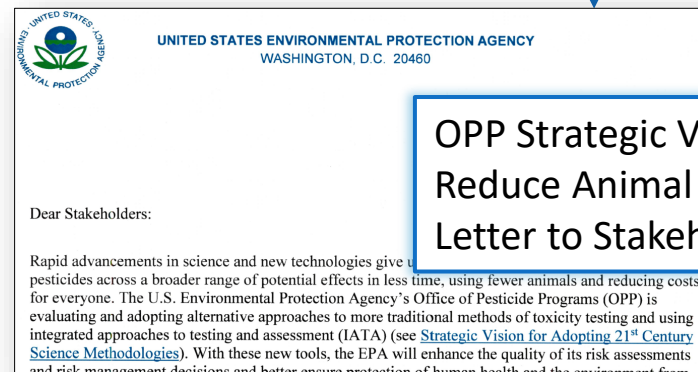
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2018

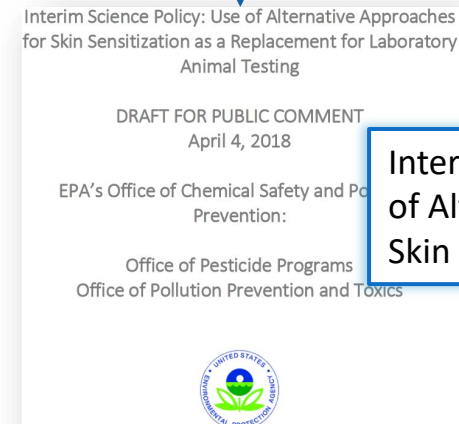
2019



Directive to Prioritize Efforts to Reduce Animal Testing



OPP Strategic Vision to Reduce Animal Testing: Letter to Stakeholders



Interim Science Policy on Use of Alternative Approaches for Skin Sensitization

ANIMAL REDUCTION MILESTONES (CONT.)



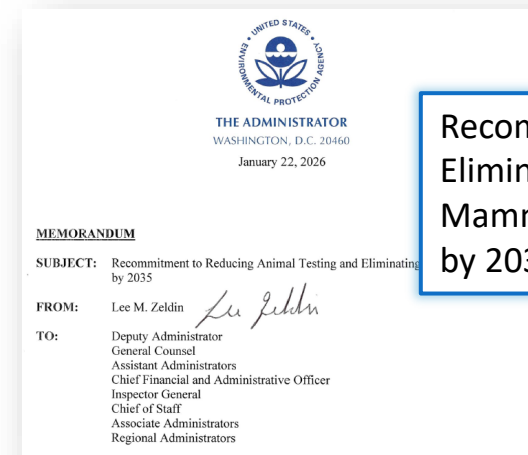
EPA New Approach Methods (NAMs) Work Plan

2020

2022

2024

2026



Recommitment to Eliminate Mammalian Testing by 2035



Prospective & Respective Evaluation of Eye Irritation Potential of Agrochemical Formulations

Prospective and Retrospective Evaluation of the Eye Irritation Potential of Agrochemical Formulations

DATA NEEDS FOR PESTICIDE REGISTRATION

TABLE—TOXICOLOGY DATA REQUIREMENTS

Guideline Number	Data Requirements	Use Pattern		Test substance to support		Test Note No.
		Food	Nonfood	MP	EP	
Acute Testing						
870.1100	Acute oral toxicity - rat	R	R	TGAI and MP	TGAI, EP, and possibly diluted EP	1, 2
870.1200	Acute dermal toxicity	R	R	TGAI and MP	TGAI, EP	1, 2, 3
870.1300	Acute inhalation toxicity - rat	R	R	TGAI and MP	TGAI and EP	4
870.2400	Primary eye irritation - rabbit	R	R	TGAI and MP	TGAI and EP	3
870.2500	Primary dermal irritation	R	R	TGAI and MP	TGAI and EP	1, 3
870.2600	Dermal sensitization	R	R	TGAI and MP	TGAI and EP	3, 5

- EPA registered pesticide products require acute toxicity testing to inform the precautionary statements, first aid statements, and signal word on the product label.
- Data requirements for Manufacturing-Use Products (MP) and End-Use products (EP) are outlined in 40 CFR §158.500.
 - Test notes indicate conditions where data may or may not be required.
 - Requirements may be satisfied by submitting new studies, citing existing data, or providing data waivers, etc.
- 40 CFR §158.30 provides flexibility in implementing data requirements for regulatory judgments

PRECAUTIONARY LABELING & SIGNAL WORD

EPA Toxicity Category	Category I	Category II	Category III	Category IV
Acute Oral	LD50 ≤50mg/kg	LD50 >50 – 500 mg/kg	LD50 >500 – 5,000 mg/kg	LD50 >5,000mg/kg
Acute Dermal	LD50 ≤200mg/kg	LD50 >200 – 2,000 mg/kg	LD50 >2,000 – 5,000 mg/kg	LD50 >5,000mg/kg
Acute Inhalation	LC50 ≤0.05mg/L	LC50 >0.05 – 0.5 mg/L	LC50 >0.5 – 2 mg/L	LC50 >2 mg/l
Eye Irritation	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Corneal involvement or other eye irritation clearing in 8-21 days	Corneal involvement or other eye irritation clearing in 7 days or less	Minimal effects clearing in less than 24 hours
Skin Irritation	Corrosive (tissue destruction into the dermis and/or scarring)	Severe irritation at 72 hours (severe erythema or edema)	Moderate irritation at 72 hours (moderate erythema)	Mild or slight irritation at 72 hours (no irritation or slight erythema)
Signal Word	DANGER	WARNING	CAUTION	NONE

- The results of the acute tests determine Toxicity Categories for each route of exposure. Dermal sensitization hazard is identified as positive or negative.
- Product labels require a signal word based on the most severe toxicity category.
- In addition, Precautionary and First Aid Statements are assigned for each route of exposure.
- Example of a Human Hazard Statement for product with the highlighted profile (non-sensitizer):

WARNING. Causes substantial but temporary eye injury. May be fatal if inhaled. Harmful if swallowed or absorbed through skin. Do not get in eyes or on clothing. Do not breathe vapor. Wear protective eye wear such as goggles, face shield, and/or shielded safety glasses. Wear long-sleeved shirt and long pants, socks, shoes, and waterproof gloves. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.

ALTERNATIVE APPROACH TO FULFILL ACUTE ORAL DATA REQUIREMENTS

- OPP initiated the “Mixtures Equation Pilot Program to Reduce Animal Testing”^A in 2016 to assess the utility of the GHS mixtures equation to predict the acute oral and acute inhalation toxicity categories for formulated pesticides in lieu of conducting new animal testing.
- Pesticide registrants provided data on product formulations (compositional information and data on individual ingredients). The submissions included GHS mixtures calculation paired with *in vivo* acute tests on the whole formulation.



^A [HTTPS://WWW.EPA.GOV/PESTICIDE-REGISTRATION/MIXTURES-EQUATION-PILOT-PROGRAM-REDUCE-ANIMAL-TESTING](https://www.epa.gov/pesticide-registration/mixtures-equation-pilot-program-reduce-animal-testing)

USING THE GHS MIXTURES EQUATION

- Chapter 3.1^B of the GHS presents a tiered approach to classify mixtures for acute toxicity:
 1. Classify based on data for the mixture
 2. Apply bridging principles to classify from data on similar mixtures
 3. Classify based on ingredients with known toxicity in the mixture using the **GHS additivity formula**.
- GHS additivity formula *aka* “mixtures equation” is used to determine the acute toxicity estimate (ATE) of a mixture using available data on individual components of the mixture.

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

C_i = concentration of ingredient

n = ingredients from 1 to n

ATE_i = acute toxicity estimate of ingredient i

ATE_{mix} = acute toxicity estimate of the mixture

**GLOBALLY HARMONIZED
SYSTEM OF CLASSIFICATION
AND LABELLING OF
CHEMICALS (GHS)**

Eleventh revised edition

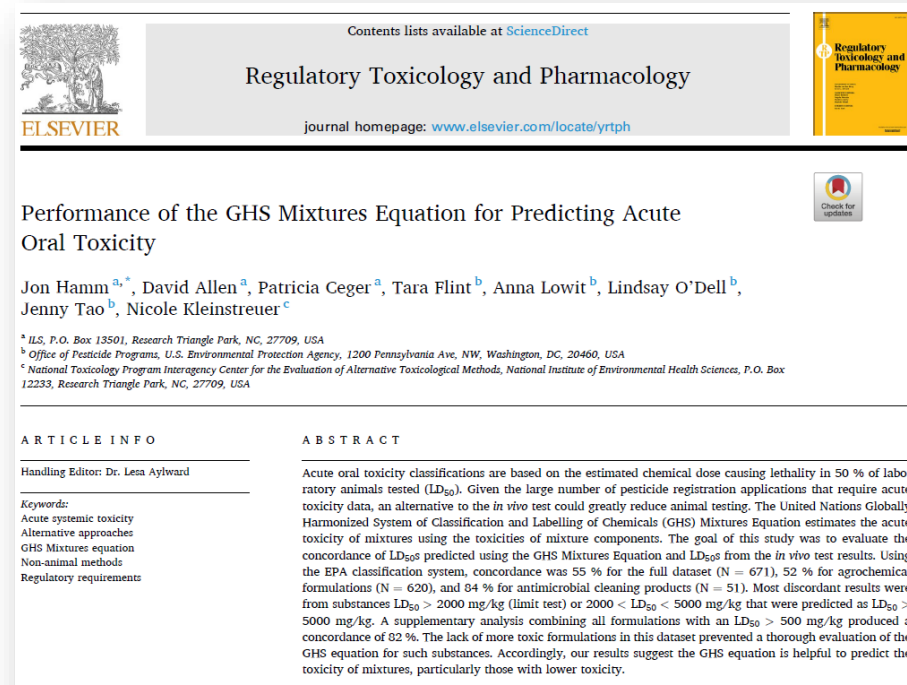


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Geneva, 2025

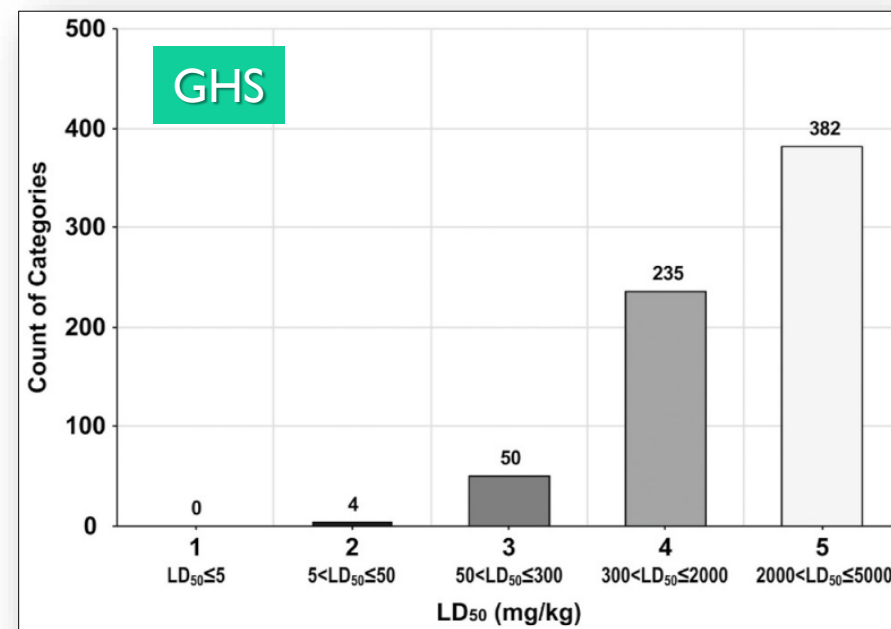
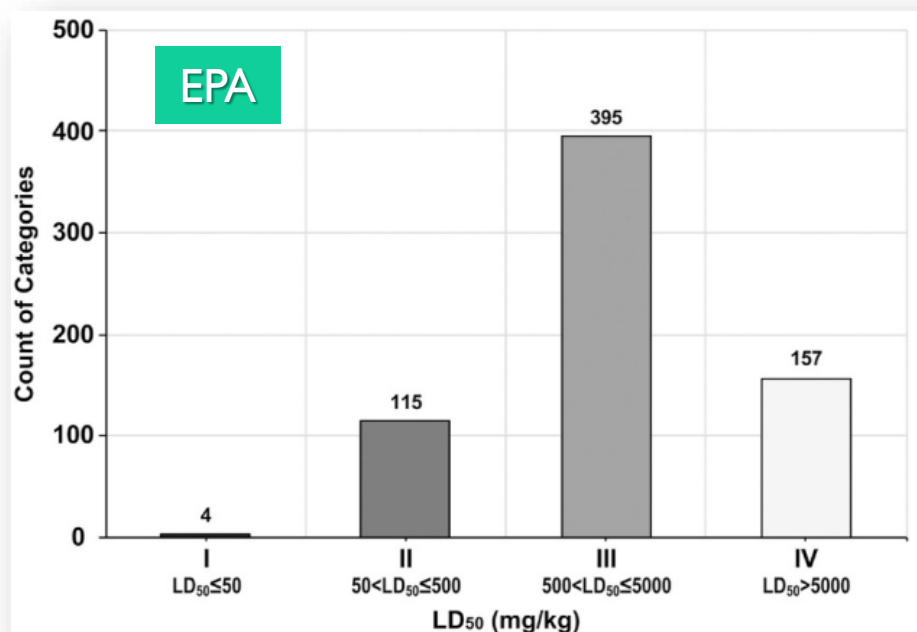
^B[HTTPS://UNECE.ORG/SITES/DEFAULT/FILES/2025-09/GHS_REV11E.PDF](https://unece.org/sites/default/files/2025-09/GHS_REV11E.PDF)

PERFORMANCE OF THE GHS MIXTURES EQUATION PREDICTIONS

- EPA collaborated with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICETAM) to evaluate the data collected in the pilot program.
- Five companies submitted data through the pilot program; three companies submitted data directly to NICETAM.
 - 618 agrochemicals; 51 antimicrobial cleaning products
- The concordance analysis of the paired *in vivo* data and predicted LD₅₀ values for both EPA and GHS classification was published in Hamm, et.al^C.



GHS MIXTURE EQUATION PILOT– DATA SET



- Majority of formulations included in retrospective were classified as EPA Cat III or IV based on *in vivo* data (550/669) or GHS Category 4, 5, or not classified (617/669).

CONCORDANCE ANALYSIS OF EPA CATEGORIES

- Within-class concordance for EPA Toxicity Categories I-IV was 75%, 26%, 50%, and 87% respectively.
- Predictions for EPA Category IV formulations ranged from 85% - 95% concordant.
- GHS predictions for EPA Category III were 50% concordant, of which 163 formulations were predicted in Tox Cat IV and 35 formulations were predicted in Tox Cat I/II when compared to *in vivo* data.

1) Full data set

<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%

2) Agrochemical formulations

<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	192	157	50%
IV	0	1	17	99	85%
Total	8	66	270	276	52%

3) Antimicrobial formulations

<i>In vivo</i> Classification	EPA Additivity Classification				Within-class Concordance
	I	II	III	IV	
I	0	0	0	0	NA
II	0	0	0	0	NA
III	0	0	5	6	45%
IV	0	0	2	38	95%
Total	0	0	7	44	84%

CONSIDERATIONS FOR TOXICITY CATEGORY III DISCORDANCE

Acute Oral Toxicity Categories for OPP and GHS Classification Systems							
LD50 range (mg/kg)	5	50	300	500	2000	5000	>5000
OPP	Category I		Category II		Category III		Category IV
GHS	Category 1	Category 2	Category 3	Category 4		Category 5	Not Classified

- Notably, the boundary dose between GHS 4/GHS 5 is 2000 mg/kg and is a recommended dose for limit testing according to OECD 420 and OECD 425.
- However, based on EPA categorization, products tested at a limit dose of 2000 mg/kg are conservatively classified as EPA Category III although the discrete LD₅₀ may be in either Toxicity Category III or Category IV.
- The discordance noted in Category III predictions (50%) was impacted by the 2000 mg/kg boundary dose, therefore an additional analysis was conducted combining Toxicity Category III & IV (LD₅₀>500 mg/kg).

CONCORDANCE ANALYSIS – LOW TOXICITY MIXTURES

- Supplementary analysis was conducted which combined all formulations with LD₅₀>500 mg/kg
- 79% (128/163) of the formulations predicted by the GHS mixtures equation as Tox Cat IV were classified with *in vivo* data LD₅₀>2000 mg/kg or 2000 <LD₅₀<5000 mg/kg
- Predictions for formulations with LD₅₀>500 mg/kg were 93% - 100% concordant.

1) Full data set

<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	515	93%
Total	8	66	596	82%

2) Agrochemical formulations

<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	464	93%
Total	8	66	545	80%

3) Antimicrobial formulations

<i>In vivo</i> LD ₅₀	Additivity LD ₅₀ Prediction (mg/kg)			Within-class Concordance
	≤50	>50 to ≤500	>500	
≤50	0	0	0	NA
>50 to ≤500	0	0	0	NA
>500	0	0	51	100%
Total	0	0	51	100%

EPA CONCLUSIONS

- In August 2026, EPA posted a notice to stakeholders^D summarizing the findings and conclusions on the OPP NAMs website.
 - <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-new-approach-2>
- The GHS mixtures equation can sufficiently identify low acute oral toxicity pesticide formulations, generally LD₅₀>500mg/kg, with no additional *in vivo* testing necessary.
- Although the pilot program did not specifically include biopesticide formulations, this approach could be considered for those formulations on a case-by-case basis.
- Due to the limited data available on formulations with higher toxicity in the retrospective analysis, further analysis is needed to determine if/how the GHS mixtures equation may be utilized across all hazard categories.



^D<https://www.epa.gov/system/files/documents/2026-08/utility-of-ghs-mixtures-equation-20260827.pdf>



CONSIDERATIONS FOR USING THE GHS MIXTURES EQUATION TO PREDICT ACUTE ORAL TOXICITY OF A PESTICIDE

INPUTS TO GHS MIXTURE EQUATION

- Section 3.1.3.6^B of the GHS details the criteria for consideration to determine the acute toxicity estimate (ATE) of a mixture. In summary the calculation includes:
 1. Ingredients are included if present in the mixture at $\geq 1\%$ w/w, with some exceptions for ingredients with known toxicity.
 2. The ATE of ingredients are included if they fall within a GHS category.
 3. Ingredients with toxicity range values are converted to point estimates and included as the ATE_i in the formula.
 4. Nontoxic ingredients are not included (e.g. water, sugar).
 5. Ingredients with limit test data >2000 mg/kg are generally not included.
 6. Modified formula is used for mixtures when ingredients of unknown toxicity total $>10\%$ (Section 3.1.3.6.2.3)

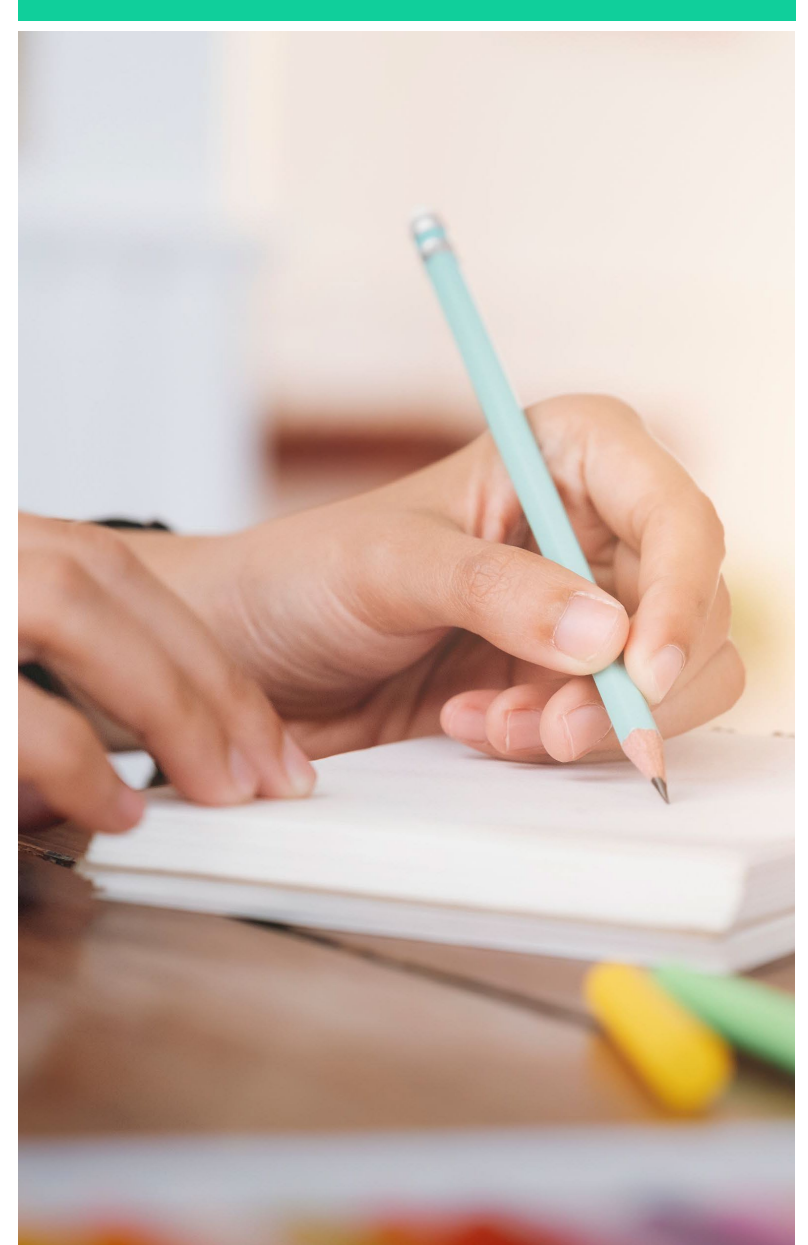
^B[HTTPS://UNECE.ORG/SITES/DEFAULT/FILES/2025-09/GHS REV11E.PDF](https://unece.org/sites/default/files/2025-09/GHS%20Rev11E.pdf)

GENERIC EXAMPLE OF GHS MIXTURES EQUATION

Component	ATE _i (LD ₅₀)	Concentration of ingredient (C _i)	Include in additivity equation	Comment
Active Ingredient	50 mg/kg < LD ₅₀ ≤ 300 mg/kg	2%	Yes	Converted point estimate LD ₅₀ = 100 mg/kg
Active Ingredient	>2000 mg/kg	10%	No	ATE of the active ingredient is based on a limit test at the upper boundary of GHS Category IV and did not demonstrate acute toxicity at this dose.
Inert Ingredient	1500 mg/kg	7%	Yes	-
Inert Ingredient	840 mg/kg	5%	Yes	-
Inert Ingredient	15,000 mg/kg	6%	No	Component of known toxicity does not fall within GHS Toxicity Categories (LD ₅₀ range 0-5000 mg/kg) and is not included in the calculation.
Inert Ingredient	Nontoxic ³	70%	No	Presumed nontoxic ingredients are not included (e.g., water, sugar).

PREPARING A GHS MIXTURES SUBMISSION TO EPA

- A. Consult the GHS to determine applicability of the equation for a proposed formulation.
- B. Include citations for acute oral toxicity data for each component of the formulation which are publically available.
- C. Include study data for proprietary data on components of the formulation.
- D. Provide a justification for LD50 selections when multiple data sources are available.
- E. Consider submitting additional evidence to support borderline classification predictions.





THANK YOU!



SUBMIT
QUESTIONS TO:
pesticidequestions
@EPA.GOV

REFERENCES

- Hamm J, Allen D, Ceger P, Flint T, Lowit A, O'Dell L, Tao J, Kleinstreuer N. (2021). Performance of the GHS mixtures equation for predicting acute oral toxicity. *Regul Toxicol Pharmacol.* 125:105007. doi: 10.1016/j.yrtph.2021.105007
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- U.S. EPA. (2026). Utility of the GHS Mixtures Equation for Acute Oral Toxicity Classification for Pesticide Formulations. <https://www.epa.gov/system/files/documents/2026-08/utility-of-ghs-mixtures-equation-20260827.pdf>