Utility of the GHS Mixtures Equation for Acute Oral Toxicity Classification for Pesticide Formulations

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I. Introduction

In 2016, the U.S. Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) announced the GHS Mixtures Equation Pilot, an initiative to evaluate the utility of the Global Harmonized System of Classification and Labelling (GHS) additivity formula (herein referred to as the "GHS mixtures equation") as an alternative to conducting acute oral toxicity studies on pesticide formulations. The pilot program was launched as part of OPP's strategic vision to reduce the reliance on animal testing to assess acute effects (referred to as the "6-pack") of pesticides (EPA, 2016a). The purpose of this document is to summarize the results of the retrospective analysis of the pilot program data and provide details to stakeholders on the utility of the GHS Mixtures Equation for acute oral toxicity classification in lieu of additional vertebrate animal testing.

II. Background

The acute toxicity data requirements inform the hazard categories, precautionary and first aid statements, and personal protective equipment required on a pesticide label. The four acute oral hazard toxicity categories (Category I through Category IV) for pesticides are established in 40 CFR § 156.62. Internationally, many organizations use the GHS for hazard classification and labelling. Although OPP's acute oral toxicity categories differ from GHS, there are instances where the OPP and GHS categorization schemes overlap (Figure 1).

Figure 1: Comparison of OPP and GHS Acute Oral Toxicity Categories

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

The GHS mixtures equation was developed to calculate the toxicity of mixtures based on weighted contributions of each ingredient in a formulation (United Nations, 2023). The acute toxicity estimate (ATE) of the mixture or formulation (ATE_{mix}) is calculated as the sum of the ATE values for each relevant ingredient (ATE_i) using the equation presented in Figure 2.

Figure 2: GHS Formula to Calculate the Acute Toxicity Estimate (ATE) of Mixtures

$$\frac{100}{ATEmix} = \sum_{n} \frac{C_i}{ATE_i}$$

C_i = concentration of ingredient
n = ingredients from 1 to n
ATE_i = acute toxicity estimate of ingredient i

GHS employs a tiered testing approach for acute toxicity classification (United Nations, 2023). In scenarios where acute toxicity data are not available for similar mixtures, the GHS mixtures equation is used to classify the mixture according to one of three scenarios:

1. Data are available for the ATE of all relevant ingredients in the mixture.
2. Data are available to derive the ATE of all relevant ingredients in the mixture.
3. Data are not available for all relevant ingredients in the mixture. (Note: a modified formula is used for mixtures when components of unknown toxicity are present at greater than 10%.)

Figure 3 presents an example of a nondescriptive mixture where the ATE of each component of the mixture is known and the ATE of the mixture is determined using all relevant ingredients with LD_{50} values within the GHS categories (1-5). Notably, in the GHS guidance (United Nations, 2023), if the ATE of an ingredient is from a limit dose test at the upper boundary of GHS Category 4 (LD_{50}>2000 mg/kg), this ingredient is not included in the calculation. In addition, the guidance specifies converted acute toxicity point estimates are to be included in the additivity equation if data available for an ingredient is a range (i.e., 300 mg/kg < LD_{50} <2000 mg/kg would convert to a point estimate of 500 mg/kg bw). In the case illustrated below, the ATE of each component of the mixture is known and relevant ingredients are included in the additivity equation to determine the predicted ATE of the mixture of 5785 mg/kg.

Figure 3: ATE of a Mixture with ingredients of known toxicity.

Component	ATE_i (LD_{50})	Concentration of ingredient (C_i)	Include in additivity equation	$\frac{C_i}{ATE_i}$
Active Ingredient	300 mg/kg	2%	Yes	0.007
Active Ingredient	>2000 mg/kg[1]	10%	No	N/A
Inert Ingredient	1500 mg/kg	7%	Yes	0.005
Inert Ingredient	840 mg/kg	5%	Yes	0.006
Inert Ingredient	15,000 mg/kg[2]	6%	No	N/A
Inert Ingredient	Nontoxic[3]	70%	No	N/A
				ATE_{mix} = 5785 mg/kg

[1]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.

[2]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.

[3]Presumed nontoxic ingredients are not included (e.g., water, sugar).

III. Performance of the GHS Mixtures Equation for Predicting Acute Oral Toxicity

Data from the pilot program consisted of *in vivo* acute oral lethal dose (LD_{50})[1] tests on 671 pesticide formulations. These data on the whole formulations were paired with the predicted LD_{50} derived from the GHS mixtures equation using data on individual ingredients of the formulation. Agrochemical formulations comprised the majority of the submissions (n=620), with the remainder consisting of antimicrobial cleaning product (AMCP) formulations (n=51). Additionally, the data set was heavily weighted towards formulations with low acute oral toxicity since 552 formulations were classified based on *in vivo* data as either EPA Toxicity Category III or Toxicity Category IV. OPP partnered with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to analyze the results of the GHS pilot submissions. A retrospective analysis of the concordance of the paired *in vivo* tests on the formulations and GHS mixture equation predicted LD_{50} values was published in 2021 (Hamm, *et al*, 2021). The publication included analysis of both EPA and GHS classification systems, however, only the concordance according to EPA classification has been extracted from the publication for this document. Figure 4 compares the toxicity classification resulting from the *in vivo* test on the formulation with the classification derived from the GHS mixtures equation for all formulation types; the number of formulations where the toxicity classification of both approaches aligned are shaded. Separate analyses were also performed for agrochemical formulations only (Figure 5) and AMCP formulations only (Figure 6).

Figure 4: Concordance of Acute Oral Toxicity Categories for All Formulations

In Vivo Test Classification	GHS Mixtures Equation 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%

Figure 5: Concordance of Acute Oral Toxicity Categories for Agrochemical Formulations Only

In Vivo Test Classification	GHS Mixtures Equation 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%

[1] Dose of test substance expected to cause 50% lethality in tested animals via the oral route

Figure 6: Concordance of Acute Oral Toxicity Categories for AMCP Formulations Only

In Vivo Test Classification	GHS Mixtures Equation Classification				Within-class Concordance
	I	II	III	IV	
I	0	0	0	0	N/A
II	0	0	0	0	N/A
III	0	0	5	6	45%
IV	0	0	2	38	95%
Total	0	0	7	44	84%

Concordance Analysis for Low Toxicity Mixtures

Formulations predicted by the GHS mixtures equation as EPA Toxicity Category IV (LD_{50} >5000 mg/kg) were 87% concordant overall with the corresponding *in vivo* classification (Figure 4). When these mixtures were considered separately based on their formulation type, the concordance for Toxicity Category IV was 85% for agrochemical formulations (Figure 5) and 95% for AMCPs (Figure 6). Formulations predicted by the GHS mixtures equation as EPA Toxicity Category III (LD_{50} > 500 mg/kg and LD_{50}≤5000mg/kg) were 50% concordant. The GHS mixtures equation predicted a less conservative toxicity category (Category IV) for 163 formulations and more conservative toxicity categories (Category I/II) for 35 formulations when compared to *in vivo* data.

As noted in the retrospective analysis, 79% (n=128) of the 163 formulations predicted in Toxicity Category IV were classified based on *in vivo* LD_{50} values between 2000-5000 mg/kg or based on a limit test of LD_{50}>2000 mg/kg. Therefore, a supplementary analysis combining all LD_{50} values within EPA Toxicity Categories III and IV (LD_{50}>500 mg/kg) was conducted since formulations with LD_{50}>2000 mg/kg would be conservatively labeled as EPA Toxicity Category III. Based on this supplementary analysis, the predicted LD_{50} was 93% concordant with the *in vivo* results (Figure 7). The GHS mixtures equation predicted a higher LD_{50} (>500 mg/kg) for 81 formulations classified as EPA Toxicity Category II *in vivo* (LD_{50}>50 to ≤500 mg/kg) resulting in 26% concordance for formulations in Toxicity Category II (n=30/115).

Figure 7: Concordance of Acute Oral Toxicity Categories for All Formulations Combining Toxicity Categories III and IV (>500 mg/kg)

In Vivo Test LD_{50} (mg/kg)	GHS Mixtures Equation Predicted LD_{50} (mg/kg)			Within-class Concordance
	I	II	III/IV	
I	3	1	0	75%
II	4	30	81	26%
III/IV	1	35	515	93%
Total	8	66	596	82%

IV. Retrospective Analysis Conclusions

The GHS Mixtures Equation Pilot and subsequent retrospective analysis aligns with OPP's vision to evaluate alternatives to vertebrate animal testing and assess their applicability to inform regulatory decisions. Based on the results of the retrospective analysis, the Agency has determined that the GHS additivity equation can sufficiently identify low acute oral toxicity pesticide formulations, generally LD_{50}>500mg/kg, with no additional *in vivo* testing necessary. The domain of applicability for the GHS Mixtures Equation Pilot program included conventional and antimicrobial formulations only. Although the pilot program did not specifically include biopesticide formulations, this approach could be considered for those formulations on a case-by-case basis.

Lastly, 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.

VI. Consideration and Use of GHS Mixtures Equation for Acute Oral Toxicity Classification of Pesticide Formulations

The 40 CFR Part 158 establishes the toxicity testing required or conditionally required to assess human health and environmental effects of pesticides according to specific criteria. In addition, 40 CFR § 158.30 provides EPA flexibility to determine data needs for pesticide chemicals and 40 CFR § 158.45 allows for waivers of data requirements if the Agency has determined that sufficient information is available for regulatory decisions. Accordingly, OPP has previously issued several publications to inform pesticide registrants of data bridging principles and clarified eligibility criteria for submission of data waivers for acute toxicity data requirements for pesticide technical active ingredients and formulations (EPA 2012; EPA 2016b; EPA 2016c; EPA 2020). Several of the data bridging principles described in OPP guidance documents are similar to those outlined in the GHS guidance.

The Agency has determined that pesticide formulations expected to be of low acute oral toxicity (Toxicity Category III or IV) are eligible to submit the GHS mixtures equation prediction or "GHS ATE_{mix}" in lieu of performing new *in vivo* testing of the formulation. Pesticide registrants are encouraged to review the comprehensive criteria outlined in the GHS guidance document (Chapter 3.1 Acute Toxicity) to determine the applicability of the GHS mixtures equation for deriving the GHS ATE_{mix} for a proposed formulation (United Nations, 2023). For example, a formulation with limited to no acute oral data available on the individual ingredients would not be an appropriate candidate to use the GHS equation to predict the acute oral toxicity of the product. Furthermore, as noted in the GHS guidance, when the concentration of ingredients in a mixture without data are >10%, the additivity equation should be modified to account for the total concentration of unknown ingredients.

At this time, pesticide registrants should submit the GHS ATE_{mix} prediction data to meet the acute oral toxicity data requirement following the Agency's current registration procedures.

The submission must include the GHS ATE_{mix} of the proposed formulation and citations to available acute oral toxicity data on each individual ingredient in the mixture. For example, registrants may cite EPA decision documents (i.e., risk assessments on active and/or inert ingredients) or utilize the National Toxicology Program's Integrated Chemical Environment (ICE) to source data on individual ingredients. Relevant data on proprietary ingredients or data not publicly available should accompany the submission for review by the Agency. All ingredients with LD_{50} values within the GHS categories (1-5) should be included in the GHS mixtures calculation, however, data from limit tests (e.g. LD_{50}>2000 mg/kg) should be excluded (see example in Figure 3). When multiple acute oral data sources are available for an individual ingredient, the most conservative LD_{50} estimate should be selected for the GHS mixtures calculation unless appropriate justification is provided. The Agency will evaluate the accuracy of the cited data and may consider information on individual ingredients within Agency databases when assessing the proposed GHS ATE_{mix}.

The GHS ATE_{mix} may be considered a stand-alone replacement for the *in vivo* study; however, scenarios may arise where a weight-of-evidence (WOE) would be needed to justify a low toxicity determination. For instance, the reliability of the GHS ATE_{mix} prediction decreases for a mixture which contains ingredients of unknown toxicity at a total concentration greater than 10%; therefore, additional lines of evidence may be necessary to support classification in certain cases. Additionally, existing *in vivo* data or other lines of evidence on a related mixture may be coupled with the GHS ATE_{mix} to justify a low toxicity classification for a formulation that would not otherwise be considered adequate. Relatedly, when the GHS ATE_{mix} is close to the boundary dose between Category II and III (i.e., 500 mg/kg), robust supporting evidence should be included given the implications of a more conservative signal word (Caution to Warning). Similarly, a dose of 1500 mg/kg is used for child-resistant packaging[2] requirements and restricted use classification[3] and, therefore, would also warrant robust supporting evidence. In circumstances where the Agency determines the GHS ATE_{mix} prediction paired with other lines of evidence does not sufficiently support the toxicity category for a proposed mixture, a more conservative classification may be warranted. Registrants are encouraged to contact the Agency to discuss the suitability of the proposed GHS ATE_{mix} prediction and other supporting evidence for novel products prior to submission.

The acute oral toxicity classification resulting from the GHS mixture equation prediction is evaluated on a case-by-case basis taking into consideration all available lines of evidence. The Chemistry and Acute Toxicology Science Advisory Council (CATSAC) may be consulted to ensure consistent application of the GHS mixtures equation prediction. CATSAC is a cross-divisional, multi-disciplinary group of scientists in OPP charged with deliberation of acute toxicity bridging

[2] 40 CFR § 157.22 specifies products permitted for residential uses must be sold in child-resistant packaging if the acute oral LD_{50} is 1500 mg/kg or less.

[3] 40 CFR § 152.170 states products intended for residential or institutional use will be considered for restricted use classification if the pesticide, as diluted for use, has an acute oral LD_{50} of 1500 mg/kg or less.

rationales, data waivers, and alternative testing methods; thus, the council is integral to the implementation of new approach methodologies (NAMs) for pesticide formulations.

VII. Association with Acute Dermal Toxicity Waivers

In 2016, OPP published, "Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations & Supporting Retrospective Analysis". The retrospective analysis assessed the results of paired acute oral toxicity and acute dermal toxicity tests for 592 formulated pesticides. The analysis indicated that hazard classification based on acute oral toxicity data was equal to or more protective of the acute dermal classification for 562 of 592 formulations or approximately 95% (EPA, 2016c). Based on this assessment, the Agency concluded dermal lethality tests may be waived for pesticide formulations.

Considering the 2016 acute dermal retrospective analysis in conjunction with the concordance of the GHS mixtures equation prediction for Toxicity Category III and IV formulations, the Agency expects scientifically sound rationales using the GHS mixtures equation to support low oral toxicity would also support waiving dermal acute toxicity requirements. Similar to acute oral toxicity classification supported by the GHS ATE_{mix} prediction, the Agency will evaluate all available lines of evidence to determine eligibility for acute dermal waiver requests in this scenario.

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