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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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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.

1. Introduction

Agrochemical formulations are complex mixtures composed to optimize biological activity and aid in handling and delivery of the active ingredient to its target pest. To accomplish these tasks, formulations enable chemical interaction between the active ingredient and other components in the formulation. This interaction can result in an increased absorption of the active ingredient to the pest target (leaf or insect) and/or alter the handling and stability of the active ingredient. These same interactions that enhance the utility of the active ingredient also may have other interactions such as altered mammalian toxicity (Damalas and Eleftherohorinos, 2011).

In accordance with various regulatory requirements, agrochemical formulations are typically tested in a battery of six in vivo studies to obtain a profile of the formulation's acute toxicity. These six studies are comprised of three studies that analyze acute systemic toxicity (oral, dermal and inhalation), as well as three studies that analyze irritation and sensitization properties (eye irritation, dermal irritation and dermal sensitization). The resulting data provide a basic understanding of acute effects and serve as a starting point for human hazard and risk assessments (USEPA, 2002a,b).

These acute in vivo studies are used to classify the formulation according to US-EPA classification criteria or the UN Global Harmonized System of Classification and Labelling of Chemicals (UN-GHS). The resulting hazard classification dictates the hazard communication contained on the formulation's product label, the personal protective equipment (PPE) prescribed for the user and transportation requirements.

An agrochemical formulation is tested as the undiluted end-use product. Modern acute toxicity testing is often conducted on a single species using the acute toxic class (ATC) method, where dose levels are set to line up with the thresholds for classification (whether from GHS, USEPA or other regulatory authorities). The resulting endpoint from such assays is a range of toxicity (e.g. LD_{50} > 500 mg/kg bw and < 2000 mg/kg bw) and is interpreted as an UN GHS Category or USEPA Category (Table 1). The modern guidelines used for conducting acute assays on agrochemical formulations are OECD 423 (OECD, 2002) and USEPA OPPTS 870.1100 for oral toxicity (USEPA, 2002a,b); OECD 402

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Table 1
Classification Criteria for oral, inhalation and dermal acute toxicity; UN GHS (UN, 2015); USEPA (USEPA, 2014).

UN-GHS	USEPA
Acute oral toxicity (ATE/LD_{50} in mg/kg bw)	
0 < Cat 1 ≤ 5	0 < Cat I ≤ 50
5 < Cat 2 ≤ 50	
50 < Cat 3 ≤ 300	50 < Cat II ≤ 500
300 < Cat 4 ≤ 2000	
	500 < Cat III ≤ 5000
2000 < Cat 5[b] ≤ 2000	Cat IV > 5000
NC > 5000	
Acute dermal toxicity (ATE/LD_{50} in mg/kg bw)	
0 < Cat 1 ≤ 50	0 < Cat I ≤ 200
50 < Cat 2 ≤ 200	
200 < Cat 3 ≤ 1000	200 < Cat II ≤ 2000
1000 < Cat 4 ≤ 2000	
2000 < Cat 5[b] ≤ 5000	2000 < Cat III ≤ 5000
NC > 5000	Cat IV > 5000
Acute inhalation toxicity[a] (ATE/LC_{50} in mg/L)	
0 < Cat 1 ≤ 0.05	0 < Cat I ≤ 0.05
0.05 < Cat 2 ≤ 0.5	0.05 < Cat II ≤ 0.5
0.5 < Cat 3 ≤ 1.0	0.5 < Cat III ≤ 2.0
1.0 < Cat 4 ≤ 5.0	
	Cat IV > 2.0
Cat.5[b]/NC > 5.0	

NC not classified.
[a] Dusts and mists.
[b] Used for sensitive populations or when substances are anticipated to have acute oral/dermal LD_{50} in the 2000–5000 mg/kg bw range or equivalent doses for inhalation exposure.

$$ATE_{mix} = \frac{100}{\sum_{i=0}^{n} \frac{Ci}{ATE_i}}$$

ATE_{mix} = Acute Toxicity Estimate (e.g. LD_{50} or LC_{50}) of mixture
ATE_i = Acute Toxicity Estimate (e.g. LD_{50} or LC_{50}) of ingredient
C_i = Concentration of ingredient i
i = Individual relevant ingredient; from 1 to n
n = Number of ingredients

Fig. 1. GHS Additivity formula.

Table 2
Classifications of Active Ingredients in the data set.

Active Ingredient		Acute Classification					
		Oral		Inhalation		Dermal	
		UN-GHS	US-EPA	UN-GHS	US-EPA	UN-GHS	US-EPA
1	Herbicide	4	II	NC	IV	NC	IV
2		4	III	NC	IV	NC	III
3	Fungicide	NC	IV	2	II	NC	IV
4		4	II	2	II	NC	III
5	Insecticide	3	II	2	II	3	II
6		3	II	3	III	NC	III
7		4	II	3	III	NC	III
8	Plant growth regulator	4	II	5	IV	NC	III

NC: Not classified.

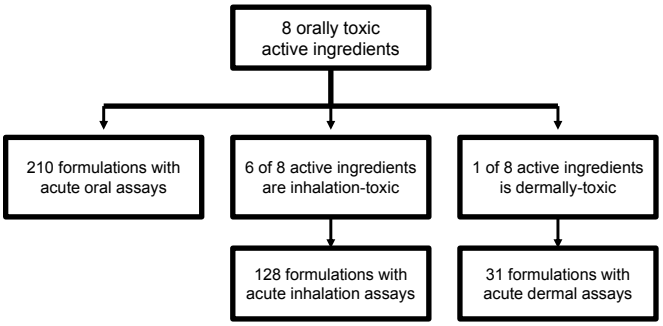

Fig. 2. Selection of data set for retrospective analysis.

(OECD, 1987b) and USEPA OPPTS 870.1200 for dermal toxicity (USEPA, 1998a); and OECD 403 (OECD, 2009) and USEPA OPPTS 870.1210 for inhalation toxicity (USEPA, 1998b).

To date, there are not widely accepted replacements for the in vivo acute systemic toxicity assays (oral, dermal and inhalation toxicity). In vitro approaches have been considered and critically reviewed for oral and inhalation toxicity (Schrage et al., 2011; Buesen et al., 2016; Sauer et al., 2013; Moore et al., 2013). The UN GHS guidance also allows for an estimate of acute toxicity of a mixture using a harmonic mean algorithm, which accounts for the various contribution each component will have to the toxicity of the mixture (UN, 2015). This approach uses a formula commonly referred to as the GHS additivity formula (see Fig. 1).

What is referred to as the "GHS additivity formula" was presented by Hoel in 1987 (Hoel, 1987) and based upon a study with acute oral testing of 27 industrial solvents in paired mixtures. (Smyth et al., 1969). Hoel stated that use of the additivity equation "assumes that chemicals are not interactive". In Hoel's words, the chemicals must have a "similar joint action" for the formula to correctly estimate toxicity. The GHS guidance (UN, 2015) does not emphasize Hoel's caution on the application of the GHS additivity formula.

A robust retrospective analysis of interaction (or non-interaction) of chemicals in a formulation requires that at least one of the components in a formulation has measurable toxicity. As a simple mechanism to collect such a data set, we analyzed data from agrochemical formulations that contained one of several active ingredients that are classified for acute oral toxicity. Using the resulting data set, we compared the Acute Toxicity Estimates (ATEs) calculated using the GHS Additivity Formula with the experimentally-derived in vivo acute toxicity values.

2. Materials and methods

This data set was based upon eight agrochemical active ingredients that are known acute oral toxicants, either when tested alone, and/or consistently when formulated. These eight active ingredients included herbicides (2), fungicides (2), insecticides (3) and a plant growth regulator (1). For the purposes of this paper, an acute toxicant is defined as a substance with measurable mortality in the acute in vivo assay at a dose less than a limit dose (i.e. 2000 mg/kg bw for oral and dermal exposure and 5 mg/L for inhalation exposure). The classification of the eight active ingredients is summarized in Table 2. Due to the proprietary nature of the data, these active ingredients are not disclosed, but were coded 1 through 8. Of these eight orally-toxic active ingredients, six were inhalation toxicants (measurable mortality with an LC_{50} less than a limit dose) (Table 2: No. 3, 4, 5, 6, 7, 8) and only one was a dermal toxicant (measurable mortality with an LD_{50} less than a limit dose) (Table 2: No. 5).

The resulting data set for retrospective analysis includes 210 formulations tested in acute oral assays, 128 formulations tested in acute inhalation assays and 31 formulations tested in acute dermal assays (Fig. 2). This selection process ensures that at least one component of