GHS ADDITIVITY APPROACH TO CLASSIFY MIXTURES BASED ON INGREDIENT TOXICITY

A CASE EXAMPLE: AGROCHEMICAL FORMULATIONS

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Solutions for the Growing World

Purpose of this presentation

- Acute inhalation toxicity for mixtures
- The theory of additivity
- The GHS additivity formula
- Case example:
  - > requirements for agrochemical formulations
  - > Comparison of existing *in vivo* results with the GHS additivity formula
  - > Our (DAS) proposed approach
  - > Current regulatory acceptance and future implementation
- Conclusions

Inhalation toxicity of mixtures: Background

- Airborne particles exposure is found in specific products (mixtures) applied in form of by liquid spray or powder/dust dispersion
  - > Industrial chemicals, agrochemicals, Beauty or house care, medical applications etc.

- Mixture registration may require inhalation toxicity testing
  - > Requirements differ globally, by geography and by industrial sector
  - > In some geographies, standard requirements may still apply:
    - i.e. acute or repeated dose *in vivo* testing
  - > In others, requirements are conditional and determined by exposure scenarios:
    - Severe local irritation and corrosivity
    - Low volatility
    - Particle size
    - Dilution
  - > Data may be used for Classification & Labeling purpose (i.e. GHS or similar).

Scientific question: the theory of additivity

- Often little information is available on toxicity mechanisms of mixture components
- Theory of additivity: a bit of history
  - > Finley, 1954 → Mathematical model for mixture toxicity prediction based on additivity assumption
  - > Pozzani, 1956 → 34/36 industrial chemical mixture studies predicted with Finley theory
  - > Smith, 1969 → Confirm previous finding

    Estimate that ca. 5% of combinations have less or greater than additive effects
- <u>For Acute systemic toxicity (high doses), additivity can be assumed</u>
- At doses < NOAELs, additivity may over predict toxicity, likely reflecting differences in Mode of Action (Feron, 1995; Borgert, 2004)

The GHS additivity formula

- Computational method from UN GHS (Globally Harmonized System) classification system based on theory of additivity:
  - > Predicts mixture toxicity without experiments
    - Use composition information and toxicity of single components
    - Prediction of acute systemic toxicity, in terms of toxicity classes for classification and labeling purpose
  - > Usable as stand alone non animal replacement method in some geographies (i.e. EU CLP; NZ, AUS regulations on AgChem formulations)
  - > Also recognized in transport regulations (UN, IATA etc…)
  - > Minimal cost/effort

The GHS additivity formula

- The formula:

$$\frac{100}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

where:

(b) The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using:

— the LD_{50}/LC_{50} where available,

— the appropriate conversion value from Table 3.1.2 that relates to the results of a range test, or

— the appropriate conversion value from Table 3.1.2 that relates to a classification category.

Information Gathering

- Source of information
  - > MSDS
  - > Robust Databases with regulatory acceptance (EChA inventory, Actor etc…)

- The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using:
  - > LD 50 /LC 50 where available,
  - > the appropriate conversion value from Table x that relates to the results of a range test, or
  - > the appropriate conversion value from Table x that relates to a classification category

Exposure routes	Classification Category or experimentally obtained acute toxicity range estimate	Converted acute toxicity point estimate (see Note 1)
Oral (mg/kg body-weight)	0 < Category 1 ≤ 5 5 < Category 2 ≤ 50 50 < Category 3 ≤ 300 300 < Category 4 ≤ 2 000	0,5 5 100 500
Dermal (mg/kg body-weight)	0 < Category 1 ≤ 50 50 < Category 2 ≤ 200 200 < Category 3 ≤ 1 000 1 000 < Category 4 ≤ 2 000	5 50 300 1 100
Gases (ppmV)	0 < Category 1 ≤ 100 100 < Category 2 ≤ 500 500 < Category 3 ≤ 2 500 2 500 < Category 4 ≤ 20 000	10 100 700 4 500
Vapours (mg/l)	0 < Category 1 ≤ 0,5 0,5 < Category 2 ≤ 2,0 2,0 < Category 3 ≤ 10,0 10,0 < Category 4 ≤ 20,0	0,05 0,5 3 11
Dust/mist (mg/l)	0< Category 1 ≤ 0,05 0,05 < Category 2 ≤ 0,5 0,5 < Category 3 ≤ 1,0 1,0 < Category 4 ≤ 5,0	0,005 0,05 0,5 1,5

Note 1

These values are designed to be used in the calculation of the ATE for classification of a mixture based on its components and do not represent test results.

The actual calculation

$$\frac{100}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

- Examples with increasing complexity

Hazard category	Classified components	Conc. % of substance	LD_{50}/LC_{50} or ATE	Calculation / total concentration of all substances in hazard category
Oral LD_{50}:	Contains no classified substances	0	Not applicable	Not applicable

Dermal LD_{50}:	Benzenesulfonic acid, mono-C11-13-branched alkyl derivs., calcimu salt (From coformulant Y)	4.596	1100	$\frac{4.596}{1100} = 0.0042$ Then $\frac{100}{0.0042}$ = LD_{50} 23809

Oral LD_{50}:	Ethoxylated Fatty Alcohol (Synperonic 13/10)	4.36	500	$\frac{4.36}{500} + \frac{8.99}{1530} = 0.0145$
	Cyclohexanone	8.99	1530	Then $\frac{100}{0.0145} = LD_{50}$ 6896

Inhalation LC_{50}:	Pyraclostrobin Polyether modified trisiloxane (Break Thru S233) Cyclohexanone 2-ethylhexan-1-ol (From Coformulant X)	6.05 4.84 8.99 3.486	0.58 1.08 11 1.5	$\frac{6.05}{0.58} + \frac{4.84}{1.08} + \frac{8.99}{11} + \frac{3.486}{1.5} = 18.0537$ Then $\frac{100}{18.0537} = LC_{50}$ 5.539

Case example: toxicity of formulations: “the 6-Pack”

- Registration of a formulation requires toxicity testing
- The main aim is predict Classification, labeling, PPE, first aid, transport, etc…
- The final classification may limit the registrability of a formulation
- The global regulatory requirements for formulation registration is a suite of 6 animal study using approximately 60 animals: the “6-pack”

Guideline Number	Data Requirements	Estimated Animal use
Acute Testing		
870.1100	Acute oral toxicity - rat	3-9 rats
870.1200	Acute dermal toxicity	10 rats
870.1300	Acute inhalation toxicity - rat	10 rats
870.2400	Primary eye irritation - rabbit	3 rabbits
870.2500	Primary dermal irritation	3 rabbits
870.2600	Dermal sensitization	31 mice (LLNA)

The analysis

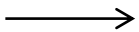
Does it work for agrochemical mixtures?

We have retrospectively reviewed 225 formulations (all types)

Product Class											
Herbicides		Insecticides		Fungicides		Fumigants		Nitrification		Blanks (no active)	
160		37		18		5		2		3	
Formulation Types											
Liquids								Gel	Solids		
SL	EC	SC	EW	SE	OD	CS	Others		WG	GR	WP
52	51	33	19	14	10	6	9	1	24	3	3

Comparison: *in vivo* data vs. calculation

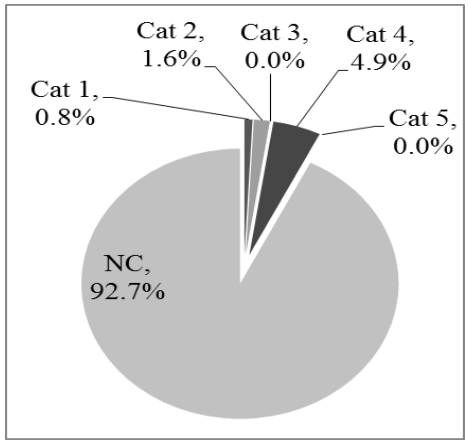
Is the classification consistent?



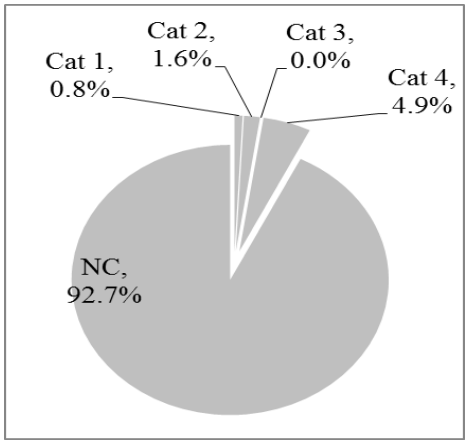
The database composition

123 acute inhalation studies (122 liquid/dust aerosol+1vapour)

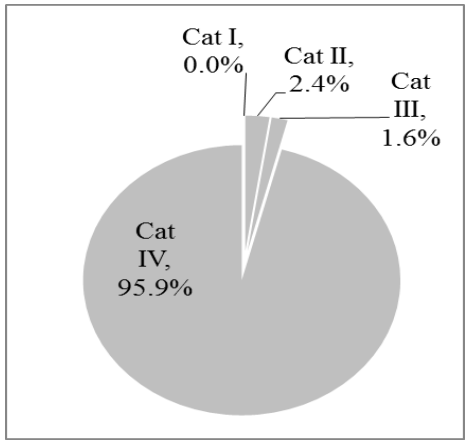
GHS



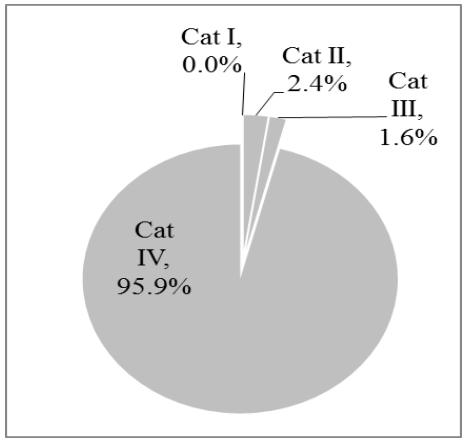
CLP



EPA



ANVISA



Endpoint	ATE thresholds	GHS	CLP	EPA	ANVISA
Acute inhalation toxicity	0	0 < Cat 1 ≤ 0.05	0 < Cat 1 ≤ 0.05	0 < Cat I ≤ 0.05	0 < Cat I ≤ 0.05
(ATE/LC_{50} in mg/L air)	0.05	0.05 < Cat 2 ≤ 0.5	0.05 < Cat 2 ≤ 0.5	0.05 < Cat II ≤ 0.5	0.05 < Cat II ≤ 0.5
	0.5	0.5 < Cat 3 ≤ 1.0	0.5 < Cat 3 ≤ 1.0	0.5 < Cat III ≤ 2.0	0.5 < Cat III ≤ 2.0
	1.0	1.0 < Cat 4 ≤ 5.0	1.0 < Cat 4 ≤ 5.0		
	2.0			Cat IV > 2.0	Cat IV > 2.0
	5.0	Cat 5*/Not classified > 5.0	Not classified > 5.0		

* used for subastance that may pose a hazard to vulnerable populations

Calculation method

Predictions are very accurate

UN GHS/ CLP		Category (ATE calculation)						
		1	2	3	4	5	NC	Total
Category (Animal data)	1	**0**	1	0	0	0	0	1
	2	0	**2**	0	0	0	0	2
	3	0	0	**0**	0	0	0	0
	4	0	2	0	**1**	0	3	6
	5	0	0	0	0	**0**	0	0
	NC	0	0	0	1	0	**113**	114

Matches: 94.3.%
Underestimation: 3.3 %
Overestimation: 2.4 %

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US EPA/ ANVISA		Category (ATE calculation)				
		I	II	III	IV	Total
Category (Animal data)	I	**0**	0	0	0	0
	II	0	**2**	1	0	3
	III	0	1	**0**	1	2
	IV	0	1	0	**117**	118

Matches: 96.7 %
Underestimation: 1.6 %
Overestimation: 1.6 %

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Calculation method

Accuracy and specificity are always very high, across endpoints

Classification system	Threshold used for negatives vs positive	Accuracy	Sensitivity	Specificity	Sample size	TP/FN	TN/FP	Positive predictive value	Negative predictive value
		%	%	%	n	n	n	%	%
Acute Oral Toxicity									
GHS cat 5/EPA Cat IV	5000 mg/Kg bw	78.9	68.4	88.5	199	65/30	92/12	84.4	75.4
CLP cat 4/ANVISA Cat IV	2000 mg/Kg bw	86.9	69.8	91.2	213	30/13	155/15	66.7	92.3
Acute Dermal Toxicity									
GHS cat 5/EPA Cat IV	5000 mg/Kg bw	92.7	60.0	93.7	179	3/2	163/11	21.4	98.8
CLP cat 4/ANVISA Cat IV	2000 mg/Kg bw	99.5	100.0	99.5	207	2/0	204/1	66.7	100.0
Acute Inhalation Toxicity									
GHS cat 4/CLP cat 4	5.0 mg/L air	96.7	66.7	99.1	123	6/3	113/1	85.7	97.4
EPA cat IV/ANVISA cat IV	2.0 mg/L air	98.4	80.0	99.2	123	4/1	117/1	80.0	99.2

Other findings – relationship with oral toxicity

Orally non-toxic (i.e. non classified) formulations are unlikely to be toxic via inhalation route

B)

		Acute inhalation toxicity (LC_{50} in mg/L air)					
		$0 < LC_{50} \leq 0.05$	$0.05 < LC_{50} \leq 0.5$	$0.5 < LC_{50} \leq 1.0$	$1.0 < LC_{50} \leq 5.0$ (GHS cat 4)	$LC_{50} > 5.0$* (GHS NC)	Total
Acute oral toxicity (LD_{50} in mg/Kg bw)	$0 < LD_{50} \leq 5$	0	0	0	0	0	0
	$5 < LD_{50} \leq 50$	1^	0	0	0	0	1
	$50 < LD_{50} \leq 300$	0	2	0	2	1	5
	$300 < LD_{50} \leq 2000$ (GHS cat 4)	0	0	0	1	17	18
	$LD_{50} > 2000$ (GHS NC)	0	0	0	3	96	99

* LC_{50}> 5.0 or no mortality at the Maximum Attainable Concentration
^ vapour formulation

True life exposure scenarios…

- LC50 was below 5.0, 2.0 or 1.0 mg/L air in 7.3%, 4.1% or 2.4% of the case (based on a 4-hour continuous rat exposure),
- Human exposure during a 4-hour time period: 5 m3 air, assuming a specific human breathing rate of 1.25 m3/hour (Derelanko and Hollinger; 1995).
- Concentrations of 1.0, 2.0 or 5.0 mg/L (equivalent to 1000, 2000, or 5000 mg/m3), would result in exposures of 5000, 10000 and 25000 mg.
- 1000, 2000 and 5000 mg/m3 corresponds to the concentration of PM10 dust particle, expected, respectively, in a normal, strong or severe dust storm, (Hoffmann, 2008; ISO, 1995)!
- Exposure to concentrate AgChem formulation is extremely limited in time (essentially during mixing and loading), most likely to occur in open spaces, and potentially uses a closed transfer system,
- When testing is justified (i.e. high volatitlity, dust content etc) a concentration of 1.0 mg/L air represents a worst case scenario.

Our proposed approach for Agrochemical formulation

- Step 1) Evaluate the need for hazard characterisation.
  - > Consider Waiving criteria (OECD, 2016)
  - > Consider oral toxicity information if available

- Step 2) If quantitative hazard characterisation is needed, perform a calculation using the GHS additivity formula.
  - > If LC_{50} > 1.0 mg/L air, accept results as negative. No PPE required (EPA, 2016a).
  - > If LC_{50} < 1.0 mg/L air (expected in approximately 2.4% of cases), re-consider the weight of evidence and physicochemical characteristics:
    - sufficient information to assign the correct respiratory protection device?

- Last resort: *in vivo* test, with a reduced number of animals, only if strictly necessary to determine the correct PPE to be used.
  - > Testing above 1.0 mg/L air (for aerosols) not recommended for AgChem formulations

Implementation

> Internal acceptance

- R&D use (formulation design)
- Regulatory use:
  - used in all EU-only Business cases
  - used as a predictive tool before any *in vivo* study to proactively act on animal welfare.

>Improving acceptance globally

> 2 publications in preparation

> Dissemination to EPA

- EPA will soon require concurrent submission of calculation for systemic toxicity endpoints for a full substitution
- This should be concurrent to a switch from EPA categories to GHS categories, LIMITED TO these 3 acute systemic endpoints.

> Presentation to congresses

> Participation in OECD and EChA working groups

> Work with animal welfare association for implementation in global developing regulations

Thank you!

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