Calculating the toxicity of plant protection products

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10-30-2019

Outline

Plant protection products (Formulations) complexity

Alternative evaluation approaches

The GHS Additivity Formula

- Performance
- Internal implementation strategy
- Current regulatory acceptance

Conclusions

Purpose: Acute Toxicity Testing

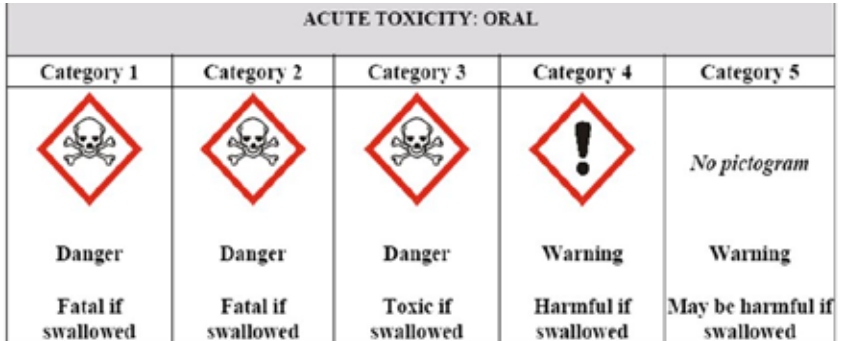
Identification of intrinsic hazard properties of chemicals or end-use products upon shorter-term exposure

Basis for hazard communication

- Classification (e.g. EPA or GHS category; LD50 mg/Kg)

	GHS	CLP	EPA	ANVISA
Acute oral toxicity (LD50 mg/Kg)	0<Cat 1≤5 5<Cat 2≤50 50<Cat 3≤300 300<Cat 4≤2000 2000<Cat 5** ≤5000 Not Classified >5000	0<Cat 1≤5 5<Cat 2≤50 50<Cat 3≤300 300<Cat 4≤2000 Not Classified .>2000	0<Cat I≤50 50<Cat II≤500 500<Cat III≤5000 Cat IV>5000	0<Cat I≤20 20<Cat II≤200 200<Cat III≤2000 Cat IV>2000

- Product label statements (e.g. signal words – level of hazard)
  - Danger, Warning



Purpose: Acute Toxicity Testing

Inform risk management decisions to protect human health

- Personal Protective Equipment (PPE)
- Transportation requirements
- Use restrictions
- Generic first-aid measures

Dose level selection for sub-chronic and other studies

The classification may impact registrability of a formulation

Acute toxicity testing: “The 6-Pack”

The global regulatory requirements for formulation registration is a suite of 6 animal studies using approximately 60 animals

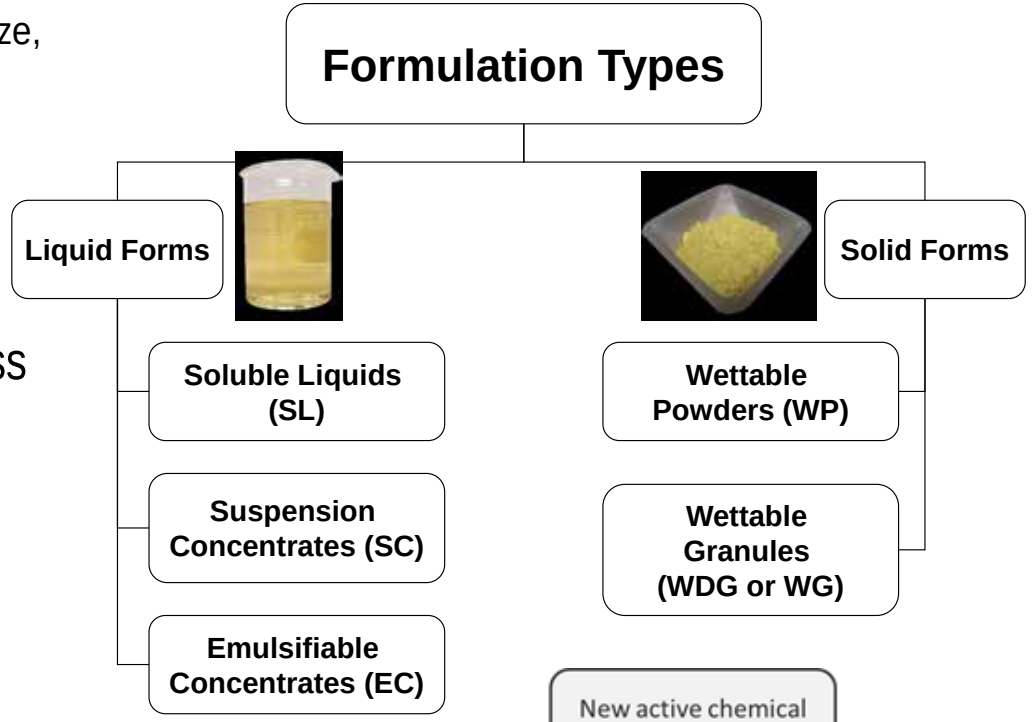
	Guideline Number	Data Requirements	Estimated Animal use
	Acute Testing		
Acute systemic toxicity	870.1100	Acute oral toxicity - rat	3-9 rats
	870.1200	Acute dermal toxicity	10 rats
	870.1300	Acute inhalation toxicity - rat	10 rats
Acute contact toxicity	870.2400	Primary eye irritation - rabbit	3 rabbits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

Vast majority of acute toxicity studies received by EPA are conducted on formulations

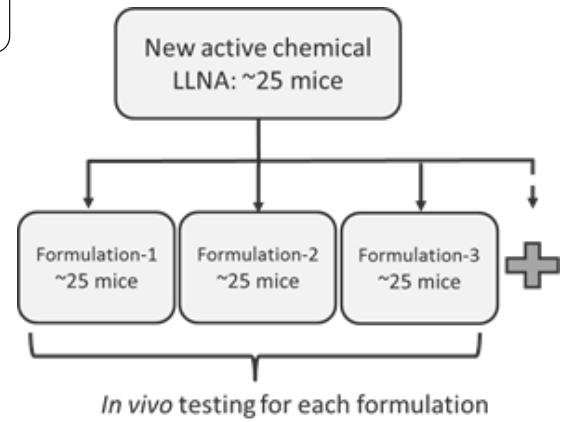
Plant Protection Product Complexity

- Active ingredient(s) + co-formulants
  - Surfactants , fillers, solvents, thickeners, biocides, anti-freeze, odorants, stabilizers, dyes and pigments
    - Optimize delivery of active ingredient(s)

- Alternative methods should be assessed across multiple formulation types
  - E.g. liquid vs dry; water vs solvent based



- Effective implementation of alternative methods may lead to:
  - Development of sustainable products
  - Greater reduction in animal usage



In vitro alternatives for plant protection products

6-pack endpoints	*In vitro* test (if available)	*Applicability to Plant Protection Products*
Acute oral	Not available yet	• The OECD 432 may have good negative prediction for chemicals not needing classification (JRC, 2013) • Not verified on Agrochemical formulations
Acute dermal	Not available yet	• Product specific evidence from dermal absorption available only for active ingredients
Acute inhalation	Not available yet	-
Dermal irritation	OECD TG 430, 431, 435, 439	• Testing ongoing on Agrochemical formulations
Ocular irritation	OECD TG 437, 491, 492	• Testing ongoing on Agrochemical formulations
Dermal sensitisation	Defined approach from several *in vitro* tests	• Defined approaches not agreed for PPP

Alternatives: What routes can we take?

- Evidence-/exposure-based testing and waiving
- Read Across strategy
- GHS/CLP additivity formula approach

Exposure considerations

- Relevant routes of exposure:

A) Mix & Load (open system) (Concentrate)

Accidental occupational exposure to concentrate end-use product

- Routes: contact (skin, eye) and inhalation
- Labelling & PPE

B) Hand-held (Dilution)

Occupational or residential exposure to agrochemical actives

- Routes: oral, dermal or inhalation (mostly due to dilutions, applications)
- PPE, when required

Exposure and evidence-based waiving and Read-Across

- Framework set and criteria laid down by OECD in 2016

  OECD ENV/JM/MONO(2016)32

- Major common waiving criteria:
  - Exposure-based waiving:

Waivers
Physical state/properties (e.g. volatility, extreme pH)
Product size/design prevents exposure
Study not technically feasible (e.g. aerosol generation)
Properties of AI (e.g. sensitizer; dermal penetration)

Bridging/Read-Across
Is there a similar existing formulation with definitive data? • Same physical form • Similar concentrations of AI or more dilute • Similar co-formulants

  - Consider alternative test(s) with good negative prediction (i.e. 3T3 NRU)
  - Evidence-based waiving for dermal toxicity
    - If the test chemical has shown no adverse effects in an acute oral toxicity test up to 2000 mg/kg bw
    - If the oral LD50 of test chemical is less than 300 mg/kg bw – classified consistent with GHS Cat as oral hazard
    - For materials with lower dermal absorption, waiver possibilities depending on oral LD50 (challenging for formulations)

Snapshot of global requirements and recent regulatory changes

Use of alternatives instead of animals is possible in some regulatory frameworks:

Country	Animal tests (6-pack)	*In vitro* tests or Exposure-based waiving	Read across	GHS Calculation
ANZ	+	+	+	+
EU	+	+	+ / ?	+ / ?
USA	+	pilot program	+	pilot program
CAN	+	pilot program	+	pilot program
Brazil	+	+ / ?	+	x
Other LA	+	x	+ (in some countries)	x
Asian countries	+	x	x	x

EU - Art 62 of 1107/2009:

- Enter into force Dec 2016: legal obligation to perform alternative approaches, if available
- However, concerns from some MS and prefer *in vivo* studies

US-EPA/CAN-PMRA:

- 2016/2017: Pilot programs launched on A) waiving of the acute dermal toxicity; B) use alternatives to animal tests (calculations or *in vitro*)
- ANVISA (2019): May consider acute inhalation waivers

The GHS additivity formula

- Computational method from UN GHS (Globally Harmonized System) classification system based on theory of additivity:
  - Predicts mixture toxicity for C&L without conducting experiments
    - Use composition information and toxicity of single components
    - Prediction of acute systemic toxicity, in terms of toxicity classes for C&L
  - 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

Systemic Toxicity- Additivity Formula

Formula:

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

ATE	=	Acute Toxicity Estimate
Ci	=	Concentration of Ingredient i
I	=	Individual Ingredient I
N	=	Number of ingredients

Information needed

- Acute toxicity of mixture components or ATE (**A**cute **T**oxicity **E**stimate)
- Concentrations of mixture components

Ingredients

- Include: Ingredients with a known acute toxicity which fall into any GHS category
- Ignore: Non-toxic ingredients

Systemic Toxicity- Additivity Formula

- Accounts for the contribution of each component to the toxicity of the mixture
- All ingredients treated equally, doesn’t give more weightage to active ingredient(s)
- Does not consider the type of solvent (dosing vehicle). Assumes the use of same solvent for all co-formulants.
  - May alter bioavailability (Cmax and AUC), which may affect systemic toxicity
- Assumes that chemicals are not interactive

Sources of information

- MSDS
- Robust Databases with regulatory acceptance (EChA inventory, Actor etc…)
- LD50 /LC50 where available,
- The appropriate conversion value from Table that relates to the results of a range test, or
- The appropriate conversion value from Table 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

Examples with increasing complexity

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

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 $\frac{100}{0.0042}$ = LD50 23809
Oral LD_{50}:	Ethoxylated Fatty Alcohol (Synperonic 13/10) Cyclohexanone	4.36 8.99	500 1530	$\frac{4.36}{500} + \frac{8.99}{1530} = 0.0145$ Then $\frac{100}{0.0145}$ = LD50 6896
Inhalation LC_{50}:	Aerosols: Pyraclostrobin Polyether modified trisiloxane (Break Thru S233) 2-ethylhexan-1-ol (From Coformulant X) Vapours: Cyclohexanone	6.05 4.84 3.486 8.99	0.58 1.08 1.5 11	$\frac{6.05}{0.58} + \frac{4.84}{1.08} + \frac{3.486}{1.5} = 17.2365$ Then $\frac{100}{17.2365}$ = Aerosol LC50 5.80 $\frac{8.99}{11} = 0.817$ Then $\frac{100}{0.817.}$ = Vapour LC50 122.40

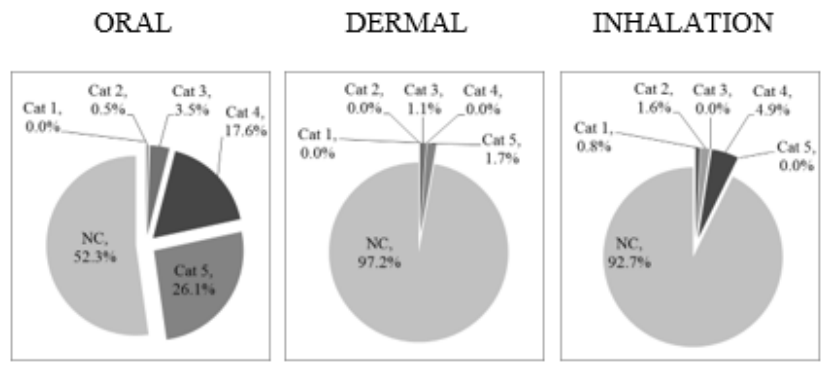
Case Study: In-house evaluation of GHS additivity approach

- A database of acute toxicity studies for 225 agrochemical formulations
  - Included solvent-based and water-based liquids and solids

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

- Acute Toxicity Estimate (ATE) of the formulation was derived using the Additivity Formula
  - Oral toxicity: >50% had LD50 higher than 5000 mg/Kg or >75% higher than 2000 mg/Kg
  - Dermal toxicity: >97% had LD50 higher than 5000 mg/Kg
  - Inhalation toxicity: >92% had LC50 higher than > 5.0 mg/L a

GHS



- In general represent lower hazard potential

In-house evaluation of GHS additivity approach

Classification system	Threshold used for negatives vs positive	Accuracy %	Sensitivity %	Specificity %	Sample size n
Acute Oral Toxicity					
GHS cat 5/EPA Cat IV	5000 mg/Kg bw	79.9	69.1	90.2	199
CLP cat 4/ANVISA Cat IV	2000 mg/Kg bw	87.8	71.1	92.3	213
Acute Dermal Toxicity					
GHS cat 5/EPA Cat IV	5000 mg/Kg bw	92.7	60.0	93.7	179
CLP cat 4/ANVISA Cat IV	2000 mg/Kg bw	99.5	100.0	99.5	207
Acute Inhalation Toxicity					
GHS cat 4/CLP cat 4	5.0 mg/L air	96.7	66.7	99.1	123
EPA cat IV/ANVISA cat IV	2.0 mg/L air	98.4	80.0	99.2	123

TP/FN: True Positives/False Negatives. TN/FP: True Negatives/False Positives

Corvaro et al., 2016

- Weaker performance in predicting oral ATE in 2000 – 5000 mg/Kg bw range for acute oral toxicity
- High accuracy and specificity for prediction of agrochemical mixture toxicity
- Integrating this approach for negative prediction may allow up to 95% reduction in *in vivo* testing

In-house evaluation of GHS additivity approach

Oral Vs Dermal toxicity:

- For single substances, acute dermal toxicity is often lower than corresponding toxicity via oral route
- The acute dermal toxicity was in the same toxicity class or in lower toxicity classes compared to the acute oral toxicity across all tested formulation types

Oral Vs Inhalation toxicity:

- The oral ATE class would predict the same or a worse case inhalation ATE in 95% of cases across all the categories
  - Orally non-toxic (i.e. non classified) formulations are unlikely to be toxic via inhalation route

Corvaro et al., 2016

GHS/CLP additivity formula: Regulatory acceptance

GHS Calculation method is currently

- An approach acceptable by EU law, Australia, New Zealand
- Potential to be legally binding in absence of further guidance (UK CRD, Nov, 2017)
- Included in global over-arching regulations on transportation

However,

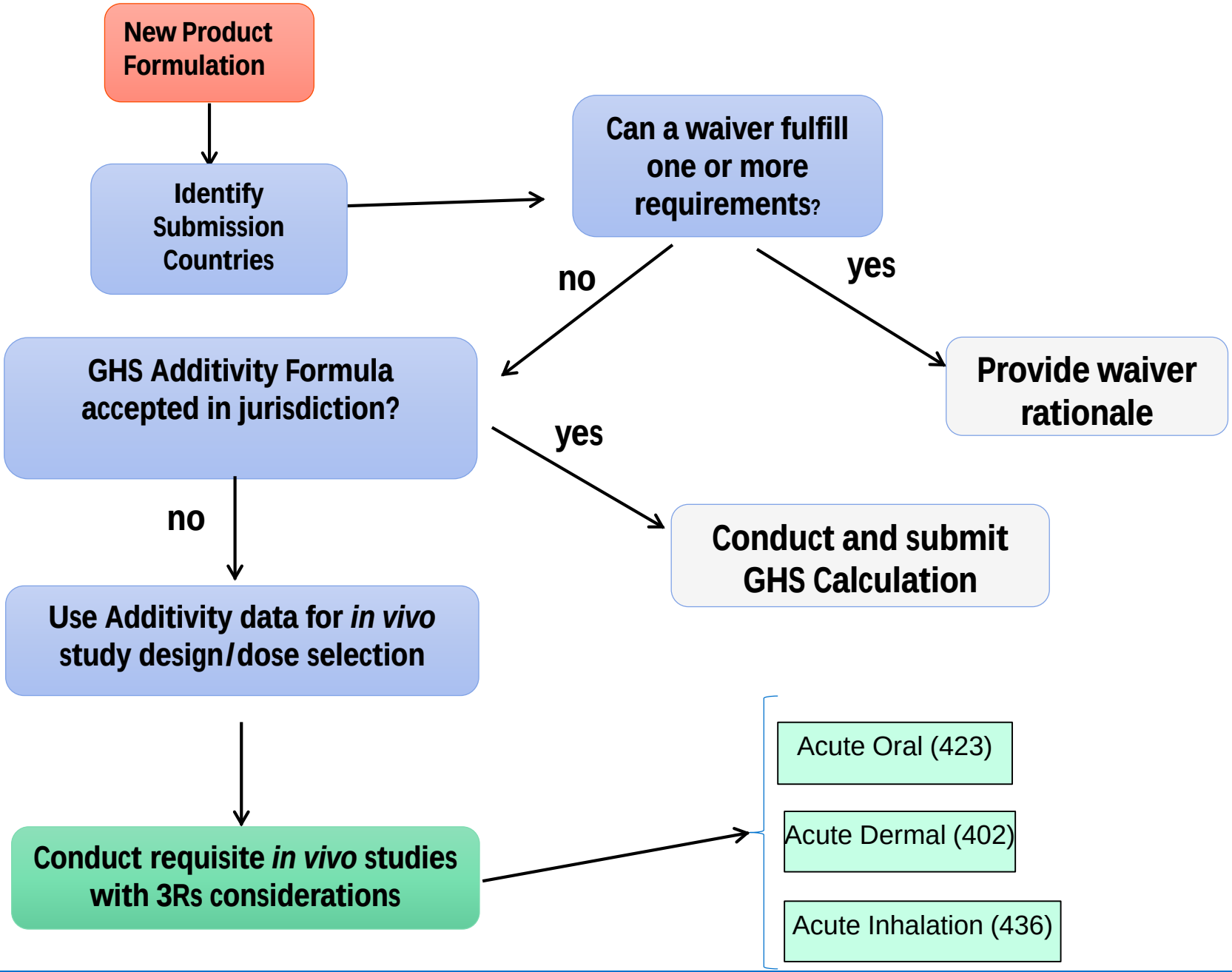
- Not yet acceptable in many other countries, including some EU member countries
- Missing a clear evidence of being satisfactory “across the board” for all endpoints/categories
- Unclear criteria on information sources (EChA DB, MSDS, etc…)

Need cross-talk between stakeholders with data, for harmonization on predictivity (strength/weaknesses) of ATE calculation

GHS additivity approach: Implementation

- **R&D use**:
  - Formulation development
    - Design,
    - Screens to prioritize the formulation with lower toxicity

- **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
  - Dose selection
  - Higher confidence for formulations with negative predictions

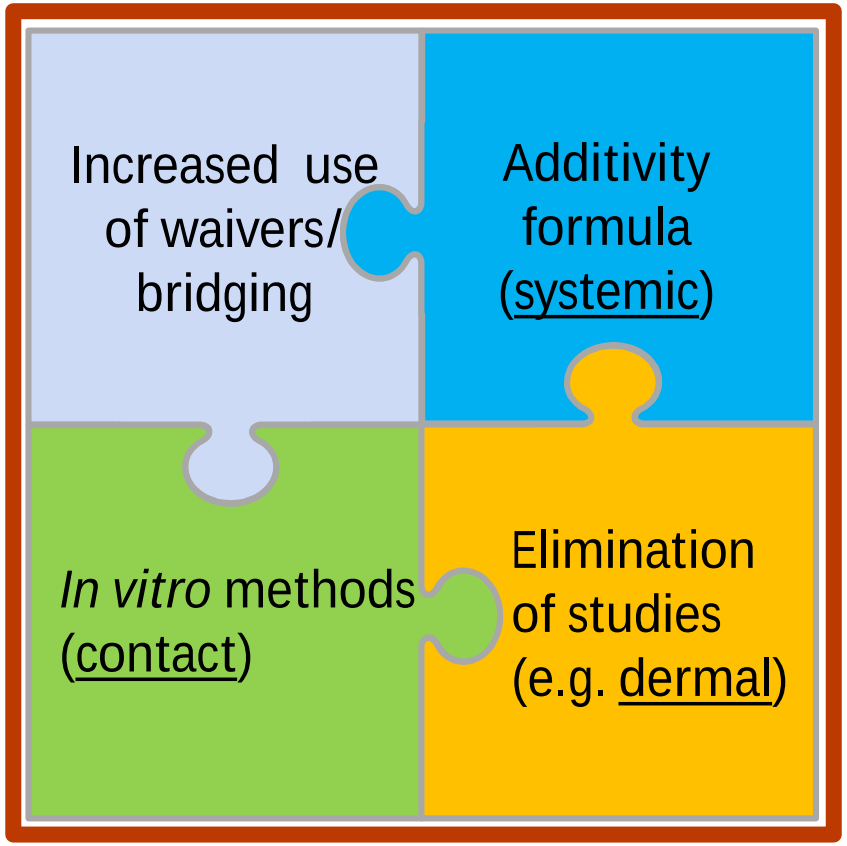

New Product Formulation
Identify Submission Countries
Can a waiver fulfill one or more requirements?
no
yes
Provide waiver rationale
GHS Additivity Formula accepted in jurisdiction?
yes
Conduct and submit GHS Calculation
no
Use Additivity data for *in vivo* study design/dose selection
Conduct requisite *in vivo* studies with 3Rs considerations
Acute Oral (423)
Acute Dermal (402)
Acute Inhalation (436)

Conclusions

- No accepted experimental stand-alone replacements for evaluating acute systemic toxicity of formulations

- Excellent performance of the GHS additivity method indicates its use as a stand-alone replacement to characterize negative outcomes

- Require cross-talk between stakeholders with data, for harmonization on predictivity (strength/weaknesses) of ATE calculation



Acknowledgements

- Sean Gehen
- Marco Corvaro
- Ricardo Acosta Amado
- Reza Rasoulpour
- Dan Wilson

Thank you!

Discussion: Evaluating mixtures for acute lethality

Challenges for evaluating acute systemic toxicity of mixtures

- Explore the applicability of GHS additivity approach to broader formulation types and industry sectors
  - Corvaro et al., 2016
    - High accuracy and specificity for prediction of formulation toxicity
  - Van Cott et al., 2018
    - Acute systemic toxicity of many formulations is not the sum of the ingredients toxicity. Ingredients in a formulation can interact to result in lower or higher toxicity than predicted by the GHS additivity formula
  - Adler-Flindt and Martin, 2019
    - Calculation method predicted 80% of the PPPs correctly
    - Cytotoxicity assays (NRU and hFF cells) did not reliably reflect differences in toxicity between AI and formulation

Challenges for evaluating acute systemic toxicity of mixtures

- Global acceptance of data generated using alternative methods
  - Lack of confidence – not all countries recognize calculation method
  - Limited verification in the literature – need for additional retrospective analysis to demonstrate applicability across chemical types and companies
  - Regional preference for certain studies
    - Inhalation studies: may be waived in EU, ANVISA
    - Dermal studies: may be waived at US EPA, PMRA
  - For global submissions – end up testing for all acute endpoints

Challenges for evaluating acute systemic toxicity of mixtures

- Mechanistic information on actives and co-formulants
  - Known MoA for some actives/chemistry classes, however, not for all (e.g. plant or soil metabolites)
  - Can we use AI MoA information in model development (for target-specific MoA) (e.g. mitochondrial toxicity, cholinesterase activity etc)

- Reproducibility of *in vivo* systemic toxicity LD50/LC50 values for PPPs
  - Less literature on animal variability. General pharmacokinetic variability in absorption
  - Current guidelines with limited animals/group and vehicle effects may impact reproducibility
    - Dermal absorption is greater in rats than in human skin
    - Inhalation – there are differences in humans vs rodents

Challenges for evaluating acute systemic toxicity of mixtures

- Lack of accurate LD50/LC50 values for co-formulants
- In additivity method, water is assumed to be the default solvent for all actives and co-formulants. Usually this information is not available from MSDS. May affect ATE predictions
  - Due to interactions between vehicle and the ingredients
  - Altered bioavailability
- Methods to evaluate interaction between co-formulants
  - Additive/synergistic effects
- Need for testing at 5000 mg/kg bw?
  - E.g., EPA Vs GHS classification (categories and scoring criteria)

Criteria for classification are still variable across geographies

Endpoint	ATE thresholds	GHS	CLP	EPA	ANVISA
Acute oral toxicity (ATE/LD_{50} in mg/Kg)	0	0 < Cat 1 ≤ 5	0 < Cat 1 ≤ 5	0 < Cat I ≤ 50	0 < Cat I ≤ 20
	5	5 < Cat 2 ≤ 50	5 < Cat 2 ≤ 50		
	20				20 < Cat II ≤ 200
	50	50 < Cat 3 ≤ 300	50 < Cat 3 ≤ 300	50 < Cat II ≤ 500	
	200				200 < Cat III ≤ 2000
	300	300 < Cat 4 ≤ 2000	300 < Cat 4 ≤ 2000		
	500			500 < Cat III ≤ 5000	
	2000	2000 < Cat 5** ≤ 5000	Not Classified .> 2000		Cat IV > 2000
	5000	Not Classified > 5000		Cat IV > 5000	
Acute dermal toxicity (ATE/LD_{50} in mg/Kg)	0	0 < Cat 1 ≤ 50	0 < Cat 1 ≤ 50	0 < Cat I ≤ 200	0 < Cat I ≤ 50
	50	50 < Cat 2 ≤ 200	50 < Cat 2 ≤ 200		50 < Cat II ≤ 200
	200	200 < Cat 3 ≤ 1000	200 < Cat 3 ≤ 1000	200 < Cat II ≤ 2000	200 < Cat III ≤ 1000
	1000	1000 < Cat 4 ≤ 2000	1000 < Cat 4 ≤ 2000		Cat IV > 1000
	2000	2000 < Cat 5** ≤ 5000	Not classified > 2000	2000 < Cat III ≤ 5000	
	5000	Not classified > 5000		Cat IV > 5000	
Acute inhalation toxicity (ATE/LC_{50} in mg/L air)	0	0 < Cat 1 ≤ 0.05	0 < Cat 1 ≤ 0.05	0 < Cat I ≤ 0.05	0 < Cat I ≤ 0.05
	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		

Challenges for evaluating acute systemic toxicity of mixtures

- What are the types of mixtures where acute tox predictions are needed?
- Building datasets that will allow models for mixture toxicity to be more effectively developed
- What are the considerations regarding mixtures composition and maintaining confidentiality? Are there tools available to allow such analyses?