

# Vision for Animal-Free Pesticide Formulation Assessments

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# Topics

- Acute toxicity testing for agrochemical formulations
- Vision for moving to animal-free approaches
  - > Waivers/Bridging
  - > GHS additivity approach (*in silico*)
  - > Non-animal alternatives (*in vitro*)
- Case-Study examples
- What is needed next

# Agrochemical Formulation Testing

## *Global testing of plant protection products (PPP)*

- **Drivers**

- ✓ Hazard ID
- ✓ C&L
- ✓ Risk assessment
- ✓ Transport

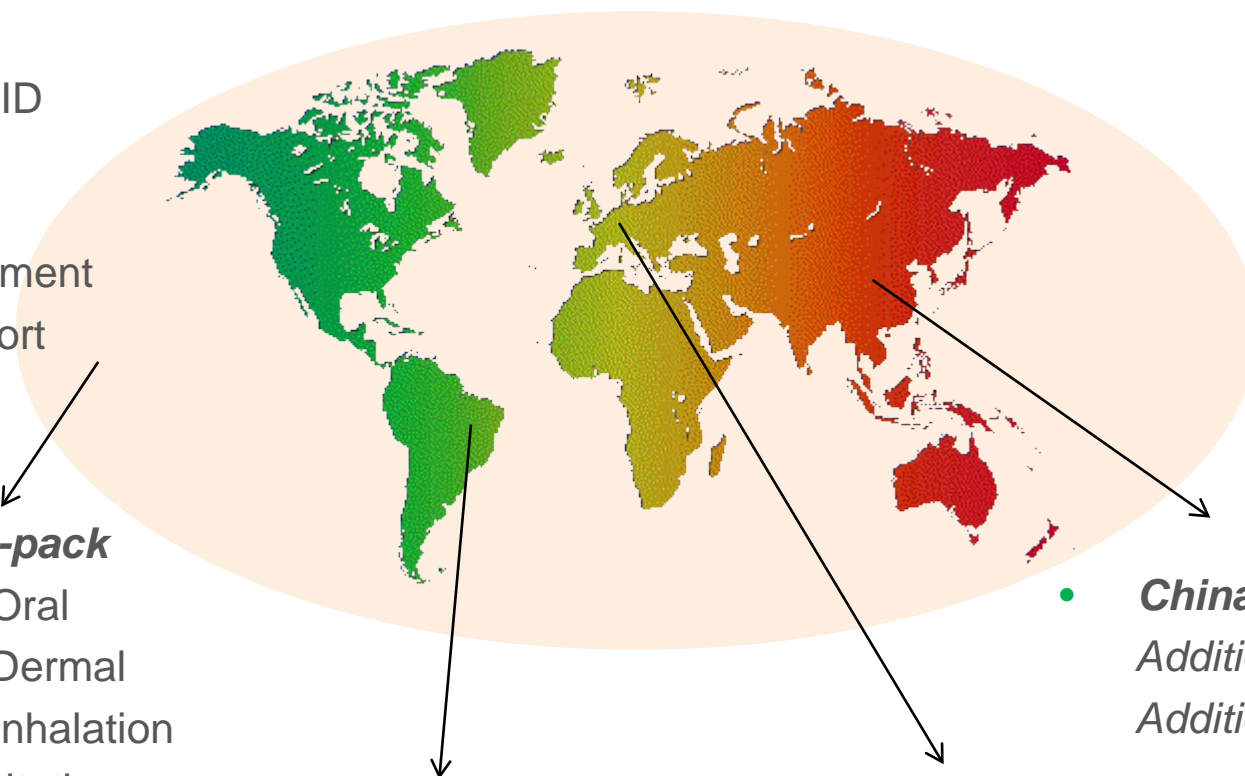
- **Global 6-pack**

- ✓ Acute Oral
- ✓ Acute Dermal
- ✓ Acute Inhalation
- ✓ Skin Irritation
- ✓ Eye Irritation
- ✓ Skin Sensitisation

- **Brazil**  
Ames test  
*In vitro* micronucleus

- **EU**  
*In vitro* Dermal Absorption

- **China (conditional)**  
Additional Buehler  
Additional Draize Test(s)



# Acute 6 Pack – Animal Use

TABLE—TOXICOLOGY DATA REQUIREMENTS

Guideline Number	Data Requirements	Use Pattern		Test substance to support	
		Food	Nonfood	MP	EP
Acute Testing					
870.1100	Acute oral toxicity - rat	R	R	TGAI and MP	TGAI, EP, and possibly diluted EP
870.1200	Acute dermal toxicity	R	R	TGAI and MP	TGAI, EP
870.1300	Acute inhalation toxicity - rat	R	R	TGAI and MP	TGAI and EP
870.2400	Primary eye irritation - rabbit	R	R	TGAI and MP	TGAI and EP
870.2500	Primary dermal irritation	R	R	TGAI and MP	TGAI and EP
870.2600	Dermal sensitization	R	R	TGAI and MP	TGAI and EP

## Estimated Animal use

3-9 rats

10 rats

10 rats

3 rabbits

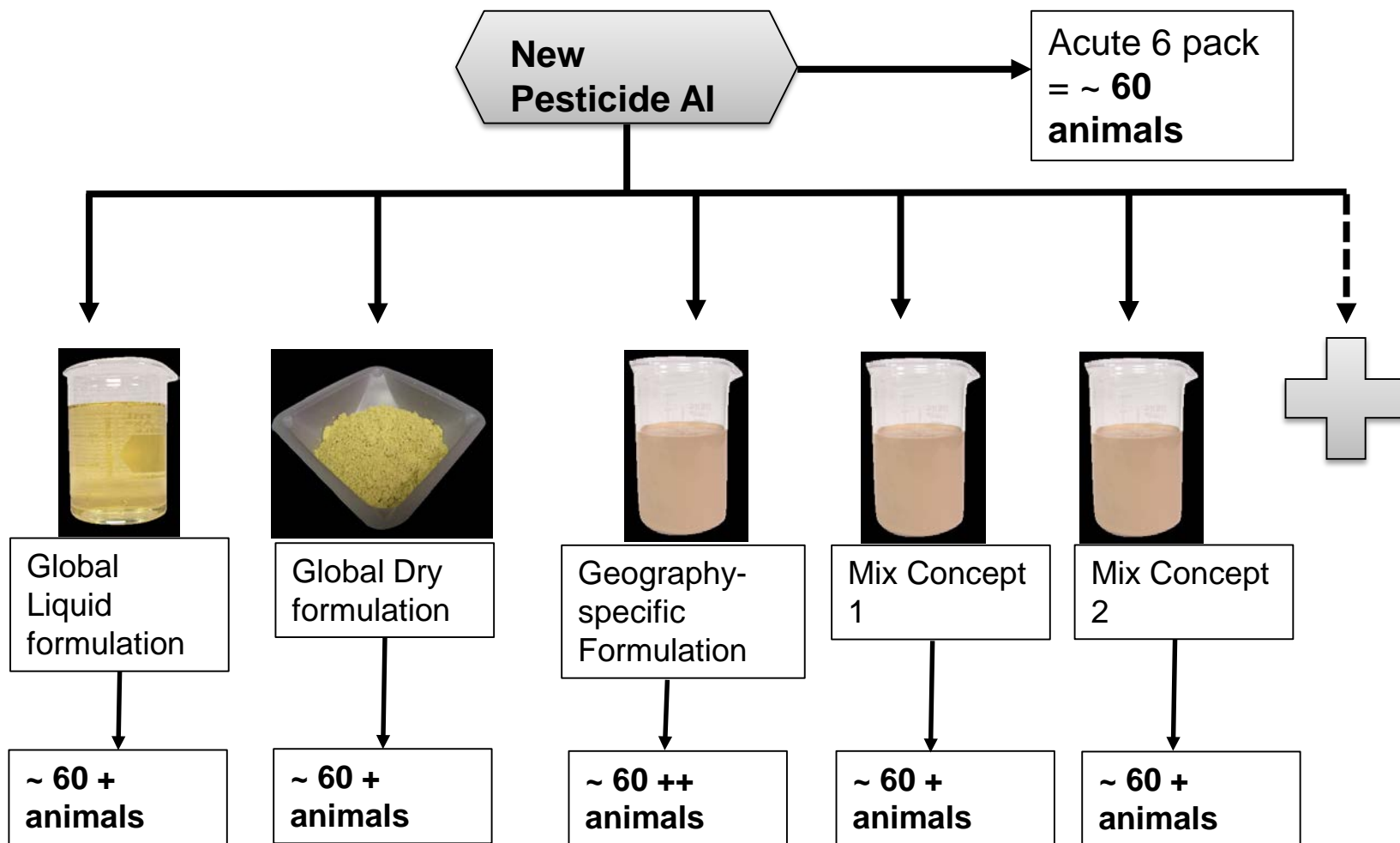
3 rabbits

31 mice  
(LLNA)

= ~ 61  
animals per 6  
pack



# Formulations- The Opportunity



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# Vision

- Eliminate animal use for assessment of acute health hazards for agrochemical formulations
- How do we make it happen?
  - > See vertebrate testing as a last resort once other options are exhausted
  - > We need a coordinated effort between Industry and Regulators
  - > Need workable approaches for all 6 endpoints

# Approach

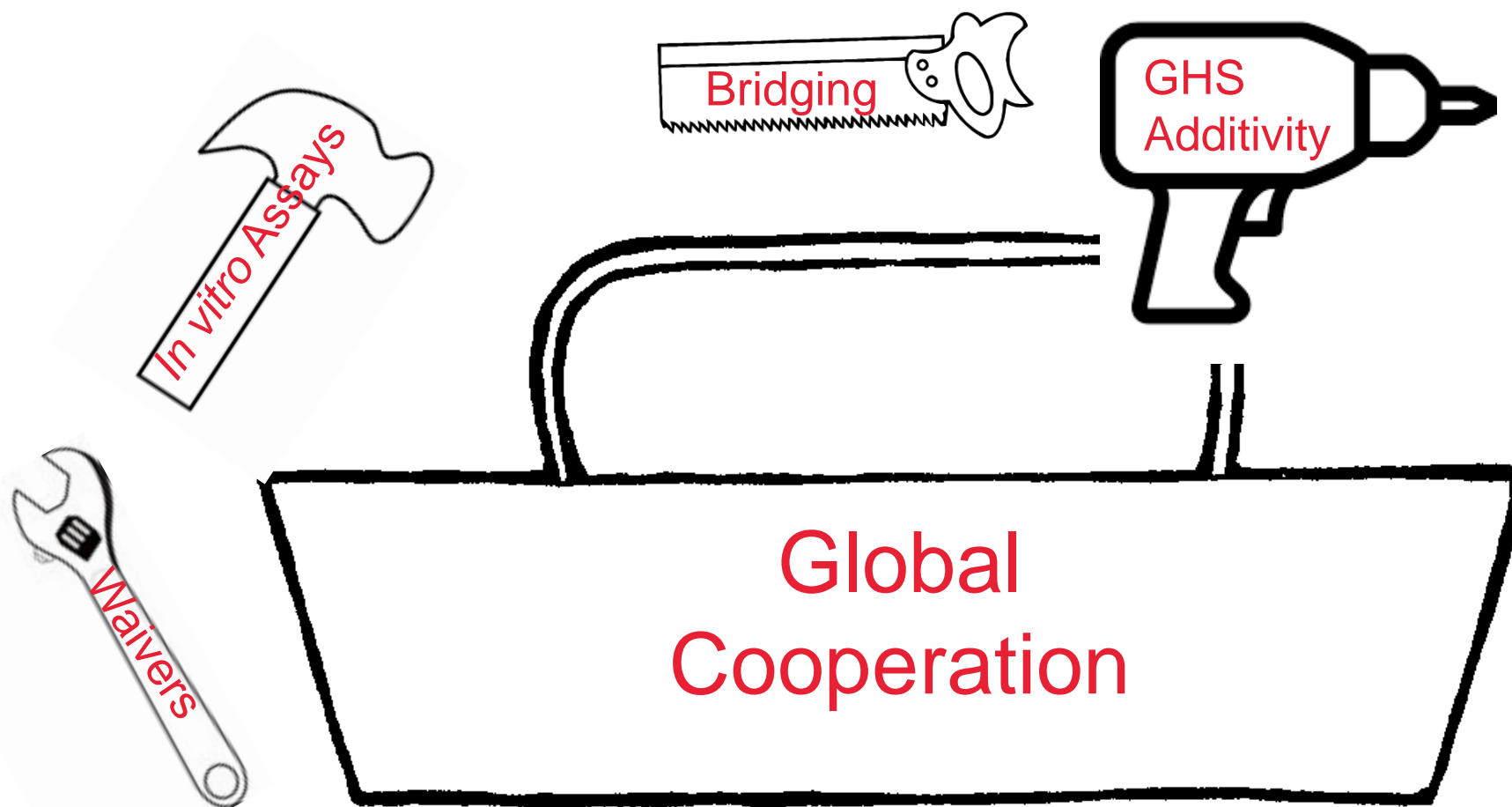
- Not a one size fits all approach
- We need the right tool for the job
- Sometimes it will take more than one tool
  - > Testing battery
  - > Integrated testing strategy



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# Use the full tool box





# Waiver and Bridging Opportunities

- EPA and PMRA have guidance documents on waiving or bridging acute toxicity studies
  - > <http://www2.epa.gov/pesticide-registration/bridging-or-waiving-data-requirements>

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

Bridging/Read-Across
Is there a similar existing formulation with definitive data? <ul style="list-style-type: none"><li>• Same physical form</li><li>• Similar concentrations of AI or more dilute</li><li>• Similar co-formulants</li></ul>
Interpolation (GHS) <ul style="list-style-type: none"><li>• A+B; C+B</li></ul>

# GHS Additivity Formula- Systemic Toxicity

- Use for classifying mixtures based on toxicity of ingredients
- Rules
  - > Include ingredients with a known acute toxicity which fall into any category of GHS
  - > Ignore: non-toxic ingredients (e.g. water); ingredients with limit-dose test and no toxicity

Ingredient	Weight %	Tox data (mg/kg)	GHS Category
Active	45%	Oral LD50: 500	4
Inert	20%	Oral LD50: 1500	4
Inert	5%	Oral LD50: 200	3
Water	30%	NA	

$$ATE_{mix} = \frac{100}{45/500 + 20/1500 + 5/200}$$

$$ATE_{mix} = 779 \text{ mg/kg (Cat. 4)}$$



# GHS Classification of Mixtures- Irritation/Sensitization

- Classification of mixture is triggered by concentration of ingredients that are classified
- Skin
  - > E.g. Skin Cat 1 ingredient  $\geq 5\%$   $\longrightarrow$  mixture classified Cat. 1
- Eye
  - > E.g. Eye Cat 1 ingredient  $\geq 3\%$   $\longrightarrow$  mixture classified Cat. 1
- Skin Sensitization
  - > E.g. Sensitizing ingredient  $\geq 1\%$   $\longrightarrow$  mixture classified

# Assessment of Additivity Method for Formulations

- Retrospective analysis conducted
  - > Comparison of results of additivity formula with classification based on *in vivo* results
  - > 226 agrochemical mixtures

Insecticide Class											
Herbicides		Insecticides		Fungicides		Fumigants		Nitrification		Blanks (no active)	
161		37		18		5		2		3	
Formulation Types											
Liquids (195)								Gel	Solids (30)		
SL	EC	SC	EW	SE	OD	CS	Others		WG	GR	WP
53	51	33	19	14	10	6	9	1	24	3	3

# Performance of Additivity Formula

**Table 1. Classification based on GHS Additivity Formula (AF) vs. various 6-Pack-based classification systems**

Endpoint	ATE criteria	Sample size@	Accuracy*	Sensitivity*	Specificity*	TP/FN *	TN/FP *
		n	%	%	%	n/n	n/n
Acute Oral Toxicity	GHS <sup>1</sup>	203	78.3	69.5	86.1	66/29	93/15
	CLP <sup>2</sup>	214	86.9	68.9	91.7	31/14	155/14
	EPA <sup>3</sup>	198	78.3	69.9	85.7	65/28	90/15
Acute Dermal Toxicity	GHS <sup>1</sup>	179	93.3	75.0	93.7	3/1	164/11
	CLP <sup>2</sup>	208	99.5	100.0	99.5	2/0	205/1
	EPA <sup>3</sup>	179	92.7	60.0	93.7	3/2	163/11
Acute Inhalation Toxicity	GHS/CLP	124	96.8	66.7	99.1	6/3	114/1
	EPA <sup>4</sup>	124	96.8	57.1	99.1	4/3	116/1
Skin Irritation	GHS <sup>5</sup>	91	67.0	76.9	63.1	20/6	41/24
	CLP <sup>6</sup>	117	70.9	32.3	84.9	10/21	73/13
Eye Irritation	GHS/CLP <sup>7</sup>	212	75.5	89.9	62.8	89/10	71/42
Skin Sensitisation	GHS/CLP/EPA <sup>8</sup>	204	64.2	58.0	69.0	51/37	80/36

## • Conclusions

- > Additivity formula should be considered as a stand-alone replacement for acute systemic toxicity
- > For topical contact toxicity, a combination of alternative approaches may be needed to improve predictions

Presented at Eurotox, 2015



# Are Acute Dermal Studies Needed at all?

*Critical Reviews in Toxicology*, 2010; 40(1): 50–83

**informa**  
healthcare

## Acute toxicity testing of chemicals—Opportunities to avoid redundant testing and use alternative approaches

Stuart Creton<sup>1</sup>, Ian C. Dewhurst<sup>2</sup>, Lesley K. Earl<sup>3</sup>, Sean C. Gehen<sup>4</sup>, Robert L. Guest<sup>5</sup>, Jon A. Hotchkiss<sup>6</sup>, Ian Indans<sup>7</sup>, Michael R. Woolhiser<sup>6</sup>, and Richard Billington<sup>8</sup>

## Can acute dermal systemic toxicity tests be replaced with oral tests? A comparison of route-specific systemic toxicity and hazard classifications under the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

Nigel P. Moore<sup>a</sup>, David J. Andrew<sup>b</sup>, Donald L. Bjerke<sup>c</sup>, Stuart Creton<sup>d,1</sup>, David Dreher<sup>e</sup>, Thomas Holmes<sup>f</sup>, Pilar Prieto<sup>g</sup>, Troy Seidle<sup>h</sup>, Tim G. Rowan<sup>i,\*</sup>

- It's time to revisit acute dermal requirement -- classification is rarely driven by this endpoint!
  - > UK Assessment of 240 active substances- Only 2 (0.8%) had more severe dermal classification compared to oral

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# Alternatives for Eye Irritation

## 1. Organotypic models

- > Hen's egg test – Chorioallantoic membrane test (HET-CAM)
- > Isolated rabbit eye test (IRE)
- > Isolated chicken eye test (ICE) (OECD 438)
- > Bovine corneal opacity and permeability test (BCOP) (OECD 437)

## 2. Cell based models

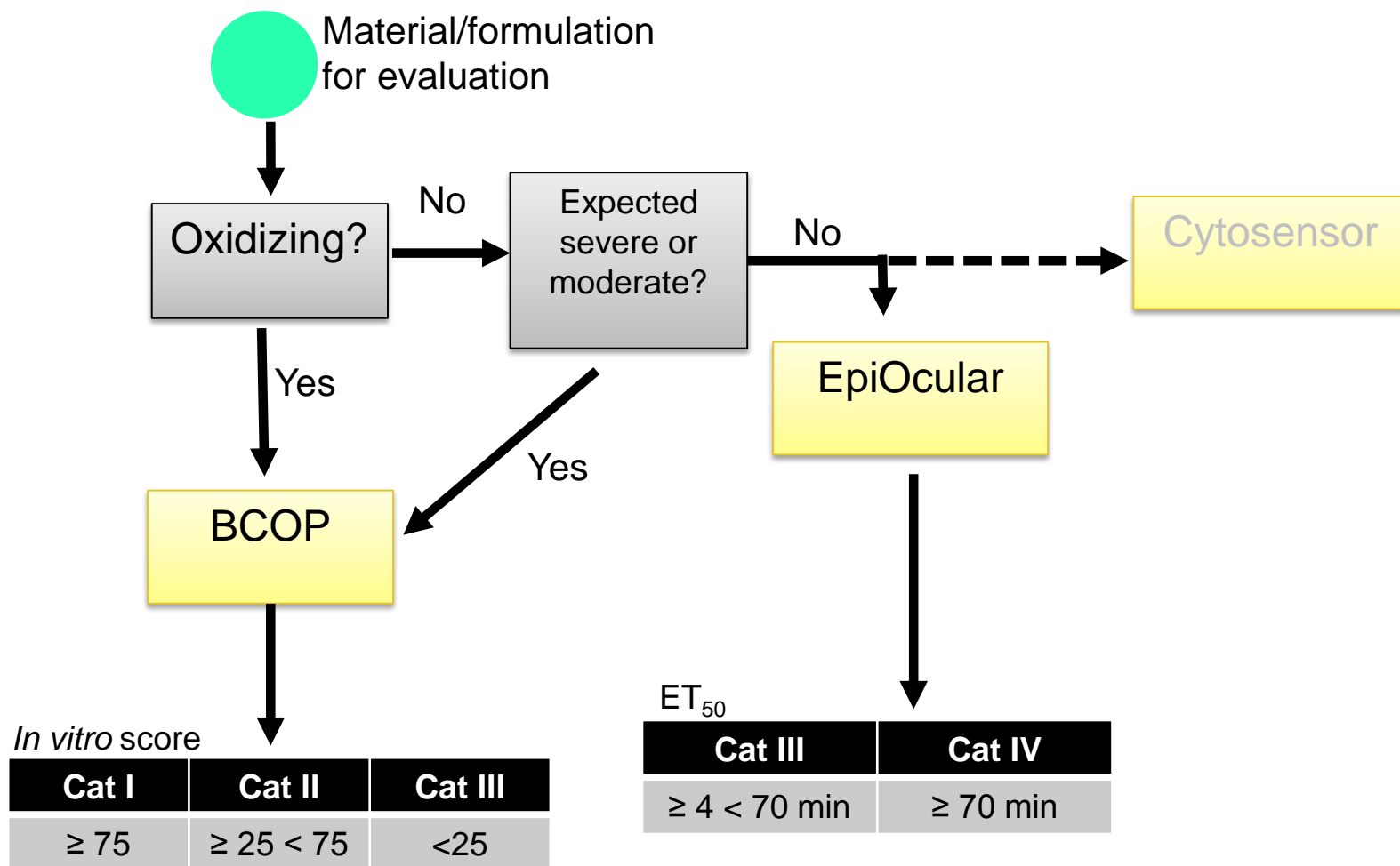
- > Red blood cell hemolysis test (RBCH)
- > Silicon Microphysiometer/Cytosensor Microphysiometer (CM)
- > Fluorescence leakage test (FL) (OECD 460)
- > Neutral red release assay (NRR)

## 3. Reconstructed human tissue models

- > EpiOcular 3D corneal assay (OECD 492)



# EPA Eye Guidance- Antimicrobial Pesticides



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# Eye Irritation – Tiered Approaches

ATLA 43, 181–198, 2015

181

## **The EpiOcular™ Eye Irritation Test is the Method of Choice for the *In Vitro* Eye Irritation Testing of Agrochemical Formulations: Correlation Analysis of EpiOcular Eye Irritation Test and BCOP Test Data According to the UN GHS, US EPA and Brazil ANVISA Classification Schemes**

**Susanne N. Kolle,<sup>1</sup> Maria Cecilia Rey Moreno,<sup>1</sup> Winfried Mayer,<sup>2</sup> Andrew van Cott,<sup>3</sup> Bennard van Ravenzwaay<sup>1</sup> and Robert Landsiedel<sup>1</sup>**

*<sup>1</sup>BASF SE Experimental Toxicology and Ecology, Ludwigshafen, Germany; <sup>2</sup>BASF SE Agricultural Products Formulation Development, Ludwigshafen, Germany; <sup>3</sup>BASF Corporation, Research Triangle Park, USA*



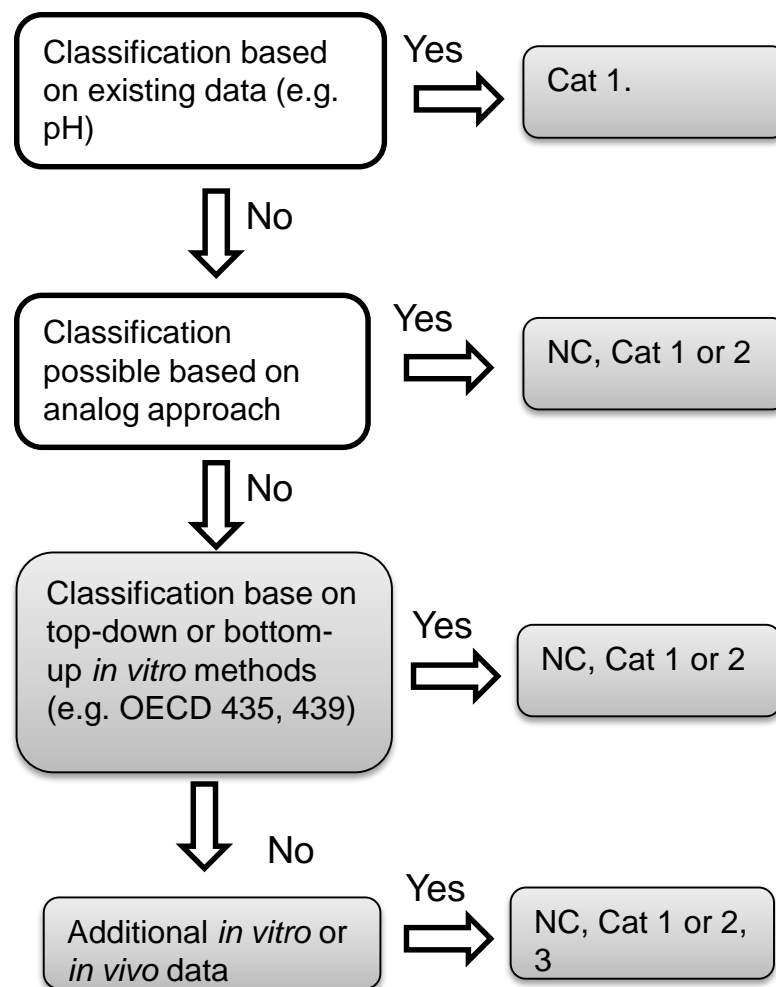
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# Eye Irritation – Tiered Approaches

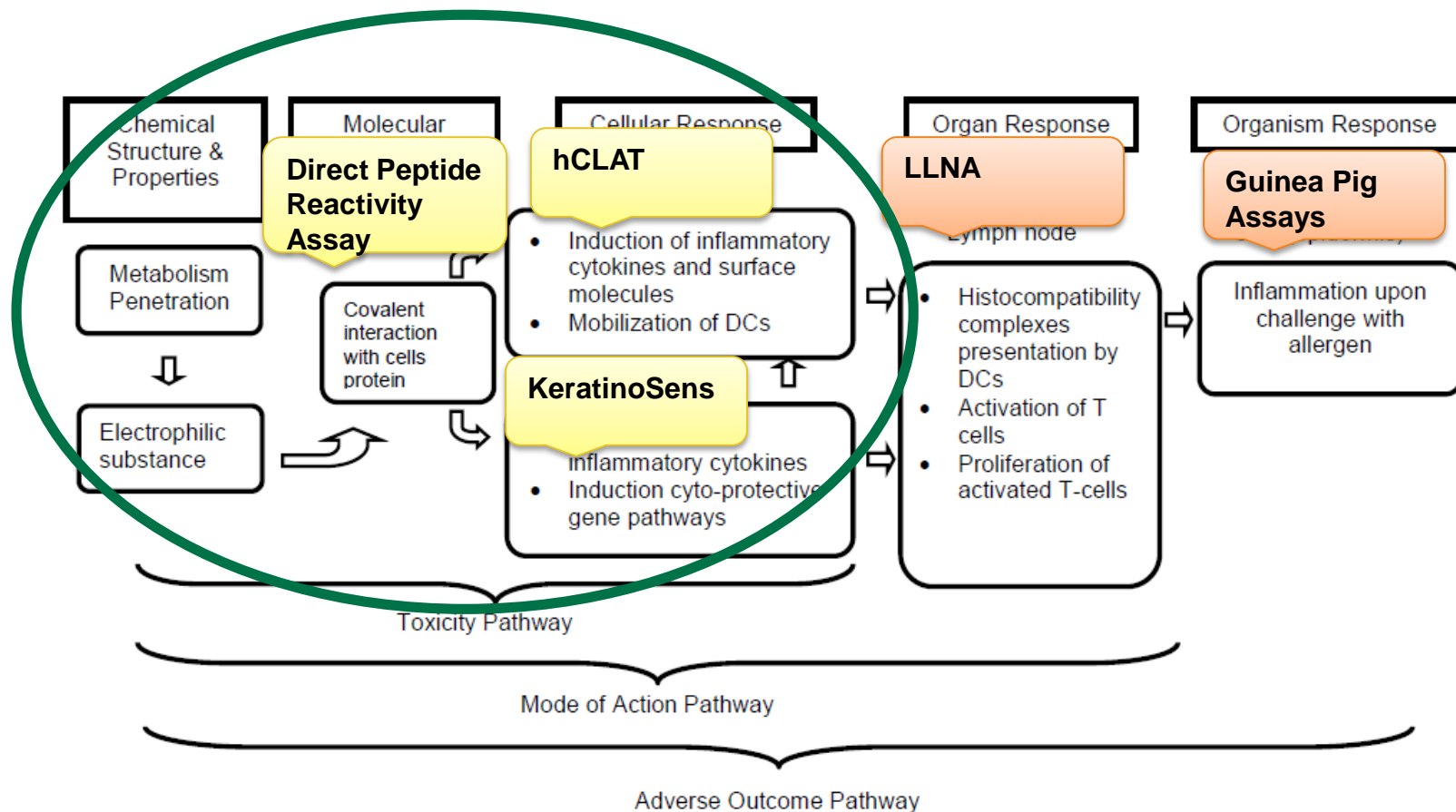
New Agrochemical formulation/co-  
formulant

# Alternatives for Skin Irritation

- OECD Guidance on IATA
  - > “Depending on country requirements, the now available validated and OECD accepted *in vitro* methods may satisfy all information requirements for skin corrosion and irritation.”



# Skin Sensitization Alternatives





Contents lists available at ScienceDirect

## Regulatory Toxicology and Pharmacology

journal homepage: [www.elsevier.com/locate/yrtph](http://www.elsevier.com/locate/yrtph)



### Application of the KeratinoSens™ assay for assessing the skin sensitization potential of agrochemical active ingredients and formulations



Raja S. Settivari<sup>a,\*</sup>, Sean C. Gehen<sup>b</sup>, Ricardo Acosta Amado<sup>b</sup>, Nicolo R. Visconti<sup>a</sup>, Darrell R. Boverhof<sup>a</sup>, Edward W. Carney<sup>a</sup>

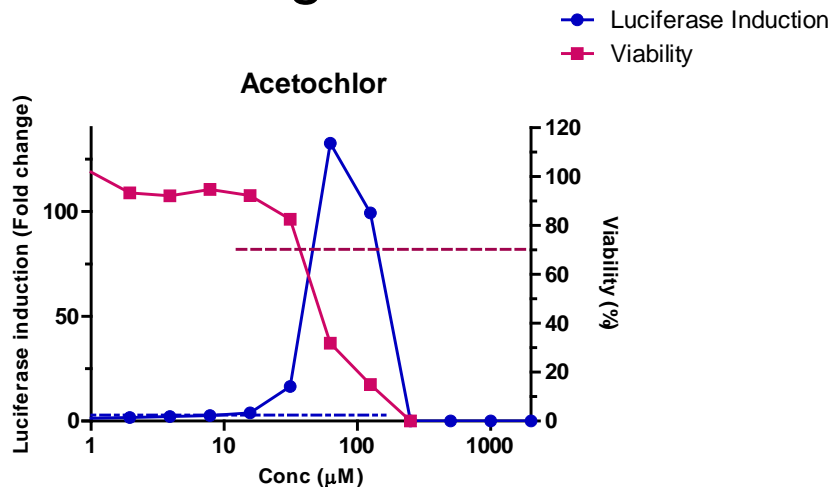
<sup>a</sup>The Dow Chemical Company, Midland, MI, United States

<sup>b</sup>Dow AgroSciences LLC, Indianapolis, IN, United States

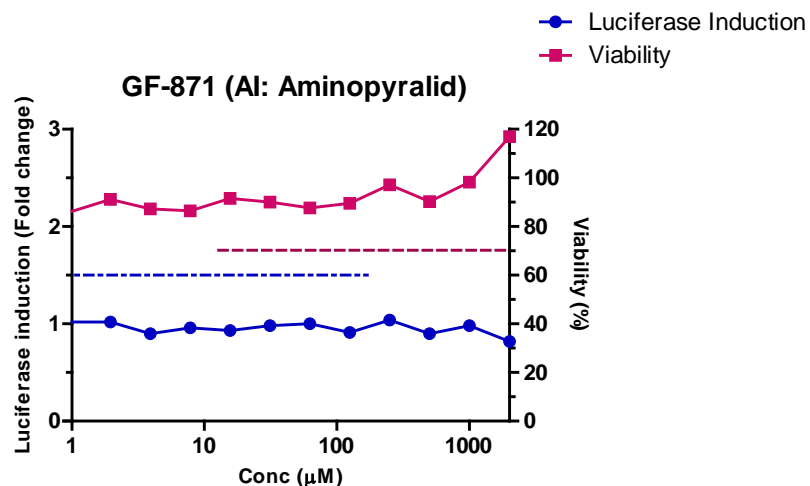
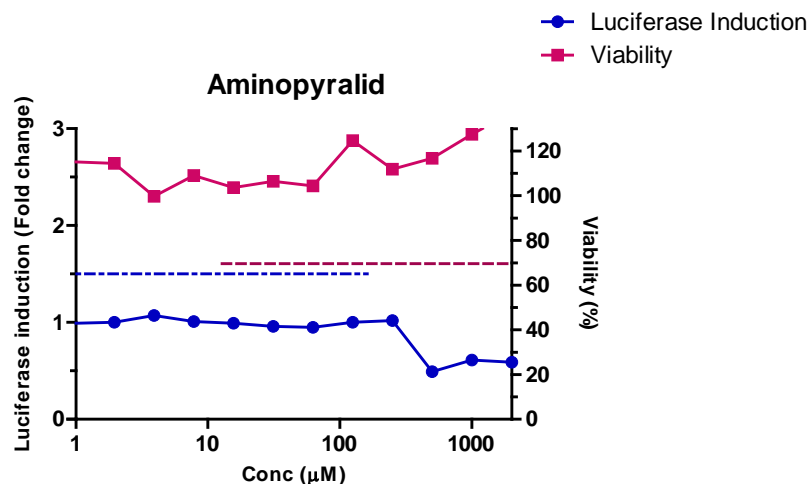
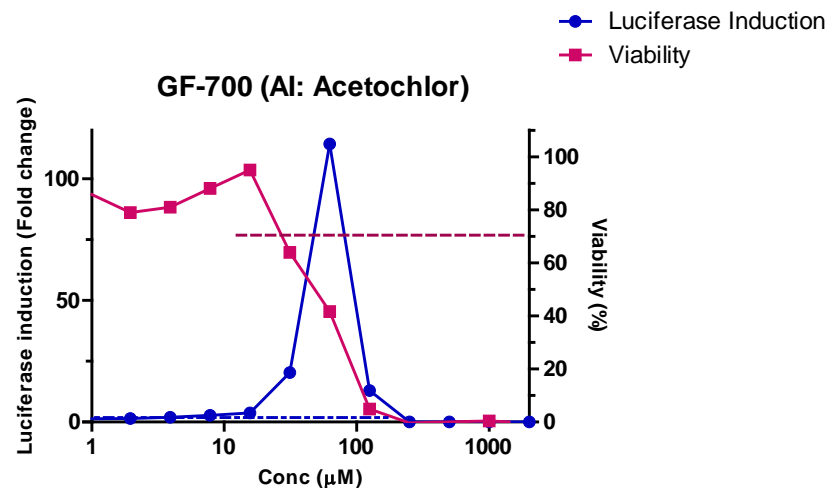


# KeratiNoSens Assay for Skin Sensitization

## Active Ingredient



## Formulation





# Skin Sensitization- Integrated Approach

Formulation	<i>In Vivo</i>	KeratinoSens	DPRA	Calculation
DAS-1	Positive	Positive	Negative	Positive
DAS-2	Positive	Positive	Positive	Positive
DAS-3	Positive	Positive	Positive	Positive
DAS-4	Positive	Positive	Positive	Positive
DAS-5	Positive	Negative	Negative	Positive
DAS-6	Positive	Negative	Positive	Positive
DAS-7	Borderline	Equivocal	NA	Negative
DAS-8	Borderline	Equivocal	NA	negative
DAS-9	Negative	Negative	Negative	Negative
DAS-10	Negative	Negative	Negative	Negative
DAS-11	Negative	Negative	Negative	Negative
DAS-12	Negative	Negative	Negative	Negative
DAS-13	Negative	Positive	Negative	Negative

# Acute 6 Pack – Proposed Alternatives

Guideline Number	Data Requirements
Acute Testing	
870.1100	Acute oral toxicity - rat
870.1200	Acute dermal toxicity
870.1300	Acute inhalation toxicity - rat
870.2400	Primary eye irritation - rabbit
870.2500	Primary dermal irritation
870.2600	Dermal sensitization

Additivity

Remove as  
default Req.

Combination  
of *in vitro*  
methods

# Suitability of Alternative Methods for Mixtures

	Method	Applicability To Mixtures/AgroChemicals
Eye Irritation	BCOP (OECD 437)	<ul style="list-style-type: none"> <li>• OECD validation data-based included 100 mixtures</li> <li>• Included in EPA Policy</li> </ul>
	EpiOcular (OECD 492)	<ul style="list-style-type: none"> <li>• Suitable for substances, mixtures, solids, liquids, semi-solids, waxes</li> <li>• Included in EPA anti-microbial Policy</li> <li>• BASF Publication (Kolle, 2015)</li> </ul>
Skin Irritation	EpiDerm (OECD 439)	<ul style="list-style-type: none"> <li>• Suitable for mixtures although limited validation data</li> </ul>
Skin Sensitization	KeratinoSens (OECD 442D)	<ul style="list-style-type: none"> <li>• Dow Publication shows applicability to agchem formulations (Settivari, 2015)</li> <li>• Limited validation (OECD) for mixtures</li> </ul>
	DPRA (OECD 442C)	<ul style="list-style-type: none"> <li>• Limited information on applicability to mixtures</li> <li>• Initial encouraging results</li> </ul>

## Example 1- Read Across

	Existing Formulation A	Existing Formulation B	New Formulation
Type	Emulsifiable Concentrate	Emulsifiable Concentrate	Emulsifiable Concentrate
AI- concentration	12%	10%	12%
Solvent	10%	12%	12%
Emulsifier	3%	3%	3%
Balance ingredient	75%	75%	73%
Acute Tox	Cat III Non-sensitizing	Cat IV Non-sensitizing	Proposed: III Non-sensitizing

- How similar is similar?
- Can *in vitro* testing be used to support read-across arguments?

## Example #2- GHS Additivity

- Can the additivity approach be envisioned to replace systemic toxicity studies under certain circumstances?
- Is an acute dermal study needed at all? Could a data package without it be considered complete? (is there a information gap?)

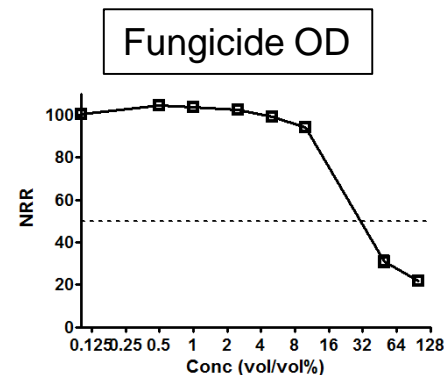
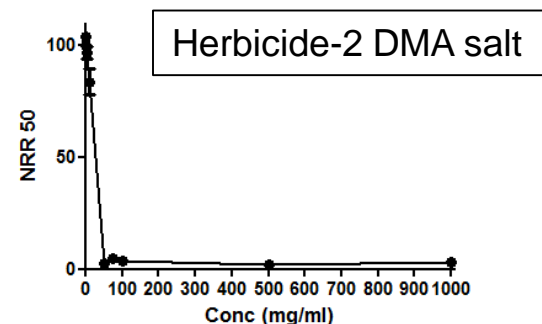
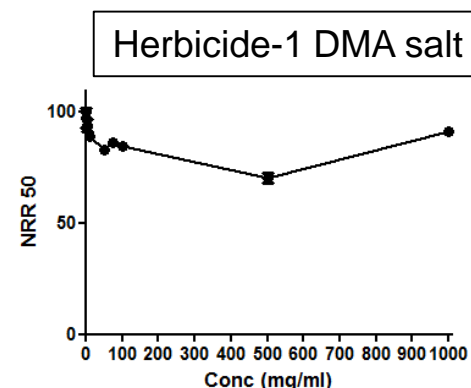
### Additivity-Based Categorization

	Herbicide Formulation	Insecticide Formulation
Acute Oral	III	II
Acute Dermal	IV	III
Acute Inhalation	IV	III



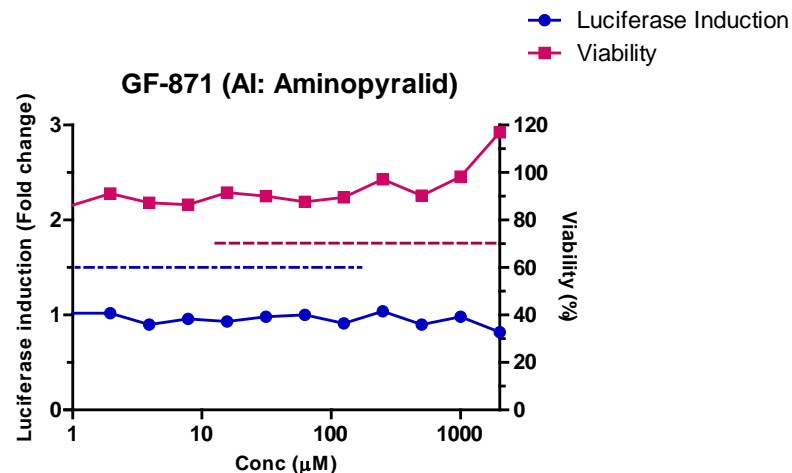
## Example #3- Eye Irritation

- In addition to EPA guidance, can other frameworks be envisioned?
- Tiered testing examples
  - > Herbicide-1 DMA salt
    - NRR: not calculable (non-irritant)
    - Draize: non-irritant
  - > Herbicide-2 DMA salt
    - NRR: 17.5 mg/mL
    - EpiOcular: < 3 (ET40)
    - Draize: strong-irritant
  - > Fungicide OD
    - NRR: 350.2 mg/mL
    - EpiOcular: > 60
    - Draize: non-irritant



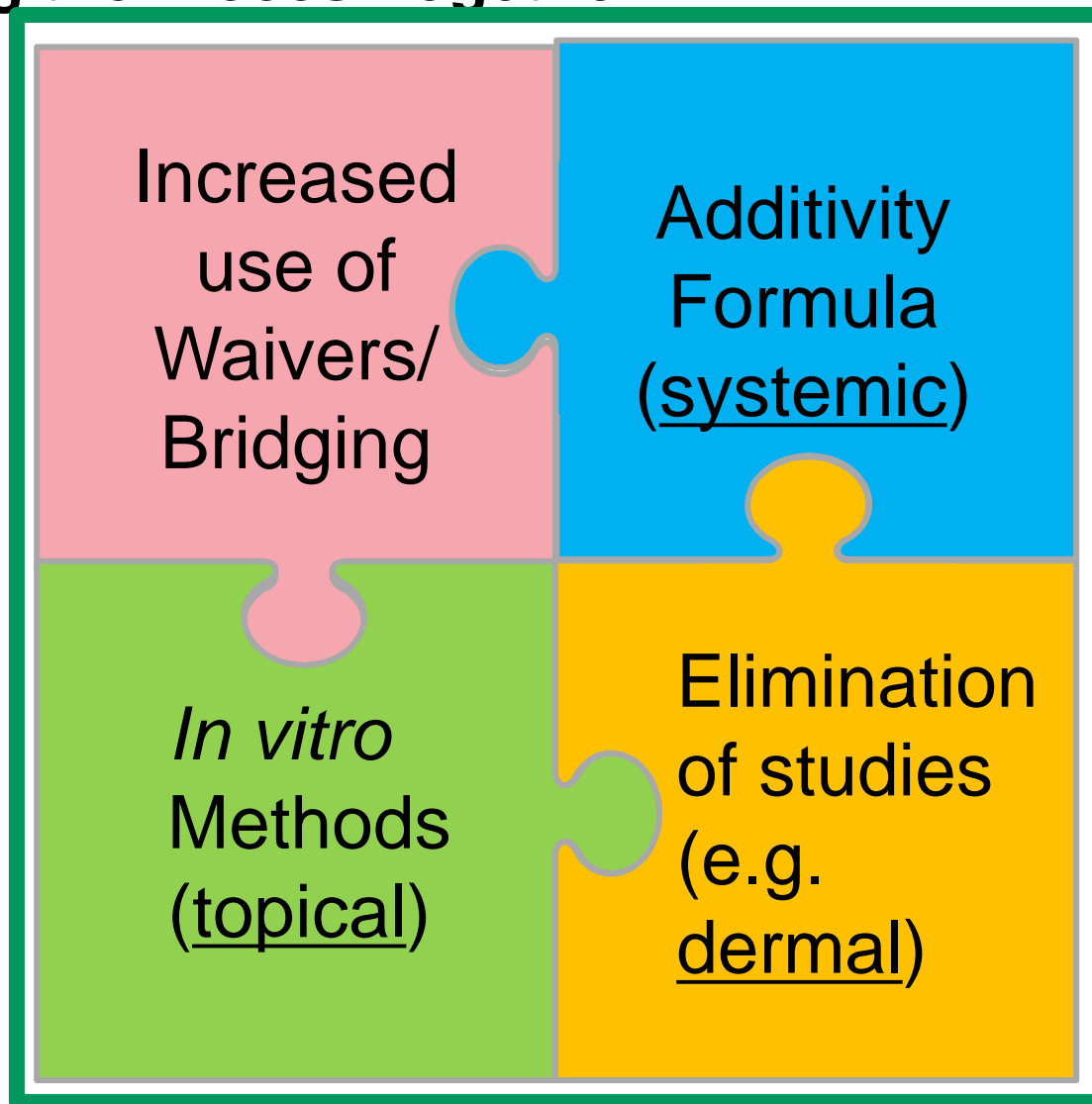
## Example #4- Skin Sensitization

- New Aminopyralid formulation
  - > AI is clearly negative for skin sensitization
  - > No Sensitizing inerts
- Questions
  - > Could a negative keratinosens result fulfill the data requirement?
  - > What additional information would be helpful?





# Putting the Pieces Together



Increased harmonization and cooperation

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# Acknowledgements

- Thanks to DAS Human Health Assessment Group, Actives to Products R&D and Dow Toxicology and Environmental Research and Consulting
  - > Ricardo Acosta Amado
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