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# Skin irritation studies for agrochemicals registration: Comparison of *in vitro* and *in vivo* results

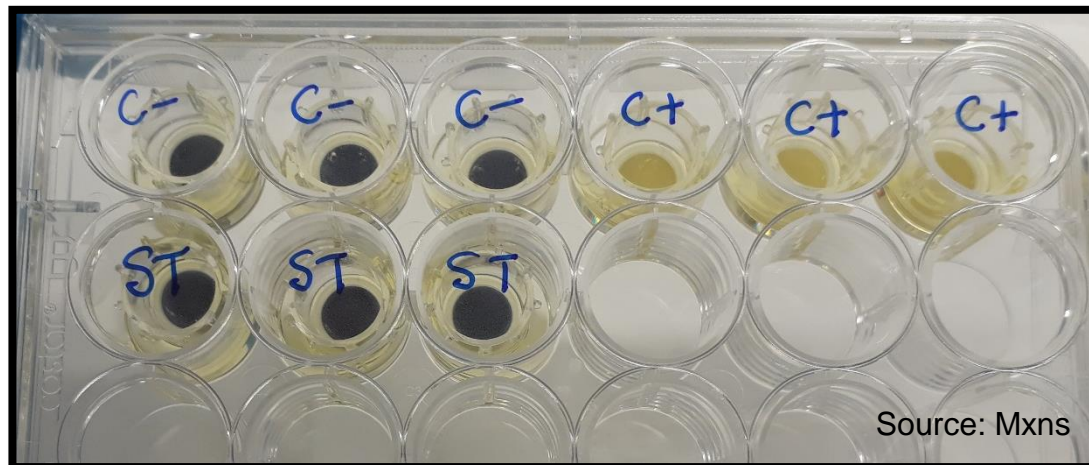
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Rodrigo De Vecchi

# Skin Irritation *In vitro* Method



- National Council To Control Animal Experimentation (CONCEA): Normative Resolution No. 17, July 03, 2014
- Brazilian Health Surveillance Agency (ANVISA) : Resolution – RDC No. 35, August 7, 2015



# Analysis of acute toxicological profile and mutagenesis



REQUIRED STUDIES	<i>In vivo</i>	<i>In vitro</i>
Acute Oral	OECD 423	OECD 420; <b><u>423</u></b> ; <b><u>425</u></b> ; <b><u>129</u></b> .
Acute Dermal	OECD 402	OECD 432; 428.
Acute Inhalation	OECD 403	----
Irritation/Corrosive (Dermal)	OECD 404	OECD 430; <b><u>431</u></b> ; 435; <b><u>439</u></b> .
Irritation/Corrosive (Eye)	OECD 405	OECD <b><u>437</u></b> ; 438; 460.
Skin Sensitization	OECD 406	OECD <b><u>429</u></b> ; 442A; <b><u>442B</u></b> .
Bacterial Reverse Mutation Test	OECD 471	----
Micronucleus Test	OECD 474	OECD <b><u>487</u></b>

# Skin Irritation *In vivo* Method



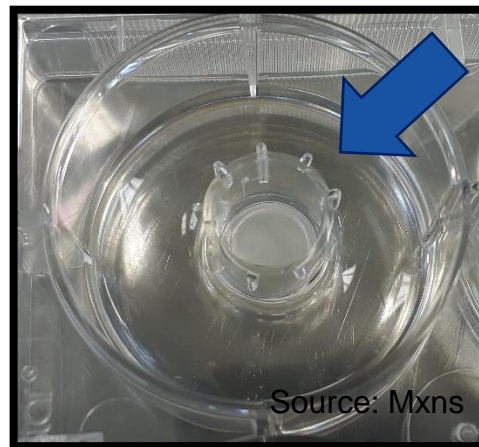
- Methodology – OECD, 404 (2015).



# Skin Irritation *In vitro* Method



- RHE model: normal human keratinocytes cultured for 17-days on an inert 0.5 cm<sup>2</sup> polycarbonate filter at the air-liquid interface;
- Presents a histological morphology comparable to the *in vivo* human tissue







## ■ OBJECTIVE

- Perform a comparison of results obtained from *in vivo* skin irritation studies and their respective *in vitro* replacement in agrochemicals with a high degree of purity.



# Material and Methods: *In vitro* Method



<b>Test Substance</b>	<b><i>In vivo</i> classification</b>	<b>Aspect</b>	<b>Color</b>	<b>Purity</b>
Pyraclostrobin Technical	Class II - Irritant	Solid	Cream	98%
Glyphosato Technical	Not classified	Solid	White	95%
Fipronil Technical	Not classified	Solid	White	98%
Imidacloprid	Not classified	Solid	Cream	97%
Azoxystrobin Technical	Not classified	Solid	White	98%
Acephate	Not classified	Solid	White	95%



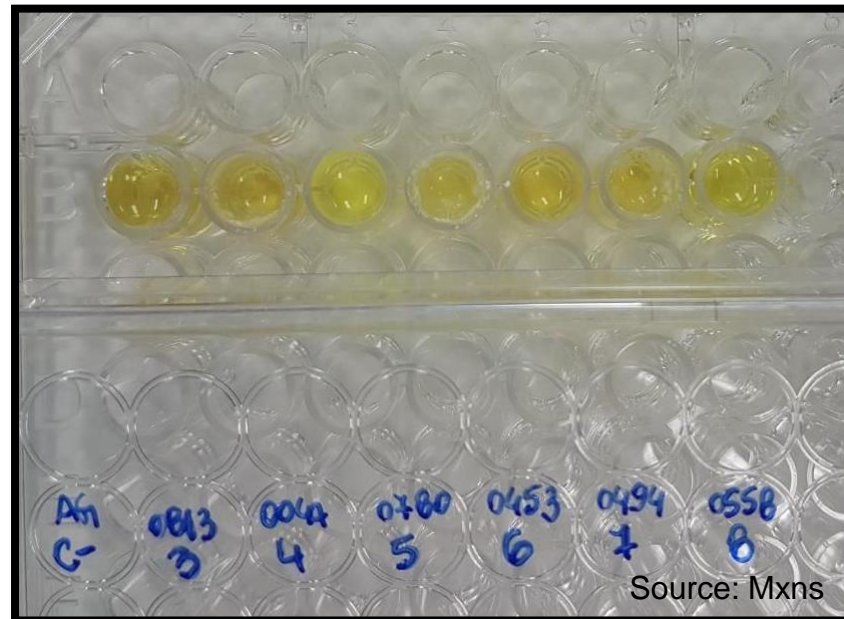
- Experimental procedure:
  - RHE – SkinEthic™;
  - Controls:
    - Positive control – 16  $\mu\text{L}$ ;
      - SDS
    - Negative control – 16  $\mu\text{L}$ ;
      - PBS
  - Test Substance – 16 mg (or 32 mg/cm<sup>2</sup>).



# Tests for additional controls:



1. MTT Reduction: 16 mg of test item in 300  $\mu$ L MTT solution (1 mg/mL); Incubate for 3 hours at 37  $^{\circ}$ C.

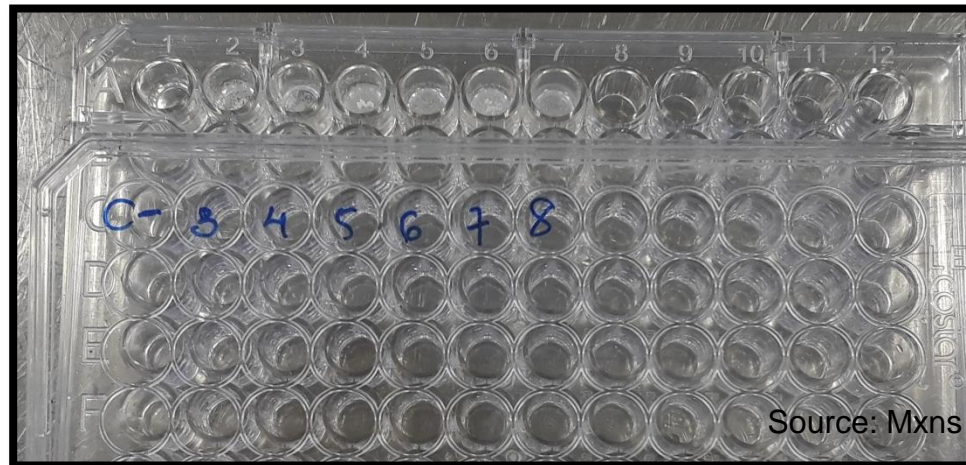


Non-interaction of test items with vital dye

# Tests for additional controls:



2. Evaluation of tissue staining: 10 mg of test item in 90  $\mu\text{L}$  of water; Incubate for 30 minutes at room temperature



No capacity to color the tissues

# Skin Irritation *In vitro* Method



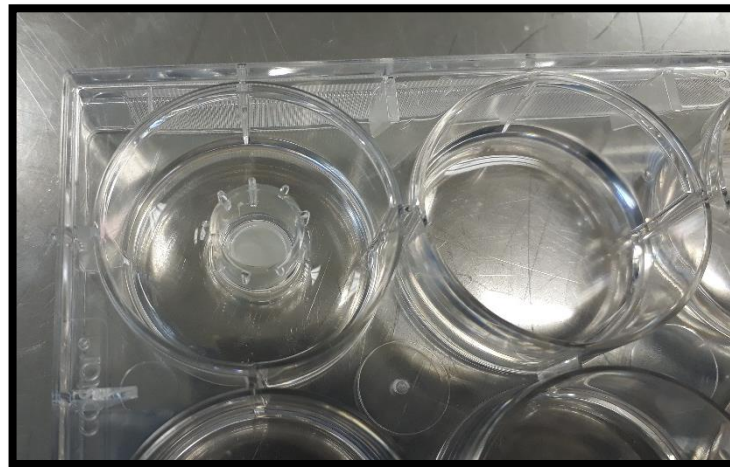
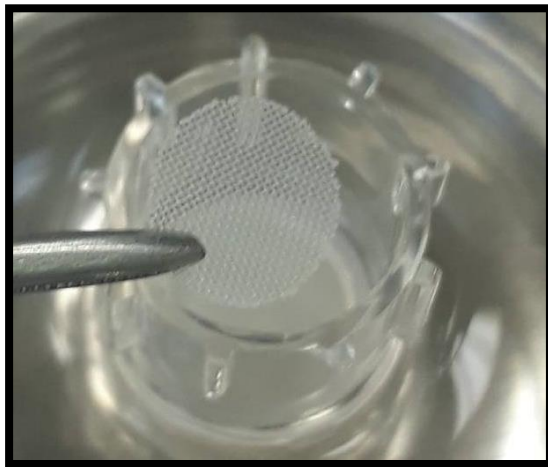
- Tissue maintenance: 1 mL of growth medium (SGM);  
4 hours at 37 °C,  $5 \pm 1\%$  CO<sub>2</sub> and  $\geq 90\%$  humidity.



# Skin Irritation *In vitro* Method



- Test Substance exposure: 300  $\mu\text{L}$  of maintenance medium (SMM); Incubated for 42 min at room temperature.
- Recovery phase: 2 mL of SGM; 42 hours at 37 °C,  $5 \pm 1\%$   $\text{CO}_2$  and  $\geq 90\%$  humidity.

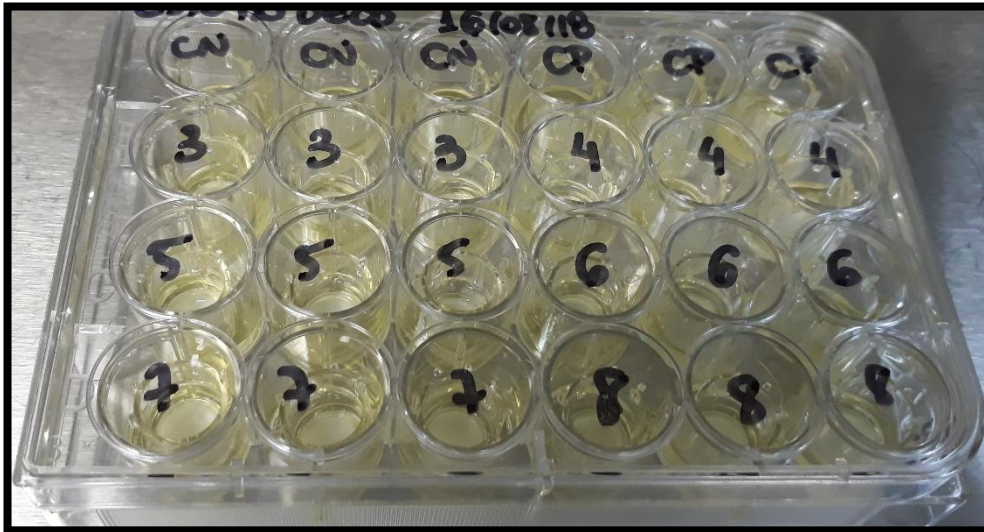




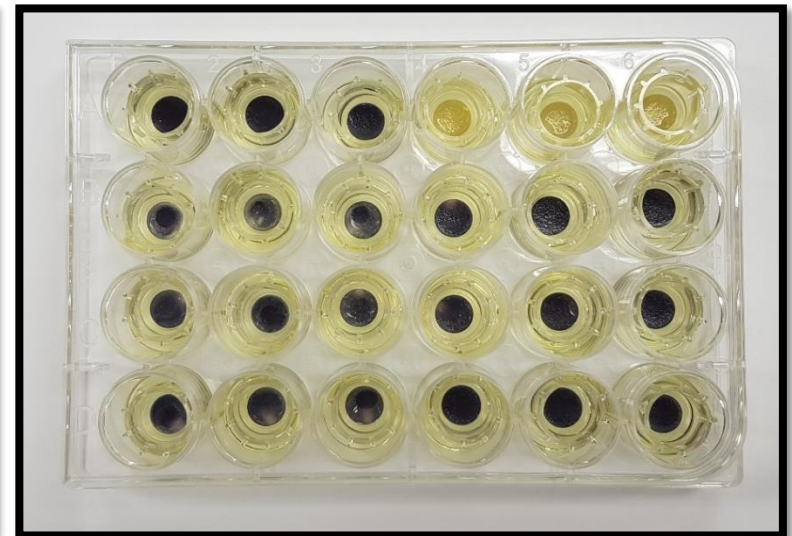
# Skin Irritation *In vitro* Method



- MTT (1 mg/mL) Conversion: 300  $\mu$ L of SMM; 3 hours at 37 °C, 5  $\pm$  1% CO<sub>2</sub> and  $\geq$  90% humidity



Before Incubation

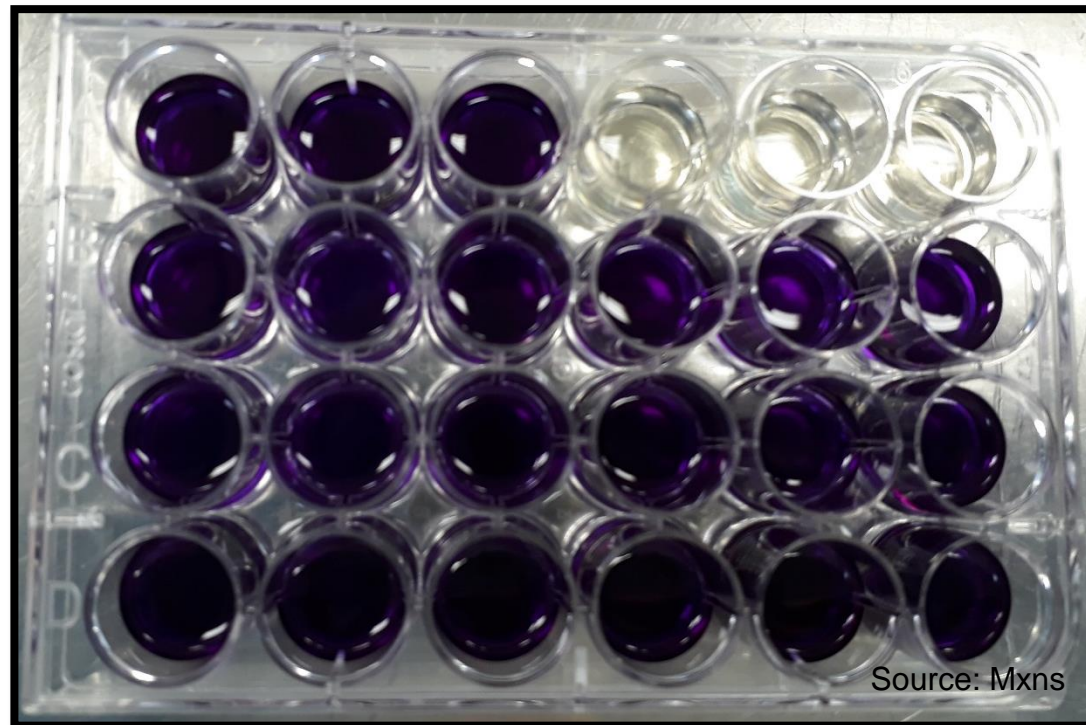


After Incubation

# Skin Irritation *In vitro* Method



- Formazan Extraction: Isopropanolol; 2 hours at room temperature; under slight stirring and protected from light

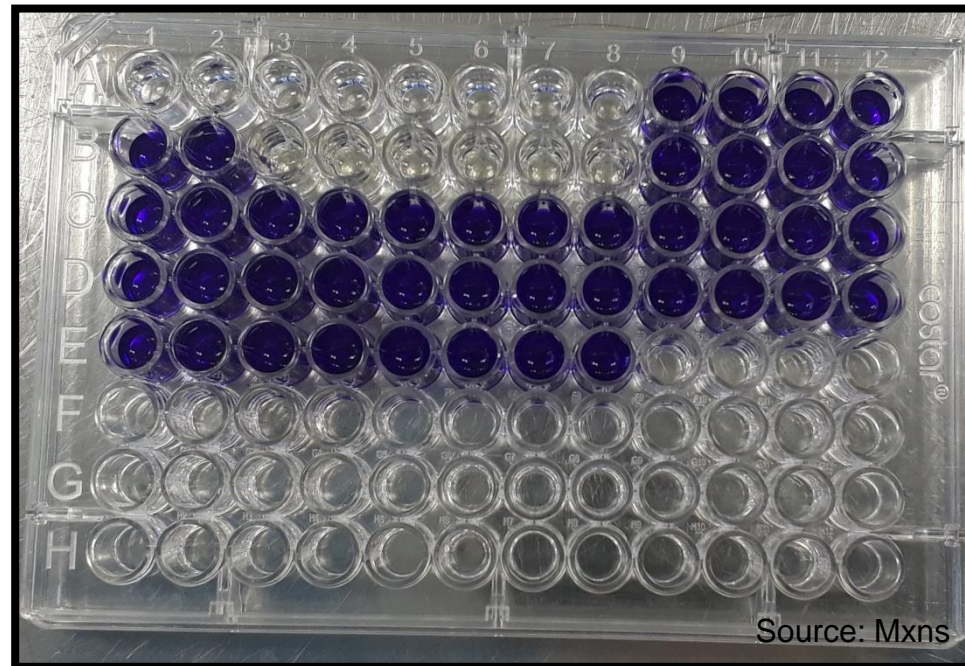




# Skin Irritation *In vitro* Method



- Read in a Spectrophotometer: Optical Density ( $OD_{570}$ )



# ■ RESULTS

- Acceptance Criteria (OECD 439):
  - Negative Control:  $OD_{570}$  of its replicates between 0.8 and 3.0.
  - Positive Control:  $OD_{570} \leq 40\%$  (replicates).
  - Test Substance: The standard deviation (SD) should be  $\leq 18\%$ .



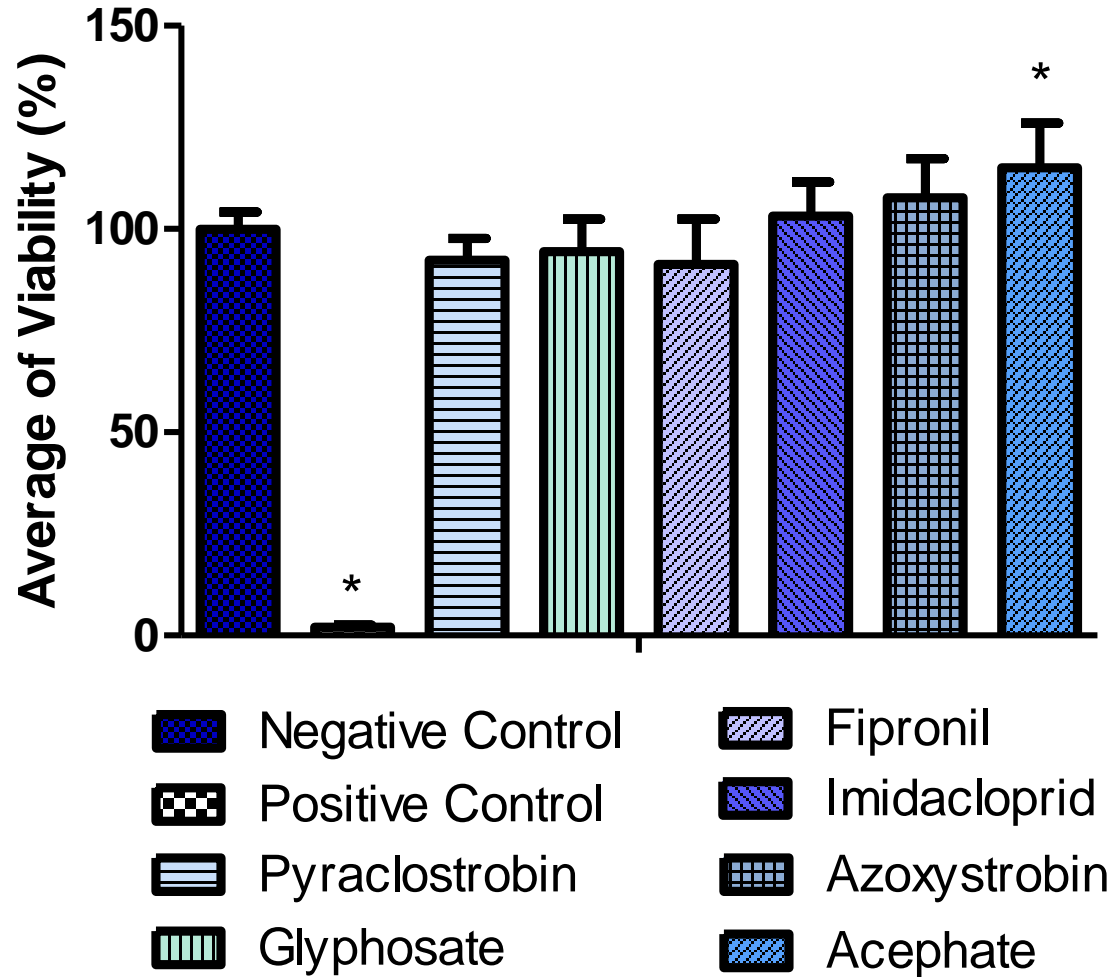
# Results



Group	Viability (%)		
	Average		SD
Negative Control	100,00	±	4,13
Positive Control	2,07*	±	0,49
Pyraclostrobin Technical	92,26	±	5,39
Glyphosato Technical	94,49	±	7,96
Fipronil Technical	91,37	±	11,13
Imidacloprid	103,17	±	8,43
Azoxystrobin Technical	107,70	±	9,66
Acephate	115,01*	±	11,10

\* p < 0,05 vs Negative Control

# Results



\*  $p < 0,05$  vs Negative Control; One-way ANOVA, Dunnet post Test



- Irritation potential of test substance is determined according to the EU classification (R38 or no label).
- Cell viability above 50 %: Non Irritant
- Agrochemical Points: test substance characteristics (i.e. color, aspect); additional controls.



- The preliminary results obtained in this study shown a correlation of **83%**.
- SkinEthic™ RHE model can be used as a complementary strategy for safety assessment of agrochemicals as an alternative to animal testing.
- The results of this study are promising with regard to the evaluation of inclusion of this test method in an **integrated acute toxicity data package for agrochemicals.**



# THANKS



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# THANK YOU!

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