Crop protection industry approach on alternative methods

Andreia Latorre
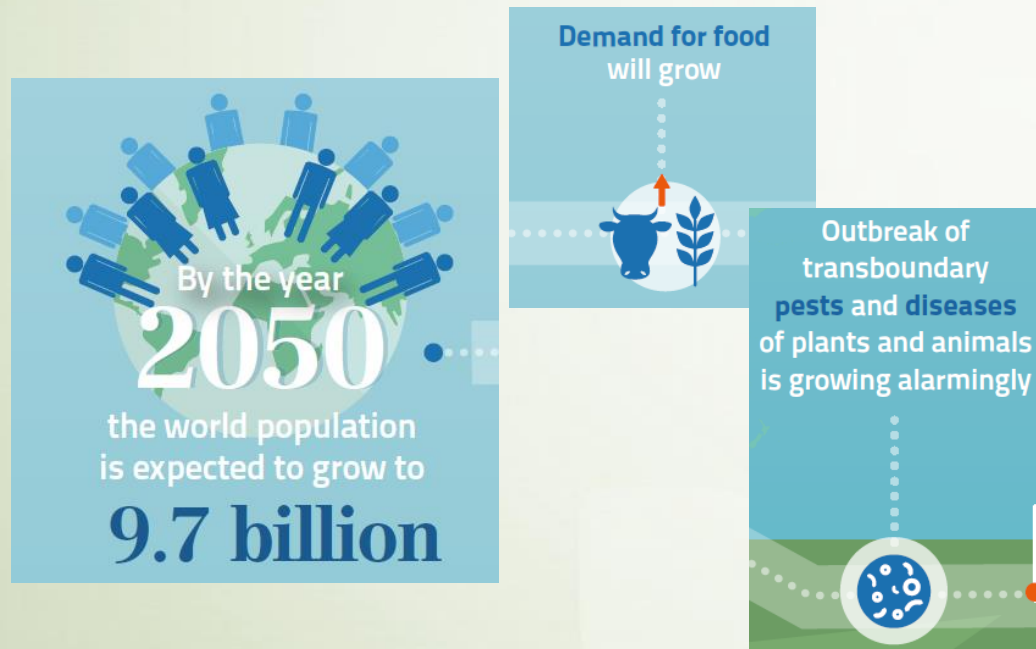
Regulatory Toxicologist Latam at Bayer

24 August 2018

Crop protection industry

The future of food and agriculture

Demand for food will grow

Outbreak of transboundary pests and diseases of plants and animals is growing alarmingly

By the year 2050 the world population is expected to grow to 9.7 billion

FAO, 2017. http://www.fao.org/3/a-i6887e.pdf

Industry and Regulation

⇨ Commitment with innovation in plant protection products that are safe for human health and environment.

⇨ Before any pesticide can be approved for use, all safety data related to human health and the environment must be submitted to regulatory authorities for their review.

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Pesticides are strictly regulated to ensure their safe uses

Submit all safety data related to human health and the environment to regulatory authorities

Industry

BRAZIL
Authorization procedure involving three official departments of the Brazilian government:

MAPA
Ministério da Agricultura e do Abastecimento

Ministry of Agriculture, Livestock and Supply

IBAMA MMA

Brazilian Institute of Environment and Renewable Natural Resources

ANVISA

Brazilian Health Regulatory Agency

http://portal.anvisa.gov.br/registros-e-autorizacoes/agrotoxicos/produtos/registro

EUROPEAN UNION
Authorization procedure involving three partners:

Member State

efsa
European Food Safety Authority

European Commission

https://www.efsa.europa.eu/en/interactive_pages/pesticides_authorisation/PesticidesAuthorisation

Brazilian Legislation
Advances and Challenges on Alternative Methods

National Council to Control Animal Experimentation (CONCEA)

⬇

NR 17/2014 - **National recognition** of validated alternative methods

⬇

Established **obligatory replacement** of traditional methods in **5 years** after the recognition by CONCEA

⬇

NR 18/2014 - recognized 17 alternative methods to animal use

1. Skin irritation and corrosion
   ⇨ OECD TG 430 / 431 / 435 / 439
2. Ocular irritation and corrosion
   ⇨ OECD TG 437 / 438 / 460
3. Phototoxicity
   ⇨ OECD TG 432
4. Skin Absorption
   ⇨ OECD TG 428
5. Skin sensitization
   ⇨ OECD TG 429 / 442A / 442B
6. Acute Toxicity
   ⇨ OECD TG 420 / 423 / 425 / GD 129
7. Genotoxicity
   ⇨ OECD TG 487

Brazilian Legislation
Advances and Challenges on Alternative Methods

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Brazilian Legislation
Advances and Challenges on Alternative Methods

NR 18/2014 - recognized 17 alternative methods to animal use; **obligatory replacement** of traditional methods in **September 2019**

NR 31/2016 - recognized 7 alternative methods to animal use; **obligatory replacement** of traditional methods in **August 2021**

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Brazilian Legislation
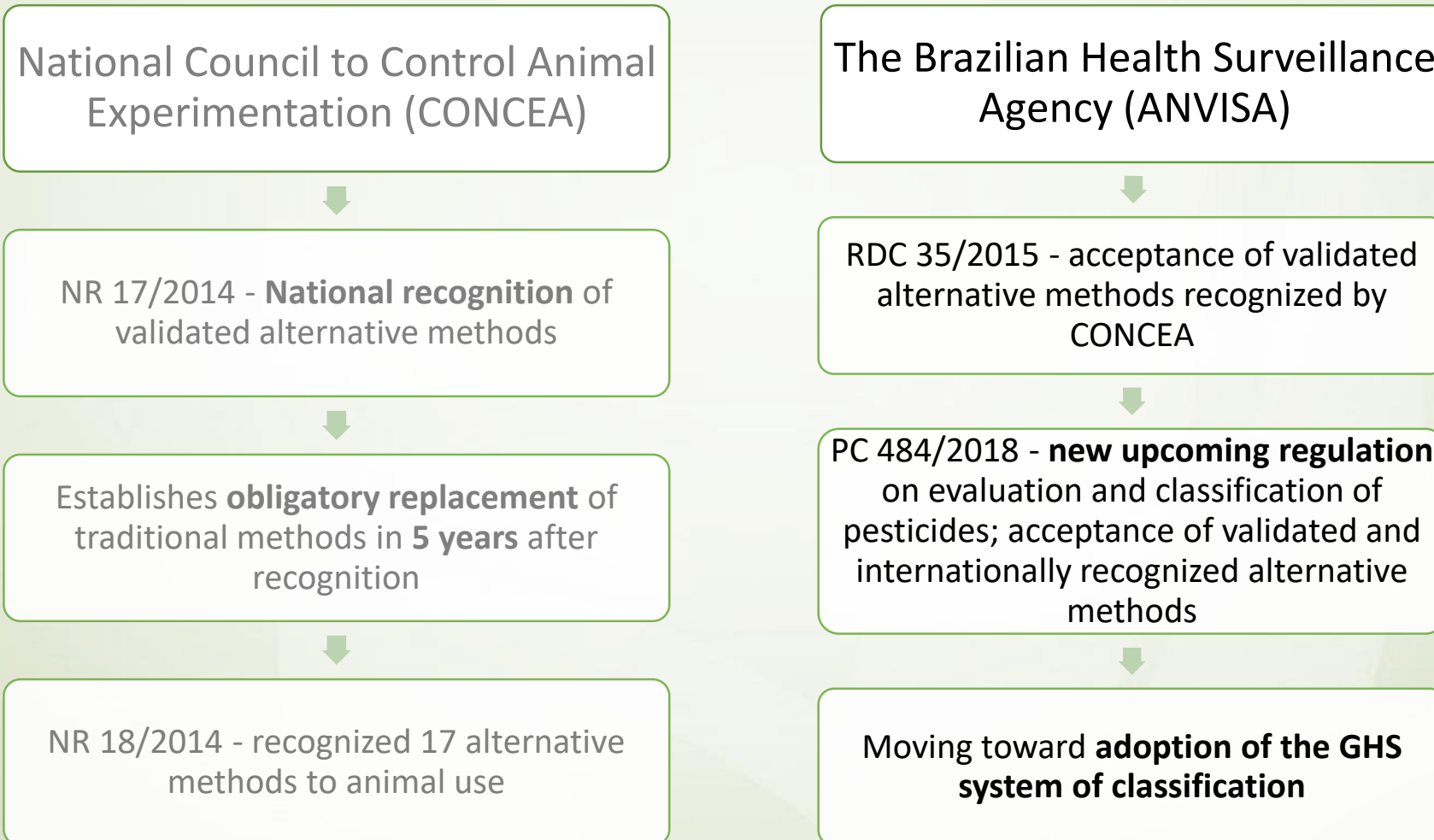## Advances and Challenges on Alternative Methods

NR 18/2014 - recognized 17 alternative methods to animal use; **obligatory replacement** of traditional methods in **September 2019**

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In order to replace animal data this TG need to be performed with human skin. Not permitted by the current Brazilian Legislation!

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Brazilian Legislation
Advances and Challenges on Alternative Methods

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↓

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The Brazilian Health Surveillance Agency (ANVISA)

↓

RDC 35/2015 - acceptance of validated alternative methods recognized by CONCEA

↓

PC 484/2018 - **new upcoming regulation** on evaluation and classification of pesticides; acceptance of validated and internationally recognized alternative methods

↓

Moving toward **adoption of the GHS system of classification**

Brazilian Legislation
Advances and Challenges on Alternative Methods

The Brazilian Health Surveillance Agency (ANVISA)

RDC 35/2015 - acceptance of validated alternative methods recognized by CONCEA

A single alternative test method can usually not substitute an *in vivo* test method.

There is no currently integrated test strategy that is fully validated for pesticides.

PC 484/2018 - **new upcoming regulation** on evaluation and classification of pesticides; acceptance of validated and internationally recognized alternative methods

Moving toward **adoption of the GHS system of classification**

ANDEF Technical and Scientific Group

Crop Protection Industry has been investing resources in order to develop integrated test strategies that permit an accurate evaluation of pesticides toxicity and that comply with regulatory requirements.

Members: Andreia Latorre (Bayer - coord.), Karen Cazarin (BASF), Priscila Fagundes (Syngenta), Rachel Figueiredo (FMC), Camila Coria (ANDEF), Andreia Ferraz (ANDEF)

- *Adhoc group*: international experts from companies to scientifically support task force actions and help in the compilation and use of data generated by Industry globally.

Waiving of acute dermal toxicity test

Rat Oral Hazard Category

(mg/kg)

Rat Dermal Hazard Category (mg/kg)	Category 1 ≤ 5	Category 2 >5 - 50	Category 3 >50 - 300	Category 4 >300 – 2,000	Category 5 >2,000 – 5,000	NC >5,000	Total
Category 1 ≤ 50	0	0	0	0	0	0	0
Category 2 >50 - 200	0	0	0	0	0	0	0
Category 3 >200 – 1,000	0	0	2	2	0	0	4
Category 4 >1,000 - 2,000	0	0	0	1	1	1	3
Category 5 >2,000 - 5,000	1	1	12	38	115	23	190
NC >5,000	0	0	8	32	44	70	154
Total	1	1	22	73	160	94	351

⇨ Retrospective analysis of GHS oral and dermal acute toxicity classifications for **351 formulations registered in Brazil**. *These data were provided by 6 companies in 2017: Bayer, Dow, DuPont, Iharabras, Syngenta and BASF.*

⇨ 188 (53.6%) formulations; same hazard category in both studies

⇨ 136 (38.7%) formulations; oral toxicity over-predicted dermal toxicity

⇨ 27 (7.7%) formulations; dermal LD_{50} values determined a more severe classification than oral LD_{50} values

Waiving of acute dermal toxicity test

Difference between the limit doses selected for conducting each acute toxicity tests of the same formulations could result in dermal LD_{50} values classified as a hazard Category 5, while oral LD_{50} values are not classified.

Rat Dermal Hazard Category 5 (> 2,000 – 5,000 mg/kg)	
Limit dose	Acute systemic toxicity from dermal exposure
< 5,000 mg/kg	below 5,000 mg/kg
21	2

⇨ 21 formulations the limit dose for the acute dermal test was 2,000 or 4,000 mg/kg, while for the acute oral test, the limit dose chosen was 5,000 mg/kg.

⇨ Overall, the oral hazard category was the same as, or over-predicted, the dermal hazard category for 345 (out of 351) or 98.3% of formulations.

⇨ **These findings corroborate the conclusion of US EPA (Nov. 2016) and PMRA (Mar. 2017) on waiving acute dermal toxicity studies.**

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Waiving of acute dermal toxicity test

US Environmental Protection Agency
Office of Pesticide Programs

Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations & Supporting Retrospective Analysis

November 9, 2016

https://www.epa.gov/sites/production/files/2016-11/documents/acute-dermal-toxicity-pesticide-formulations_0.pdf

Health Canada | Santé Canada — Your health and safety... our priority. | Votre santé et votre sécurité... notre priorité.

Regulatory Proposal   PRO2017-02

Acute Dermal Toxicity Study Waiver

https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/cps-spc/alt_formats/pdf/pest/part/consultations/pro2017-02/pro2017-02-eng.pdf

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In vitro testing strategy for eye irritation
Challenges

⇨ Pesticides formulations are very complex mixtures

⇨ No *in vitro* testing strategy has been validated as a full replacement for Draize rabbit eye test (OECD TG 405)

⇨ No validated *in vitro* method to detect **persistence of effects**

It is a common driver of Cat 1 classification for pesticides

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In vitro testing strategy for eye irritation

⇨ Integrated Approach on Testing and Assessment (IATA) No. 263 (2017)

for serious eye damage/irritation
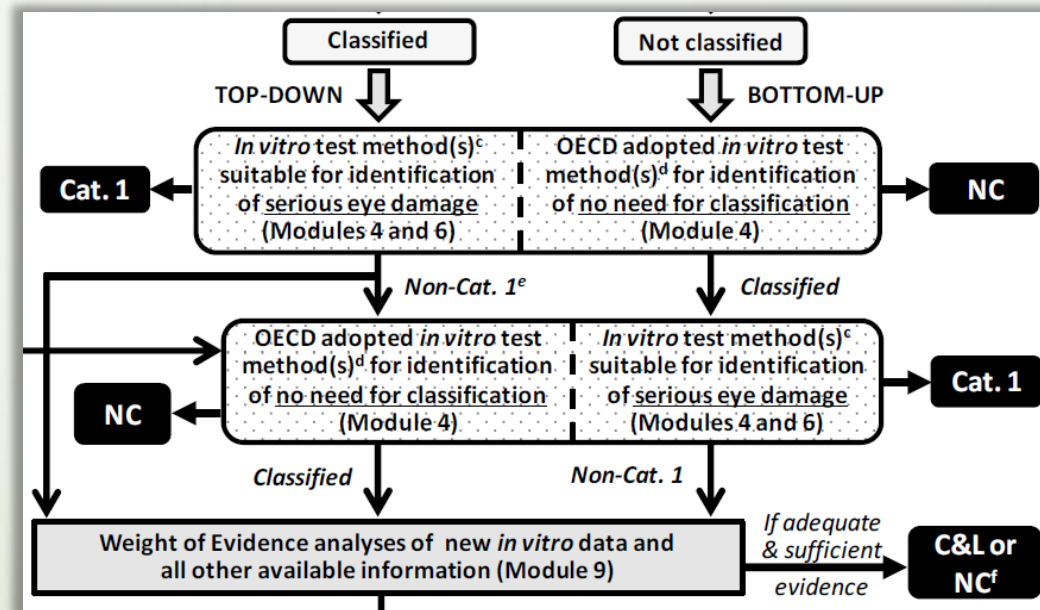
Applicability of *in vitro* eye irritation methods for pesticides

Test 👁	ICE (OECD TG 438) 🐔	EIT (OECD TG 492) 🧫	BCOP (OECD TG 437) 🐄
Test System	*Ex vivo* Chicken eyes	RhCE tissues	*Ex vivo* Bovine eyes
Indication	Bottom-up and Top-down	Bottom-up	Bottom-up and Top-down
In vitro UN GHS Classification	No Category No prediction Category 1	No Category No prediction	No Category No prediction Category 1

⇨ Bayer data on ICE test (*unpublished*).

⇨ BASF data on ICE, EIT and BCOP tests (Kolle, S.N. et al., *ATLA* 43, 181-198, 2015; Kolle, S.N. et al., *Regulatory Toxicology and Pharmacology* 85, 33-47, 2017).
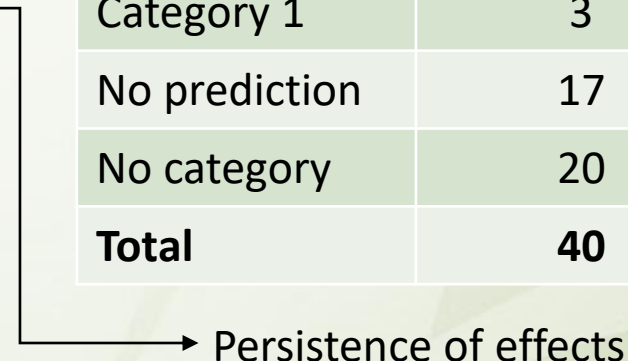
Applicability of ICE test for pesticides
(OECD TG 438 → Bottom-up and Top-down)

⇨ **Bayer** paired *in vivo* (405) and *in vitro* (438) data for **40 formulations**:

✓ 2 out 10 formulations identified as Category 1;

✓ 18 out 24 formulations identified as No Category;

✗ 1 Cat 1 and 1 Cat 2 → No Category;

✗ 1 Cat 2 → Cat 1.

OECD 405	Total
Category 1	10
Category 2	6
No category	24
Total	**40**

OECD 438	Total
Category 1	3
No prediction	17
No category	20
Total	**40**

Persistence of effects

Applicability of ICE test for pesticides
(OECD TG 438 → Bottom-up and Top-down)

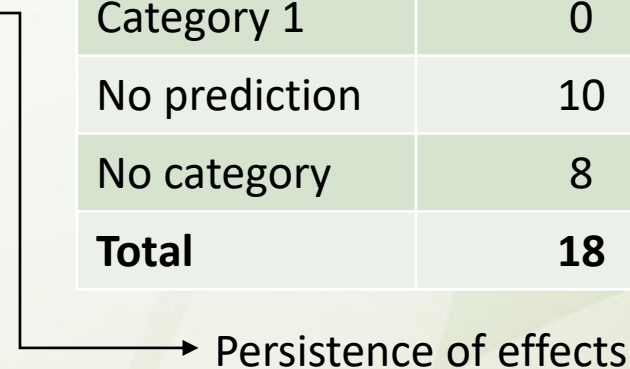⇨ **Bayer** paired *in vivo* (405) and *in vitro* (438) data for **18 active ingredients**:

✓ 7 out 14 active ingredients identified as No Category;

✗ 1 Cat 1 → No Category.

OECD 405	Total
Category 1	1
Category 2	3
No category	14
Total	**18**

OECD 438	Total
Category 1	0
No prediction	10
No category	8
Total	**18**

Persistence of effects

Applicability of ICE test for pesticides
(OECD TG 438 → Bottom-up and Top-down)

Overall conclusions on applicability of **ICE test for pesticides**:

⇨ can be used in testing strategies for both formulations and active ingredients;

⇨ higher sensitivity to identify pesticides not requiring classification;

⇨ can be used as initial step within a **Bottom-up testing approach**.

Applicability of ICE test for pesticides
(OECD TG 438 → Bottom-up and Top-down)

⇨ **BASF** paired *in vivo* (405) and *in vitro* (438) data for **10 formulations**:

    ✓ 1 out 5 formulations classified as Category 1.

Persistence of effects

Table 5
ICE Results (slit-lamp examination).[a]

Formu-lation ID	UN GHS Cat	ICE					
		Swelling %	Opacity	Fluorescein retention	ICE classes[b]	Irritation index[c]	Predicted classification (UN GHS)[d]
11	1	19	2.2	2	III;III;III	103	NP /2A
12	1	21	2.3[e]	2	III;III;III	107	NP /2A
13	1	12	1.8	2	II;III;III	88	NP /2A
17	1	17	2.7[f]	2.7	II;IV;IV	125	1 /1
18	1	13	1.8[g]	2	II;III;III	89	NP /2A
30	2A	18	1.8	2	II;III;III	94	NP /2A
34	2A	15	2	1.7	II;III;III	89	NP /2A
42	2B	12	1.7[f]	2	II;III;III	86	NP /2A
48	2B	10	2	2	II;III;IIII	90	NP /2A
58	NI	14	1.5	2	II;II;III	84	NP /2B

[a] NI, non-irritant (not classified); NP, no prediction can be made.
[b] On the basis of the severity of the observed findings ICE classes for corneal swelling, corneal opacity and fluorescein retention were determined as I = no effect, II = slight effect, III = moderate effect and IV = severe effect as described in OECD TG 438 (OECD, 2013b).
[c] *Irritation Index = maximum mean corneal swelling + maximum mean opacity score*20 + mean fluorescein retention score*20.*
[d] Using the prediction model described in OECD TG 438 (OECD, 2013b) /the extended prediction model described in the materials and methods section.
[e] Erosion of epithelium in two corneas.
[f] Loosening of epithelium in one or two corneas.
[g] Immediate opacity score 2 during administration; wrinkling of epithelium in one cornea.

S.N. Kolle et al. / Regulatory Toxicology and Pharmacology 85 (2017) 33–47

Applicability of BCOP test for pesticides
(OECD TG 437 → Bottom-up and Top-down)

⇨ **BASF** paired *in vivo* (405) and *in vitro* (437) data for **11 formulations**:

✓ 1 out 3 formulations identified as No Category;

✗ 1 Cat 1 and 1 Cat 2A → No Cat.

Persistence of effects

Table 4
BCOP and modified BCOP protocol results.[a]

Formu-lation ID[b]	UN GHS Cat	OECD TG 437 Liquid protocol: 10 min exposure with the formulation applied neatly							
		Opacity		Permeability *15		HSI	IVIS		Predicted classification (UN GHS)
		Mean	SD	Mean	SD	Median	Mean	SD	
11	1	3.5	1.6	0.11	0.08	II	3.7	1.5	NP
12	1	8.9	1.3	7.27	5.50	n.e.	16.2	4.8	NP
13	1	8.6	1.1	1.30	1.25	II	9.9	0.9	NP
18	1	1.5	0.8	0.99	0.82	II	2.5	0.9	NI
30	2A	6.0	1.3	0.49	0.32	IV	6.5	1.4	NP
34	2A	-0.4	1.3	2.79	3.19	II	2.4	2.3	NI
42	2B	6.9	1.9	0.97	0.52	III	7.8	2.1	NP
48	2B	10.9	2.7	1.55	1.01	n.e.	12.4	2.3	NP
55	NI	14.6	1.2	2.19	1.85	n.e.	16.8	1.1	NP
58	NI	12.9	0.7	0.94	0.86	II	13.8	1.4	NP
63	NI	0.8	0.3	0.00	0.07	I	0.0	0.2	NI

S.N. Kolle et al. / Regulatory Toxicology and Pharmacology 85 (2017) 33–47

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Applicability of EIT (EpiOcular™) test for pesticides
(OECD TG 492 → Bottom-up)

⇨ **BASF** paired *in vivo* (405) and *in vitro* (492) data for **27 formulations**:

✓ 4 out 8 formulations identified as No Category;

Table 7
EpiOcular™ ET50 Neat Protocol results.

Formulation ID	UN GHS cat	Relative viability [%]						ET50 [min]	Predicted classification (UN GHS)[a]
		3 min		30 min		60 min			
		Mean	Inter-tissue difference	Mean	Inter-tissue difference	Mean	Inter-tissue difference		
55	NI	81.7	0.2	10.8	2.7	3.7	0.3	8.4	Cat 2
58	NI	65.0	3.2	16.4	2.4	9.5	2.3	6.1	Cat 2
63	NI	87.2	6.1	49.2	1.9	20.2	10.5	28.4	Cat 2
106	NI	79.8	17.3	35.2	0.7	16.0	5.1	14.0	Cat 2
107	NI	89.3	1.1	98.4	2.6	103.7	5.9	>60	NI
108	NI	108.6	7.4	85.6	15.2	52.8	11.4	>60	NI
109	NI	100.4	0.4	100.2	1.1	101.9	2.0	>60	NI
110	NI	106.5	2.0	87.7	6.3	57.3	3.3	>60	NI

S.N. Kolle et al. / Regulatory Toxicology and Pharmacology 85 (2017) 33—47

Applicability of EIT (EpiOcular™) test for pesticides
(OECD TG 492 → Bottom-up)

⇨ **BASF** paired *in vivo* (405) and *in vitro* (492) data for **97 formulations**:

    ✓ 31 out 43 formulations identified as No Category;

Table 7: Predictive capacity of the EpiOcular-EIT to predict ocular non-irritant agrochemical formulations according to UN GHS (relative viability cut-off 60%) by formulation type

	All formulation types	EC formulations	SC formulations
Sensitivity	91% (49/54)	100% (31/31)	86% (12/14)
Specificity	72% (31/43)	20% (1/5)	91% (21/23)
Accuracy	83% (80/97)	89% (32/36)	89% (33/37)
False negatives	9% (5/54)	0% (0/31)	14% (2/14)
False positives	28% (12/43)	80% (4/5)	9% (2/23)
Positive predictive value	80% (49/61)	89% (31/35)	86% (12/14)
Negative predictive value	86% (31/36)	100% (1/1)	91% (21/23)

EC: Emulsifiable concentrate   SC: Suspension concentrate

S.N. Kolle *et al.* ATLA 43, 181–198, 2015

Final Considerations

⇨ Prediction of non-irritant pesticides

- EpiOcular™ or

- ICE test

⇨ Prediction of severe irritant pesticides

- additional non-animal methods are needed that enabled to evaluate the persistence of effects

Crop protection industry is committed to develop safe products fostering animal-free tools.

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