

# ADVANCING EYE IRRITATION ASSESSMENT WITH NON-ANIMAL METHODS FOR AGROCHEMICALS: PROGRESS AT US EPA



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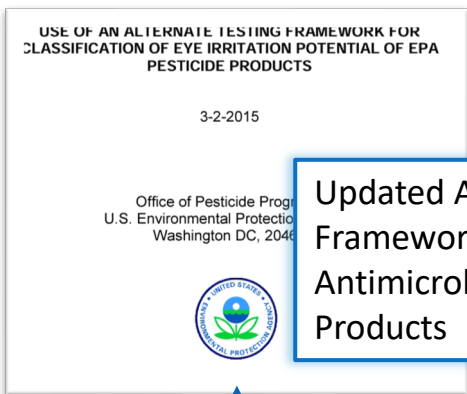
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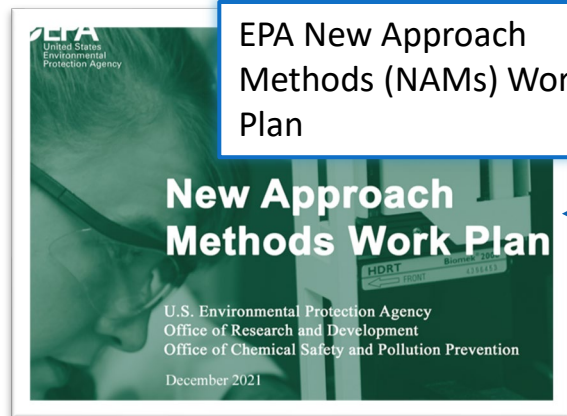
# OVERVIEW

- Background & Milestones
- Human relevance of alternative testing methods
- Alternative testing framework for antimicrobial cleaning products
- Defined approaches for conventional agrochemical formulations
- Animal reduction metrics
- Next steps & Summary

# BACKGROUND & MILESTONES



Updated Alternative Testing Framework for Antimicrobial Cleaning Products



EPA New Approach Methods (NAMs) Work Plan

2015

2016

2017

2018

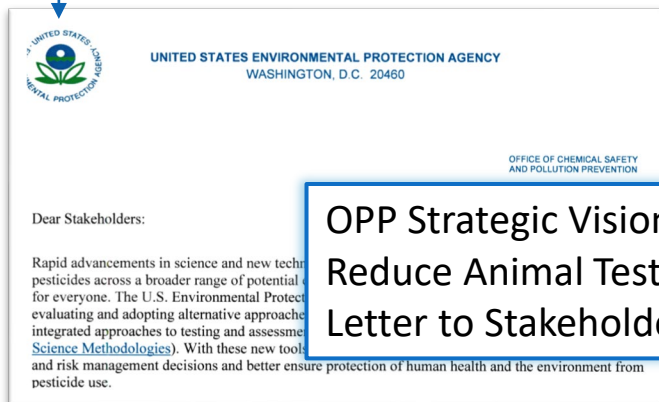
2019

2020

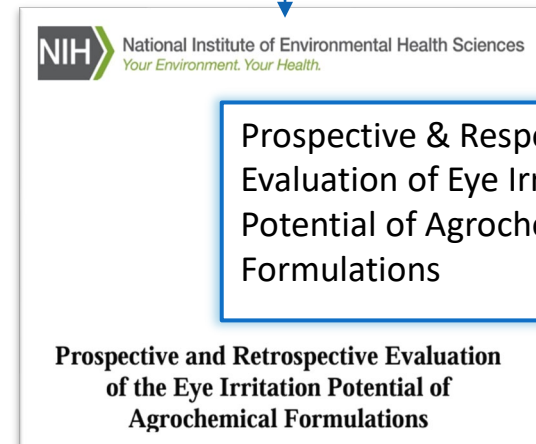
2021

2022

2023



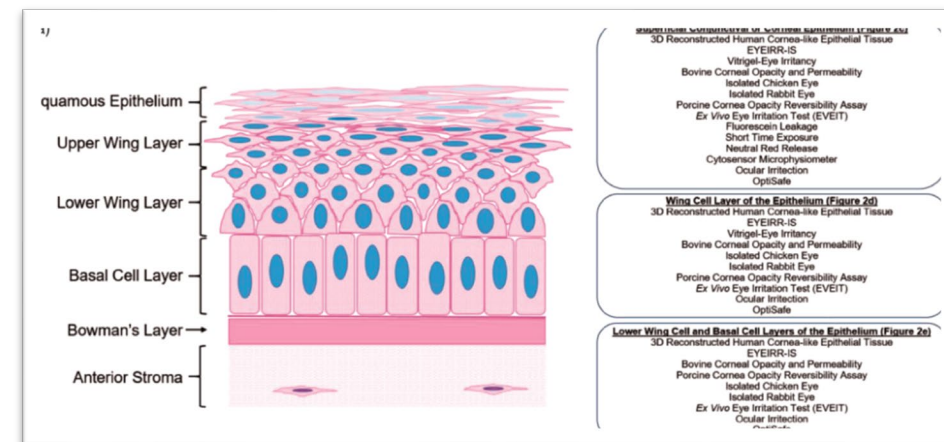
OPP Strategic Vision to Reduce Animal Testing: Letter to Stakeholders



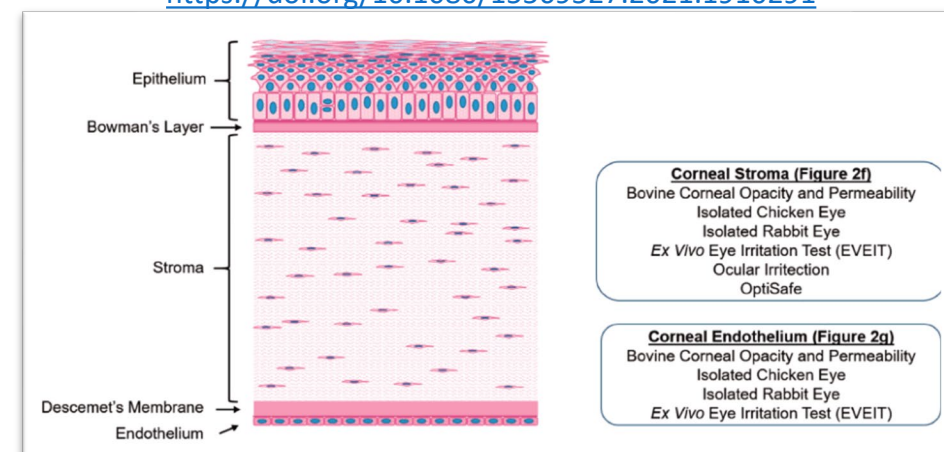
Prospective & Respective Evaluation of Eye Irritation Potential of Agrochemical Formulations

# HUMAN RELEVANCE OF EYE IRRITATION NAMs

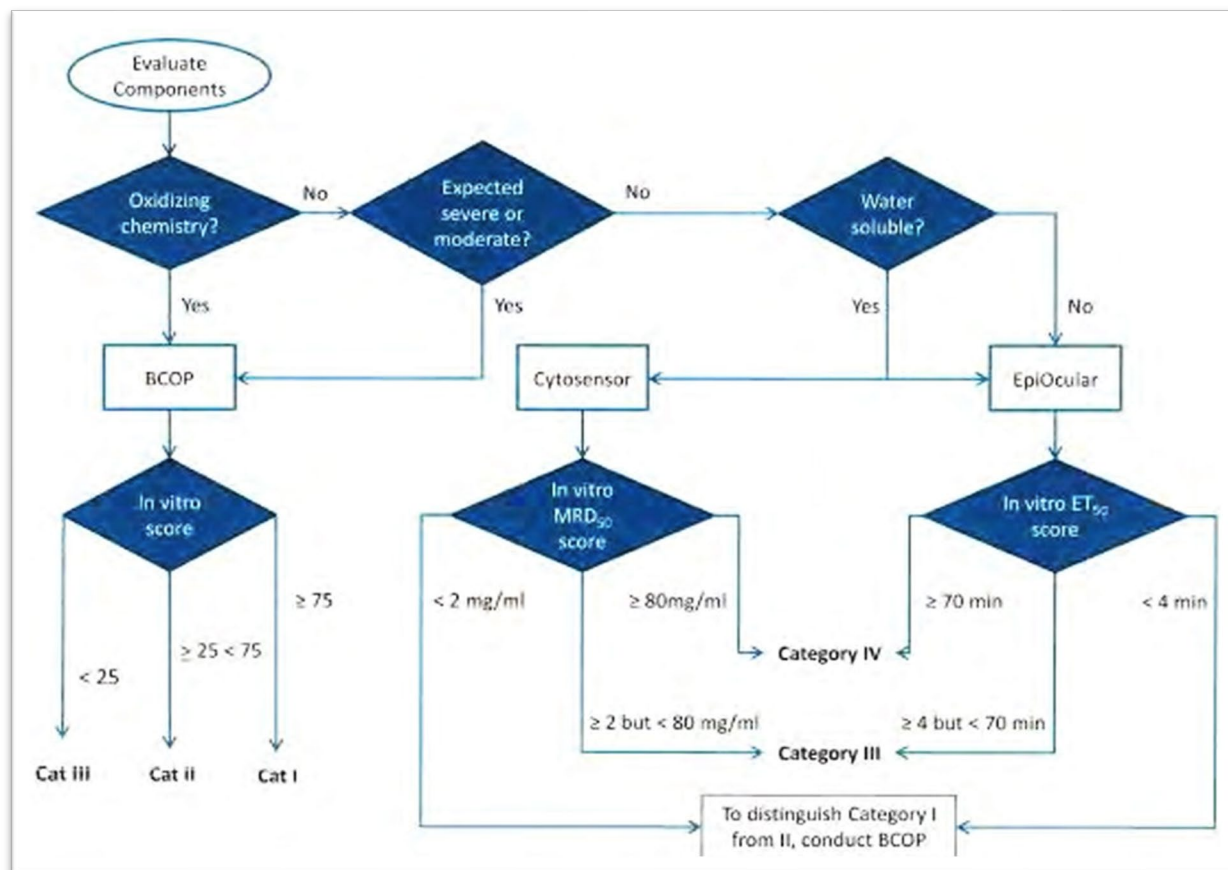
- The 2021 publication in Cutaneous and Ocular Toxicology, Clippinger et al., reviewed the test methods available to assess eye irritation outside of a living animal.
- Evaluated the human relevance of the *in vivo* rabbit study and *in vitro* assays.
  - Comparison of human, rabbit, porcine, chicken, and bovine corneas.
  - Describes strengths and uncertainties of the *in vivo* and *in vitro* assays.
  - Proposes an adverse outcome pathway for eye irritation.
  - Concludes that many *in vitro/ex vivo* methods are equivalent or scientifically superior to the rabbit test for hazard identification.



<https://doi.org/10.1080/15569527.2021.1910291>



# ALTERNATIVE TESTING FRAMEWORK: ANTIMICROBIAL CLEANING PRODUCTS (AMCPs)



- Testing framework for assessing eye irritation potential of antimicrobial cleaning products using three *in vitro/ex vivo* assays.
- The policy was updated in 2015 to include three eye irritation categories predicted in the (bovine corneal opacity and permeability (BCOP) assay).
- This approach currently considered on a case-by-case basis for other classes of pesticides.
- OPP is currently receiving paired *in vivo* and *in vitro* data on agrochemical formulations.

# DEFINED APPROACHES (DAs) FOR EPA CLASSIFICATION OF AGROCHEMICAL FORMULATIONS

- Published in 2023, “Defined approaches to classify agrochemical formulations into EPA hazard categories using Epiocular™ reconstructed human corneal epithelium and bovine corneal opacity and permeability assays”, presented two defined approaches (DA) utilizing two accepted OECD test guidelines to assess eye irritation.
  - Additional effort was needed to expand the use of alternative assays to other types of pesticides, outside of antimicrobial cleaning products.
- The project was a collaboration between NICEATM<sup>1</sup>, PETA Science Consortium, Institute for In Vitro Sciences, and EPA. Common conventional agrochemical formulation types with historical *in vivo* data spanning all EPA categories were selected for analysis (29 formulations total).

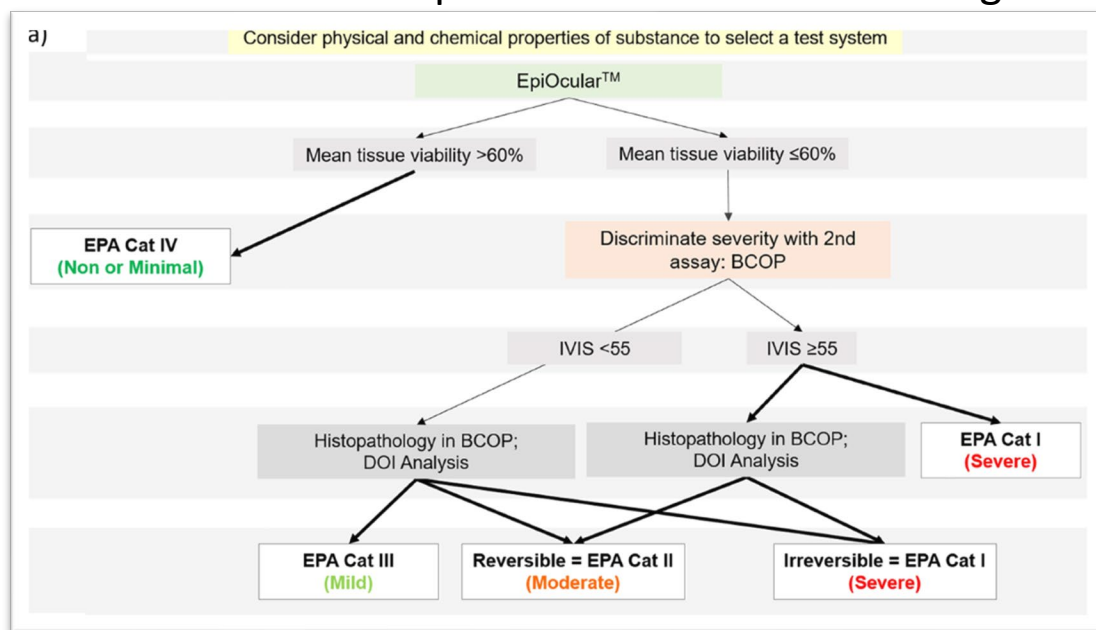
EPA Category	Criteria	Personal Protective Equipment (PPE), Signal Word, and Precautionary Statement	Number of Formulations
I	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Goggles face shield, or safety glasses. DANGER. Corrosive. Causes irreversible eye damage	7
II	Corneal involvement or irritation clearing in 8–21 days	Goggles face shield, or safety glasses. WARNING. Causes substantial but temporary eye injury	7
III	Corneal involvement or irritation clearing in 7 days or less	Protective eyewear if appropriate. CAUTION. Causes moderate irritation	8
IV	Minimal effects clearing in less than 24 hours	No signal word or statement required	7
Total	–	–	29

<sup>1</sup> National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

# ASSESSING IRRITATION ACROSS EPA TOXICITY CATEGORIES

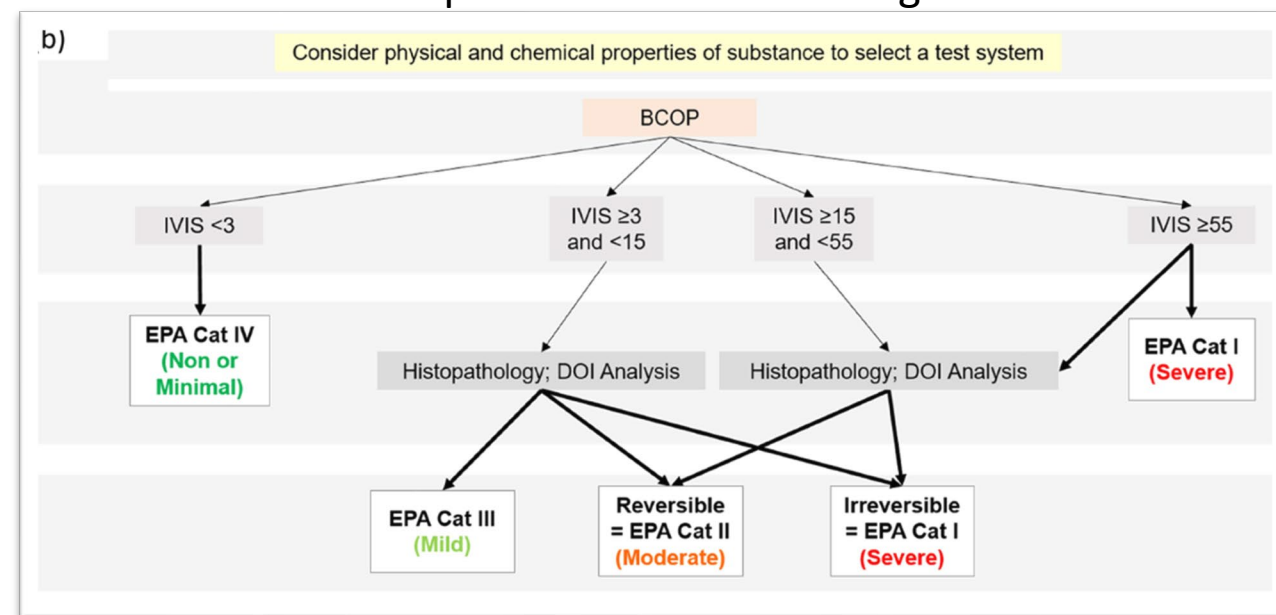
## DA – EO + BCOP

- For formulations predicted to be non-irritating



## DA – BCOP

- For formulations predicted to be irritating



- The cut-off values for EpiOcular (EO) and BCOP follow OECD TG 492 and OECD TG 437.
- In the BCOP, if the *in vitro* irritation score (IVIS) is ≥15 and <55, histopathology is conducted to assess the depth and degree of injury of the cornea.
- A test substance with IVIS >15 cannot be classified as EPA Category III.



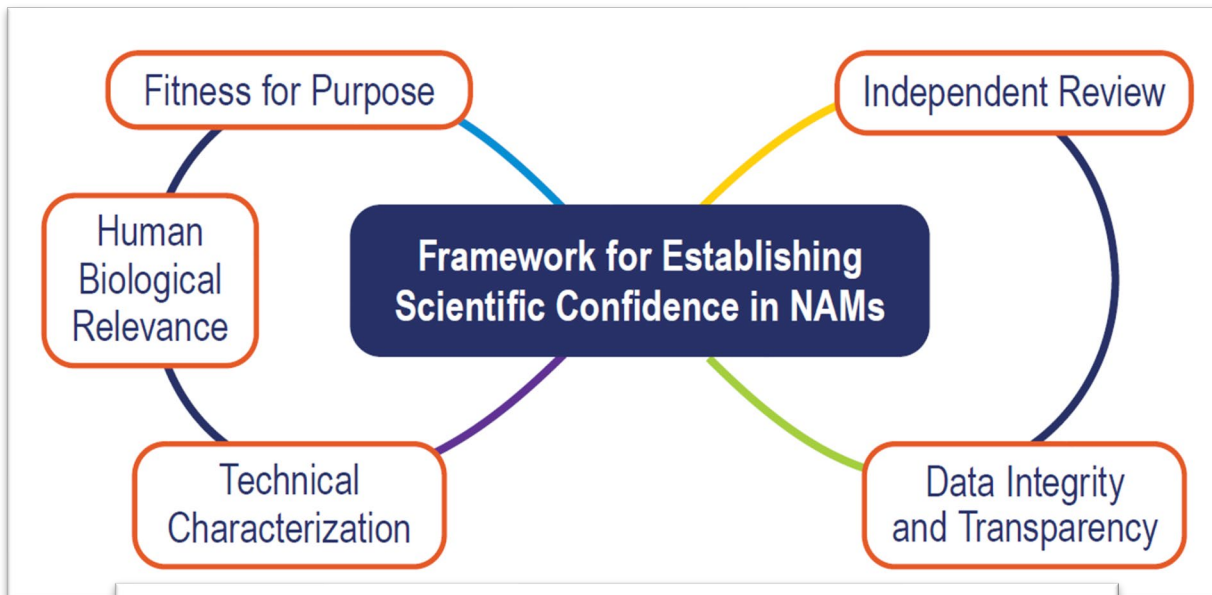
# ALIGNMENT ACROSS APPROACHES

- Concordance of the results were compared across all three approaches for the 29 formulations.
- For 17 formulations tested (58.6%), all approaches aligned.
- For 26 formulations tested (89.6%), at least two approaches aligned.
- For 21 formulations tested (72.4%), the DAs were aligned.
  - Misalignment in DAs for formulation Q would trigger a change in personal protection equipment (PPE).
- Specific results for formulation K → no alignment between approaches

FORMULATION K: SOLUBLE LIQUID		
EO + BCOP → CAT III	BCOP → CAT IV	IN VIVO → CAT II
MEAN CELL VIABILITY 15.35% IN EPIOCLAR, THUS EXCLUDING CAT IV	IIVS SCORE = 0, HISTOPATHOLOGY/DOI WAS MINIMAL	1/3 ANIMALS SHOWED CORNEAL OPACITY, CLEARING BY DAY 14; EFFECTS IN 2/3 ANIMALS CLEARED BY DAY 4

Formulation code	Formulation type	Predicted EPA classification using DA-EO+BCOP	Predicted EPA classification using DA-BCOP	Predicted EPA classification based on historical <i>in vivo</i> rabbit eye data
D	EC	I	I	I
G	EC	I	I	I
J	EC	I	I	I
F	SL	I	I	I
H	SL	I	I	I
I	SL	I	I	I
V	SL	I	I	III
U	EC	II	II	II
X	EC	II <sup>a</sup>	II <sup>a</sup>	II
R	SL	II	II	II
AB	EC	II	II	III
K	SL	III	IV	II
Q	SL	II <sup>a</sup>	IV	II
AC	EC	III	III	III
W	SL	III	III	III
E	EC	III	III	I
L	EC	III	IV	III
S	SL	III	IV	III
O	SL	III	IV	IV
Y	EC	III	IV	II
AA	EC	III	IV	II
A	EC/ME	IV	IV	IV
B	SC	IV	IV	IV
C	SC	IV	IV	IV
N	SC	IV	IV	IV
P	SC	IV	IV	IV <sub>9</sub>
M	SL	IV	IV	IV
T	SC	IV	IV	III
Z	EC	IV	III	III

# WEIGHT OF EVIDENCE EVALUATION



Archives of Toxicology  
<https://doi.org/10.1007/s00204-022-03365-4>

REVIEW ARTICLE

**A framework for establishing scientific confidence in new approach methodologies**

Anna J. van der Zalm<sup>1</sup> · João Barroso<sup>2</sup> · Patience Browne<sup>3</sup> · Warren Casey<sup>4</sup> · John Gordon<sup>5</sup> · Tala R. Henry<sup>6</sup> · Nicole C. Kleinstreuer<sup>7</sup> · Anna B. Lowit<sup>6</sup> · Monique Perron<sup>8</sup> · Amy J. Clippinger<sup>1</sup>

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- The proposed DAs were assessed according to the scientific confidence framework published in Archives of Toxicology in 2022.

## ***Fit for purpose***

- DAs developed specifically for EPA classification using scientifically advanced and internationally accepted methodologies.

## ***Human biological relevance***

- EO uses Reconstructed human Cornea-like Epithelium (RhCE), which models barrier function and epithelial cell death.
- BCOP is a full thickness model that can assess mechanistic effects, depth of injury in the epithelium, stroma, and endothelium.

## ***Technical characterization***

- EO and BCOP have been assessed for reliability and reproducibility compared to the *in vivo* test.

## ***Data Integrity, transparency, and peer review***

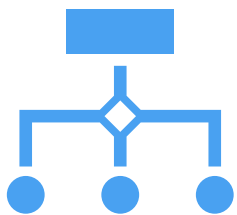
- Methodologies presented in the DAs are accepted OECD test guidelines, testing laboratories were blinded to all formulation details, and study results were subjected to independent peer-review for publication.

# OCSPN NAMS METRICS

- OPP and OPPT are currently tracking submissions of alternative methods which replace *in vivo* data on pesticides and industrial chemicals.
- Currently OPP metrics on animal reduction are published on the website; publication of OPPT metrics are in progress.
- NAMs represent a small portion of data submitted to OPP and OPPT for these toxicity endpoints.
  - Both offices typically receive hundreds of applications/year.
- Paired *in vitro* and *in vivo* eye irritation tests are currently submitted for conventional pesticides.

Non-animal Test Methods						
Fiscal Year	Eye Irritation Tests		Skin Irritation Tests		Skin Sensitization Tests	
	OPP	OPPT	OPP	OPPT	OPP	OPPT
<b>2018</b>	19	45	11	56	1	20
<b>2019</b>	12	40	7	49	0	19
<b>2020</b>	13	42	7	52	3	31
<b>2021</b>	32	39	28	54	12	23
<b>2022</b>	17	43	13	38	7	17
<b>Total</b>	<b>93</b>	<b>209</b>	<b>66</b>	<b>249</b>	<b>23</b>	<b>110</b>

## NEXT STEPS



- In addition to the assays utilized in the defined approaches mentioned, there are other accepted OECD TGs available to assess eye irritation.
  - Relatedly, a retrospective analysis was published in 2024 (Choksi, et al.) of 192 formulations from CropLife Brasil companies, 70% of which were reclassified as non-irritants based on the GHS concentration threshold approach. This project presented a bottom-up testing strategy using one of four methods (BCOP, RhCE, Isolated Chicken Eye (ICE), GHS CT) to classify non-irritants.
- Often pesticides are registered in the US *and* globally; therefore, continued efforts to harmonize the acceptance of NAMs are needed.
- The Agency will continue to build confidence in the alternative testing strategies to replace the *in vivo* test to predict eye irritation hazard in agrochemical pesticides and work with stakeholders on development and implementation of NAMs.
- As new NAMs-related documents are published, EPA will assess the progress and extent of adoption of these approaches over the years and evaluate any trends.



**THANK YOU!**

# REFERENCES

- Clippinger, A. J., Raabe, H. A., Allen, D. G., Choksi, N. Y., van der Zalm, A. J., Kleinstreuer, N. C., ... Lowit, A. B. (2021). Human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations. *Cutaneous and Ocular Toxicology*, 40(2), 145–167. <https://doi.org/10.1080/15569527.2021.1910291>
- van der Zalm, A. J., Daniel, A. B., Raabe, H. A., Choksi, N., Flint Silva, T., Breeden-Alemi, J., ... Clippinger, A. J. (2024). Defined approaches to classify agrochemical formulations into EPA hazard categories developed using EpiOcular™ reconstructed human corneal epithelium and bovine corneal opacity and permeability assays. *Cutaneous and Ocular Toxicology*, 43(1), 58–68. <https://doi.org/10.1080/15569527.2023.2275029>
- van der Zalm, A.J., Barroso, J., Browne, P. *et al.* A framework for establishing scientific confidence in new approach methodologies. *Arch Toxicol* 96, 2865–2879 (2022). <https://doi.org/10.1007/s00204-022-03365-4>
- Choksi N, Latorre A, Catalano S, Grivel A, Baldassari J, Pires J, Corvaro M, Silva M, Ogasawara M, Inforzato M, Habe P, Murata R, Stinchcombe S, Kolle SN, Masinja W, Perjessy G, Daniel A, Allen D. Retrospective evaluation of the eye irritation potential of agrochemical formulations. *Regul Toxicol Pharmacol*. 2024 Jan;146:105543 <https://doi.org/10.1016/j.yrtph.2023.105543>