Identifying Eye Irritation Hazards Without Using Animals

CIR Expert Panel Meeting
03 June 2024

www.iccs-cosmetics.org

ICCS
INTERNATIONAL COLLABORATION ON COSMETICS SAFETY

Presentation Outline

1. What is ICCS?
2. 80 Years of the Draize Eye Test
3. Requirements for Eye Irritation Testing
4. Evaluating NAMs with an Imperfect Reference Method
5. The Nonanimal Methods Toolbox for Eye Irritation
6. Complete Replacements: Defined Approaches for Eye Irritation

ICCS | INTERNATIONAL COLLABORATION ON COSMETICS SAFETY

Working Towards Animal-Free Science, Together

A non-profit organization with a mission:

Accelerate the global acceptance of animal-free science for human and environmental safety assessment of cosmetics and their ingredients through

Science, **Education & Training**, and **Regulatory Engagement**

Global, **multi-stakeholder** organization headquartered in the U.S.

Dedicated to advancing animal-free safety assessments for cosmetic products and their **ingredients**

Covering both **human health** and **environmental** safety

ICCS Members and Growing…

27 Cosmetic Product and Ingredient Manufacturers

Amorepacific
BASF
Beiersdorf
Chanel
Colgate
Coty
Croda
Estée Lauder
Edgewell
Evonik
Haleon
Henkel
IFF
Innospec
Inolex
Kao
Kenvue (J&J)
L'Oréal
LVMH
Oriflame
P&G
Reckitt
Shiseido
Syensqo
Takasago
Unilever
Wella

10 Cosmetic & Chemical Trade and Research Associations

CAC, Cosmetics Alliance Canada

CE, Cosmetics Europe

CTPA, Cosmetic, Toiletry, Perfumery Assoc. (UK)

EFfCI, European Federation for Cosmetic Ingredients

FCA, Fragrance Creators Association

IFRA, International Fragrance Association

JCIA, Japan Cosmetic Industry Association

CASIC, Latin American Cosmetic, Personal Care and Home Care Industries Association

PCPC, Personal Care Products Council (PCPC)

RIFM, Research Institute for Fragrance Materials

5 NGOS

CFI, Cruelty Free International

HSI, Humane Society International

IIVS, Institute for In Vitro Sciences

PCRM, Physicians Committee for Responsible Medicine

PSCI, Peta Science Consortium International

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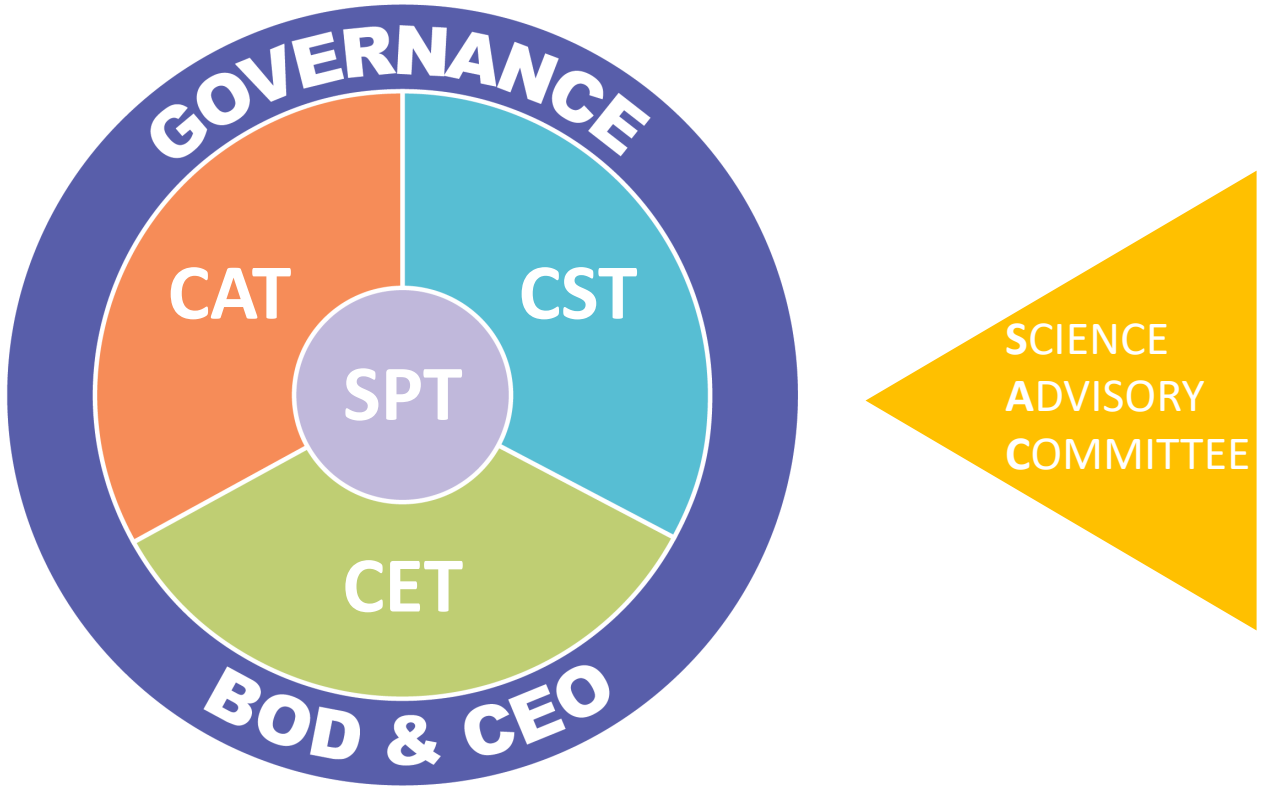
ICCS Pillars to Accomplish our Mission:
Regulatory Engagement, Science, Education & Training



Representative		Organization
Government/Regulatory Agencies		
Tomasz	Sobanski	European Chemicals Agency (ECHA)
Tara	Barton-MacLaren	Health Canada
Marize	Campos Valadares	Universidade Federal de Goiás/ ANVISA (Brazilian Regulatory)
Alison	Harrill	US Environmental Protection Agency (US EPA)
Katie	Paul-Friedman	US Environmental Protection Agency (US EPA)
Validation Bodies of Governments		
Maurice	Whelan	European Commission Joint Research Center/ ECVAM
Takao	Ashikaga	Japanese Ceneter for Validation of Alternative Methods (JaCVAM)
Nicole	Kleinstreuer	US National Center for the Evaluation of Alternative Toxicological Methods (NICEATM/ICCVAM)
Octavio	Presgrave	Brazilian Center for Validation of Alternative Methods (BraCVAM)
Research Institutes and Experts		
Carole	Yauk	University of Ottawa
Kristin	Schirmer	Swiss Federal Institute of Aquatic Science and Technology (EAWAG)
Nathalie	Burden	UK National Center for the 3Rs (NC3RS)
Scott	Belanger	Independent environmental expert
Charlie	Menzie	Independent environmental expert

Science Advisory Committee

ICCS Strategy – Core Principles

All ICCS activities should adhere to the following:

1. Prioritize activities focused on addressing **regulatory needs of cosmetics**
2. **Collaborate** with relevant stakeholders to leverage existing regulatory, scientific and educational resources, **avoiding duplication of effort and maximizing harmonization**
3. Develop broadly accessible tools and resources to enable robust animal-free safety assessments that **protect human and environmental health**
4. Strive for **integration of human and environmental** NAMs into NGRA frameworks where possible

Core Science Team (CST): Aim & Objectives

Build a Robust Scientific Toolbox

Chair: V. Poulsen (L'Oréal); Vice-chair: A. Schepky (Beiersdorf)

1 **Identify** regional stakeholder & regulatory needs

2 **Prioritize** activities where ICCS can have best impact

3 **Develop** ICCS as a Center of Competence in NGRA

Delivery Teams

- Human Health Effect
- Environmental Effects
- Human Health Exposure
- Environmental Exposure and Fate
- Safety Decision (Case Studies)

CST Strategic Elements:

- Develop robust NAMs and **NGRA frameworks**
- Maintain a balanced portfolio for continued **deliverables**
- Projects focused on **cosmetics safety** at all stages of the product pipeline: screening/prioritization to regulatory decision making
- Collaborate with CAT and CET to ensure activities and deliverables **fulfill appropriate regulatory needs**

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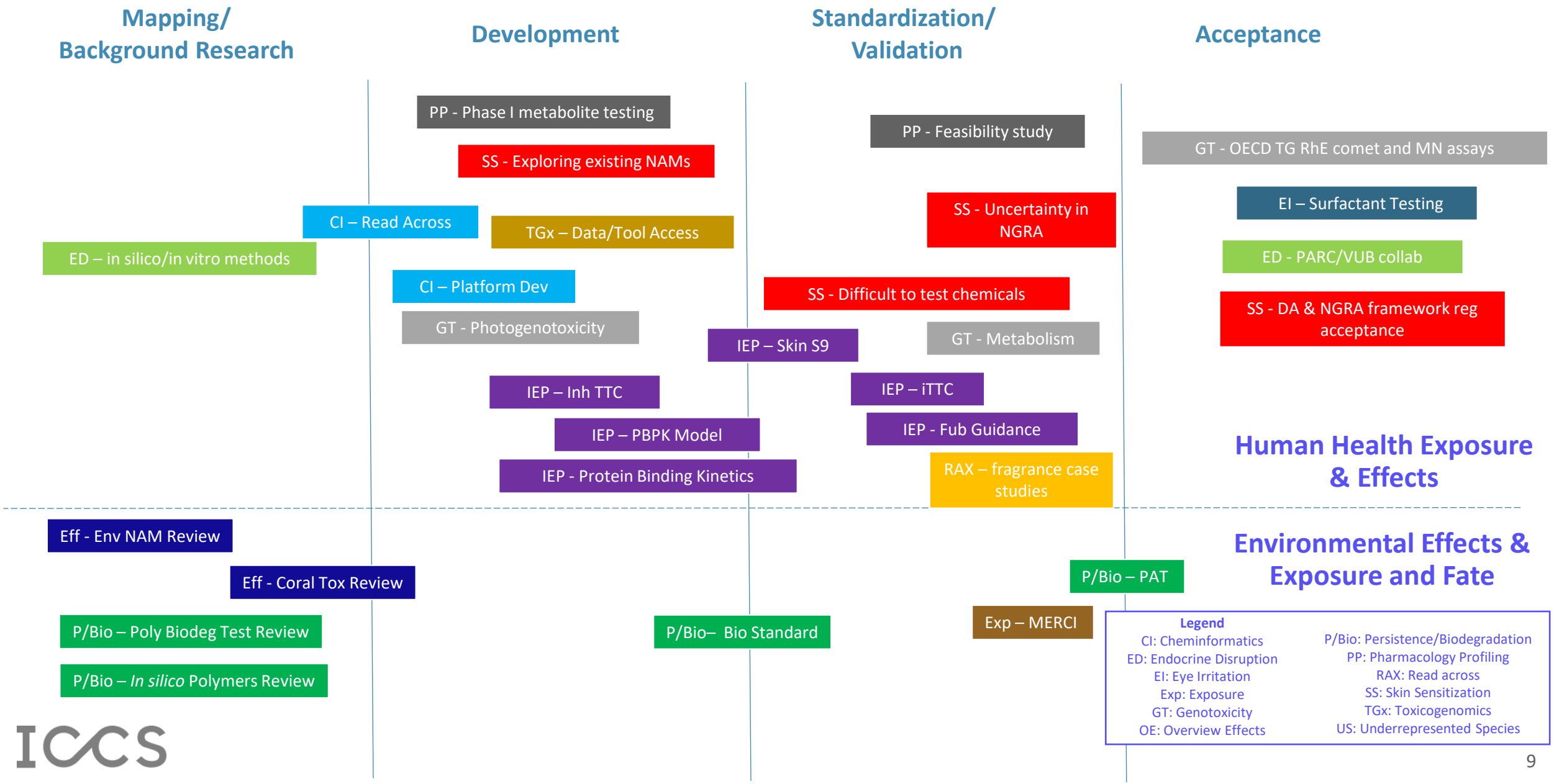
30 ongoing ICCS Core Science Team (CST) projects



Core Acceptance Team: Aim & Objectives

Accelerate Uptake of Animal-Free Safety Assessments for Cosmetics
Chair: K. Sullivan; Vice-chair: G. Maxwell

1 **Standardization & Validation** of animal-free safety assessment approaches for regulatory use

2 **Regulatory Acceptance** of NAMs and Next Generation Risk Assessment (NGRA) frameworks for Cosmetics

3 **Global Alignment** of Cosmetic and Chemical regulatory information requirements

Multi-stakeholder collaborations (e.g. ICCS) are needed to efficiently address these strategic challenges

CAT Strategic Elements:

- **Increase regulator confidence** in accepting NAMs and NGRA frameworks by engaging in scientific dialog, in coordination with relevant national and regional trade associations and validation bodies
- **Promote** the use of NAMs and NGRA frameworks by providing input into new guidance/regulation of cosmetics
- Aid CST and CET in **prioritization** by ensuring that regulatory needs are identified and communicated

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Core Education Team: Aim & Objectives

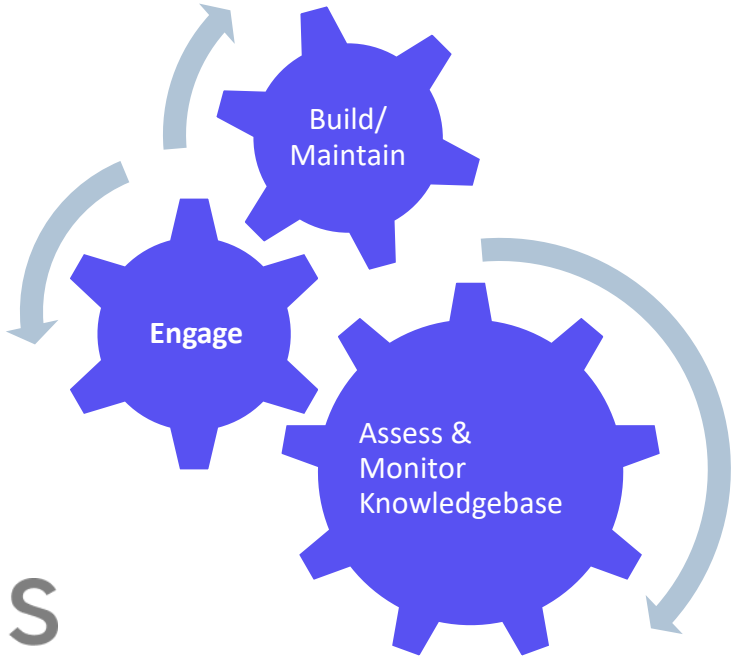
Support and Design Continuous Educational Programs
Chair: B. Montemayor; Vice-chair: F. Rapolla

1 **Understand** education needs from various stakeholders

2 **Facilitate** awareness & engagement with existing activities

3 **Fill gaps** through coordination with Acceptance & Science Teams

An Iterative Approach



CET Strategic Elements

- Facilitate dialog to **share experiences** to **educate** and **build confidence** in the use of NAMs and NGRA frameworks
- Determine **educational needs** for key internal and external stakeholders
- **Identify and support existing** education & training activities and initiatives, seeking to **complement**, not **duplicate**
- Identify, in concert with CAT and CST, education & training gaps and develop materials for the **areas of greatest need**
- Promote education & training of all **CST/CAT activities** to further uptake and use

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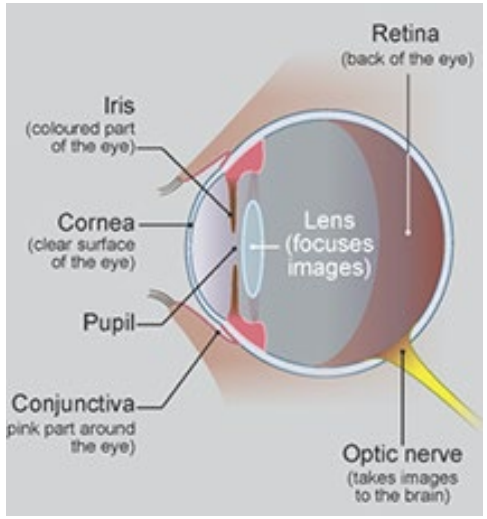
1944: Protecting Public Health with Available Tools

In vivo rabbit eye test

- Primary *in vivo* method (developed in 1944)
- Accepted by CPSC 1995; EPA 1998; OECD 2012
- Test substance placed in lower conjunctival sac
- Cornea, Iris, Conjunctiva evaluated
- Animal observed daily over 21 days after exposure

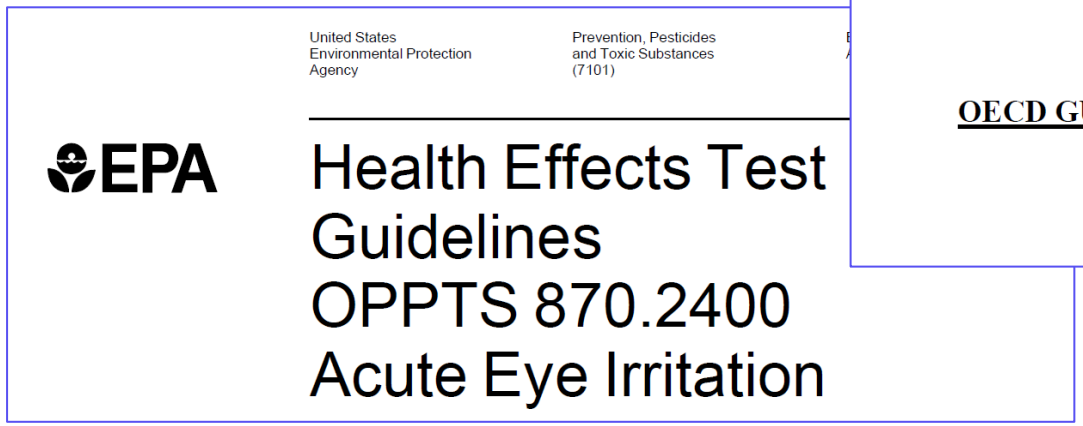


https://www.pdsa.org.uk/

Pharmacokinetic factor	Human eye	Rabbit eye
Tear volume (µL)	7.5	7.0–30.0
Tear turnover rate (µL/min)	0.5–2.2[a]	0.6–0.8
Spontaneous blinking rate (times/min)	15	4–5
Nictitating membrane	Absent	Present
pH of tears	7.14–7.82	7.14–7.82
Milliosmolarity of tears (mOsm/L)	305	305
Corneal thickness (mm)	0.52	0.40
Corneal diameter (mm)	15	12
Aqueous humor volume (µL)	310	310
Aqueous humor turnover rate (µL)	1.53	1.53

[a]Range depending on blinking rate and conjunctival sac volume

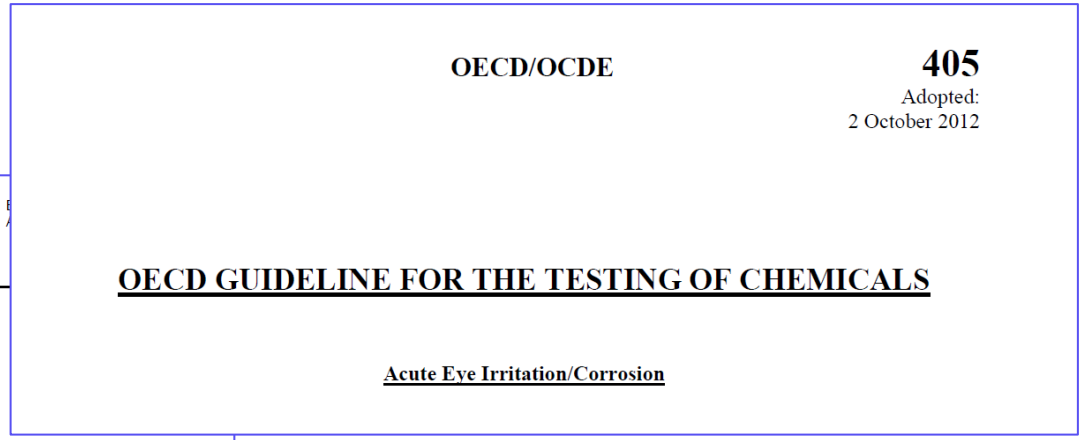
Anatomical and physiological difference in human eye and New Zealand rabbit
Zafar et al., 2016 doi: 10.1007/978-3-319-47691-9_9


United States Environmental Protection Agency

Prevention, Pesticides and Toxic Substances (7101)

EPA

Health Effects Test Guidelines
OPPTS 870.2400
Acute Eye Irritation


OECD/OCDE

405
Adopted:
2 October 2012

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Acute Eye Irritation/Corrosion

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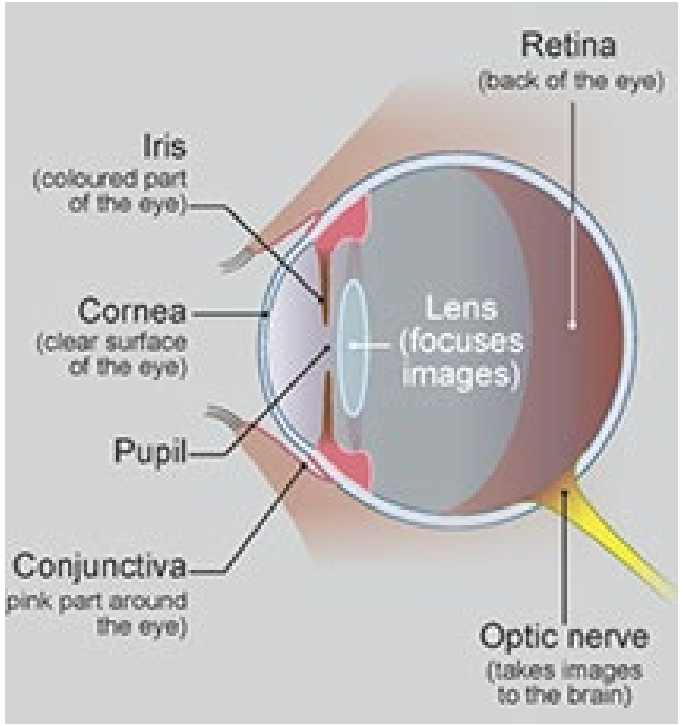
Rabbit Eye Test Scoring

Cornea, iris, and conjunctiva are subjectively evaluated and scored

- Corneal opacity (CO)
  - 1 = Scattered or diffuse area – details of iris visible
  - 2 = Easily discernible translucent areas – details of iris slightly obscured
  - 3 = Opalescent areas, no details of iris visible, size of pupil barely discernable
  - 4 = Opaque – iris not visible
- Iris
  - 1 = Folds above normal, congestion, swelling, circumcorneal injection (any one or all of there, or combination of any thereof), iris still reacting to light
  - 2 = No reaction to light, hemorrhage, gross destruction (any one or all of these

- Conjunctival redness (CR)
  - 1 = Vessels definitely injected above normal
  - 2 = More diffuse, deeper crimson red, individual vessels not easily discernable
  - 3 = Diffuse, beefy red

- Conjunctival chemosis (CC)
  - 1 = Any swelling above normal (includes nictitating membrane)
  - 2 = Obvious swelling with partial eversion of the lids
  - 3 = Swelling with lids about half closed
  - 4 = Swelling with lids half to completely closed



https://www.pdsa.org.uk/

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US and International Hazard Classification Systems

EPA Classification

- **Category I**: Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days.
- **Category II**: Corneal involvement or irritation clearing in 8-21 days.
- **Category III**: Corneal involvement or irritation clearing in 7 days or less.
- **Category IV**: Minimal effects clearing in less than 24 hours.

- Maximum score in any animal used for classification
- Positive: CO or IR ≥ 1 or CC or CR ≥ 2

GHS Classification

- **Category 1**: Effects on the cornea, iris or conjunctiva that are not expected to reverse or that have not fully reversed within 21 days.
- **Category 2A**: Effects on the cornea, iris or conjunctiva that fully reverse within 21 days.
- **Category 2B**: Effects on the cornea, iris or conjunctiva that fully reverse within 7 days.

Category	*In Vivo* Effect
1	≥ 1 animal with CO = 4 at any time OR ≥ 2 animals with mean* CO ≥ 3 or IR ≥ 1.5 OR ≥1 animal at day 21 with CO or IR ≥ 1 or CC or CR≥ 2
2A	≥ 2 animals with mean* CO or IR ≥ 1 or CC or CR≥ 2 which reverses within 21 days.
2B	≥ 2 animals with mean* CO or IR ≥ 1 or CC or CR≥ 2 which reverses within 7 days.

*Mean values calculated over days 1-3

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CUTANEOUS AND OCULAR TOXICOLOGY
2019, VOL. 38, NO. 2, 141–155
https://doi.org/10.1080/15569527.2018.1540494

Taylor & Francis
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REVIEW ARTICLE

Check for updates

United States regulatory requirements for skin and eye irritation testing

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ABSTRACT

Purpose: Eye and skin irritation test data are required or considered by chemical regulation authorities in the United States to develop product hazard labelling and/or to assess risks for exposure to skin- and eye-irritating chemicals. The combination of animal welfare concerns and interest in implementing methods with greater human relevance has led to the development of non-animal skin- and eye-irritation test methods. To identify opportunities for regulatory uses of non-animal replacements for skin and eye irritation tests, the needs and uses for these types of test data at U.S. regulatory and research agencies must first be clarified.

Methods: We surveyed regulatory and non-regulatory testing needs of U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) agencies for skin and eye irritation testing data. Information reviewed includes the type of skin and eye irritation data required by each agency and the associated decision context: hazard classification, potency classification, or risk assessment; the preferred tests; and whether alternative or non-animal tests are acceptable. Information on the specific information needed from non-animal test methods also was collected.

Results: A common theme across U.S. agencies is the willingness to consider non-animal or alternative test methods. Sponsors are encouraged to consult with the relevant agency in designing their testing program to discuss the use and acceptance of alternative methods for local skin and eye irritation testing.

Conclusions: To advance the implementation of alternative testing methods, a dialog on the confidence of these methods to protect public health and the environment must be undertaken at all levels.

ARTICLE HISTORY
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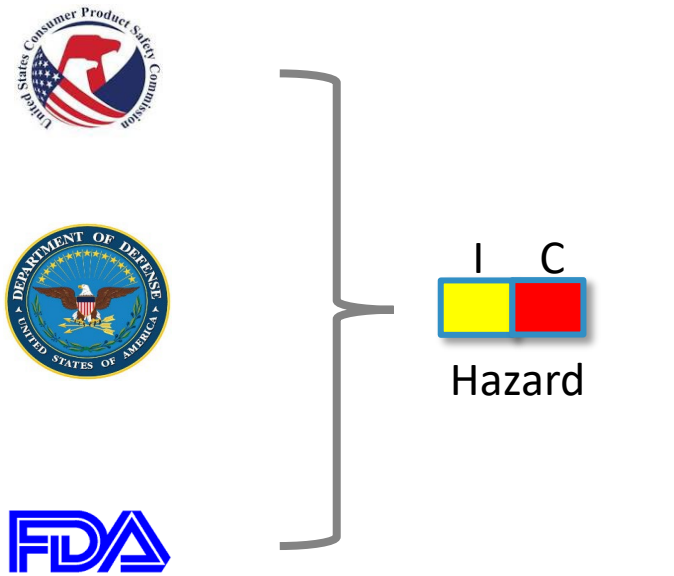
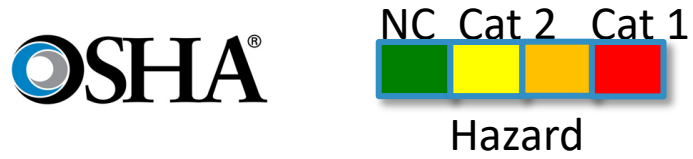
Accepted 18 October 2018

KEYWORDS
Eye irritation testing; skin irritation testing; alternative approaches; non-animal methods; regulatory requirements; corrosive



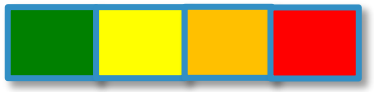


- Identifies requirements, needs, and decision contexts data for each endpoint

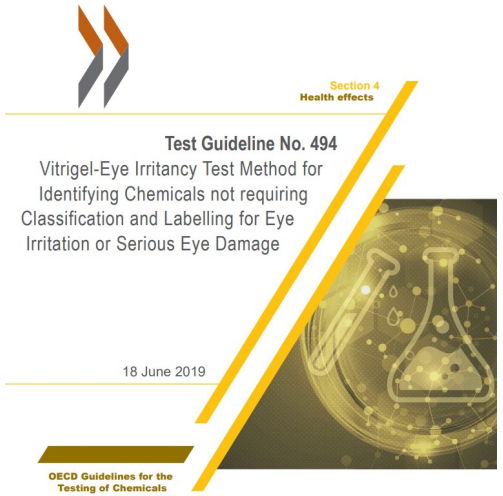
OECD Guidelines for in vitro/ex vivo eye irritation testing – Each one based on comparison to the rabbit test…

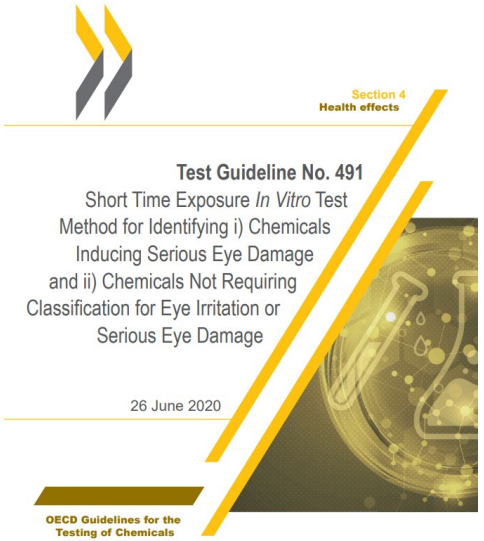
✓ ? ? ✓

IV III II I



Hazard





OECD/OCDE **438**
Adopted:
25 June 2018

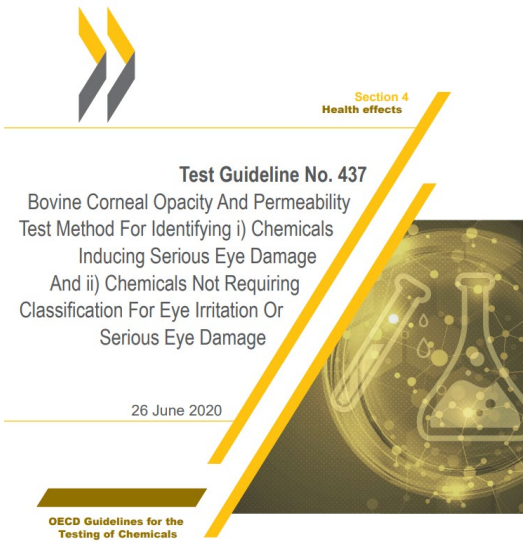
OECD GUIDELINE FOR THE TESTING OF CHEMICALS

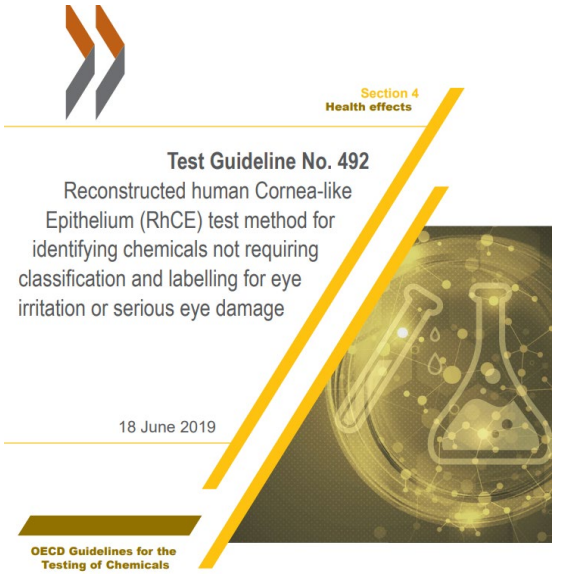
Isolated chicken eye test method for identifying I) chemicals inducing serious eye damage and II) chemicals not requiring classification for eye irritation or serious eye damage

OECD/OCDE **460**
Adopted:
9 October 2017

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants





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BETTER POLICIES FOR BETTER LIVES

Reproducibility of the Draize Eye Test

Prior type	1	2A	2B	NC	Total
1	73%	16.1%	0.4%	10.4%	46
2A	4.2%	32.9%	3.5%	59.4%	138
2B	0.2%	4%	15.5%	80.2%	86
NC	1.1%	3.5%	1.5%	93.9%	400

- ECHA database evaluation
- 491 substances with at least 2 Draize eye studies
- Conditional probabilities of Draize evaluations based on a previous test result
- Ex: 46 substances had multiple Draize test results that included at least one Category 1 response

from Luechtefeld et al., ALTEX 33(2), 2016.

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NC	1.1%	3.5%	1.5%	93.9%	400

- Some Category 1 could be NC in a subsequent test

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from Luechtefeld et al., ALTEX 33(2), 2016.

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NC	1.1%	3.5%	1.5%	93.9%	400

- Category 2A and 2B more likely to be NC than Category 2 in a subsequent test
- Category 2B and NC very similar in response

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from Luechtefeld et al., ALTEX 33(2), 2016.

Four major groups of non-animal test methods

1. *ex vivo* tissues and organ systems
2. non-cellular, *in chemico* test systems
3. *in vitro* monolayer cell culture systems
4. *in vitro* reconstructed tissue models

Relationship of *Nonanimal* Models to *In Vivo* Tissues

Cornea

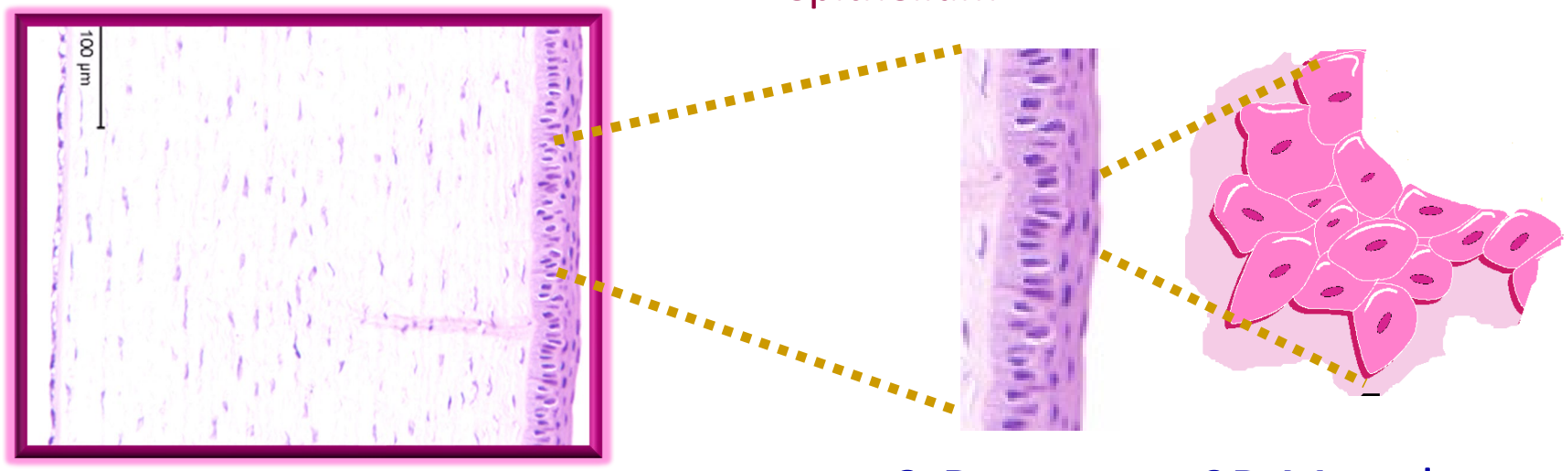
epithelium, stroma and endothelium

Epithelium

Squamous, wing, and basal epithelium

Squamous Epithelium

Cells covering cornea



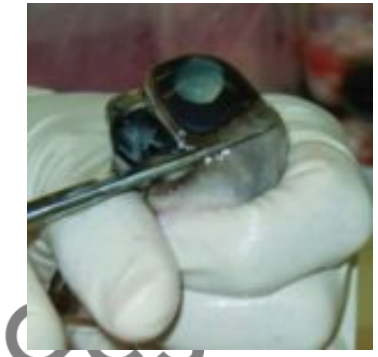
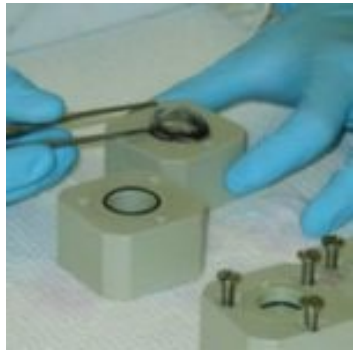
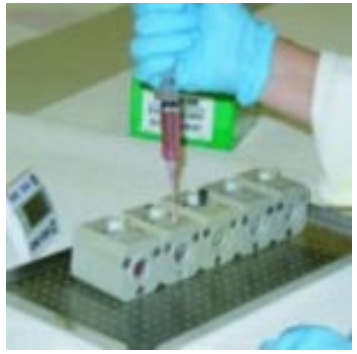
Bovine Cornea and Isolated Chicken Eye

3-D reconstructed human corneal tissue

2D Monolayer

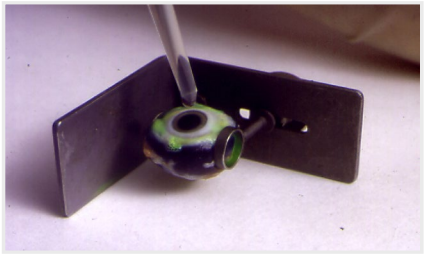
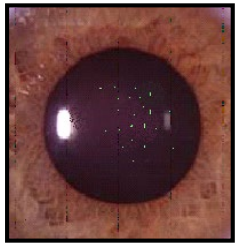
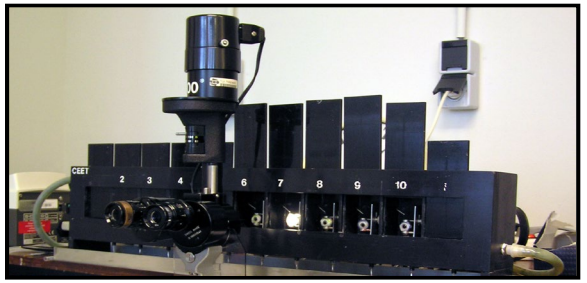
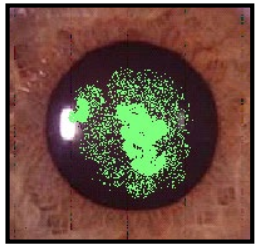
Ex vivo Organotypic Models: e.g., BCOP, ICE

- *Ex vivo* corneal models assess corneal injury (in vitro irritation score) based on:
  - Opacity (measure the amount of light transmitted through the cornea).
  - Permeability (measure the amount of fluorescein dye that penetrates through the cornea)
- Taken from animals used in food production (bovine, porcine)
- Porcine model (PORCORA) developed to assess reversibility of corneal effects based on fluorescein retention

http://iivs.org/testing-services/assays/ocular/bcop/

- *Ex vivo* whole eye models assess corneal injury based on:
  - disruption of the corneal epithelium
  - swelling of the cornea
  - corneal opacity
- Evaluate effects *ex vivo* using whole eyes taken from animals used for other purposes (e.g., food production; other research testing)

Images courtesy of Menk Prinsen

Histological Evaluation

Histopathology of progressive surfactant-induced corneal epithelial erosion and stromal swelling.

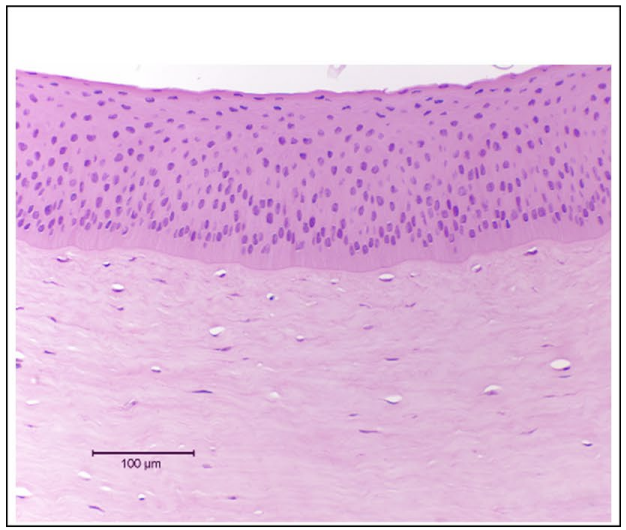


Fig a. Negative Control cornea showing intact epithelium and organized upper stroma.

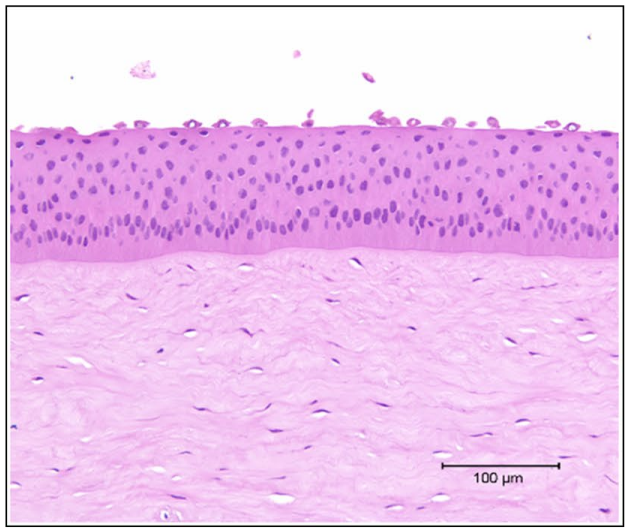


Fig b. Loss of squamous and upper wing layers, results in increases in FL_{490}.

Opacity = 1.7

FI OD490 = 0.302

IVIS = 6.2

10 minute exposure to SLS 1.5%

Superficial loss of epithelium only

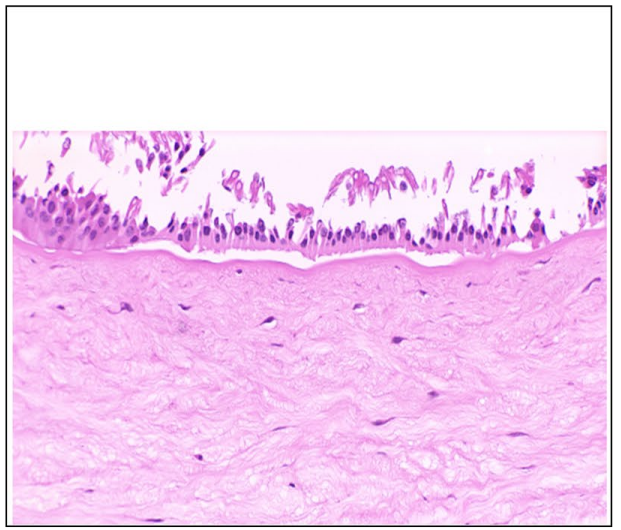


Fig c. Complete loss of epithelium results in high FL_{490}. Marked stromal edema and disorganization results in modest opacity.

Opacity = 7.7

FI OD490 = 2.540

IVIS = 45.8

30 minute exposure to SLS 5%

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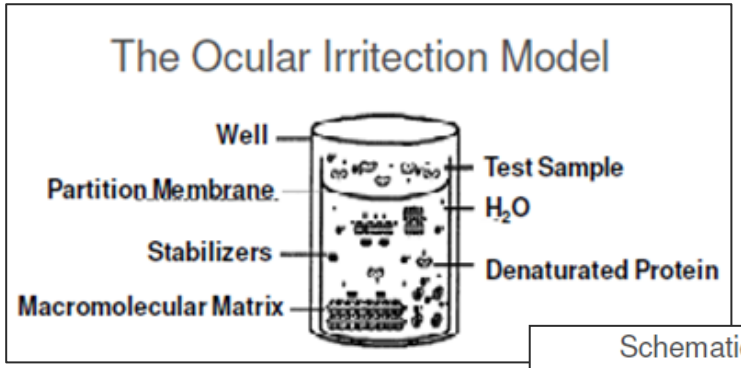
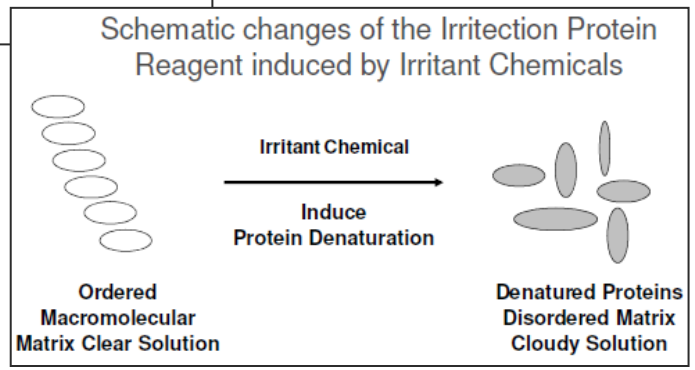
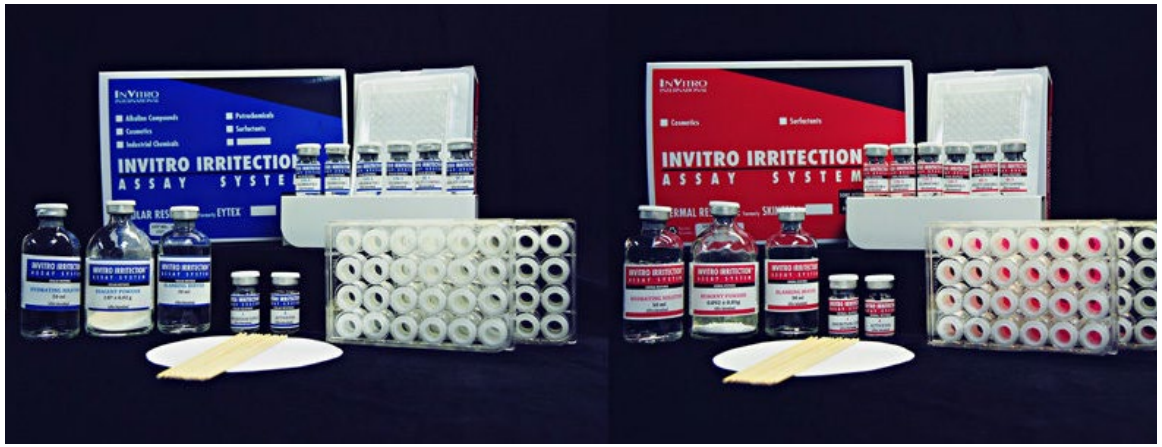
Non-cellular or In Chemico Methods (OECD TG 496)

- Do not require cell culture facility or cell culture expertise
- May be relatively inexpensive to conduct
- Test system: macromolecular matrix composed of lipids, glycoproteins, carbohydrates, low MW molecules
  - Mimics the highly ordered structure of the corneal stroma
- Standardized manufacturing or processes ensure reproducible testing platforms

OptiSafe test method







Ocular Irritection test method

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In Vitro Methods: 2D vs 3D culture systems

In Vitro Monolayer Cell Systems

Advantages

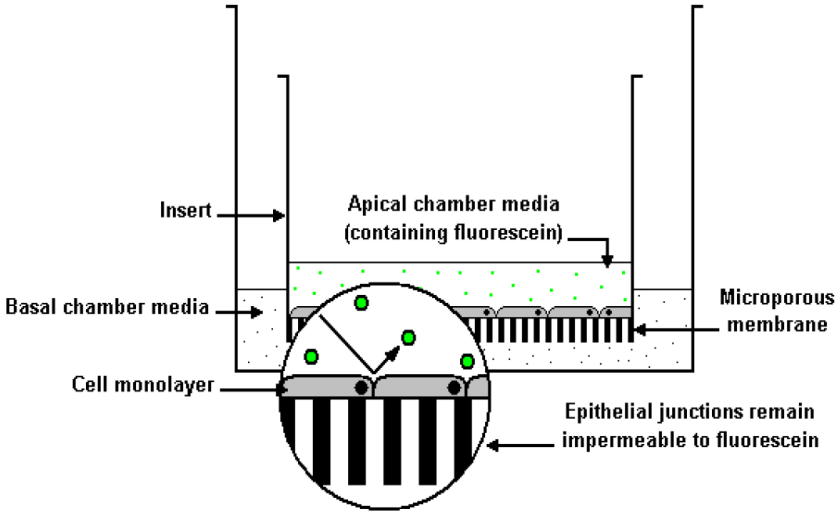
- Generally easy to conduct – primary cells and cell lines
- Generally quite rapid to execute
- Cost effective with batches of test materials
  - Can use robotics and also scale to medium/high throughput
- Machine scored endpoints
- Evaluate individual chemicals (ingredients) rather than formulations

Disadvantages

- Aqueous insoluble materials
- Dilution or buffering effects in medium which mask toxicity of the neat material
- Pharmacokinetics poorly modeled
- Little/No Tissue Barrier Function modeled

Monolayer Culture Systems: Fluorescein Leakage (TG 460)

- Assess cell damage – Top-Down approach
  - Madin Darby Canine Kidney (MDCK) cells cultured on permeable inserts
- Measures permeability to a marker dye (disruption of tight junctions)
- Assumes that chemicals causing eye damage/irritation will disrupt epithelial barrier function in the corneal epithelium and/or conjunctiva
- Limitations
  - Not recommended for Category 2, 2A, or 2B
  - Only water soluble chemicals
  - strong acids and bases, cell fixatives and highly volatile chemicals are outside of the applicability domain
  - colored and viscous chemicals may be difficult to remove
  - Tight junctions are increasingly compromised with increasing cell passage number



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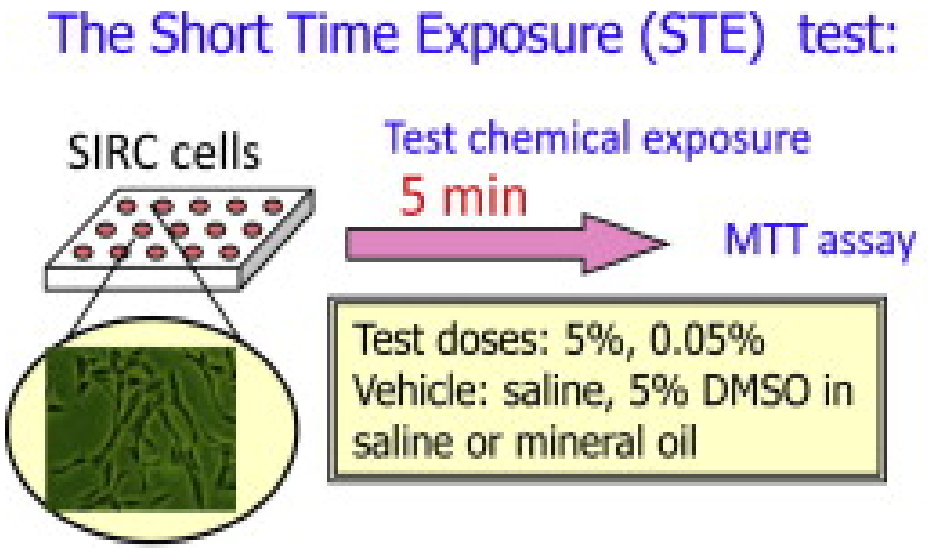
Monolayer Culture Systems: Short time exposure (STE) test (TG 491)

- Assess cell damage – top down or bottom up
  - Seruminstitute rabbit cornea (SIRC) cells cultured on microplates
- Measures cytotoxicity (% viability relative to control)
- Assumes that chemicals causing eye damage/irritation will induce cytotoxicity in corneal epithelial cells

- Limitations
  - Not suitable for test chemicals that are insoluble or cannot be uniformly suspended for at least 5 minutes in physiological saline, 5% DMSO in saline, or mineral oil
  - Solids (other than surfactants) or highly volatile substances with a vapor pressure over 6 kPa are also outside of the applicability domain

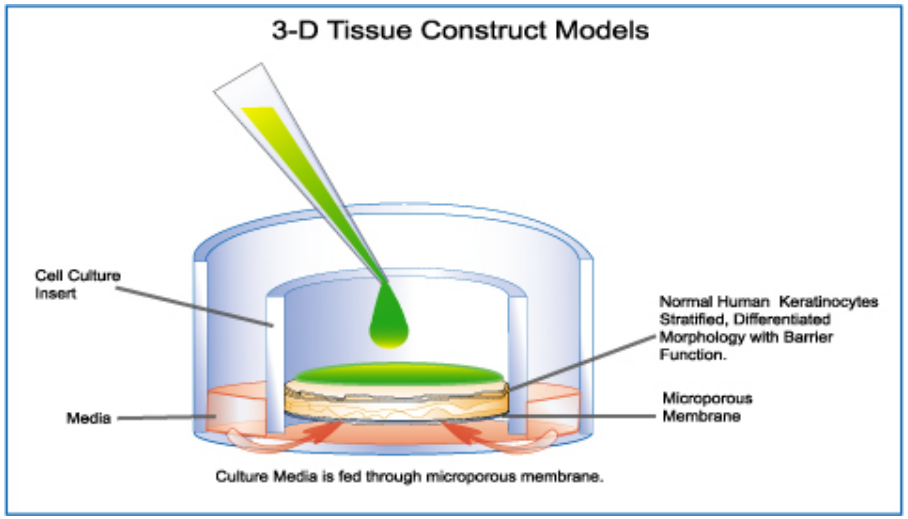


Classification with the STE score

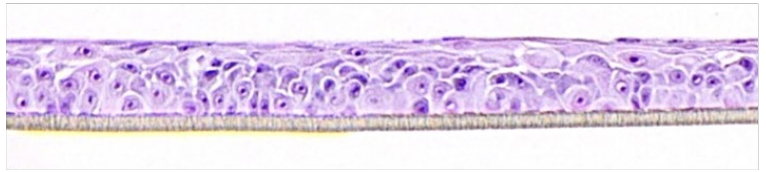
	Non Irritant (NI)	Irritant (I)
Cell viability	> 70%: Score 0	⩽70% : Score

3D Tissue RhCE Models

- Allow testing without use of live animals
- Reconstructed corneal tissues better model corneas in vivo (relative to monolayer cell systems)
- Exposure to substances as in vivo
- Standardized manufacturing ensures standard platforms
- Relevant mechanisms of action
  - Chemical permeation and cytotoxicity
- Endpoints may be machine scored
- Limitations:
  - Test chemicals and chemical mixtures that interfere with the cell viability measurements (e.g., direct MTT reducers, color interference)
  - Gases and aerosols



EpiOcular



Human Cornea

EIT test protocol comparisons

Eye Irritation Test (EIT)

- OECD TG 492: Identify test chemicals that don't require labeling ("No Label") for eye irritation (per GHS)
- Single exposure protocol

  (uses very few tissues)
- Limitations:
  - "yes/no" results – no additional information
  - Cannot discriminate Cat 1 vs Cat 2
  - May need additional test methods for irritants

Time-To-Toxicity ET50 Protocol

- Used in product development to create progressively milder/safer formulations
- Rank-order candidate formulations
- Multiple exposure times up to 24 hours
- Provides a continuum of responses across eye irritation spectrum
- Limitations:
  - Limited regulatory acceptance

regulatory classification and labeling vs. product development and stewardship

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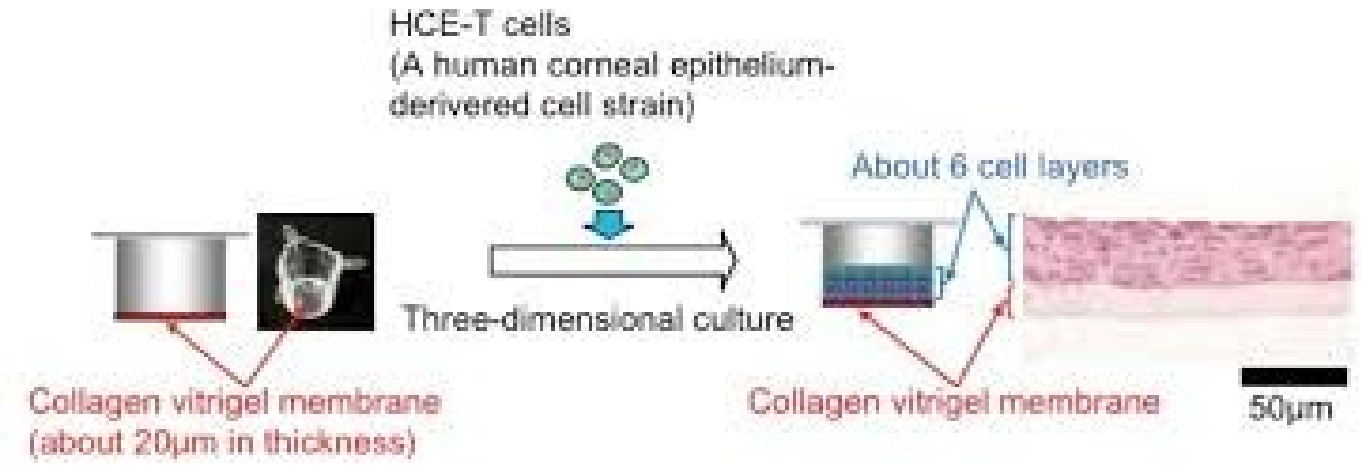
Vitrigel EIT (TG 494)

- Human immortalized corneal epithelial cells fabricated in a collagen vitrigel membrane chamber
- Assumes that chemicals causing eye damage/irritation will damage the barrier function of the corneal epithelium (TEER)
- Bottom-up approach

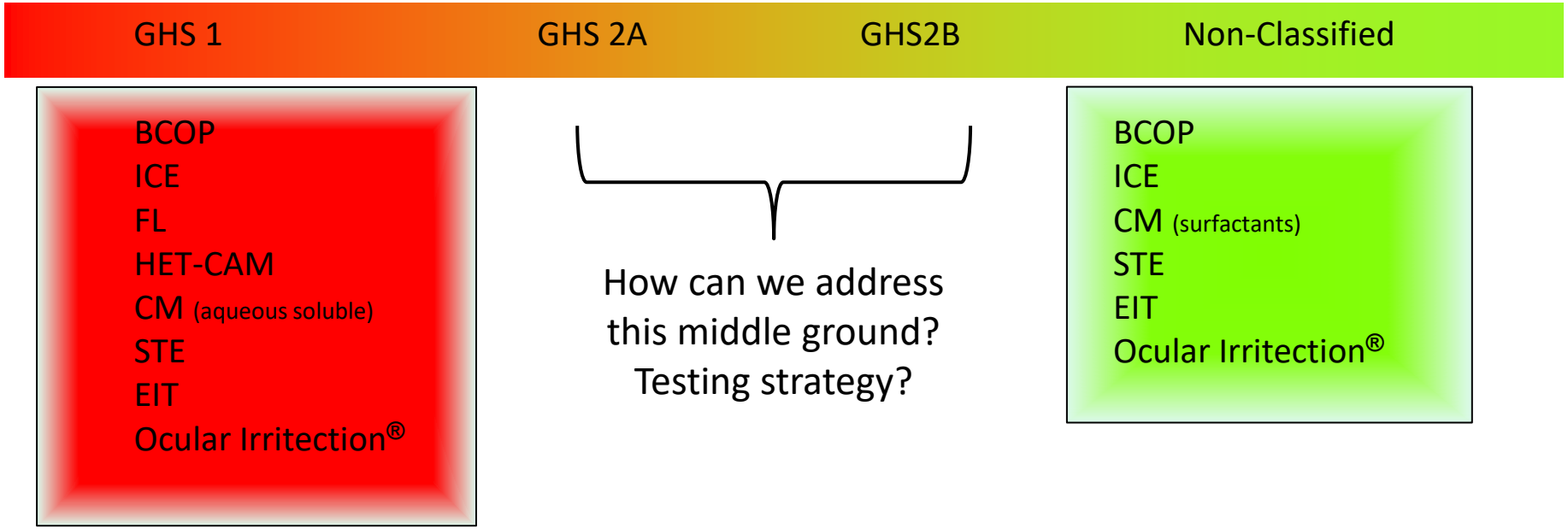
Limitations:

- Test chemicals with pH<5.0 or solids



www.naro.affrc.go.jp

On the Surface: A gap in testing approaches



GHS 2 (2A, 2B): Irritant, Reversible
GHS 1: Severe/Corrosive Irritant

Chemwatch: December 2014 Webinar

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Defined Approaches for Eye Irritation

OECD adopted full replacements

Eye hazard identification according to UN GHS

UNITED NATIONS
New York and Geneva,

No Cat.

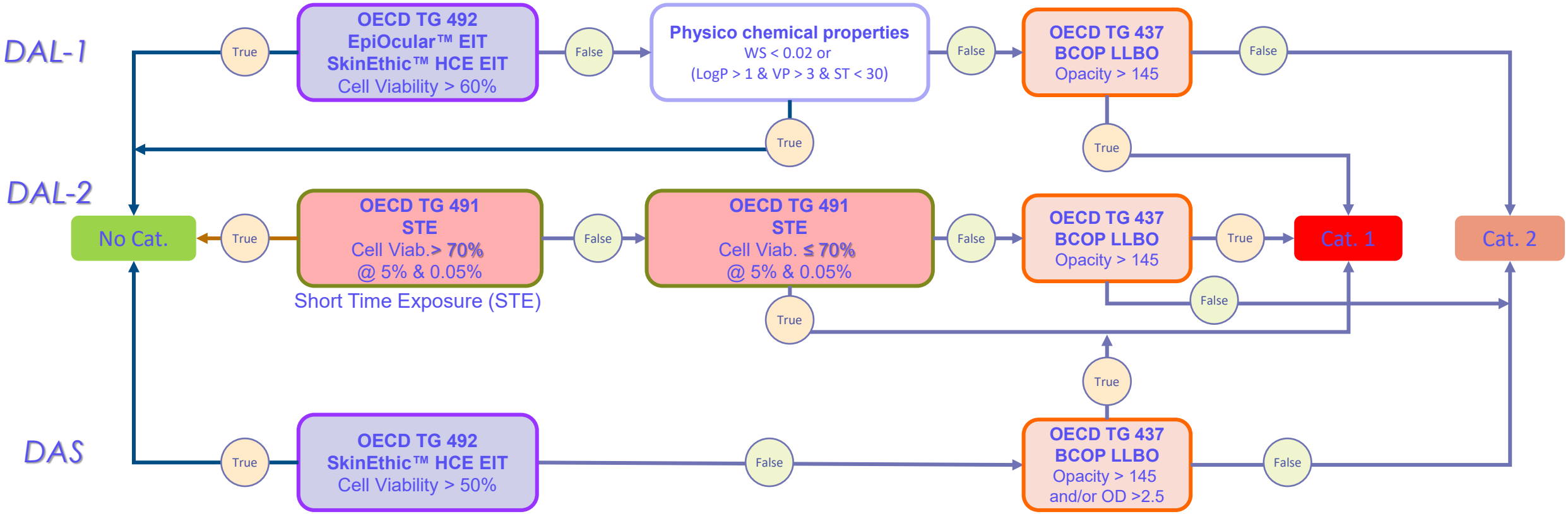
Cat. 2
Eye irritation

Cat. 1
Serious eye damage

OECD TG 492B (2022) – Stand-alone method

SkinEthic™ HCE
Time-To-Toxicity

OECD TG 467 – Defined Approaches (DAs) for Non-surfactant liquids (adopted 2022) and solids (adopted 2024)



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OECD TG 467 and Surfactants

Defined Approaches for Eye hazard identification – OECD TG 467

- Non-surfactant liquids – DAL1 and DAL-2
- Solids – DAS

These DAs are not applicable for surfactants.

- DAL-1 uses the BCOP LLBO to identify Cat. 1
  - Too many *in vivo* UN GHS Cat. 2 surfactants were predicted Cat. 1
- DAL-2 uses the BCOP LLBO and/or the STE to identify Cat. 1
  - Almost half of the *in vivo* UN GHS Cat. 1 surfactants were predicted Cat. 2 with the STE
  - Too many *in vivo* UN GHS Cat. 2 surfactants were predicted Cat. 1 with the BCOP LLBO

Development of a DA for Surfactants (DASF)

Combination of OECD adopted methods

- Integrated Approach on Testing and Assessment (IATA) for eye hazard identification was applied by combining OECD adopted test methods into a DA.
- None of the combinations resulted in acceptable performance to distinguish between the 3 UN GHS categories for eye hazard identification.
- According to the IATA, in addition to the OECD adopted *in vitro* test method, the use of optimised non-OECD adopted *in vitro* test methods might be used to identify UN GHS Cat. 1 test chemicals.

Combination OECD adopted methods and optimization of an OECD adopted method

- Defined Approach for Surfactants (DASF) combination of
  - OECD TG 492: EpiOcular™ HCE (VRM1) and SkinEthic™ HCE EIT (VRM2)
  - Modified Short Time Exposure (STE) test method

OECD TG 491			Modified STE	
Cell viability		UN GHS	Cell viability	UN GHS
at 5%	at 0.05%		at 0.5%	
> 70%	> 70%	No Cat.	> 20%	Not Cat. 1
≤ 70%	> 70%	NPCM		
≤ 70%	≤ 70%	Cat. 1	≤ 20%	Cat. 1

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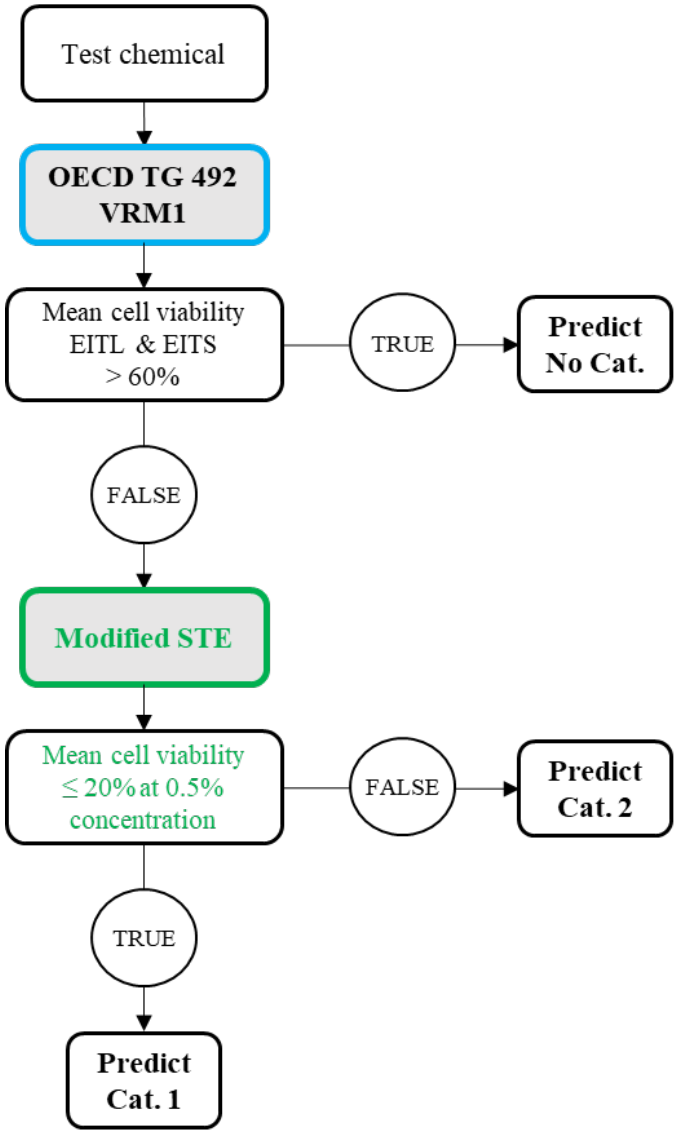
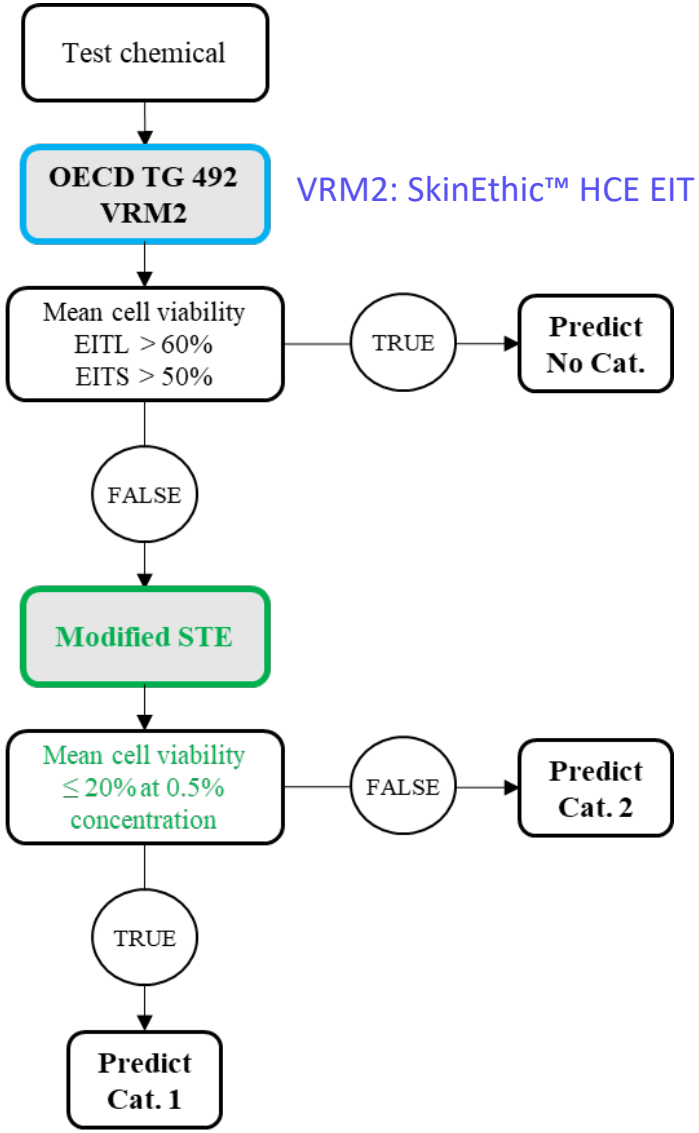
DASF: Bottom-Up Approach

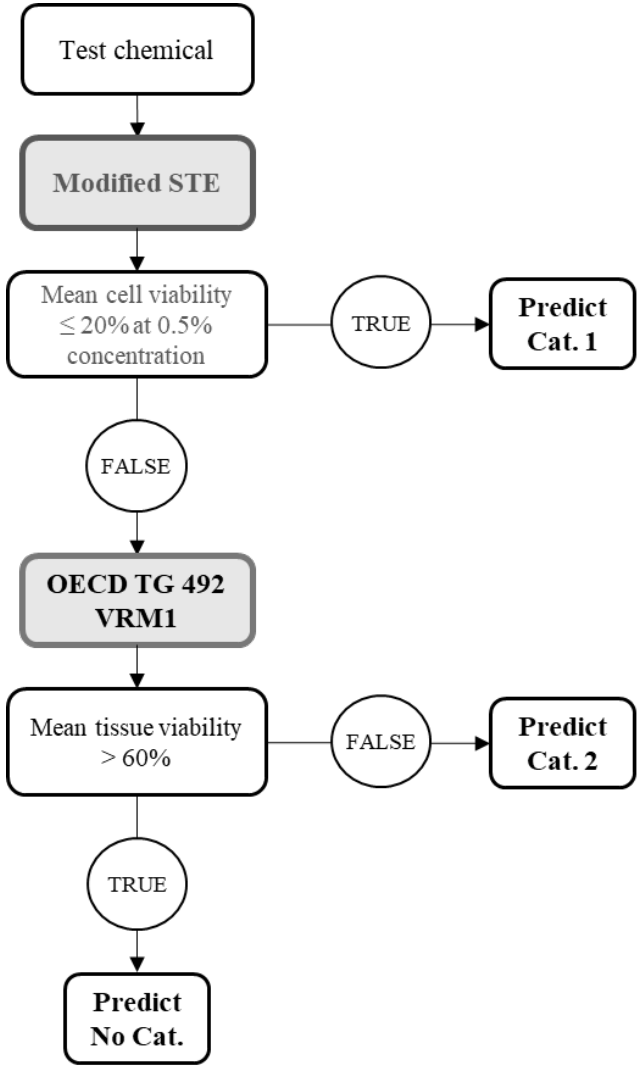
VRM1: EpiOcular™ EIT

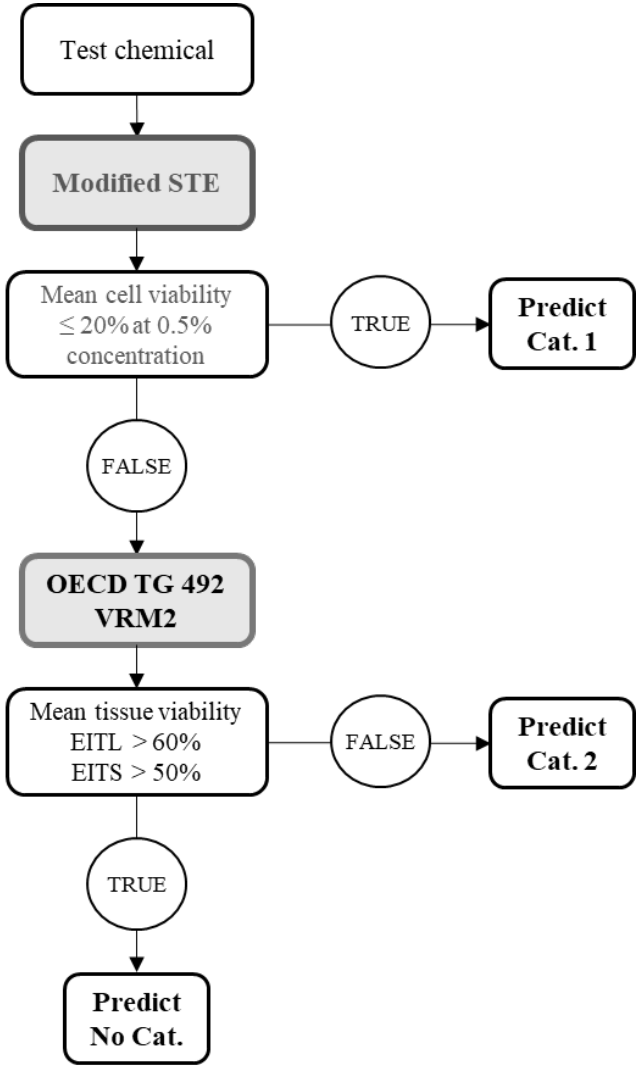


VRM2: SkinEthic™ HCE EIT



DASF: Top-Down Approach





Using mechanistic information and human relevance

- Consider strengths and limitations of all available methods with respect to:
  - their relevance to human ocular anatomy
  - the mechanisms of eye irritation/corrosion in human

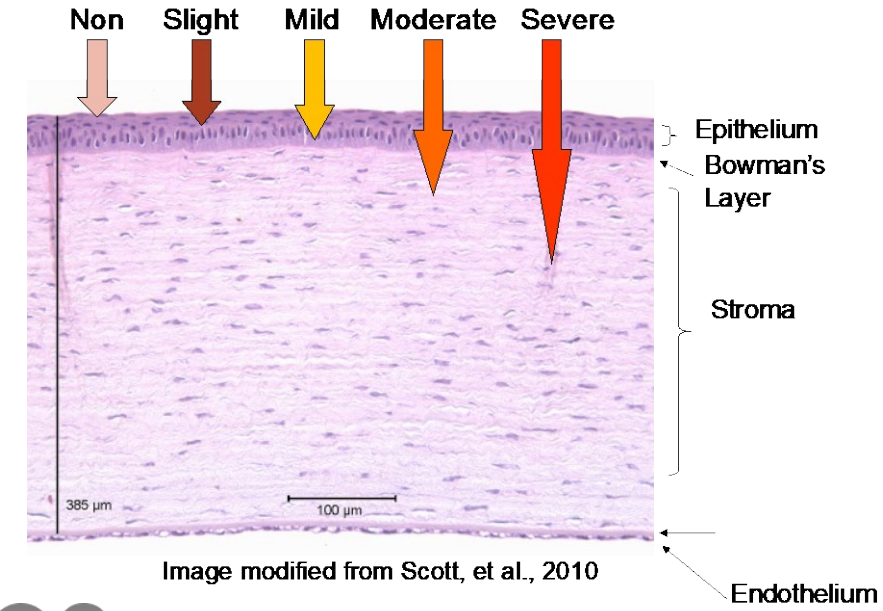


Image modified from Scott, et al., 2010

CUTANEOUS AND OCULAR TOXICOLOGY
https://doi.org/10.1080/15569527.2021.1910291

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REVIEW ARTICLE

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Human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations

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ABSTRACT

There are multiple *in vitro* and *ex vivo* eye irritation and corrosion test methods that are available as internationally harmonized test guidelines for regulatory use. Despite their demonstrated usefulness to a broad range of substances through inter-laboratory validation studies, they have not been widely adopted for testing agrochemical formulations due to a lack of concordance with parallel results from the traditional regulatory test method for this endpoint, the rabbit eye test. The inherent variability of the rabbit test, differences in the anatomy of the rabbit and human eyes, and differences in modelling exposures in rabbit eyes relative to human eyes contribute to this lack of concordance. Ultimately, the regulatory purpose for these tests is protection of human health, and, thus, there is a need for a testing approach based on human biology. This paper reviews the available *in vivo*, *in vitro* and *ex vivo* test methods with respect to their relevance to human ocular anatomy, anticipated exposure scenarios, and the mechanisms of eye irritation/corrosion in humans. Each of the *in vitro* and *ex vivo* methods described is generally appropriate for identifying non-irritants. To discriminate among eye irritants, the human three-dimensional epithelial and full thickness corneal models provide the most detailed information about the severity of irritation. Consideration of the mechanisms of eye irritation, and the strengths and limitations of the *in vivo*, *in vitro* and *ex vivo* test methods, show that the *in vitro/ex vivo* methods are as or more reflective of human biology and less variable than the currently used rabbit approach. Suggestions are made for further optimizing the most promising methods to distinguish between severe (corrosive), moderate, mild and non-irritants and provide information about the reversibility of effects. Also considered is the utility of including additional information (e.g. physical chemical properties), consistent with the Organization for Economic Cooperation and Development's guidance document on an integrated approach to testing and assessment of potential eye irritation. Combining structural and functional information about a test substance with test results from human-relevant methods will ensure the best protection of humans following accidental eye exposure to agrochemicals.

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KEYWORDS
Eye irritation; eye corrosion; EPA; agrochemicals; human-relevant; *in vitro*; BCOP; EpiOcular; ICE; neutral red release

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Evaluating Performance Relative to Consensus

Formulation Information		GHS Predictions					
Code	Type	DA: BCOP/histo	DA: EO + BCOP/histo	DA: TTL + BCOP/histo	DA: EyeIRR-IS + BCOP/histo	Historical In Vivo	Consensus
A	EC/ME	NC	NC	NC	NC	NC	**NC**
B	SC	NC	NC	NC	NC	NC	**NC**
C	SC	NC	NC	NC	NC	NC	**NC**
D	EC	1	1	1	1	1	**1**
E	EC	2B	2B	2B	1	1	**1**
F	SL	1	1	1	1	1	**1**
G	EC	1	1	1	1	1	**1**
H	SL	1	1	1	1	1	**1**
I	SL	1	1	1	1	1	**1**
J	EC	1	1	1	1	1	**1**
K	SL	NC	2B	2B	2B	2A	**2A**
L	EC	NC	2B	2B	NC	NC	**NC**
M	SL	NC	NC	NC	NC	NC	**NC**
N	SC	NC	NC	NC	NC	NC	**NC**
O	SL	NC	2B	2B	NC	NC	**NC**
P	SC	NC	NC	NC	NC	NC	**NC**
Q	SL	**2A***	2A	2A	2A	NC	**2A**
R	SL	2A	2A	1	1	2A	**1**
S	SL	**2B***	2B	2B	2B	2B	**2B**
T	SC	**2B***	NC	2B	NC	NC	**NC**
U	EC	2A	2A	2A	1	2A	**2A**
V	SL	**1†**	**1†**	**1†**	**1†**	2B	**1**
W	SL	2B	2B	2B	2B	NC	**2B**
X	EC	2A	2A	2A	1	2A	**2A**
Y	EC	**2B***	2B	2B	2B	2A	**2B**
Z	EC	2B	NC	NC	NC	NC	**NC**
AA	EC	NC	2B	2B	2B	2A	**2A**
AB	EC	2A	2A	-	-	2B	**Inconclusive**
AC	EC	2B	2B	2B	NC	NC	**Inconclusive**

Effects	GHS Classification	PPE
Corrosive	Category 1	Eye protection
Moderate irritant	Category 2A	Eye protection
Mild irritant	Category 2B	Eye protection
Non-corrosive/ minimal irritant	Not Classified	None noted

- Concordant with consensus
- Underpredicted relative to consensus, but same PPE labeling
- Overpredicted relative to consensus, but same PPE labeling
- Overpredicted relative to consensus; PPE (overprotective)
- Underpredicted relative to consensus; no PPE (underprotective)

***IVIS < 3 but histopathology analysis led to a more severe classification**
†Optional histopathology analysis would lead to a less severe classification (i.e., GHS Cat. 2A)

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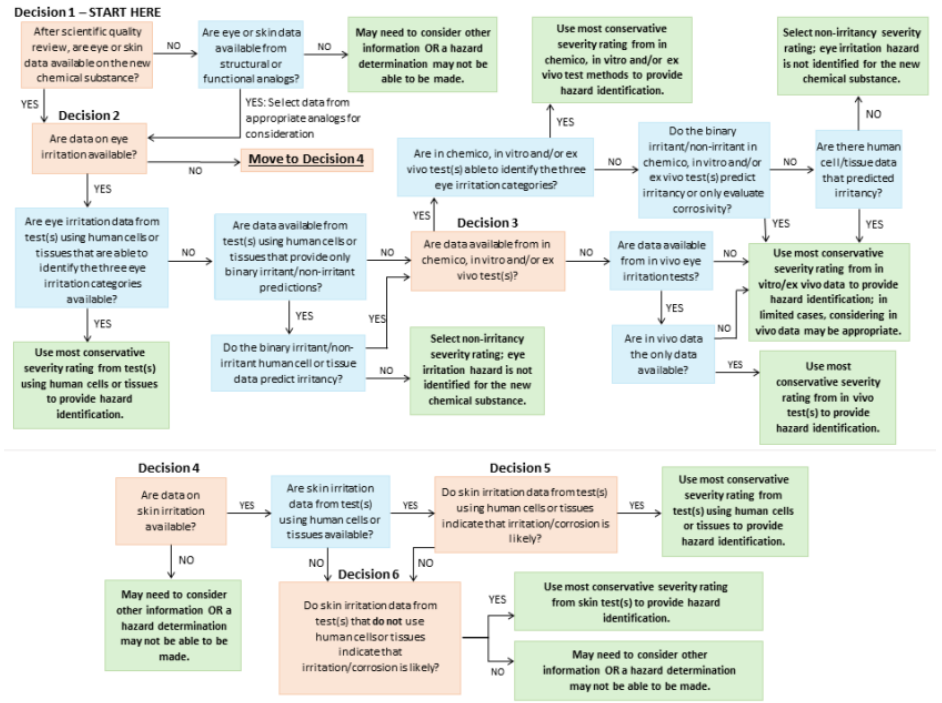
EPA OPP Strategic Vision for Adopting NAMs

To expand the applicability of NAMs to assess eye irritation hazard across the program, OPP collaborated with NICEATM and PETA Science Consortium to evaluate the performance of two proposed DAs. A scientific confidence framework was applied to assess the utility of the DAs to classify agrochemical formulations into EPA hazard categories for eye irritation. The DAs and the results of the retrospective testing from 29 agrochemical formulations are published in: Cutaneous and Ocular Toxicology, (2023)

https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-new-approach-2

- EPA OPPT New Chemicals Program Decision Framework for Hazard Identification of Eye Irritation and Corrosion



Scientific and Non-scientific Challenges to Acceptance

- Animal methods typically used as the reference data for evaluating alternatives
  - Results are variable
  - Need to characterize uncertainty and consider consensus approaches
- Data requirements vary across U.S. and global regulatory authorities and are often ambiguous
- Overcoming regulatory and institutional inertia
  - Education and training, communication with method/model developers

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