Establishing scientific confidence in new approach methodologies: eye irritation testing and beyond

SOT RASS-IVAM Joint Webinar

May 11, 2022

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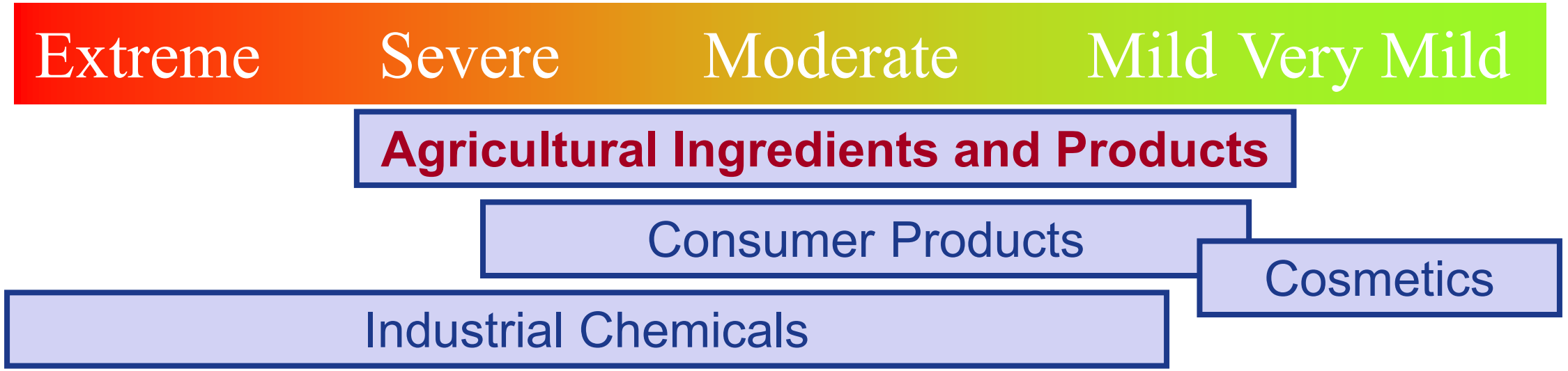
Outline

- Methods available to assess eye effects
  - Human biological relevance
  - Reproducibility
- Testing agrochemical formulations in *in vitro* and *ex vivo* eye tests
- Framework for establishing scientific confidence in NAMs

How have we traditionally conducted testing?

EPA I	EPA II	EPA III	EPA IV
Rabbit Draize Test			
GHS 1	GHS 2	Non-classified	



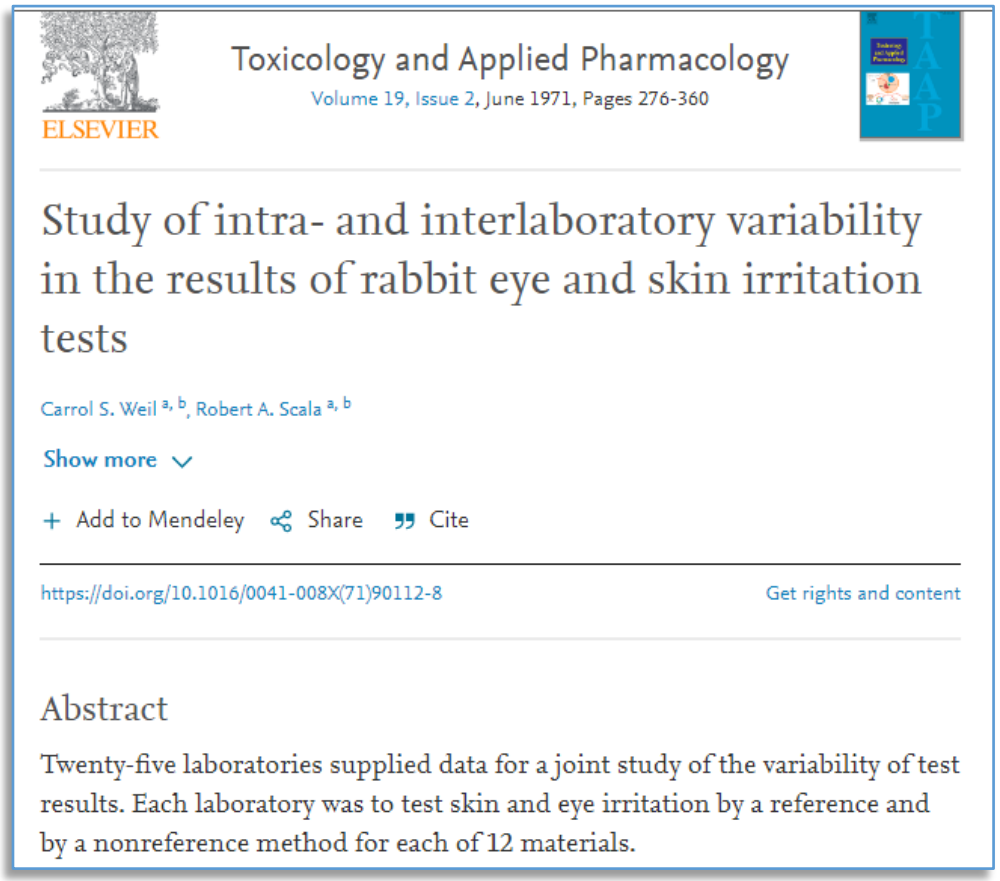
Draize Rabbit Eye Test Method

- Primary *in vivo* method (developed in 1944)
- Accepted by CPSC; EPA; OECD
- Test substance placed in lower conjunctival sac
- Cornea, Iris, Conjunctiva evaluated
- Animal observed over 21 days after exposure
- Conservative/hazard assessment – given differences between human and rabbit eyes

Draize Rabbit Eye Test Method

- Apical Endpoints
  - endpoints are observed outcomes in eyes and tissues after exposure
  - what have we learned of the Modes of Action?
- Subjectivity
  - observations are subjective, prone to inter-operator variability
- Variability
  - between replicate animals in the same test
  - within a laboratory
  - between laboratories
- Hazard and Risk Assessment
  - are the predictions relevant to human responses?

Intra- and inter-lab variability in the Draize eye irritation test



ELSEVIER

Toxicology and Applied Pharmacology
Volume 19, Issue 2, June 1971, Pages 276-360

Study of intra- and interlaboratory variability in the results of rabbit eye and skin irritation tests

Carrol S. Weil [a, b], Robert A. Scala [a, b]

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https://doi.org/10.1016/0041-008X(71)90112-8 Get rights and content

Abstract

Twenty-five laboratories supplied data for a joint study of the variability of test results. Each laboratory was to test skin and eye irritation by a reference and by a nonreference method for each of 12 materials.

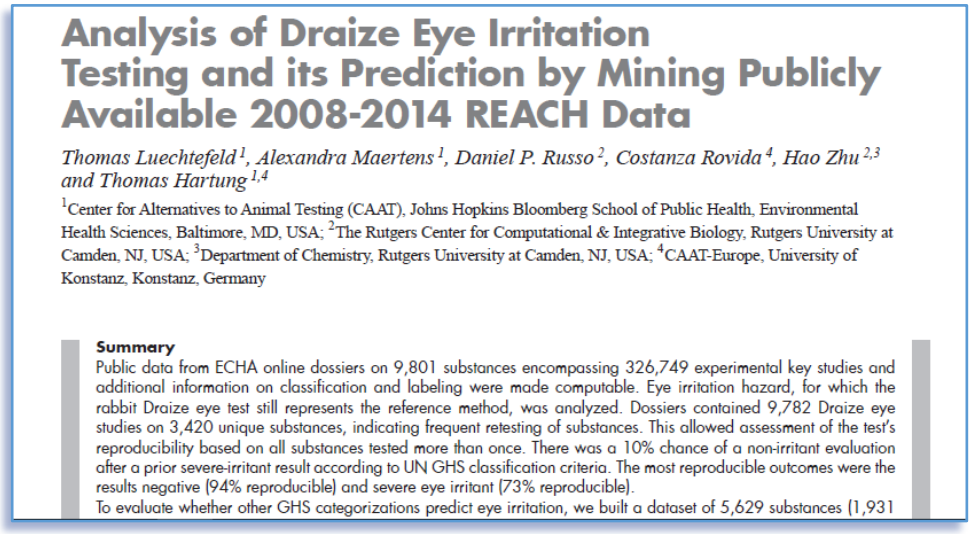
Controlled evaluation in 24 labs

- 12 chemicals tested in all labs
- standardized Draize protocol followed
- Significant variability across labs, spanning spectrum of categories
- Within lab variability in 6-animal data
- Inconsistent rank ordering of irritation
- Variability in recovery times

Some labs consistently scored unusually severe scores, while other labs consistently reported non-irritating scores

Suggests operator scoring subjectivity; variations in dose / exposure control

Reproducibility of the Draize Eye Test



Analysis of Draize Eye Irritation Testing and its Prediction by Mining Publicly Available 2008-2014 REACH Data

Thomas Luechtefeld [1], Alexandra Maertens [1], Daniel P. Russo [2], Costanza Rovida [4], Hao Zhu [2,3] and Thomas Hartung [1,4]

[1]Center for Alternatives to Animal Testing (CAAT), Johns Hopkins Bloomberg School of Public Health, Environmental Health Sciences, Baltimore, MD, USA; [2]The Rutgers Center for Computational & Integrative Biology, Rutgers University at Camden, NJ, USA; [3]Department of Chemistry, Rutgers University at Camden, NJ, USA; [4]CAAT-Europe, University of Konstanz, Konstanz, Germany

Summary

Public data from ECHA online dossiers on 9,801 substances encompassing 326,749 experimental key studies and additional information on classification and labeling were made computable. Eye irritation hazard, for which the rabbit Draize eye test still represents the reference method, was analyzed. Dossiers contained 9,782 Draize eye studies on 3,420 unique substances, indicating frequent retesting of substances. This allowed assessment of the test's reproducibility based on all substances tested more than once. There was a 10% chance of a non-irritant evaluation after a prior severe-irritant result according to UN GHS classification criteria. The most reproducible outcomes were the results negative (94% reproducible) and severe eye irritant (73% reproducible).

To evaluate whether other GHS categorizations predict eye irritation, we built a dataset of 5,629 substances (1,931

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 (UN GHS categories)
- 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

Reproducibility of the Draize Eye Test

Prior type	1	2A	2B	NC	Total
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NC	1.1%	3.5%	1.5%	93.9%	400

Most reproducible results were at the extremes

- 94% likelihood to confirm a NC prediction
- 73% likelihood to confirm a severe (GHS 1) prediction
- 10.4% of Category 1 materials predicted as NC in a subsequent test

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

Reproducibility of the Draize Eye Test

Science Education & Outreach

Prior type	1	2A	2B	NC	Total
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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
- Minimal discrimination between Category 2B and NC

  (77 of 86 substances with at least one GHS 2B result also have at least one NC prediction)
- NICEATM is now curating available rabbit eye test data to repeat this analysis (for GHS categories) and to also evaluate EPA categories

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

Sources of Test Method Variability

Science Education & Outreach

Parameters	Draize Eye Test	Non animal methods
Dosing	Dose volume may overfill cul-de-sac Spill-out commonly reported	Precise control of dose applied (±2%) No loss of dose during exposure
Exposure time	Actual exposure times variable due to spill and animal blinking/pawing	Precise control of exposure period, and dose rinse-out timing
Test system	Animal behaviors (pawing, blinking, rubbing) may affect dosing and endpoint expression; Variability among replicates	Test system conditions tightly controlled between replicates Consistency among replicates
Endpoints	Subjective apical observations	Objective machine-read data

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 humans

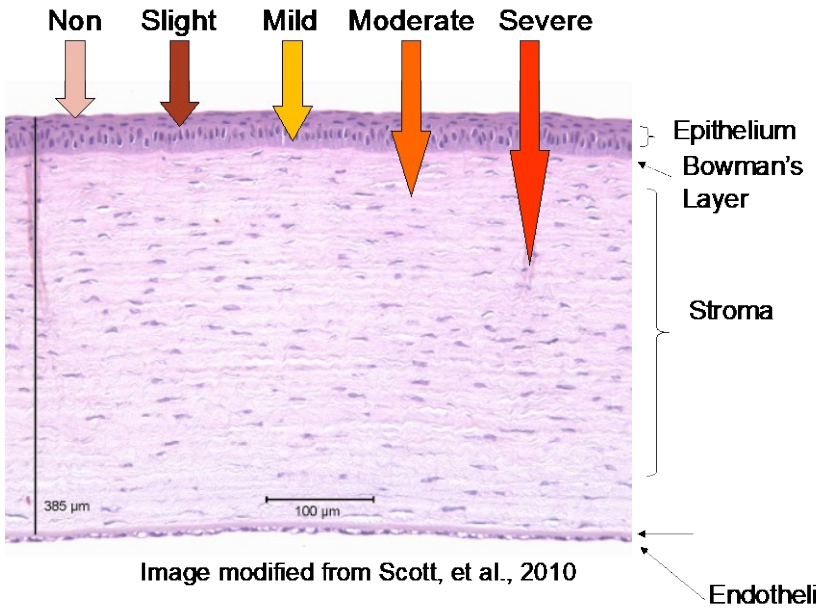

Image modified from Scott, et al., 2010

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

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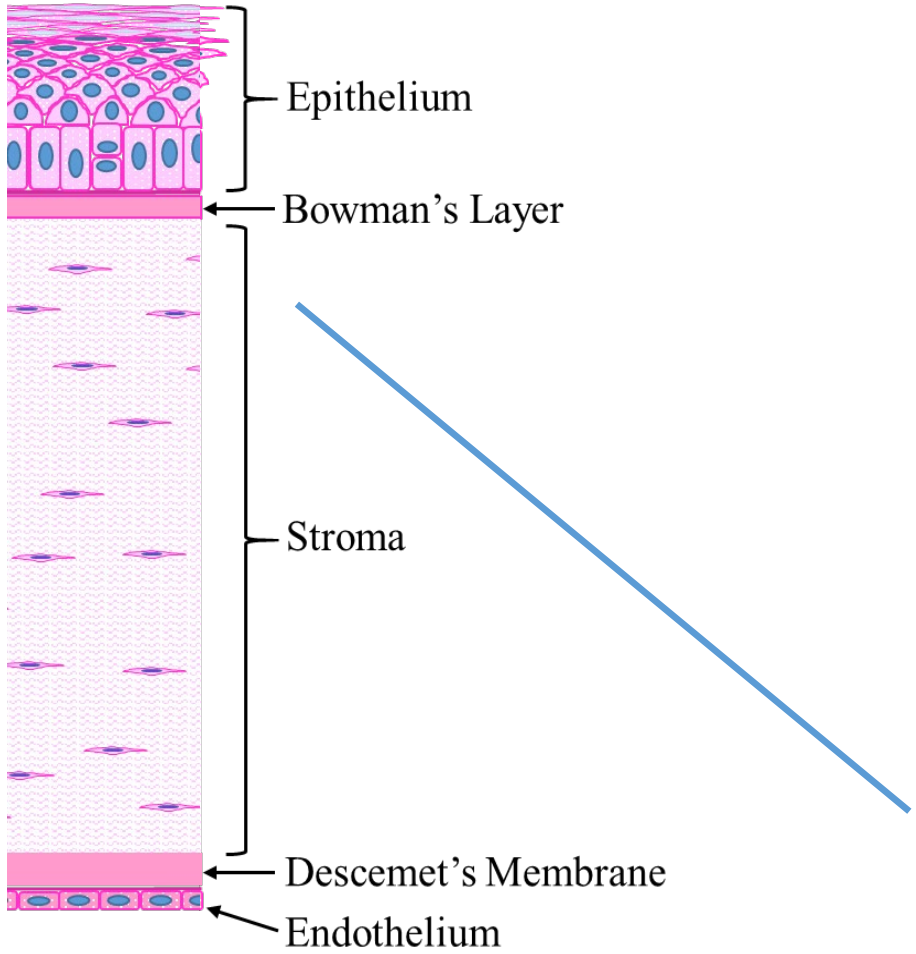
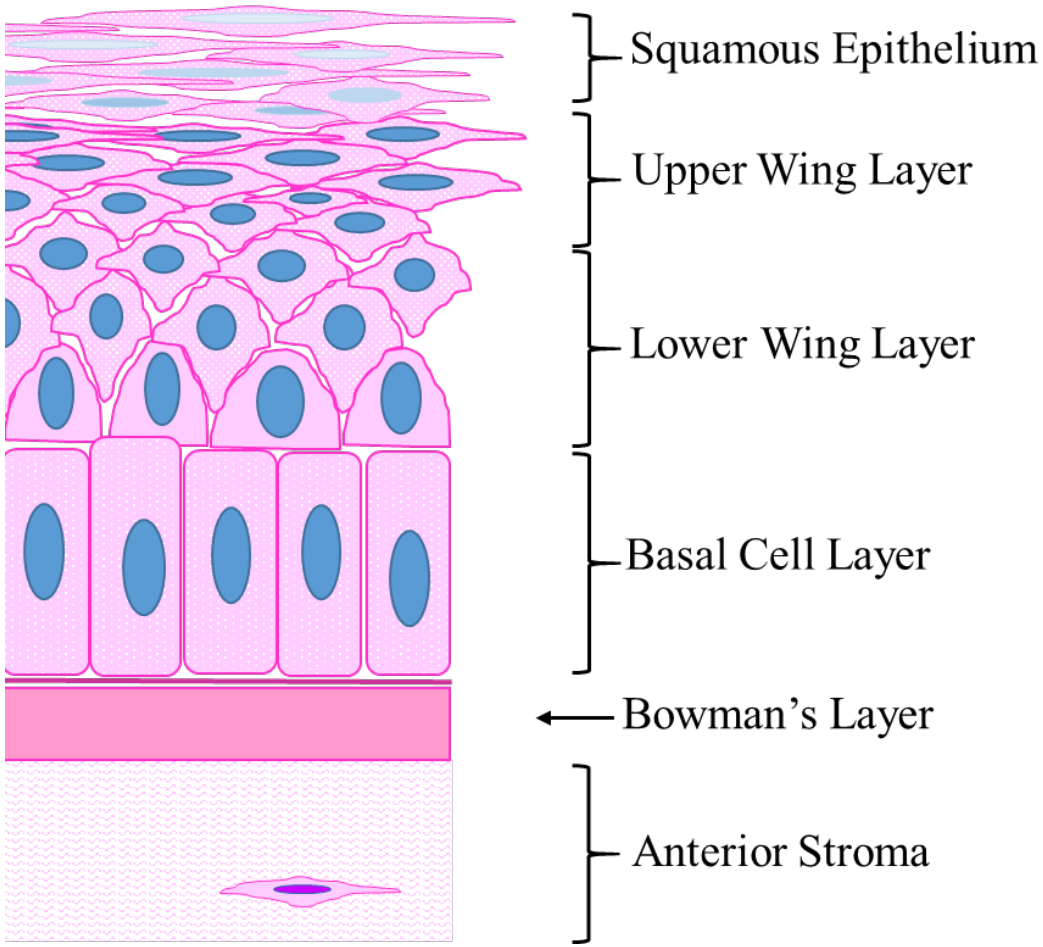
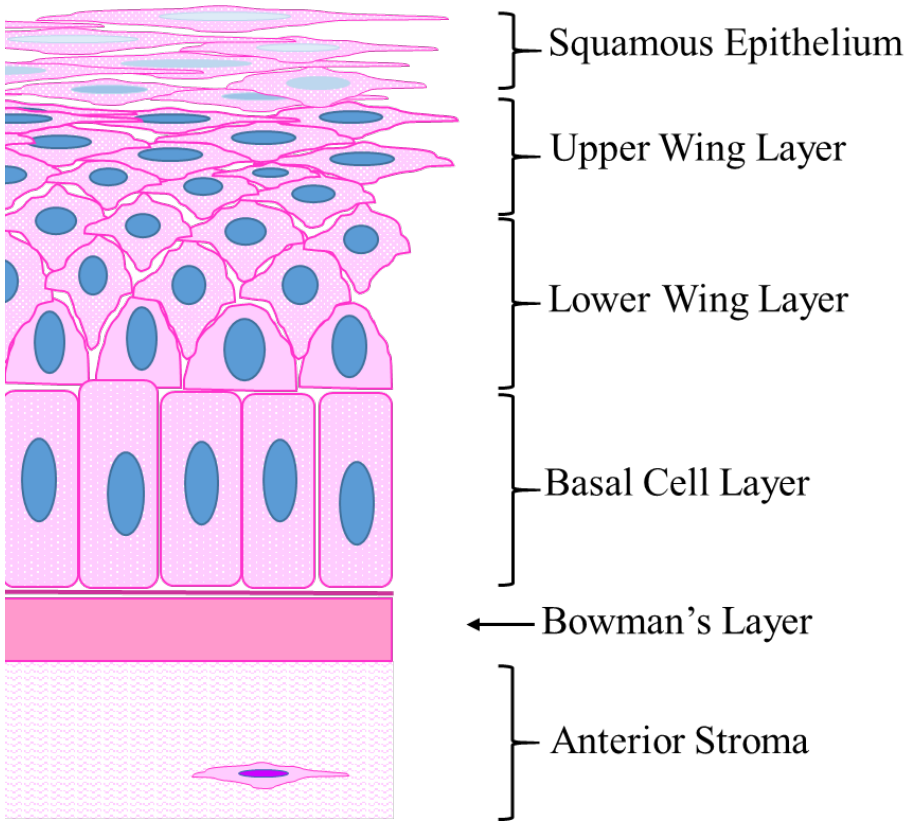
Corneal Physiology and Tissue Functions





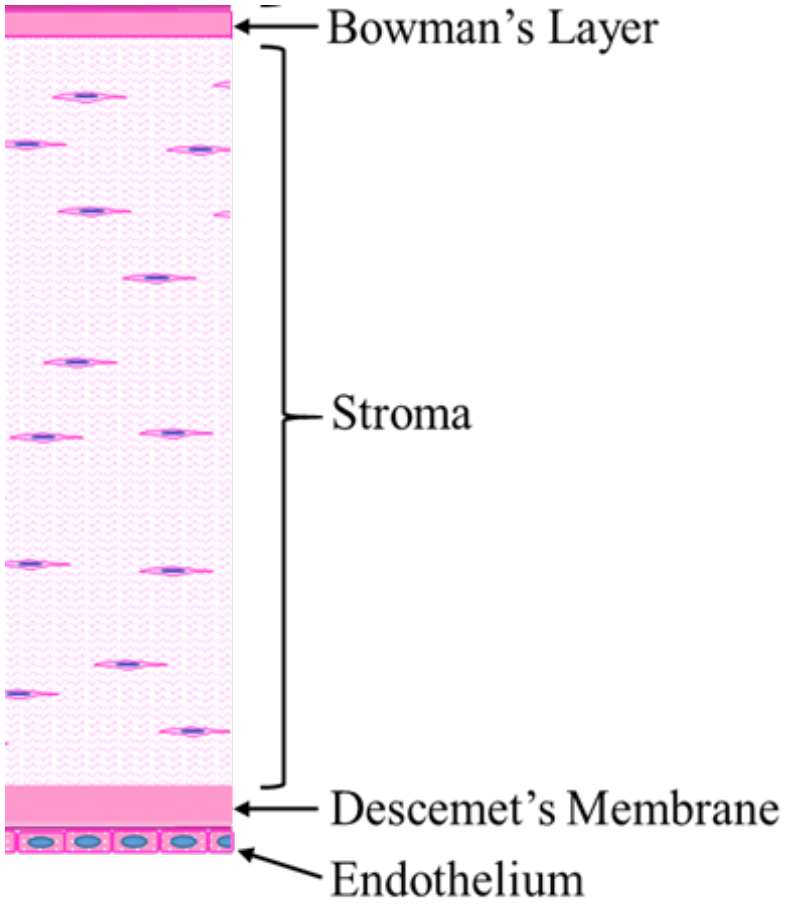
Corneal Physiology and Tissue Functions

Epithelium



- Protection from xenobiotic and foreign material insults
- Provides an optical interface
- Maintains ideal stromal hydration state
- Bowman's Layer and basal membrane provide structure and matrix for basal cell layer
- Basal cells – proliferative cells maintain basal layer matrix; are source for upward epithelial development and stratification; corneal wound healing through sheet migration and rapid proliferation
- Wing cells – intermediate cells expressing precursors of tight junctions; provide significant structural support
- Squamous cells – protective barrier / zona occludens

Corneal Physiology and Tissue Functions



Stroma and Endothelium

- Stroma: makes up 80% of the corneal cross-section
- Optical clarity and light transmission functions
- Keratocytes – sparse but networked cells involved in maintenance of organized collagen fiber bundles
- Disorganized collagen fibers result in opacities
- Disruption of keratocytes induces inflammatory response to stimulate keratocyte proliferation, migration and reestablishment of collagen fibers
- Descemet's Membrane provides structure and anchoring matrix for endothelial cell layer
- Endothelium –non-proliferative single cell layer maintains ideal stromal hydration

Depth of Corneal Injury Concept

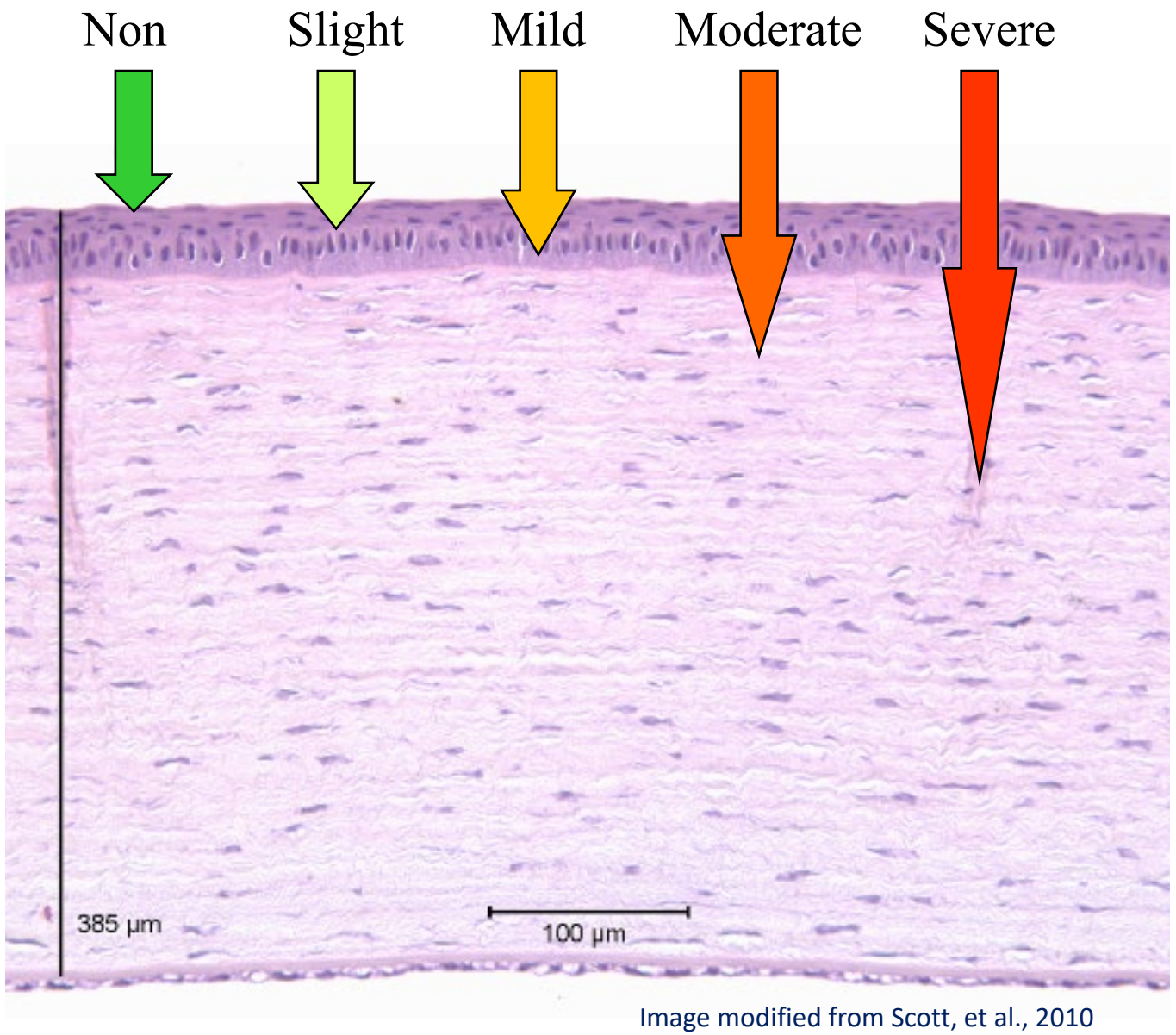


Image modified from Scott, et al., 2010

Depth of injury is predictive of the degree and duration of injury

"Regardless of the process leading to tissue damage, extent of initial injury is the principal, mechanistic factor determining the outcome of the ocular irritation"

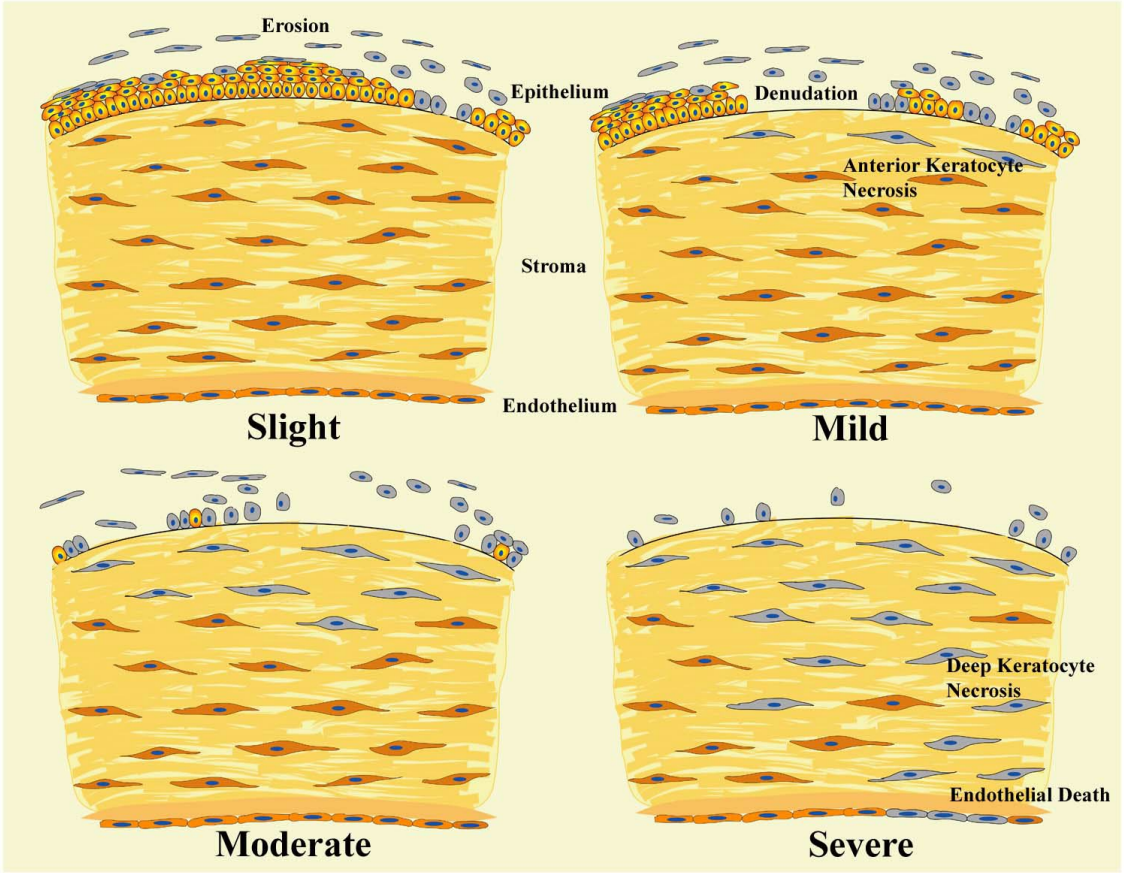
Maurer et al, 2002

In Vivo Studies - LVET

Slight Irritants (Clear by Day 1)
Injury limited to the Corneal Epithelium.

Mild Irritants (Clear by Day 7)
Injury Extends Through the Epithelium
and into the Anterior Stroma.

Severe Irritants (Never Clear)
Injury Extends Through the Epithelium and into the Deep Stroma.

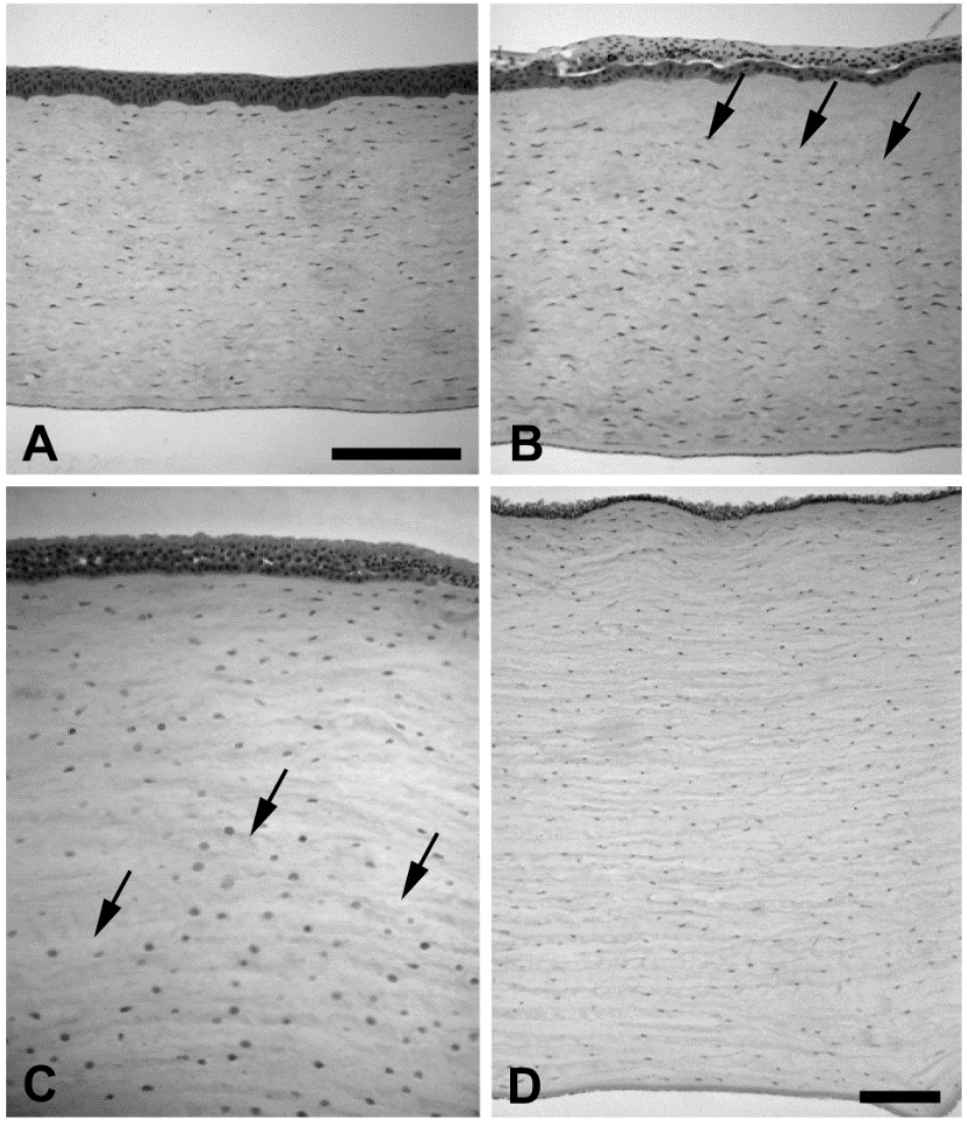


Mechanistic Basis of Ocular Irritation is Defined by the Extent of Corneal Injury.

From presentation by James V. Jester, The Eye Institute, ***University of California at Irvine***

Histologic Changes

- Ex vivo culture maintains normal corneal appearance (A).
- Mild Irritants show epithelial erosion and loss of anterior keratocytes (arrows, B & C).
- Severe irritants produce marked corneal swelling and loss of deep corneal keratocytes (D).



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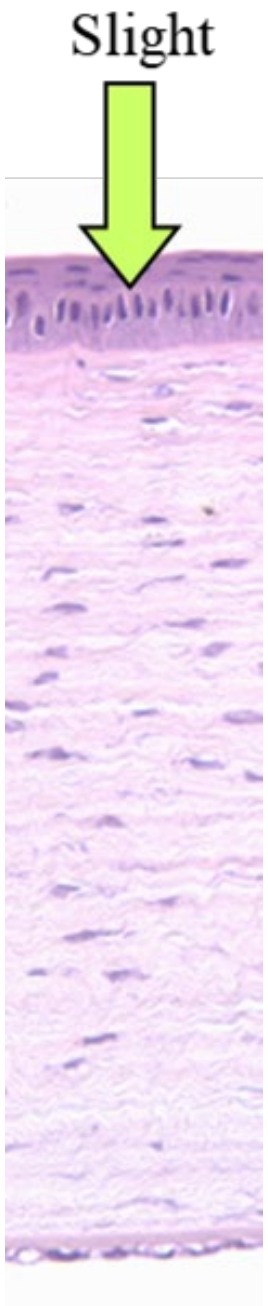


Damage Limited to the Superficial Conjunctival or Corneal Epithelium

CELLULAR RESPONSE

Upon exposure to the squamous epithelium, chemicals may induce

- cell stress responses
- release of chemokines and cytokines
- changes in relevant biomarkers
- breakdown of the tight junctions
- loss of cell to cell adhesion molecules
- changes in cell metabolism/respiration
- necrotic or apoptotic damage

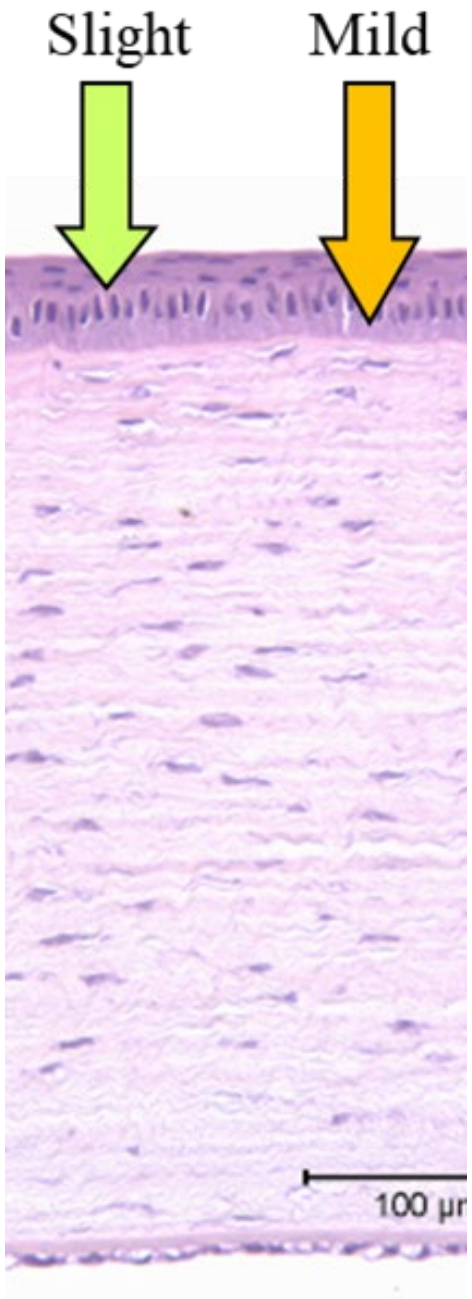
- **epithelial cell death**

ORGAN RESPONSE

- increased corneal or conjunctival permeability/loss of barrier function
- susceptibility to xenobiotics
- conjunctival hyperemia and discharge
- swelling of the conjunctival tissues
- transient and mild corneal swelling

- sloughing of superficial epithelial cells
- induction of wound healing response and basal cell regeneration/turnover
- limited inflammatory response and neutrophil migration

Rapid recovery of the corneal and conjunctival tissues typical



Damage Limited to the Wing Cell Layer of the Epithelium

CELLULAR RESPONSE

Upon penetration into the squamous epithelium and upper wing cells, or the conjunctival layers, chemicals may induce

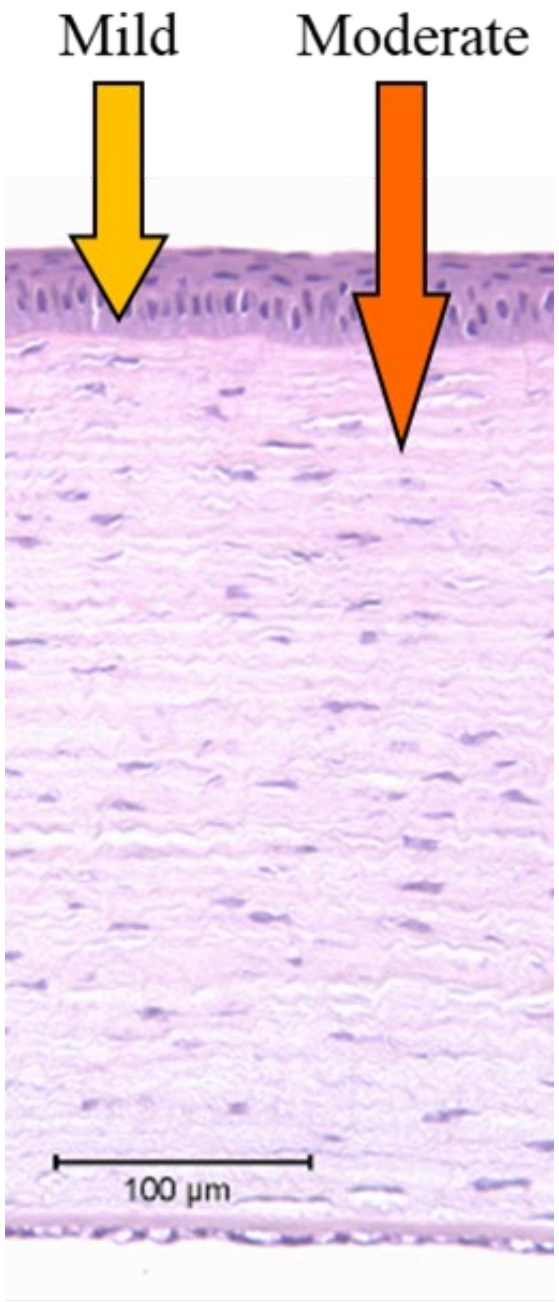
- cell stress responses
- release of chemokines and cytokines
- changes in relevant biomarkers
- breakdown of the tight junctions
- damage to the desmosomes
- loss of cell to cell adhesion molecules
- changes in cell metabolism/respiration
- necrotic or apoptotic damage

- **cell death**

ORGAN RESPONSE

- increased corneal permeability/loss of barrier function
- Increased susceptibility to xenobiotics
- corneal swelling and related opacity
- corneal opacity due to cellular/molecular denaturation/coagulation
- sloughing of mid to lower epithelial tissues
- increased induction of wound healing response and basal cell regeneration/turnover
- increased potential for inflammatory response and neutrophil migration

Recovery of the corneal and conjunctival tissues likely



Damage Into The Lower Wing Cell and Basal Cell Layers

CELLULAR RESPONSE

Upon penetration into the lower wing cells, and/or into the basal cell layers, chemicals may induce

- cell stress responses
- release of chemokines and cytokines
- loss of cell to cell adhesion and cell to basement membrane adhesion
- changes in cell metabolism/respiration
- necrotic or apoptotic damage

- **cell death**

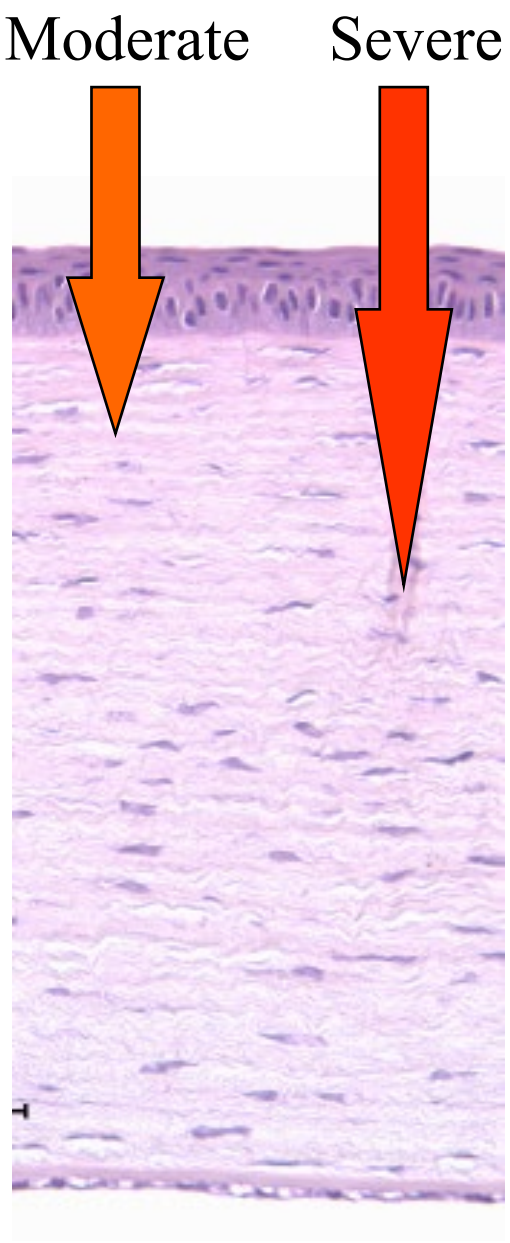
- changes in basement membrane? *

ORGAN RESPONSE

- increased corneal permeability/loss of barrier function
- susceptibility to xenobiotics
- corneal swelling and related opacity
- corneal opacity due to cellular/molecular denaturation/coagulation
- sloughing of lower epithelial tissues
- increased induction of wound healing response and basal cell regeneration/turnover increased
- inflammatory response and neutrophil migration

Recovery of the corneal tissues expected but prolonged.

* Basement membrane integrity is essential



Damage Into the Corneal Stroma

CELLULAR RESPONSE

Upon penetration through the epithelium into the corneal stroma, chemicals may induce

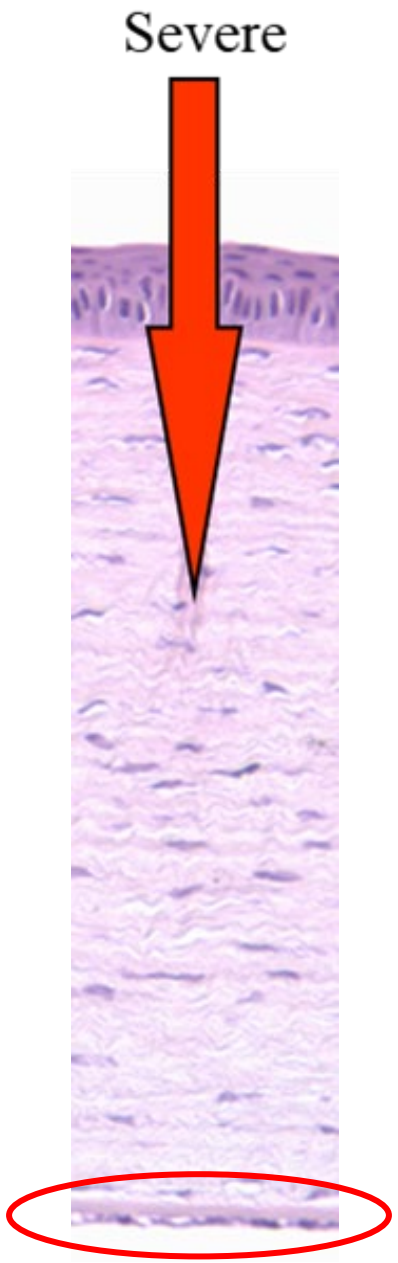
- cell stress responses
- retraction of keratocyte cell to cell network
- release of chemokines and cytokines, primarily IL-1α and TNFα
- induction of extracellular matrix / collagen synthesis
- activation of matrix metalloproteases result in loss of cell to cell adhesion and local tissue restructuring
- changes in cell metabolism/respiration
- necrotic or apoptotic damage

- **Keratocyte cell death**

ORGAN RESPONSE

- susceptibility to xenobiotics
- progressive ulceration and tissue necrosis
- notable stromal swelling and related opacity
- corneal opacity due to cellular/molecular denaturation/coagulation
- induction of wound healing response and basal cell regeneration/turnover
- recruitment of neutrophils / inflammatory response in stroma
- fibrosis resulting in disorganized collagens
- pannus and neovascularization
- loss of endothelium

Recovery becomes less likely with progression of the depth and degree of injuries



Damage involving the Corneal Endothelium

CELLULAR RESPONSE

Upon penetration through the corneal epithelium and stroma, chemicals may induce

- cell stress responses, leading to changes in cell adhesion
- release of chemokines and cytokines
- changes in relevant biomarkers
- activation of matrix metalloproteases result in loss of cell to cell adhesion and cell to Descemet's membrane adhesion
- changes in cell metabolism/respiration
- necrotic or apoptotic damage

- **Endothelial cell death**

ORGAN RESPONSE

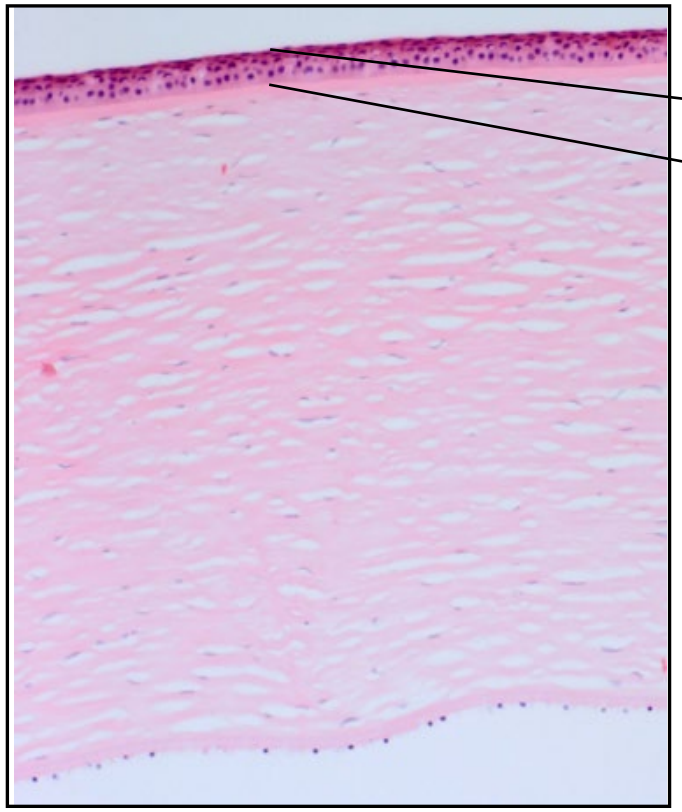
- notable lower corneal swelling and swelling-related corneal opacity
- loss of endothelium
- loss of keratocytes in lower stroma

No meaningful recovery of cornea

Test Method Relevance to Corneal Cross-sections

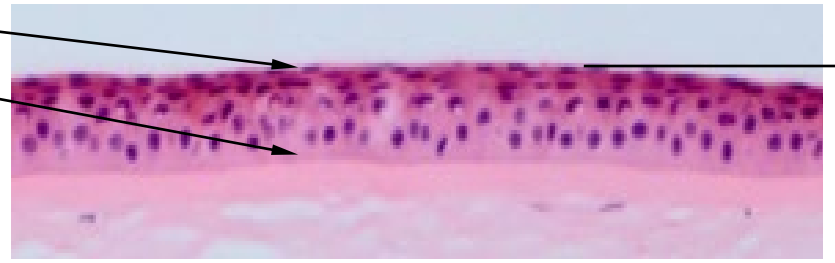
Full thickness Cornea

epithelium, stroma and endothelium



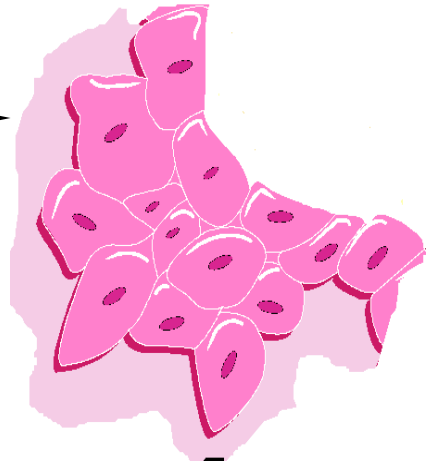
Epithelium

Squamous, wing, and basal cells



Squamous Epithelium

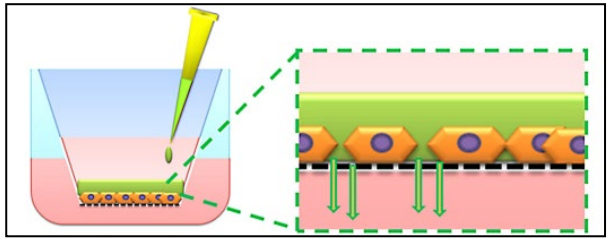
Outermost cells covering epithelium



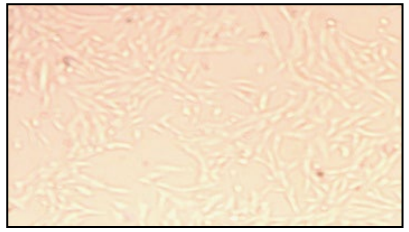
Available non-animal test methods model different portions of the cornea.

Its important to understand the relationship of those test methods to the various corneal layers to appreciate the mechanistic relevance in eye irritation assessments.

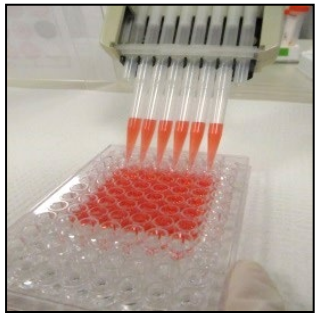
Fluorescein Leakage Assay



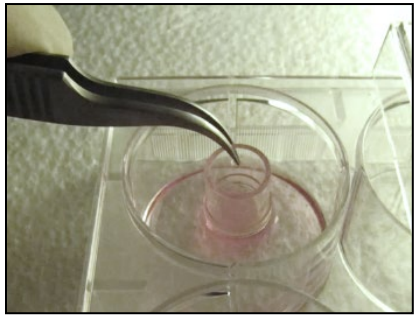
Squamous epithelium



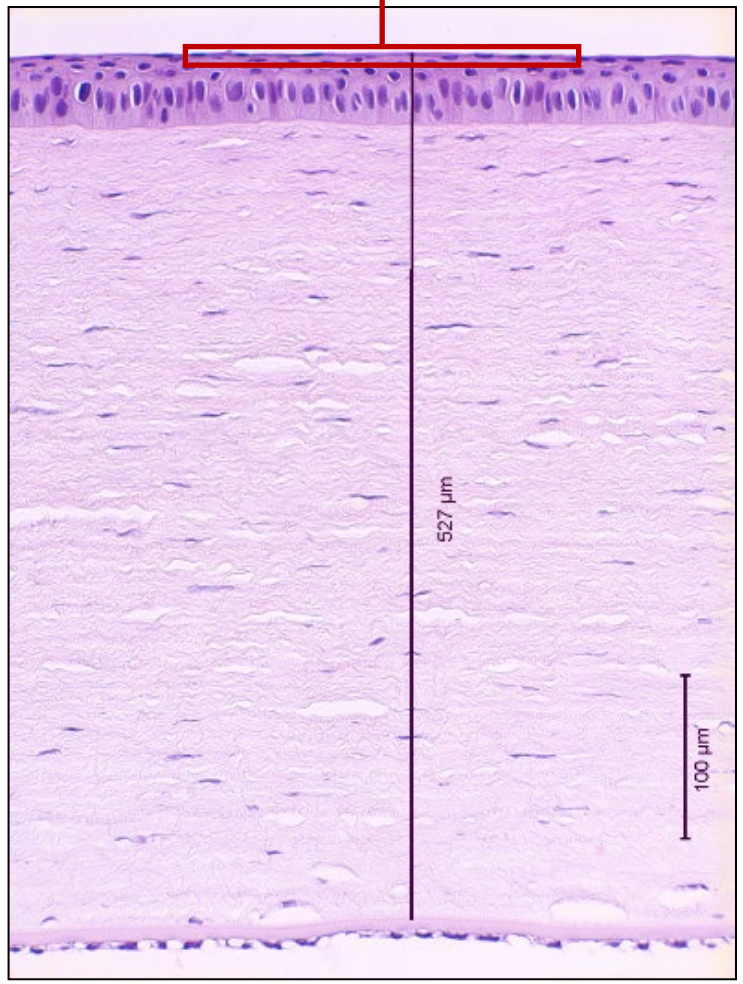
Short Time Exposure Assay

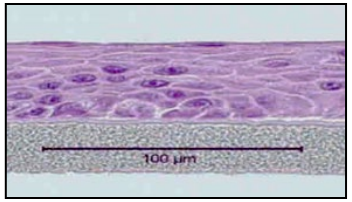


Reconstructed Human Cornea-like Epithelium Test



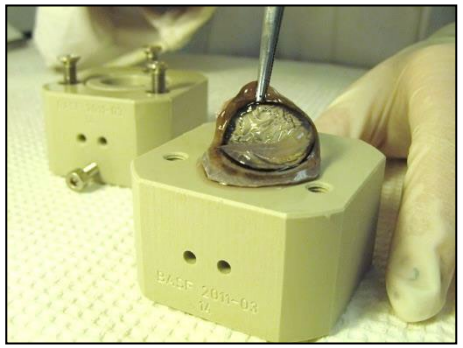
Full thickness corneal models





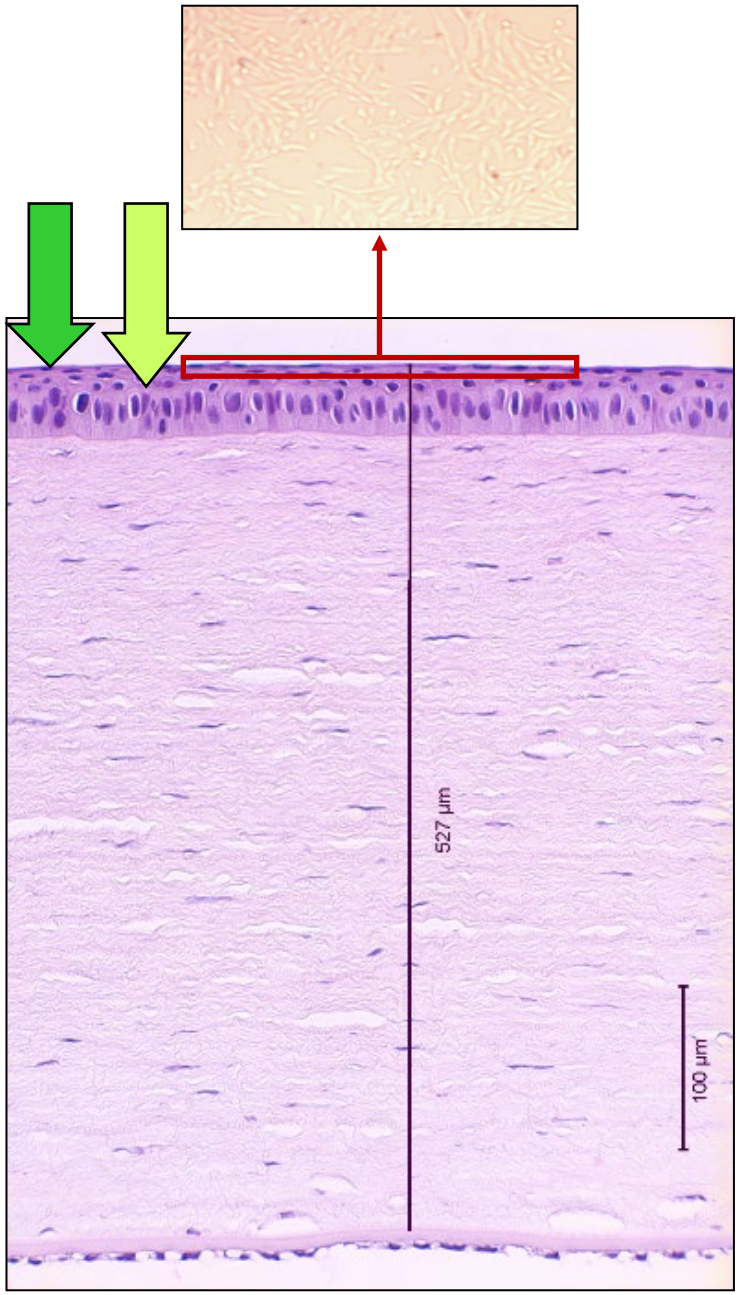
Epithelium models

Bovine Corneal Opacity and Permeability Assay

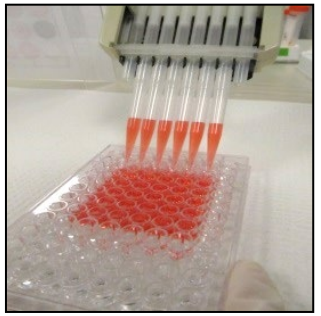


Isolated Chicken Eye Test

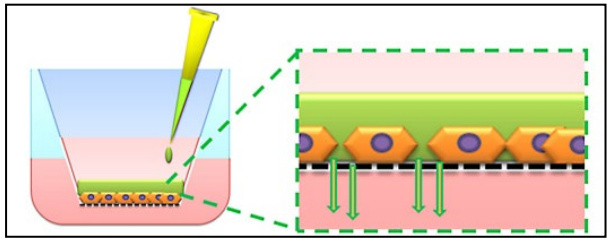
Squamous epithelium models



Short Time Exposure Assay

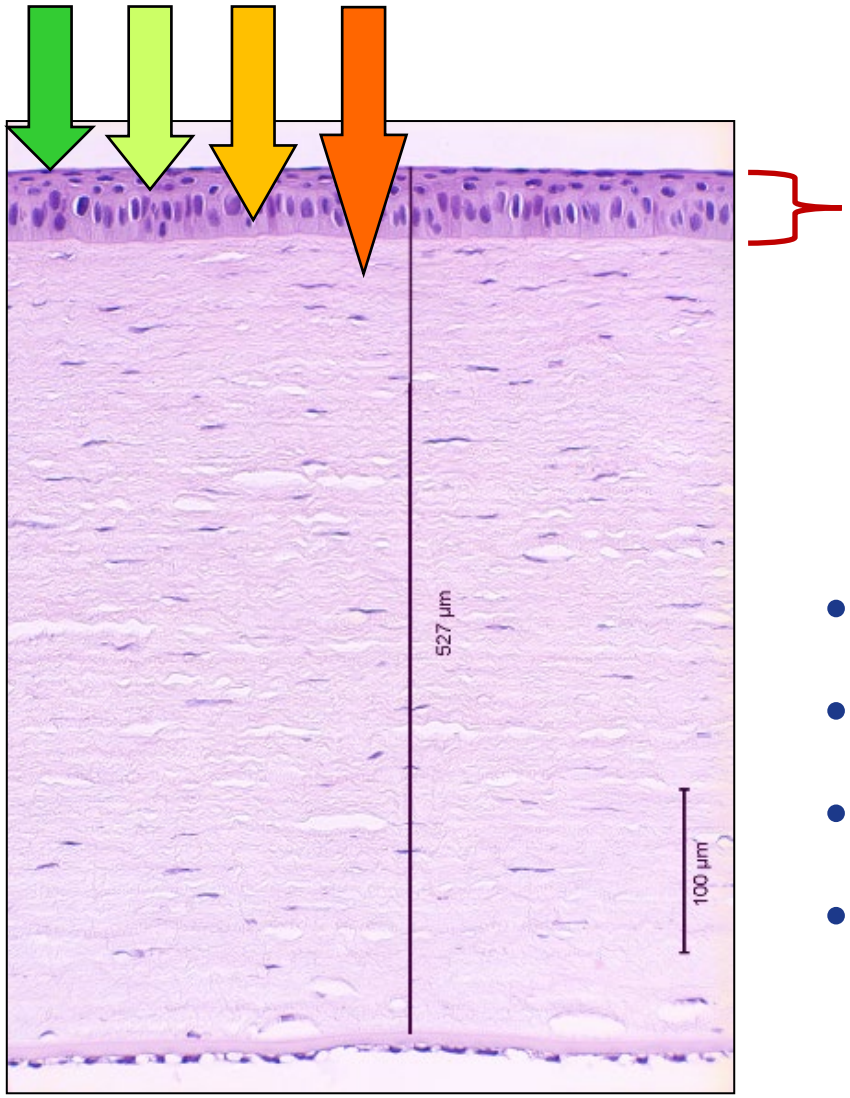


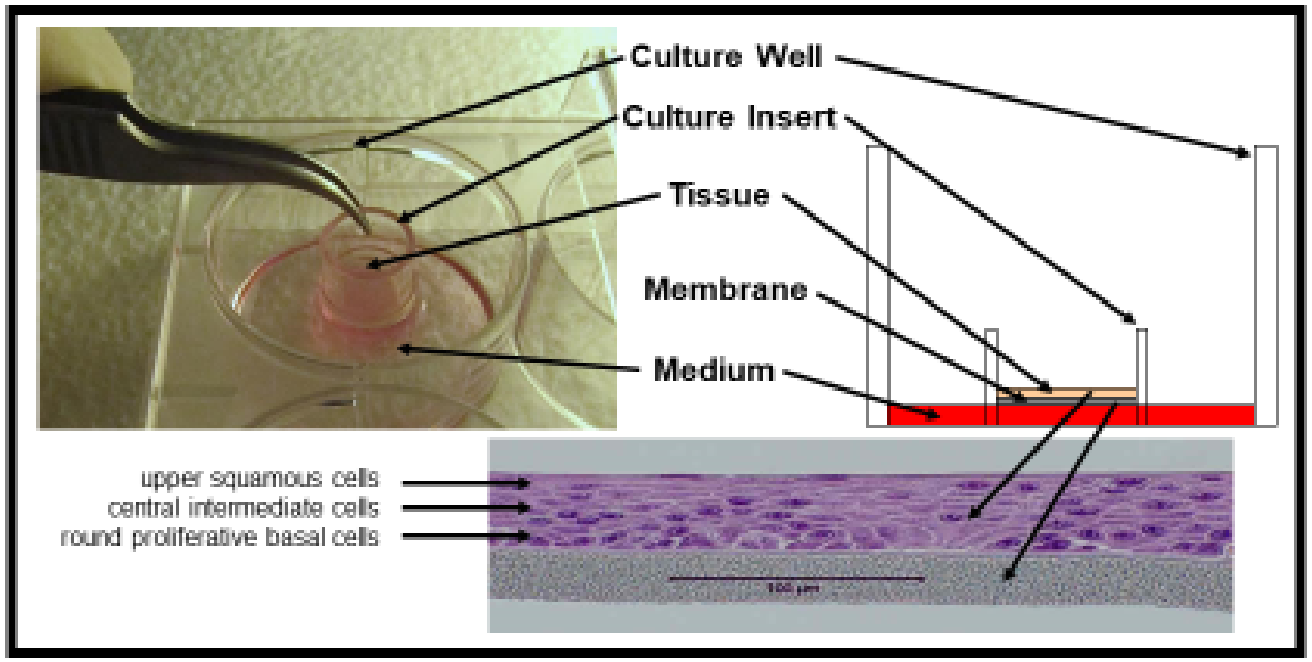
Fluorescein Leakage Assay



- Model the upper-most squamous layer
  - –Relevant to tight junction and barrier disruption
  - –Validated methods do not use human cells
- Cell viability / cell death can be determined
- Concentration-based prediction models correlate to severe and/or non-irritants
- Depth of injury not modeled
  - –Mechanistically limited to discriminating non-irritants from irritants

Reconstructed human corneal epithelium models





- Model the stratified human corneal epithelium
- Cell viability / cell death are determined
- Cytokine release / expression can be measured
- Depth of injury into epithelium modeled
  - –Discriminate among non, mild and moderate irritants

RhCE Test Method Overview

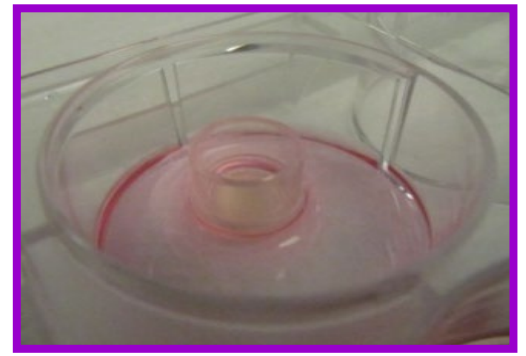
Measuring chemical-induced cell death

Tissue Treatment

Chemicals or formulations are applied without dilution to model real life exposures

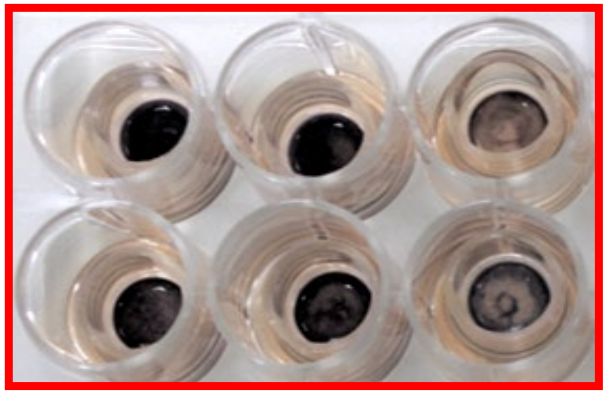
Tissue Rinsing

Post-treatment Expression Incubation

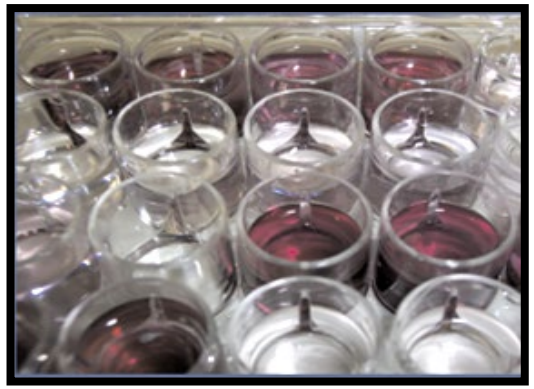


After exposure, tissues are rinsed, immersed in medium for 12 minutes, and then incubated for a post-treatment incubation

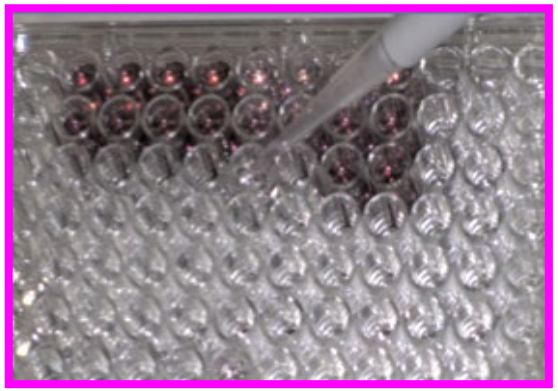
MTT Reduction



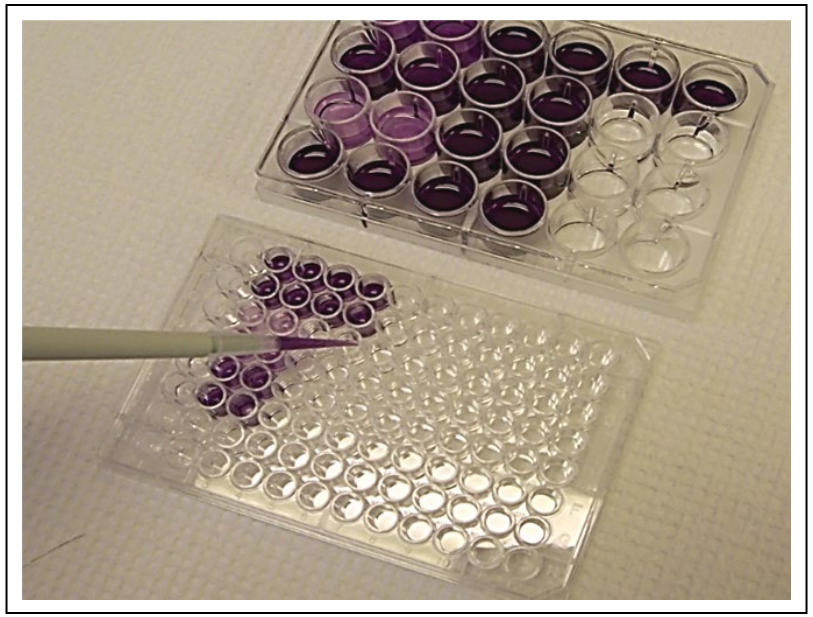
Isopropanol Extraction



Prepare aliquots for spectrophotometry



MTT endpoint for cell cytotoxicity assessment



Extracted MTT is thoroughly mixed and transferred to a 96-well plate.

The 96-well plate/MTT-isopropanol samples are quantified using a microplate reader. Optical Density (OD) at 550 to 570 nm is measured.

OD550 values are used to calculate relative viability values.

Viability is presented relative to negative control tissue values

$$\% \text{ of Control} = \frac{\text{Test Material OD550}}{\text{Negative Control OD550}}$$

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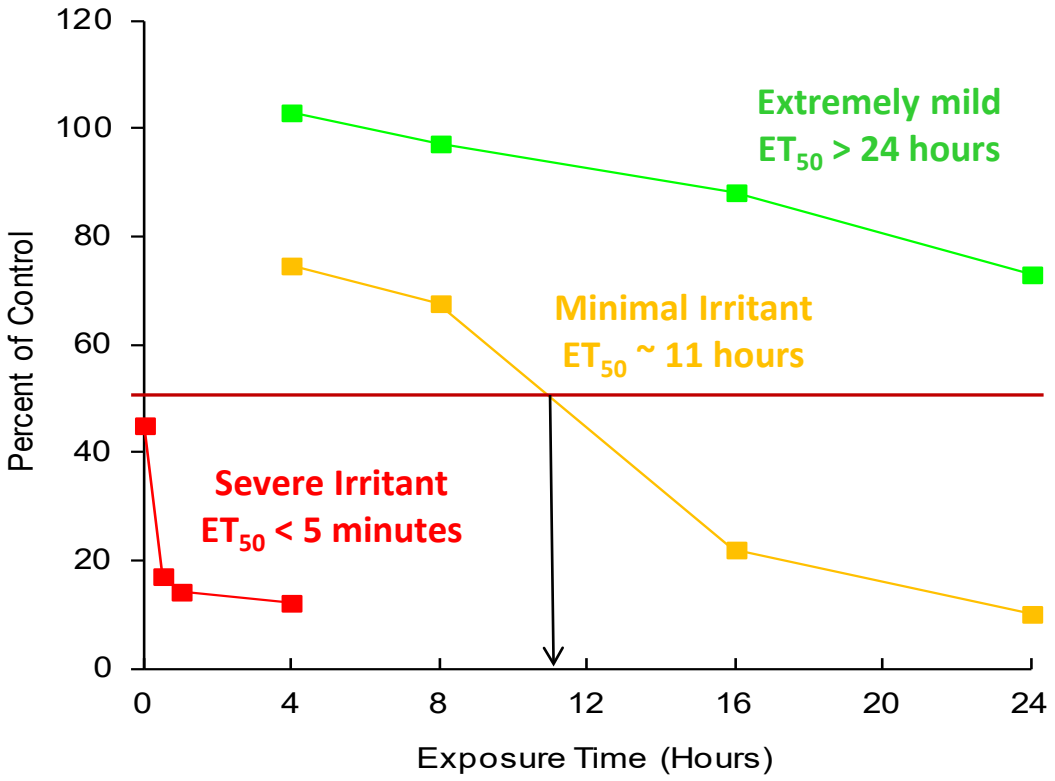
Time-to-toxicity Concept in RhCE Models



ET_{50} (estimated time to reduce viability to 50% of control), plot relative viability over exposure time

US EPA Antimicrobial Cleaning Products (AMCP)

- To discriminate between EPA III and IV or identify EPA Cat I (without further testing)
- Multiple exposure time protocol
- Continuum of responses across eye irritation spectrum
- Also used in product development to create progressively milder/safer formulations
- Rank-order candidate formulations
  - Can include benchmarks for data interpretation

Eye Irritation Test (EIT) Data Evaluation

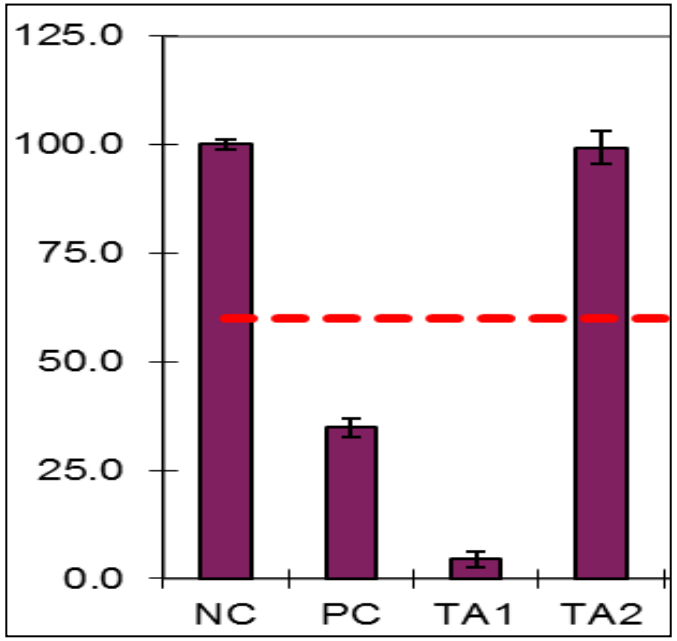
OECD TG 492 for Eye Irritation

Uses a single fixed exposure time (liquids are treated for 30 minutes; solids for 6 hours)

- Viability is assessed by MTT reduction, and the following prediction model applied

For Bottom-up strategy to identify GHS "No Category"

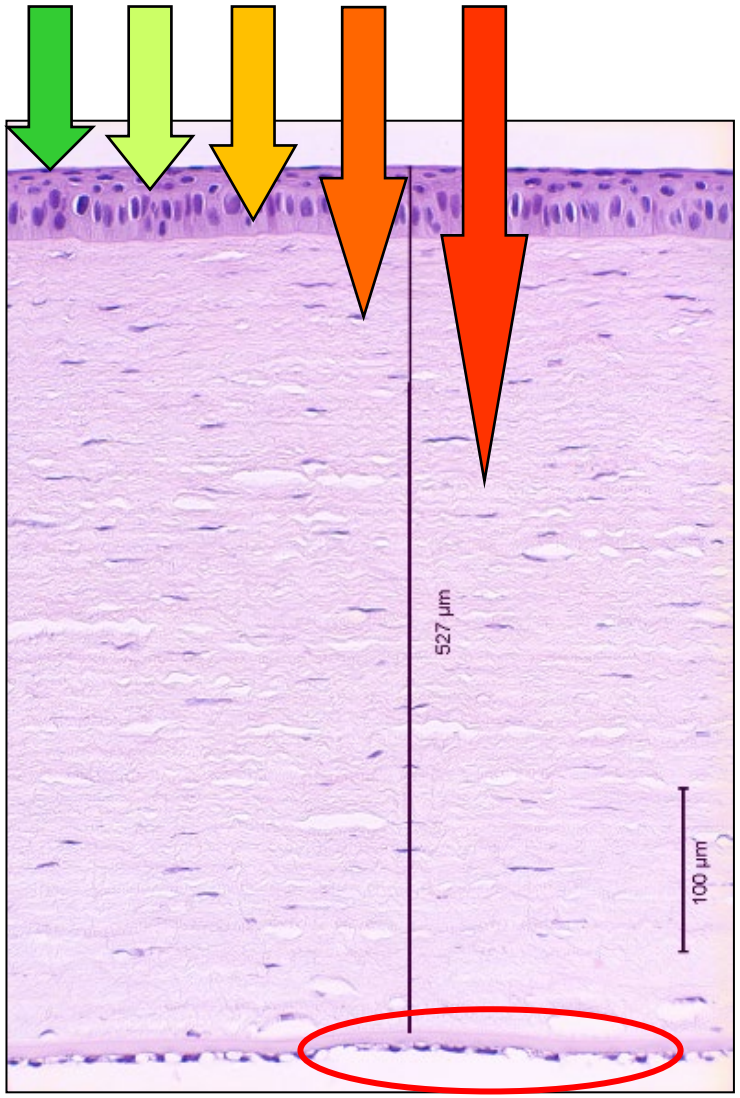
- Viability **> 60%** - test chemical does not require labeling for eye irritation/ serious eye damage (**GHS No Cat**)
- Viability **≤ 60%** - test chemical classified as requiring classification and labelling as an irritant
- **does not distinguish between GHS category 1 or 2 – further testing indicated**



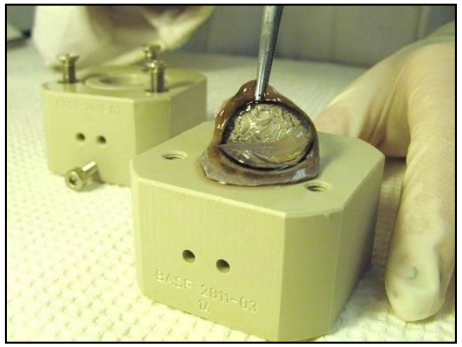
Overall Accuracy	80%
Sensitivity	96%
False Negative Rate	4% !
Specificity	63%
False Positive Rate	37%

Assay performance when used to identify chemicals that do not induce either moderate or severe eye irritation or damage (GHS No Category)

Full corneal thickness models



Bovine Corneal Opacity and Permeability Assay

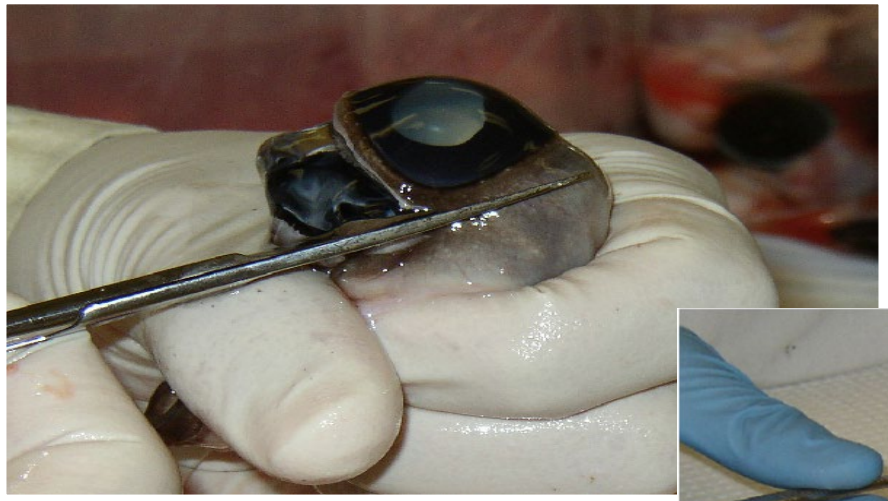


Isolated Chicken Eye Test

- Model all layers of the cornea
  - –non-human species used; human eyes are rare
- Opacity, swelling, loss of barrier measured
- Histopathology can be very helpful for DOI
- Other endpoints possible (viability, cytokine)
- Model penetration and injury in all corneal layers
  - –Discriminate among all categories

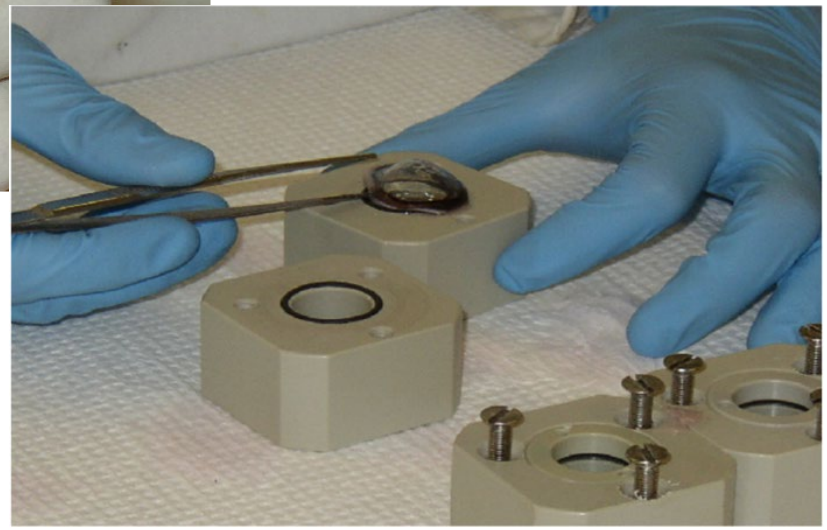
Bovine Corneal Opacity and Permeability (BCOP) - Overview

Measuring changes in corneal opacity and loss of barrier function



Bovine eyes are obtained as a byproduct of meat production

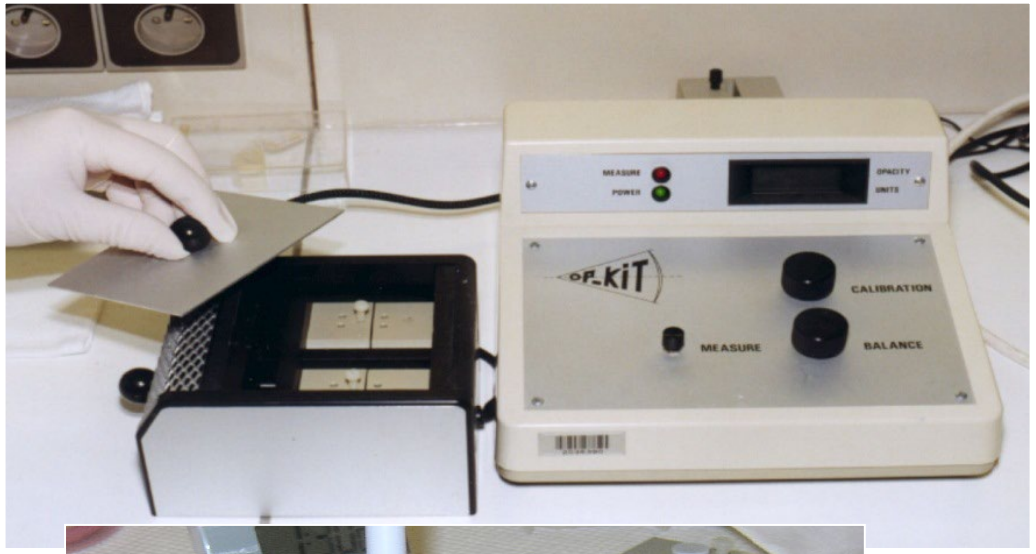
No live animals used

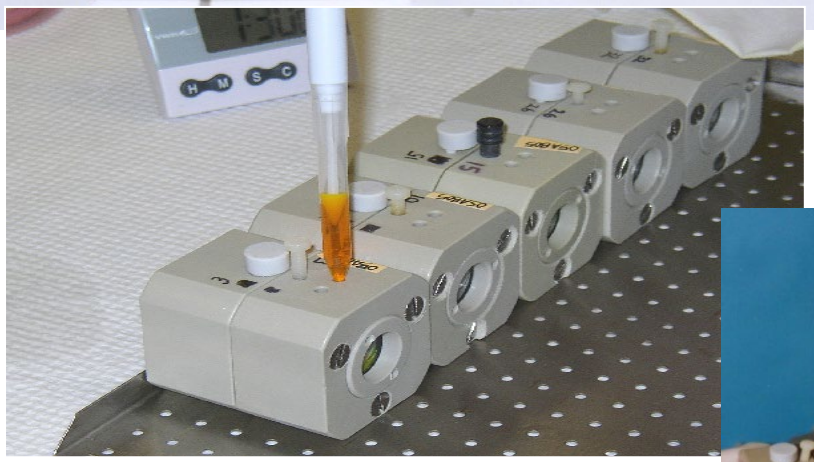


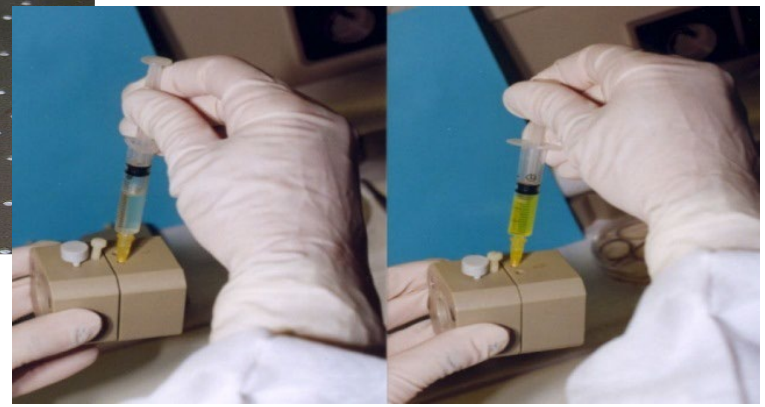
Bovine corneas are mounted in corneal chambers with glass windows. Cultured in EMEM at 32°C

Initial opacity values determined using an opacitometer

Bovine Corneal Opacity and Permeability (BCOP) - Overview







- Treat test chemical
  - 10 minutes (liquids)
  - 4 hours (solids) 20% aqueous preparation
- Rinse / incubate (2 hours for liquids)
  (expression of toxic effects)
- Read post-treatment opacity
- Induction of opacity (up to 150+ units)
- Loss of corneal barrier function

measured by determining fluorescein permeation after 90 minutes (OD490)

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BCOP Prediction Models

$$\textit{In Vitro}\ \text{Score} = \text{Opacity} + (15 \times OD_{490})$$

Prediction Model Developed by Merck*
(non regulatory use)

In Vitro Score	Predicted Irritation Potential
≤ 25	Mild
25.1 – 55	Moderate
> 55.1	Severe

Prediction Model per OECD TG 437
(for UN GHS classification and labeling)

In Vitro Score	UN GHS
≤ 3	No Category
>3 ≤ 55	No standalone prediction can be made
> 55	Category 1

The assay provides a continuum of responses across the eye irritation spectrum from mild to severe

*Sina, *et al*. (1995) **Fund. and Applied Tox.** 26:20-31.

Histological Evaluation

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

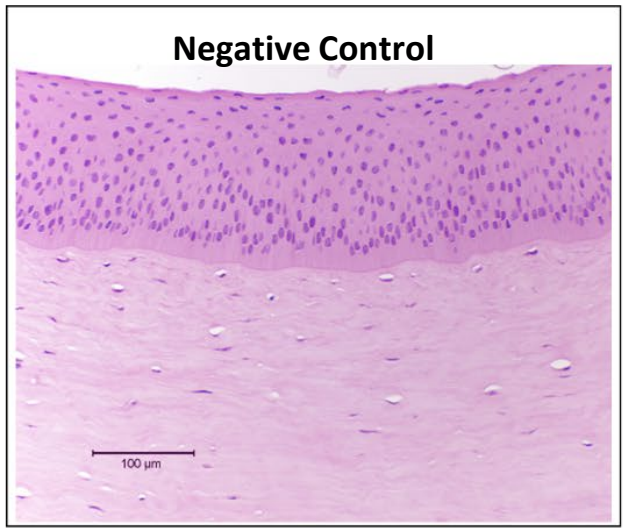


Fig a. Negative Control cornea showing intact epithelium and organized upper stroma.

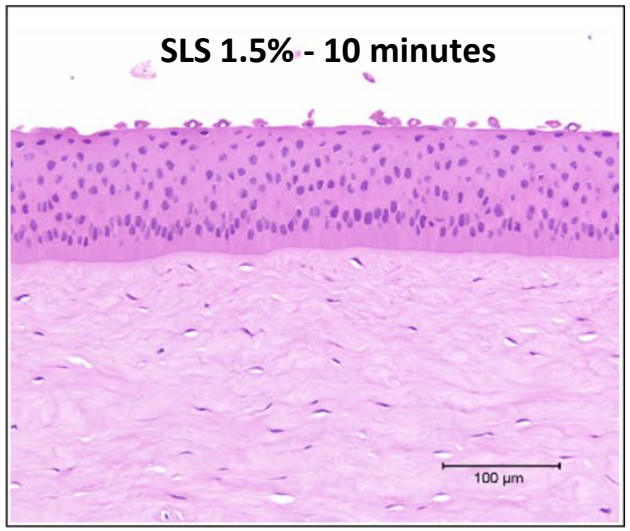


Fig b. Loss of squamous and upper wing layers, results in increases in FL_{490}.

Opacity = 1.7

FL OD490 = 0.302

IVIS = 6.2

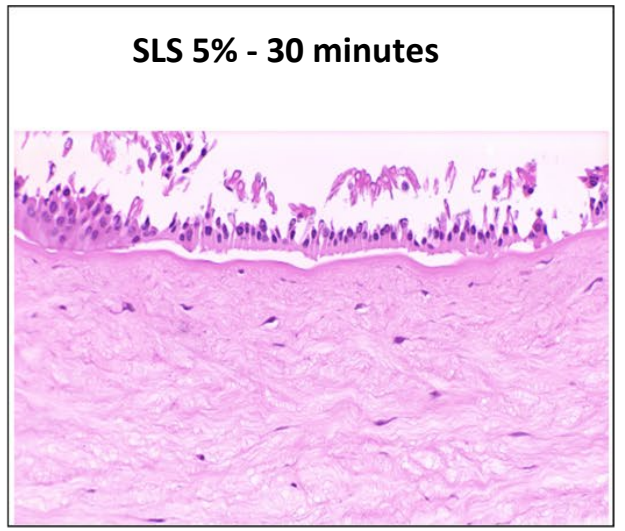


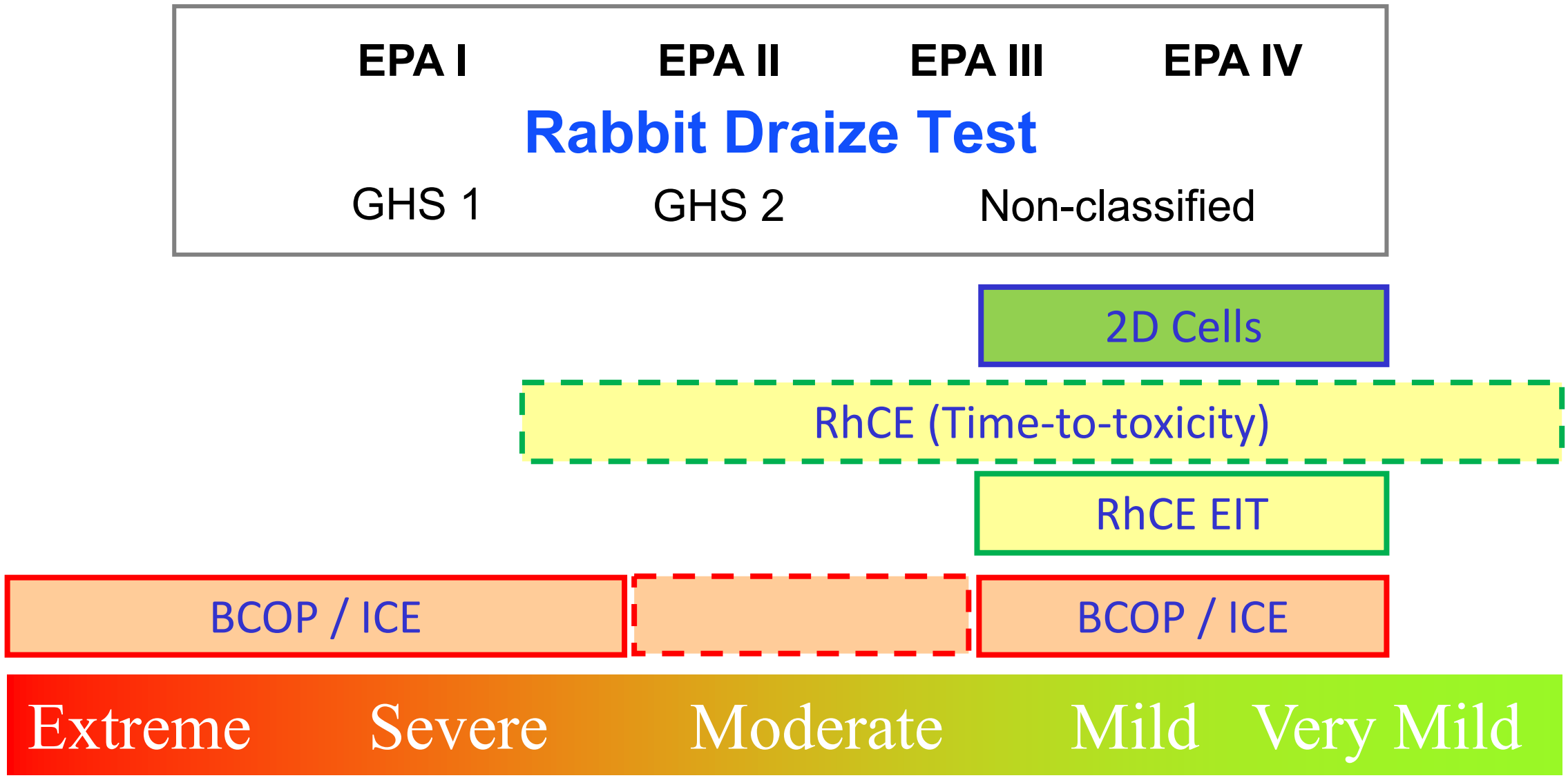
Fig c. Complete loss of epithelium results in high FL_{490}. Marked stromal edema and disorganization results in modest opacity.

Opacity = 7.7

FL OD490 = 2.540

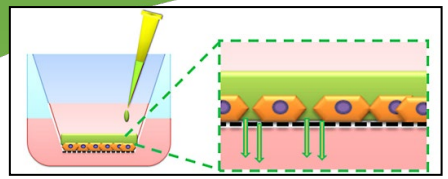
IVIS = 45.8

Assays should complement each other
(*integrate mechanisms and evidence*)

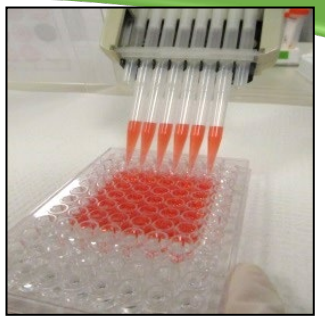


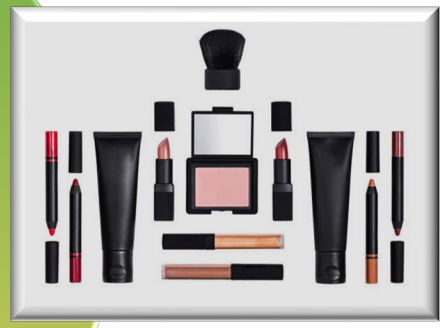
Applying Test Methods to Product Categories

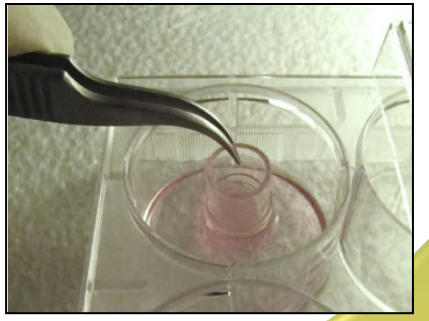
Fluorescein Leakage Assay



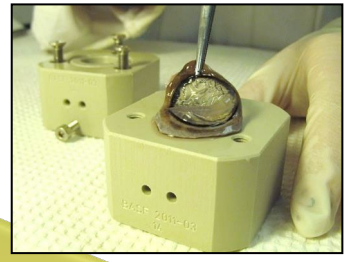
Short Time Exposure Assay



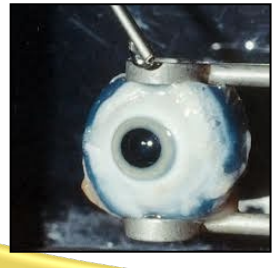




Reconstructed Human Cornea-like Epithelium Test

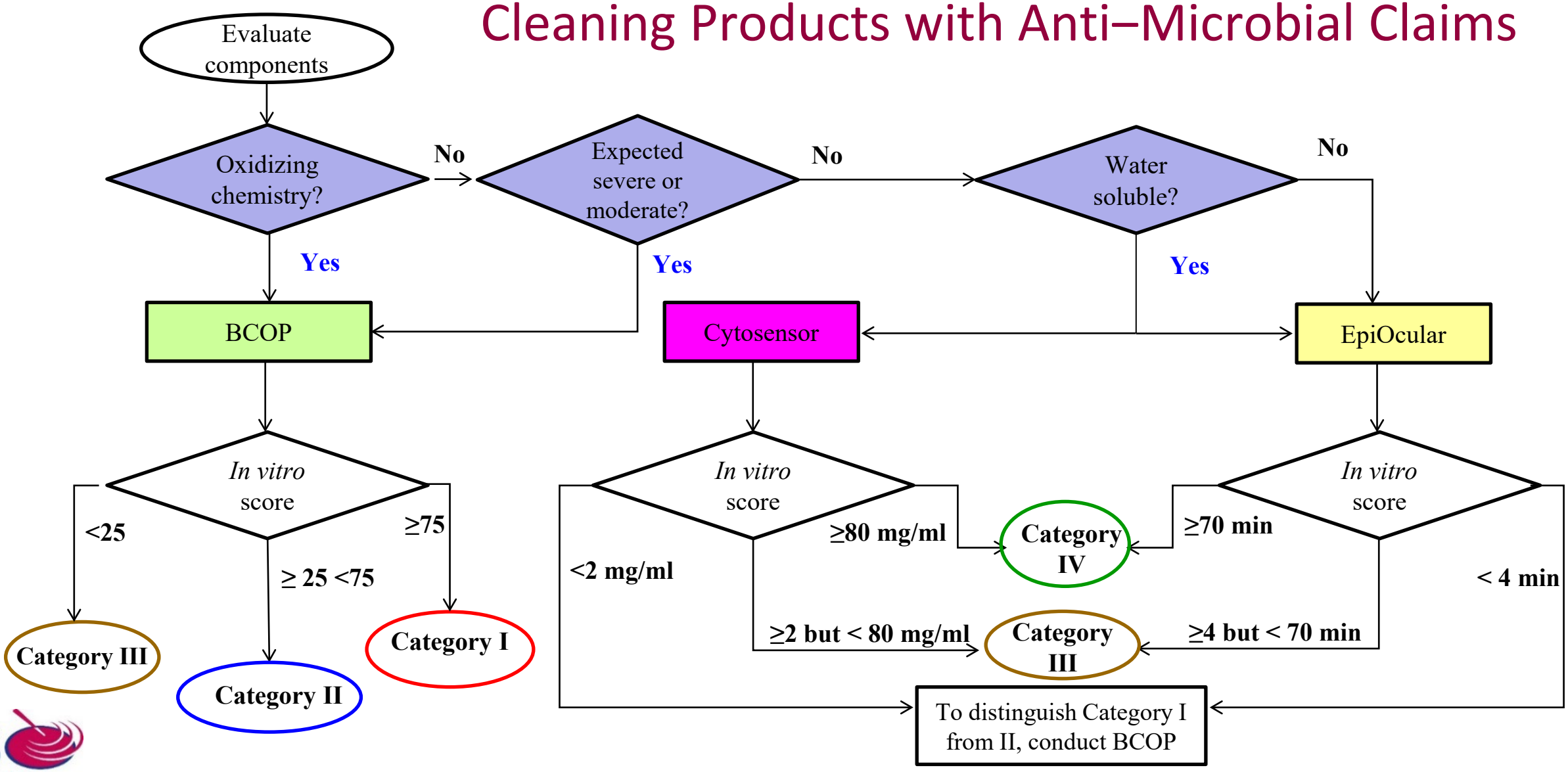


Bovine Corneal Opacity and Permeability Assay



Isolated Chicken Eye Test

EPA OPP Non-animal Testing Strategy for Cleaning Products with Anti–Microbial Claims



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USE OF AN ALTERNATE TESTING FRAMEWORK FOR CLASSIFICATION OF EYE IRRITATION POTENTIAL OF EPA PESTICIDE PRODUCTS

3-2-2015

Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington DC, 20460

Retrospective Analysis

- 232 agrochemical formulations (data analysis conducted by NICEATM*)

Prospective *In Vitro/Ex Vivo* Testing

- 28 agrochemical formulations

*NICEATM = NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

Coded formulations and existing data donated by companies

Project was co-organized by NICEATM and PETA Science Consortium International, with stakeholders from ICCVAM, EURL ECVAM, Canada's PMRA, and industry

28 agrochemical formulations

- 16 formulations – testing complete
- 12 formulations – testing ongoing

EPA category	Completed - # of formulations	Ongoing - # of formulations	Total
EPA I	7	0	7
EPA II	1	6	7
EPA III	1	6	7
EPA IV	7	0	7

Focus on:

- Emulsifiable concentrates (EC)
- Soluble liquids (SL)
- Suspension concentrates (SC)

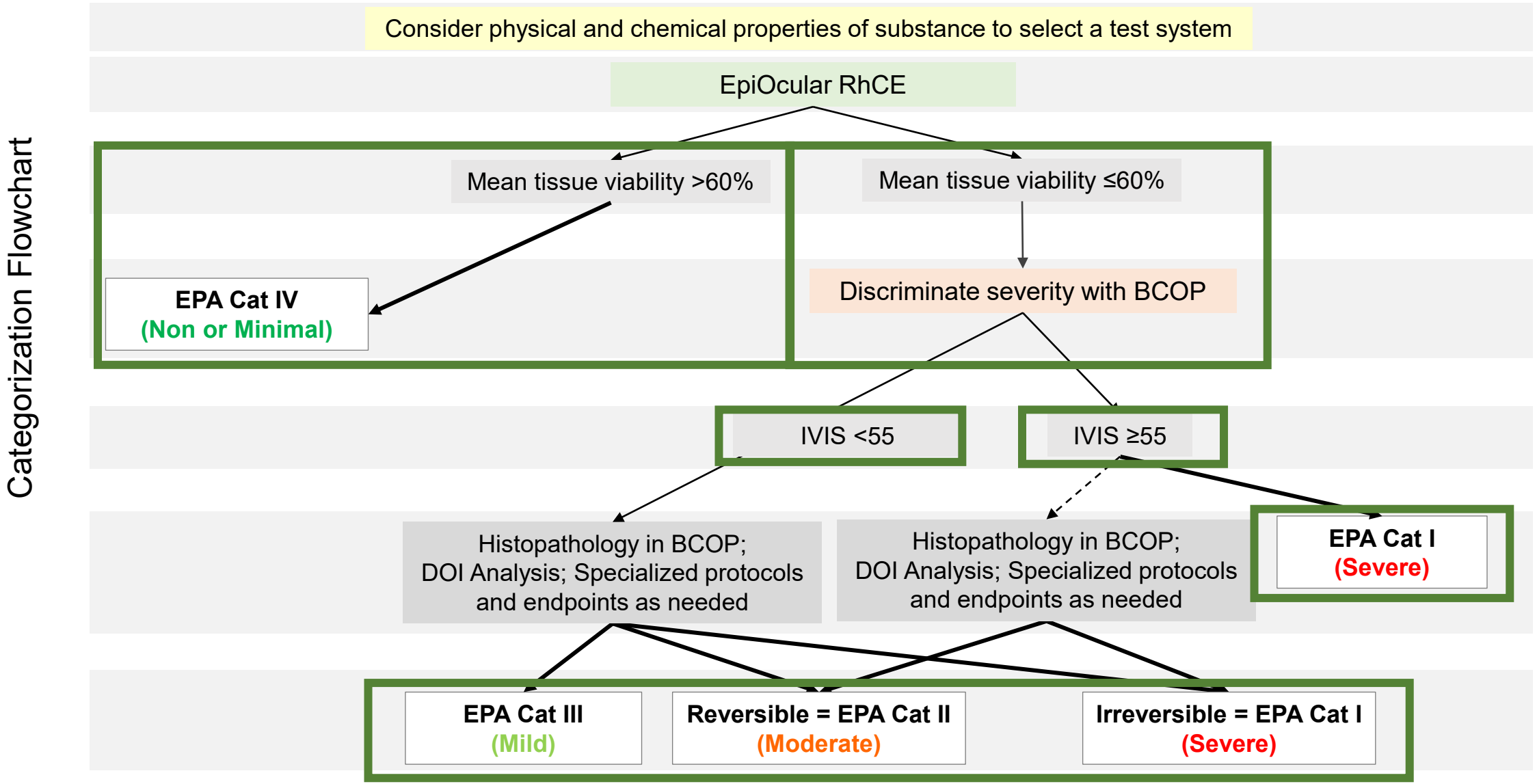
Formulation type	Completed - # of formulations	Ongoing - # of formulations	Total
EC (or EC/ME)	6	6	12
SL	6	5	11
SC	4	1	5

NICEATM report: https://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/formulationreport_2021_fd-508.pdf

In Vitro and *Ex Vivo* Methods Used

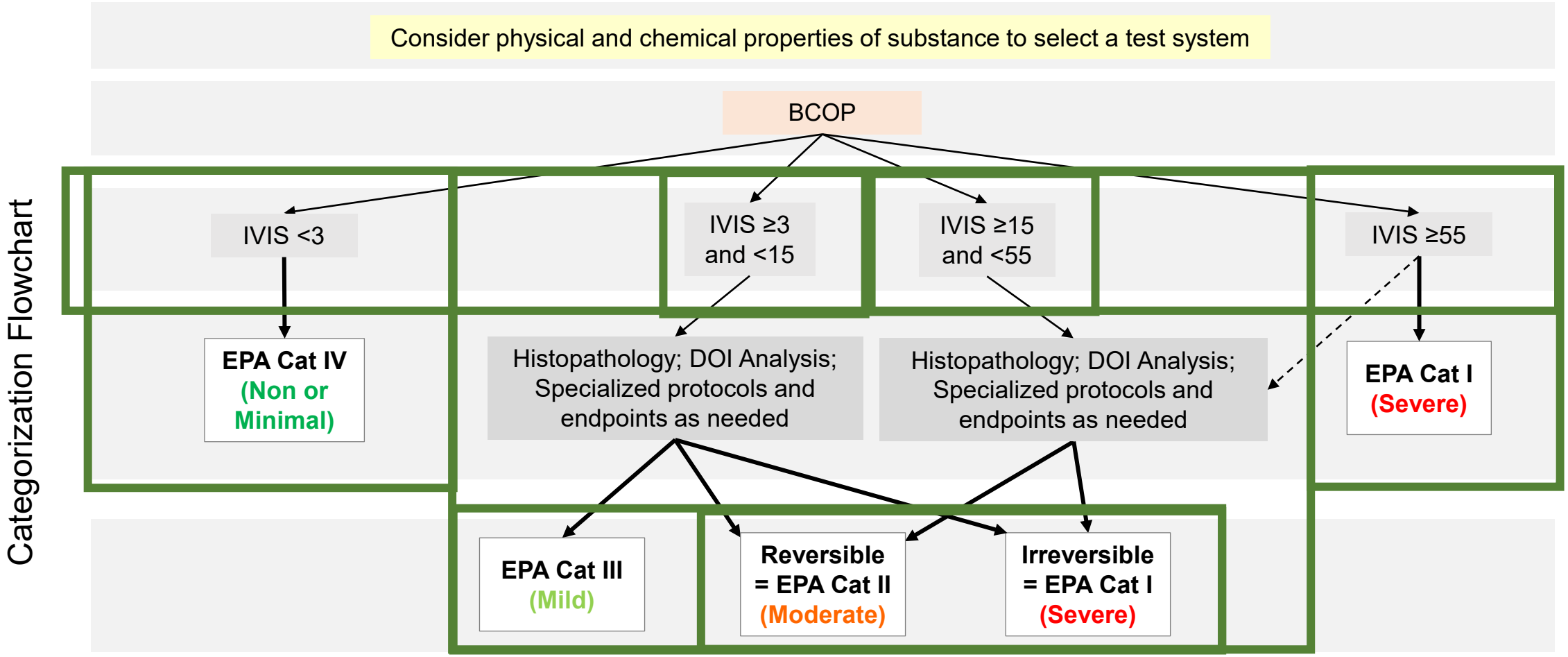
- Bovine Corneal Opacity and Permeability (BCOP) Assay
  - OECD TG 437 (+ histopathology)
  - Extended protocol (+ histopathology)
- Reconstructed Human Cornea-like Epithelial (RhCE) Tissue Models
  - OECD TG 492 (EpiOcular)
  - Time to Toxicity (EpiOcular and Skin Ethic/draft OECD TG 492B)
  - CON4EI protocol (EpiOcular)
- EYEIRR-IS RhCE
- Neutral Red Release Assay
- Isolated Chicken Eye
  - OECD TG 438
- Porcine Cornea Reversibility Assay (PorCORA)

Approach A: Defined approach for EPA hazard classification of eye irritation of agrochemical formulations using the EpiOcular and BCOP assays



IVIS – *in vitro* irritancy score

Approach B: Defined approach for EPA hazard classification of eye irritation of agrochemical formulations using the BCOP assay



IVIS – *in vitro* irritancy score

EPA Hazard Classification

	Approach A (EpiOcular + BCOP)	Approach B (BCOP)	Approach C *In Vivo* Rabbit	Animals Tested (Driving Classification)
1	I	I	I	1 (1)
2	I	I	I	6 (NR)
3	I	I	I	3 (1)
4	I	I	I	3 (1)
5	I	I	I	6 (1)
6	I	I	I	1 (1)
7	III	III	I	1 (1)
8	II	IV	II	3 (1)
9	III	IV	III	3 (NR)
10	III	IV	IV	3 (3)
11	IV	IV	IV	3 (3)
12	IV	IV	IV	3 (3)
13	IV	IV	IV	3 (NR)
14	IV	IV	IV	3 (3)
15	IV	IV	IV	3 (3)
16	IV	IV	IV	9 (9)

NR = not reported

EPA Category	Approach A (EpiOcular + BCOP)	Approach B (BCOP)	Approach C *In Vivo* Rabbit	Animals Tested (Driving Classification)
7	III	III	I	1 (1)
8	II	IV	II	3 (1)
9	III	IV	III	3 (NR)
10	III	IV	IV	3 (3)

Analysis of Draize Eye Irritation Testing and its Prediction by Mining Publicly Available 2008-2014 REACH Data

Thomas Luechtefeld [1], Alexandra Maertens [1], Daniel P. Russo [2], Costanza Rovida [4], Hao Zhu [2,3] and Thomas Hartung [1,4]

[1]Center for Alternatives to Animal Testing (CAAT), Johns Hopkins Bloomberg School of Public Health, Environmental Health Sciences, Baltimore, MD, USA; [2]The Rutgers Center for Computational & Integrative Biology, Rutgers University at Camden, NJ, USA; [3]Department of Chemistry, Rutgers University at Camden, NJ, USA; [4]CAAT-Europe, University of Konstanz, Konstanz, Germany

Prior GHS 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

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

Conclusions from Eye Testing

- Two proposed approaches are comprised of methods that are reproducible, and relevant to human mechanism and biological understanding.
- Good alignment across three approaches for 16 formulations.
- Focusing on mechanistic and human relevance, Approaches A and B are as good as or better than the rabbit test.

A framework for establishing scientific confidence in new approach methodologies

Anna J van der Zalm[a*], João Barroso[b], Patience Browne[c], Warren Casey[d], John Gordon[e], Tala R Henry[f], Nicole C Kleinstreuer[g], Anna B Lowit[h], Monique Perron[h], Amy J Clippinger[a]

[a] PETA Science Consortium International e.V., Stuttgart, Germany.
[b] European Commission, Joint Research Centre (JRC), Ispra, VA, Italy.
[c] Organisation for Economic Co-operation and Development, Hazard Assessment and Pesticides Programmes, Environmental Directorate, Paris, France.
[d] National Institutes of Health, Division of the National Toxicology Program, National Institutes of Environmental Health Sciences, Research Triangle Park, NC, USA.
[e] U.S. Consumer Product Safety Commission, Directorate for Health Sciences, Rockville, MD, USA.
[f] U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, USA.
[g] National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, Research Triangle Park, NC, USA.
[h] U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC, USA.

Unclassified ENV/JM/MONO(2005)14

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

18-Aug-2005

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/MONO(2005)14
Unclassified

OECD SERIES ON TESTING AND ASSESSMENT
Number 34

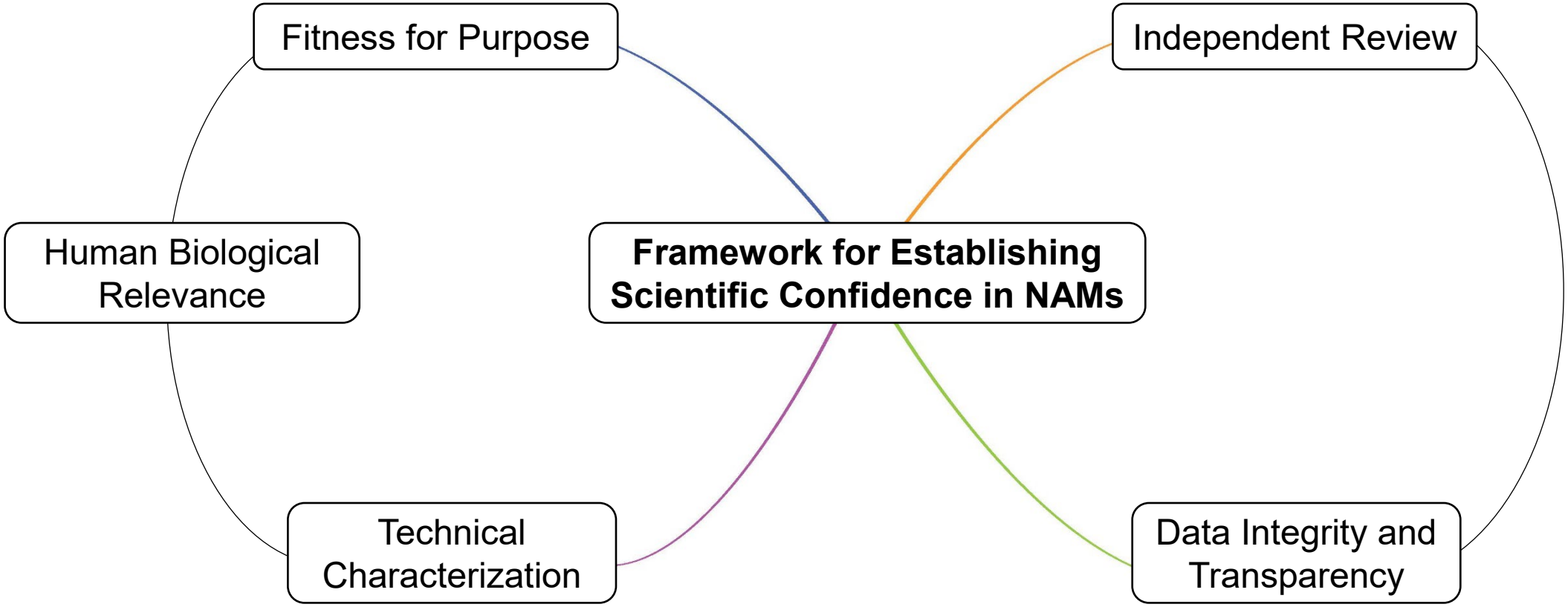
GUIDANCE DOCUMENT ON THE VALIDATION AND INTERNATIONAL ACCEPTANCE OF NEW OR UPDATED TEST METHODS FOR HAZARD ASSESSMENT

Arch Toxicol (2018) 92:611–617
https://doi.org/10.1007/s00204-017-2097-4

REGULATORY TOXICOLOGY

Standardisation of defined approaches for skin sensitisation testing to support regulatory use and international adoption: position of the International Cooperation on Alternative Test Methods

S. Casati[1] · K. Aschberger[1] · J. Barroso[1] · W. Casey[2] · I. Delgado[3] · T. S. Kim[4] · N. Kleinstreuer[2] · H. Kojima[5] · J. K. Lee[4] · A. Lowit[6] · H. K. Park[4] · M. J. Régimbald-Krnel[7] · J. Strickland[8] · M. Whelan[1] · Y. Yang[9] · Valérie Zuang[1]

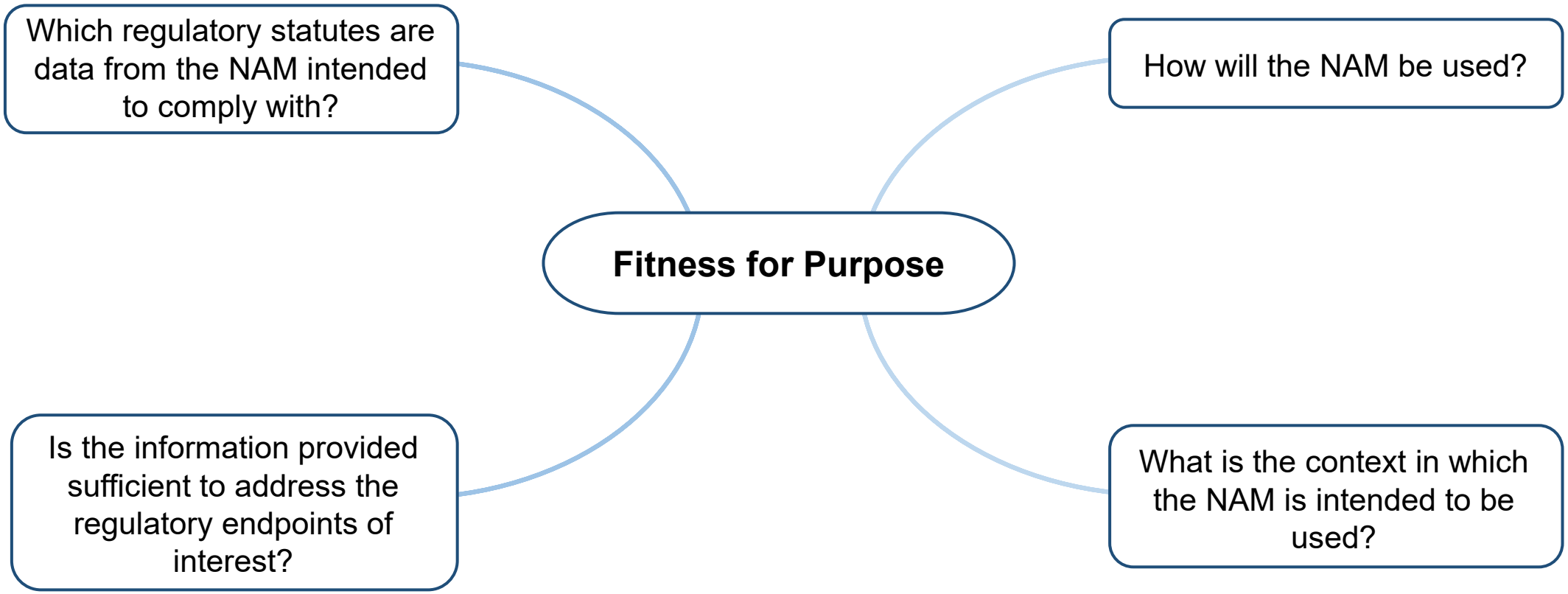

Fitness for Purpose
Independent Review
Human Biological Relevance
Framework for Establishing Scientific Confidence in NAMs
Technical Characterization
Data Integrity and Transparency

Data Integrity and Transparency

- Assess integrity and credibility of the raw data to the final report
- Communicate transparently and publicly
- Assess and describe the uncertainties

Independent Review

- Determine the appropriate level of external review



Data integrity and transparency • Independent review • Fitness for purpose • Human biological relevance • Technical characterization

Human Biological Relevance

Similarities between the physiology of, or the biology measured by, the test system, and human biology

Concordance with human responses

Technical Characterization

Describe:

- accuracy
- intra-laboratory reproducibility
- transferability
- applicability domain
- reference chemicals and controls
- limits of detection and quantification

Evaluate:

- protocol
- equipment
- computational models being used

Toxicology and Applied Pharmacology
Volume 19, Issue 2, June 1971, Pages 276-360

Study of intra- and interlaboratory variability in the results of rabbit eye and skin irritation tests

Carrol S. Weil [a, b], Robert A. Scala

Arch Toxicol (2014) 88:701–723
DOI 10.1007/s00204-013-1156-8

IN VITRO SYSTEMS

Retrospective analysis of the Draize test for serious eye damage/eye irritation: importance of understanding the in vivo endpoint under UN GHS/EU CLP for the development and evaluation of in vitro test methods

Els Adriaens · João Barroso · Chantra Eskes · Sebastian Hoffmann · Pauline McNamee · Nathalie Alépée · Sandrine Bessou-Touya · Ann De Smedt · Bart De Wever · Uwe Pfannenbecker · Magalie Tailhardat · Valérie Zuang

Arch Toxicol (2017) 91:521–547
DOI 10.1007/s00204-016-1679-x

REVIEW ARTICLE

Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: the Draize eye test Reference Database (DRD)

João Barroso[1,2] · Uwe Pfannenbecker[3] · Els Adriaens[4] · Nathalie Alépée[5] · Magalie Cluzel[6] · Ann De Smedt[7] · Jalila Hibatallah[8] · Martina Klaric[1] · Karsten R. Mewes[9] · Marion Millet[10] · Marie Templier[10] · Pauline McNamee[11]

Analysis of Draize Eye Irritation Testing and its Prediction by Mining Publicly Available 2008-2014 REACH Data

Thomas Luechtefeld [1], Alexandra Maertens [1], Daniel P. Russo [2], Costanza Rovida [4], Hao Zhu [2,3] and Thomas Hartung [1,4]

Regulatory Toxicology and Pharmacology 122 (2021) 104920
Contents lists available at ScienceDirect
Regulatory Toxicology and Pharmacology
journal homepage: www.elsevier.com/locate/yrtph

Analysis of variability in the rabbit skin irritation assay

John P. Rooney [a,*], Neepa Y. Choksi [a], Patricia Ceger [a], Amber B. Daniel [a], James Truax [a], David Allen [a], Nicole Kleinstreuer [b]

Toxicology in Vitro 34 (2016) 220–228
Contents lists available at ScienceDirect
Toxicology in Vitro
journal homepage: www.elsevier.com/locate/toxinvit

Analysis of the Local Lymph Node Assay (LLNA) variability for assessing the prediction of skin sensitisation potential and potency of chemicals with non-animal approaches

Coralie Dumont, João Barroso, Izabela Matys, Andrew Worth, Silvia Casati *

Concept Article

Uncertainties of Testing Methods: What Do We (Want to) Know About Carcinogenicity?

Martin Paparella [1], Annamaria Colacci [2] and Miriam N. Jacobs [3]

Toxicological Sciences

Evaluation of Variability Across Rat Acute Oral Systemic Toxicity Studies

Agnes L. Karmaus*, Kamel Mansouri†, Kimberly T. To*, Bevin Blake†,1, Jeremy Fitzpatrick‡,2, Judy Strickland*, Grace Patlewicz‡, David Allen*, Warren Casey†, and Nicole Kleinstreuer†

Review

A Curated Database of Rodent Uterotrophic Bioactivity

Nicole C. Kleinstreuer,[1] Patricia C. Ceger,[1] David G. Allen,[1] Judy Strickland,[1] Xiaoqing Chang,[1] Jonathan T. Hamm,[1] and Warren M. Casey[2]

Reprod Toxicol. 2018 October ; 81: 259–271. doi:10.1016/j.reprotox.2018.08.016.

DEVELOPMENT OF A CURATED HERSHBERGER DATABASE

P Browne[a], NC Kleinstreuer[b], P Ceger[c], C Deisenroth[d], N Baker[e], K Markey[f], RS Thomas[d], RJ Judson[d], W Casey[b]

Published in final edited form as:
Comput Toxicol. 2020 August 1; 15(August 2020): 1–100126. doi:10.1016/j.comtox.2020.100126.

Variability in *in vivo* studies: Defining the upper limit of performance for predictions of systemic effect levels

Ly Ly Pham[1,2], Sean Watford[1,3], Prachi Pradeep[1,2], Matthew T. Martin[1,4], Russell Thomas[1], Richard Judson[1], R. Woodrow Setzer[1], Katie Paul Friedman[1]

Scientific confidence is increased when:

Information about the model and data are publicly available to the greatest extent possible and reviewed by independent third parties

The purpose of the model is clearly identified

The technical aspects of the model have been characterized

The model captures key aspects of human biology or mechanisms of toxicity

The model shows concordance with human data or across multiple methods

- Confidence in a NAM should be determined with the species of interest (humans) in mind

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