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?

<table>
<thead>
<tr>
<th>EPA I</th>
<th>EPA II</th>
<th>EPA III</th>
<th>EPA IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit Draize Test</td>
<td>GHS 1</td>
<td>GHS 2</td>
<td>Non-classified</td>
</tr>
</tbody>
</table>

**Extreme**    **Severe**    **Moderate**    **Mild**    **Very Mild**

- **Agricultural Ingredients and Products**
- **Consumer Products**
- **Industrial Chemicals**
- **Cosmetics**
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
• **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

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

- 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

<table>
<thead>
<tr>
<th>Prior type</th>
<th>1</th>
<th>2A</th>
<th>2B</th>
<th>NC</th>
<th>Total</th>
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<tbody>
<tr>
<td>1</td>
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### Reproducibility of the Draize Eye Test

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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

Luechtelfeld et al., ALTEX 33(2), 2016.
## Reproducibility of the Draize Eye Test

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- 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

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

To link to this article: https://doi.org/10.1080/15569527.2021.1910291
Corneal Physiology and Tissue Functions

- Squamous Epithelium
- Upper Wing Layer
- Lower Wing Layer
- Basal Cell Layer
- Bowman’s Layer
- Anterior Stroma

- Epithelium
- Bowman’s Layer
- Stroma
- Descemet’s Membrane
- Endothelium
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
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 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).

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

It's important to understand the relationship of those test methods to the various corneal layers to appreciate the mechanistic relevance in eye irritation assessments.
Isolated Chicken Eye Test
Bovine Corneal Opacity and Permeability Assay

Fluorescein Leakage Assay
Squamous epithelium
Short Time Exposure Assay

Reconstructed Human Cornea-like Epithelium Test

Full thickness corneal models
Bovine Corneal Opacity and Permeability Assay

Isolated Chicken Eye Test

Epithelium models
Squamous epithelium models

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

- **Post-treatment Expression Incubation**: Prepare aliquots for spectrophotometry.

- **Isopropanol Extraction**: Isopropanol Extraction.

- **MTT Reduction**:
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

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

<table>
<thead>
<tr>
<th>Overall Accuracy</th>
<th>80%</th>
</tr>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>96%</td>
</tr>
<tr>
<td>False Negative Rate</td>
<td>4%</td>
</tr>
<tr>
<td>Specificity</td>
<td>63%</td>
</tr>
<tr>
<td>False Positive Rate</td>
<td>37%</td>
</tr>
</tbody>
</table>

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

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

Initial opacity values determined using an opacitometer

Bovine eyes are obtained as a byproduct of meat production

No live animals used
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)
BCOP Prediction Models

*In Vitro Score* = Opacity + (15 x OD$_{490}$)

**Prediction Model Developed by Merck***
(non regulatory use)

<table>
<thead>
<tr>
<th>In Vitro Score</th>
<th>Predicted Irritation Potential</th>
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<tbody>
<tr>
<td>≤ 25</td>
<td>Mild</td>
</tr>
<tr>
<td>25.1 – 55</td>
<td>Moderate</td>
</tr>
<tr>
<td>&gt; 55.1</td>
<td>Severe</td>
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</table>

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

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<th>UN GHS</th>
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<tr>
<td>≤ 3</td>
<td>No Category</td>
</tr>
<tr>
<td>&gt;3 ≤ 55</td>
<td>No standalone prediction can be made</td>
</tr>
<tr>
<td>&gt; 55</td>
<td>Category 1</td>
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The assay provides a continuum of responses across the eye irritation spectrum from mild to severe

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)

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Rabbit Draize Test

- 2D Cells
- RhCE (Time-to-toxicity)
- RhCE EIT
- BCOP / ICE

Extreme       Severe       Moderate       Mild       Very Mild
Isolated Chicken Eye Test

Bovine Corneal Opacity and Permeability Assay

Reconstructed Human Cornea-like Epithelium Test

Fluorescein Leakage Assay

Short Time Exposure Assay

Applying Test Methods to Product Categories

Isolated Chicken Eye Test

Bovine Corneal Opacity and Permeability Assay

Reconstructed Human Cornea-like Epithelium Test
EPA OPP Non-animal Testing Strategy for Cleaning Products with Anti-Microbial Claims

1. Evaluate components
   - Oxidizing chemistry?
     - No
     - Yes → BCOP
   - Expected severe or moderate?
     - No
     - Yes → Cytosensor
   - Water soluble?
     - No
     - Yes → EpiOcular

   - Category III
   - Category II
   - Category I

2. For BCOP:
   - In vitro score
     - <25
     - ≥25 <75
     - ≥75

3. For Cytosensor:
   - In vitro score
     - ≥80 mg/ml → Category IV
     - ≥2 but < 80 mg/ml
     - <2 mg/ml → Category III
     - ≥4 but < 70 min

4. For EpiOcular:
   - In vitro score
     - ≥70 min
     - < 4 min

To distinguish Category I from II, conduct BCOP.
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

Focus on:
- Emulsifiable concentrates (EC)
- Soluble liquids (SL)
- Suspension concentrates (SC)

<table>
<thead>
<tr>
<th>EPA category</th>
<th>Completed - # of formulations</th>
<th>Ongoing - # of formulations</th>
<th>Total</th>
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<td>7</td>
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<td>7</td>
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<td>6</td>
<td>12</td>
</tr>
<tr>
<td>SL</td>
<td>6</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>SC</td>
<td>4</td>
<td>1</td>
<td>5</td>
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**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

Consider physical and chemical properties of substance to select a test system

Mean tissue viability >60%

- **EPA Cat IV** (Non or Minimal)

Mean tissue viability ≤60%

Discriminate severity with BCOP

IVIS <55

- Histopathology in BCOP; DOI Analysis; Specialized protocols and endpoints as needed
- **EPA Cat III** (Mild)

IVIS ≥55

- Histopathology in BCOP; DOI Analysis; Specialized protocols and endpoints as needed
- **EPA Cat I** (Severe)

- Reversible = **EPA Cat II** (Moderate)

Irreversible = **EPA Cat I** (Severe)

IVIS – *in vitro* irritancy score
**Approach B:** Defined approach for EPA hazard classification of eye irritation of agrochemical formulations using the BCOP assay

Consider physical and chemical properties of substance to select a test system

- **BCOP**
  - **IVIS <3**
    - **EPA Cat IV**
      - (Non or Minimal)

- **IVIS ≥3 and <15**
  - Histopathology; DOI Analysis; Specialized protocols and endpoints as needed

- **IVIS ≥15 and <55**
  - Histopathology; DOI Analysis; Specialized protocols and endpoints as needed

- **IVIS ≥55**
  - **EPA Cat I**
    - (Severe)

- **EPA Cat III**
  - (Mild)

- **Reversible**
  - (Moderate)

- **Irreversible**
  - (Severe)

IVIS – in vitro irritancy score
## EPA Hazard Classification

<table>
<thead>
<tr>
<th></th>
<th>Approach A (EpiOcular + BCOP)</th>
<th>Approach B (BCOP)</th>
<th>Approach C In Vivo Rabbit</th>
<th>Animals Tested (Driving Classification)</th>
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<th>Approach C <em>In Vivo</em> Rabbit</th>
<th>Animals Tested (Driving Classification)</th>
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**Analysis of Draize Eye Irritation Testing and its Prediction by Mining Publicly Available 2008-2014 REACH Data**

Thomas Luechtefeld¹, Alexandra Maertens¹, Daniel P. Russo², Costanza Rovida¹, Hao Zhu¹,² and Thomas Hartung¹,²

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

**Prior GHS type** | 1 | 2A | 2B | NC | Total |
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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\textsuperscript{a}, João Barroso\textsuperscript{b}, Patience Browne\textsuperscript{c}, Warren Casey\textsuperscript{d}, John Gordon\textsuperscript{e}, Tala R Henry\textsuperscript{f}, Nicole C Kleinstreuer\textsuperscript{g}, Anna B Lowith\textsuperscript{h}, Monique Perron\textsuperscript{h}, Amy J Clippinger\textsuperscript{a}

\textsuperscript{a}PETA Science Consortium International e.V., Stuttgart, Germany.
\textsuperscript{b}European Commission, Joint Research Centre (JRC), Ispra, VA, Italy.
\textsuperscript{c}Organisation for Economic Co-operation and Development, Hazard Assessment and Pesticides Programmes, Environmental Directorate, Paris, France.
\textsuperscript{d}National Institutes of Health, Division of the National Toxicology Program, National Institutes of Environmental Health Sciences, Research Triangle Park, NC, USA.
\textsuperscript{e}U.S. Consumer Product Safety Commission, Directorate for Health Sciences, Rockville, MD, USA.
\textsuperscript{f}U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, USA.
\textsuperscript{g}National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, Research Triangle Park, NC, USA.
\textsuperscript{h}U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC, USA.
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. Casati¹ · K. Aschberger¹ · J. Barroso¹ · W. Casey² · I. Delgado³ · T. S. Kim⁴ · N. Kleinreuter² · H. Kojima⁵ · J. K. Lee⁶ · A. Lowit⁶ · H. K. Park⁴ · M. J. Régimbald-Krnel⁷ · J. Strickland⁸ · M. Whelan¹ · Y. Yang⁹ · Valérie Zuan¹
Assess integrity and credibility of the raw data to the final report

Communicate transparently and publicly

Assess and describe the uncertainties

Determine the appropriate level of external review

Data Integrity and Transparency

Independent Review
How will the NAM be used?

What is the context in which the NAM is intended to be used?

Is the information provided sufficient to address the regulatory endpoints of interest?

Which regulatory statutes are data from the NAM intended to comply with?

Fitness for Purpose

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

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

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