



A Modern Framework to Establish Scientific Confidence in New Methods

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FUNDING



TRAINING



WORKSHOPS
AND WEBINARS



RETROSPECTIVE
DATA REVIEWS



PUBLICATIONS
AND PRESENTATIONS

Outline

- Framework to establish scientific confidence in NAMs
- Focus on relevance and accuracy
- Example: eye irritation



ENV/JM/MONO(2005)14
Unclassified

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

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. Casati¹ · K. Aschberger¹ · J. Barroso¹ · W. Casey² · I. Delgado³ · T. S. Kim⁴ ·
N. Kleinstreuer² · H. Kojima⁵ · J. K. Lee⁴ · A. Lowit⁶ · H. K. Park⁴ ·
M. J. Régimbald-Krnel⁷ · J. Strickland⁸ · M. Whelan¹ · Y. Yang⁹ · Valérie Zuang¹

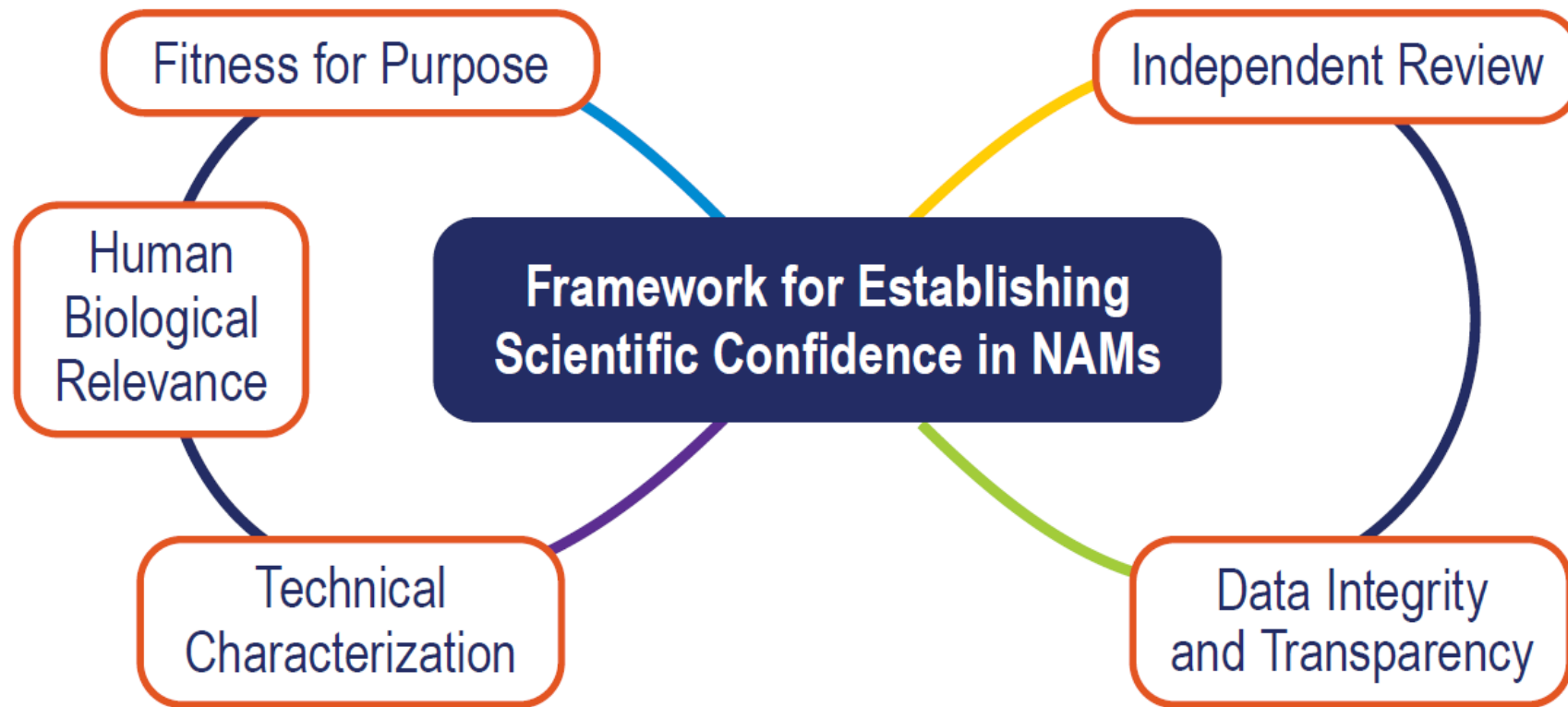


A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm¹  · João Barroso² · Patience Browne³ · Warren Casey⁴ · John Gordon⁵ · Tala R. Henry⁶ ·
Nicole C. Kleinstreuer⁷ · Anna B. Lowit⁶ · Monique Perron⁸ · Amy J. Clippinger¹

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Data Integrity and Transparency

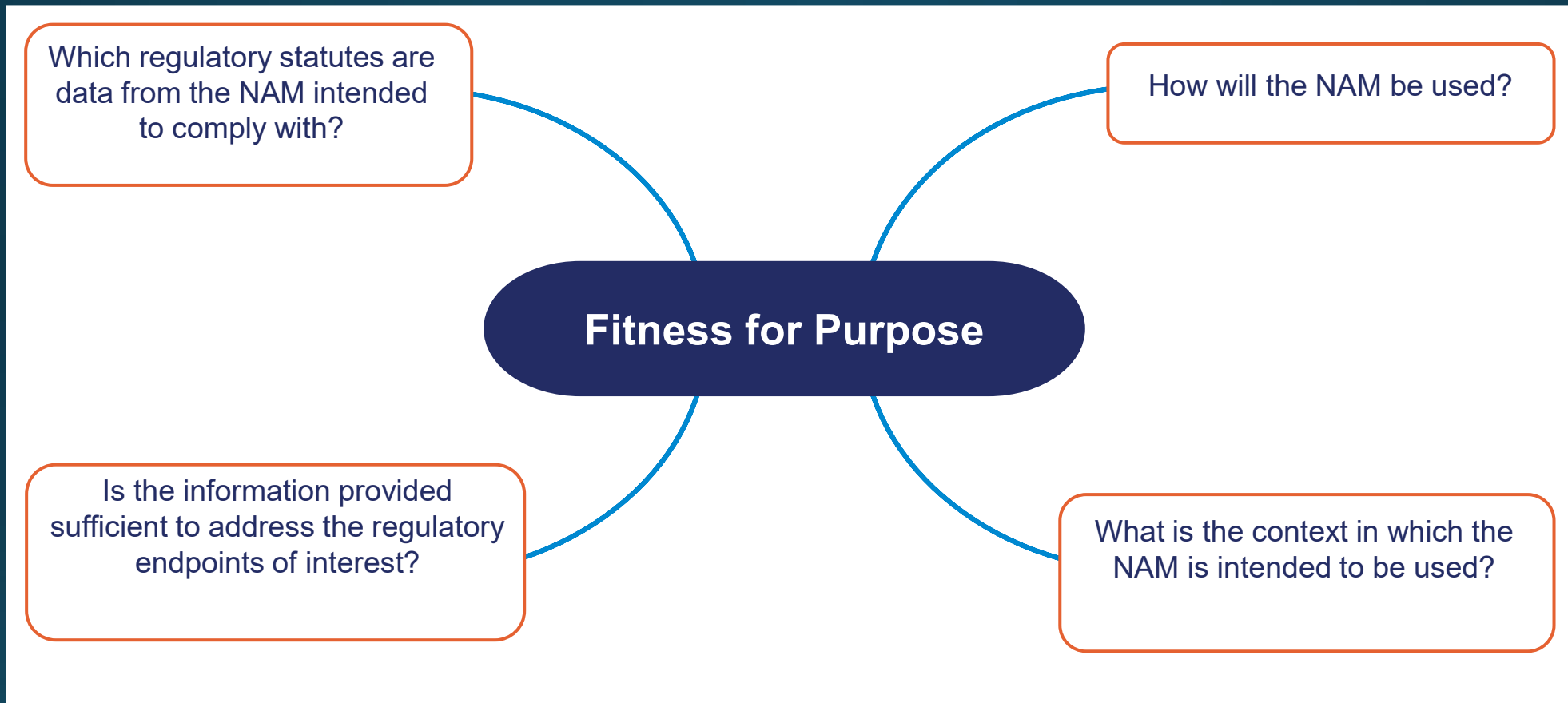
Assess integrity and credibility of the raw data to the final report

Communicate transparently and publicly

Assess and describe the uncertainties

Independent Review

Determine the appropriate level of external review



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



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}

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 Rovida⁴, 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

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[†]

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 Pfannenbecker³ · Els Adriaens⁴ · Nathalie Alépée⁵ · Magalie Cluzel⁶ · Ann De Smedt⁷ · Jalila Hibatallah⁸ · Martina Klaric¹ · Jürgen R. Mewes⁹ · Marion Millet¹⁰ · Marie Templier¹⁰ · Pauline McNamee¹¹

Toxicology in Vitro 34 (2016) 220–228

Contents lists available at ScienceDirect



ELSEVIER

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^{*}

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 endpoints 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



Concept Article

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

Martin Paparella¹, Annamaria Colacci² and Miriam N. Jacobs³

Review

A Section 508–conformant HTML version of this article is available at <http://dx.doi.org/10.1289/ehp.1510183>.

A Curated Database of Rodent Uterotrophic Bioactivity

Nicole C. Kleinstreuer¹, Patricia C. Ceger¹, David G. Allen¹, Judy Strickland¹, Xiaoqing Chang¹, Jonathan T. Hamm¹, and Warren M. Casey²

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



EPA Public Access

Author manuscript

Comput Toxicol. Author manuscript; available in PMC 2021 August 01.

About author manuscripts

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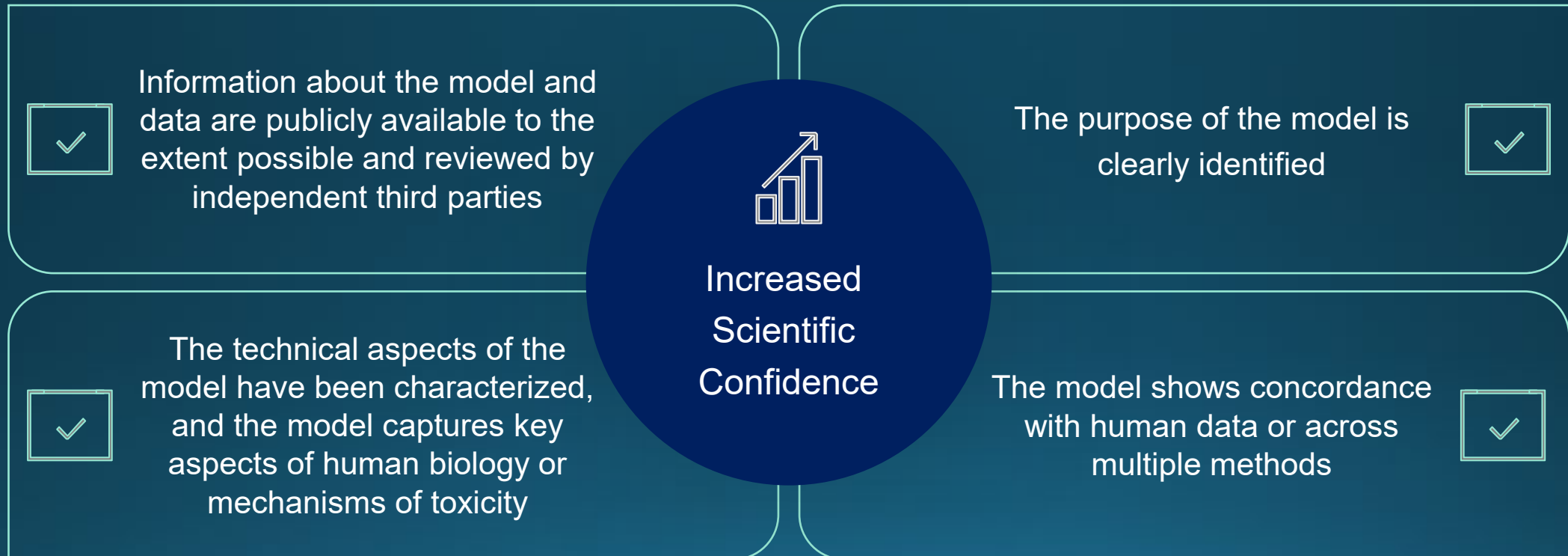
Published in final edited form as:

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 Thomas¹, Richard Judson¹, R. Woodrow Setzer¹, Katie Paul Friedman¹

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



Human-Relevant Approaches to Assess Eye Corrosion/Irritation Potential of Agrochemical Formulations

Amy J. Clippinger^{a*}, Hans A. Raabe^b, David G. Allen^c, Neepa Choksi^c, Anna van der Zalm^a, Nicole Kleinstreuer^d, João Barroso^e

^aPETA Science Consortium International e.V.; ^bInstitute for In Vitro Sciences, Inc.; ^cIntegrated Laboratory Systems, LLC; ^dNational Toxicology Program, National Institute of Environmental Health Sciences; ^eEuropean Commission, Joint Research Centre (JRC)

BACKGROUND AND OBJECTIVES

- Aim: Identify methods that will reliably protect humans following accidental eye exposure to agrochemicals.
- Multiple *in vitro* and *ex vivo* eye irritation and corrosion test methods exist, but are not routinely used for regulatory testing of agrochemical formulations due to a lack of concordance with results from the rabbit eye test.
- The rabbit Draize test:
 - has significant intra- and inter-study variability
 - evaluates qualitative apical outcomes in the rabbit eye
 - provides limited mechanistic information
 - may not elucidate MoAs that occur in humans
 - uses a longer exposure time than anticipated in humans
 - was never validated for its relevance to humans

APPROACH

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

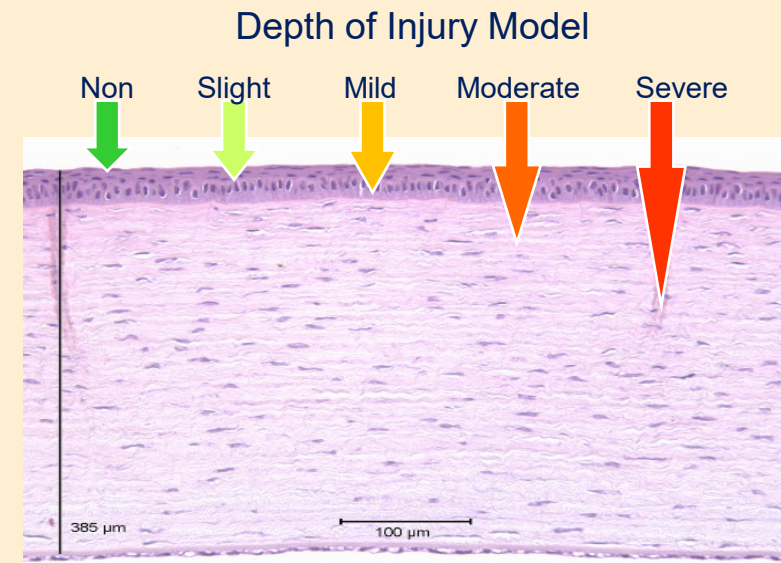


Image modified from Scott, et al., 2010

Human-Relevant Approaches to Assess Eye Corrosion/Irritation

Potential of Agrochemical Formulations

Image purchased from iStock

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MECHANISMS OF EYE IRRITATION

MOLECULAR INITIATING EVENT

- binding to cell surface proteins
- binding to cytoplasmic proteins / enzymes
- surfactant intercalation and disruption of cell membrane lipid bilayer organization
- solvent dissolution of cell membrane proteins or lipids
- solvent precipitation of cell proteins
- alkaline saponification of membrane lipids
- acid precipitation of cell proteins, enzymes and nucleic acids
- oxidative damage to cell membrane lipids
- oxidative changes in cell proteins
- solvent dissolution of nucleic acids
- solvent dissolution of cytoplasmic components
- precipitation of nucleic acids
- binding to DNA and/or RNA



CELLULAR RESPONSE

- chemical antagonism of vital enzymes, nucleic acids
- cell stress responses
- breakdown of the tight junctions
- activation of matrix metalloproteases
- changes in cell surface markers and cell-to-cell and cell-to-basement membrane adhesion molecules, desmosomes / hemidesmosomes / anchoring proteins
- breakdown of cell membrane integrity
- release of chemokines and cytokines (e.g., IL-1 α , TNF α)
- induction of secondary cytokines
- neural dendrites trigger TRPV1-type nociceptive response
- changes in cell metabolism/respiration
- changes in normal functional phenotype
- necrotic or apoptotic damage leading to cell death



ORGAN RESPONSE

- increased corneal or conjunctival permeability/loss of barrier function
- conjunctival hyperemia and discharge
- swelling of the conjunctival tissues / swelling of the eye lid tissues
- epithelial tissue swelling
- sloughing and loss of epithelial tissue layers
- corneal/stromal swelling and oedema, and swelling-related corneal opacity
- corneal opacity due to cellular/molecular denaturation/coagulation
- induction of wound healing response and basal cell regeneration/turnover
- inflammatory response and neutrophil migration
- induction of fibrosis, panus, and neovascularization
- loss of endothelium



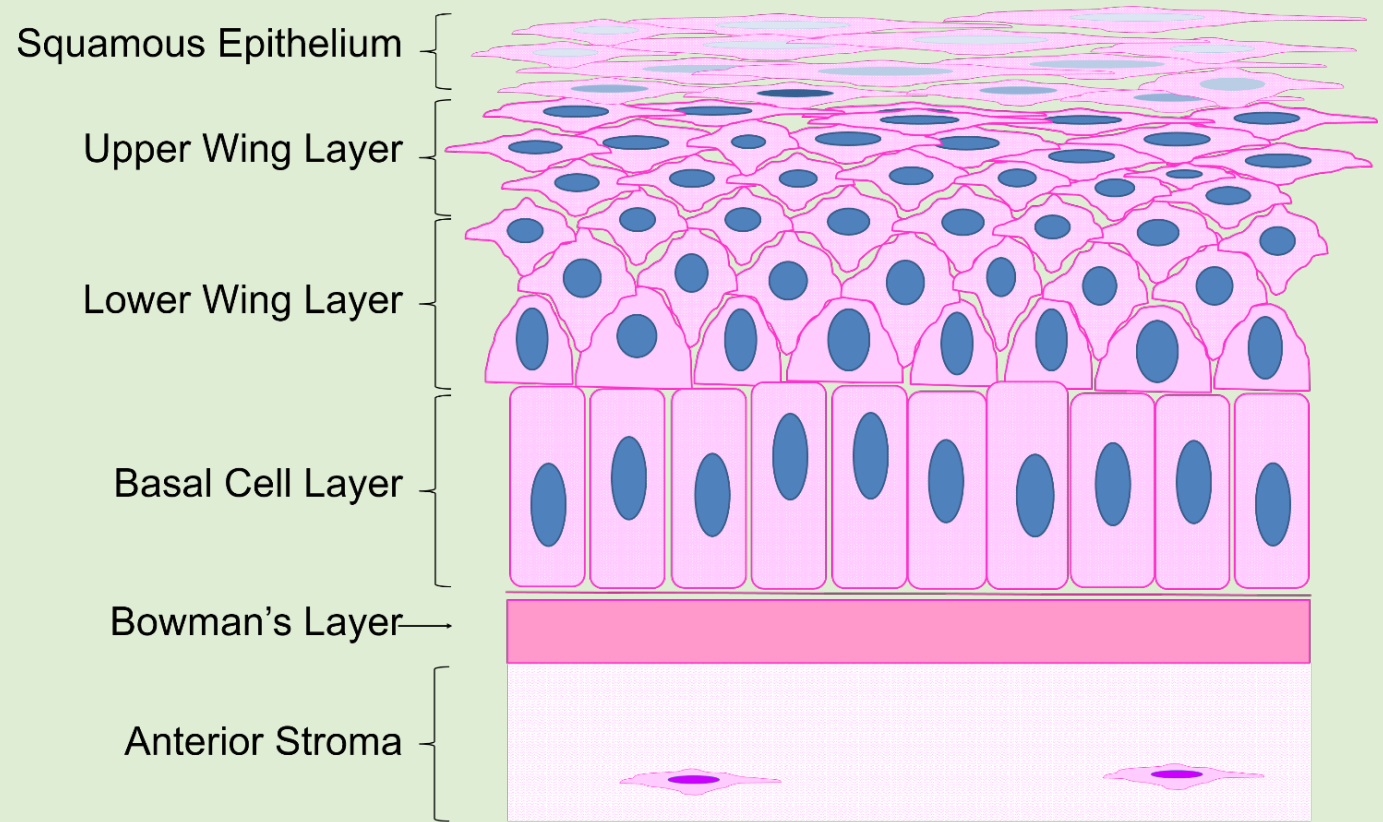
ORGANISM RESPONSE

- increased corneal or conjunctival susceptibility to xenobiotics
- pain and nociceptive responses
- induction of lachrymation
- transient or permanent loss of visual acuity

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Superficial Conjunctival or Corneal Epithelium

3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Vitrigel-Eye Irritancy
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Porcine Cornea Opacity Reversibility Assay
Ex Vivo Eye Irritation Test (EVEIT)
Fluorescein Leakage
Short Time Exposure
Neutral Red Release
Cytosensor Microphysiometer
Ocular Irritation
OptiSafe

Wing Cell Layer of the Epithelium

3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Vitrigel-Eye Irritancy
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Porcine Cornea Opacity Reversibility Assay
Ex Vivo Eye Irritation Test (EVEIT)
Ocular Irritation
OptiSafe

Lower Wing Cell and Basal Cell Layers of the Epithelium

3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Bovine Corneal Opacity and Permeability
Porcine Cornea Opacity Reversibility Assay
Isolated Chicken Eye
Isolated Rabbit Eye
Ex Vivo Eye Irritation Test (EVEIT)
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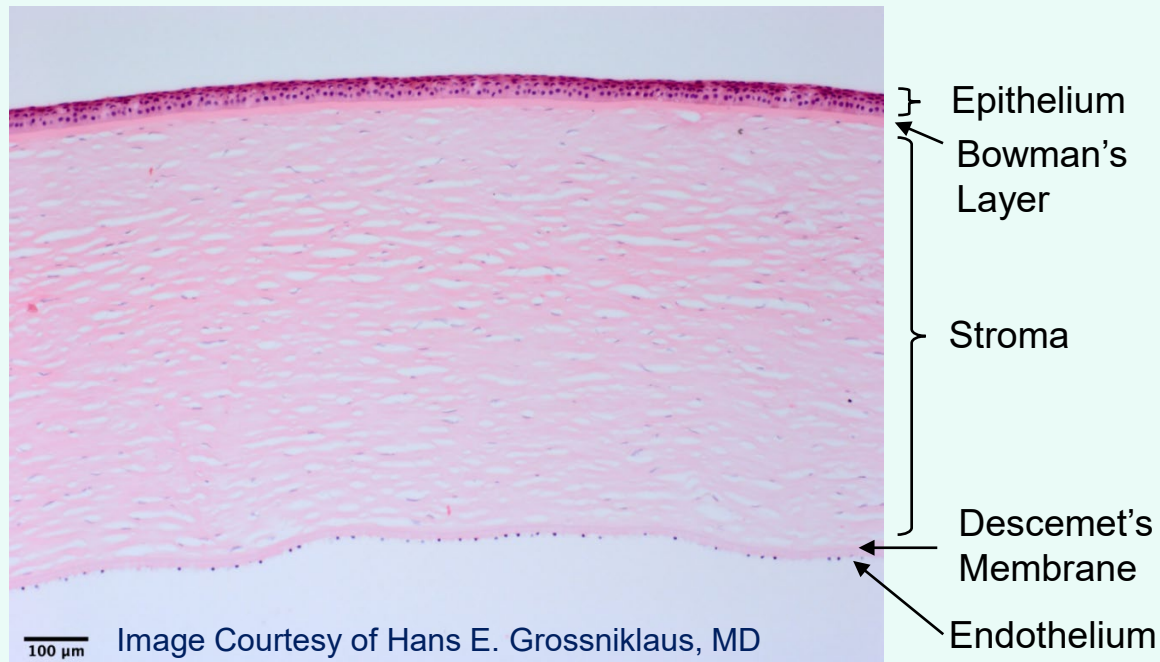
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MAIN RESULTS

- The available *in vitro* and *ex vivo* methods are as or more robust and reflective of human biology and mechanisms than the rabbit test.



IMPACT

- The rabbit test is not a suitable reference method. Rather, the scientific validity of an *in vitro/ex vivo* method should be assessed by understanding its relevance to human biology and mechanisms.
- Considering the variability of the currently used rabbit test and an understanding of human biology and mechanisms of eye irritation, data from the *in vitro/ex vivo* methods should be used at this time.

Next steps

- Eye irritation: Publication on prospective testing of agrochemical formulations in *in vitro* and *ex vivo* methods.
- Establishing scientific confidence:
 - SOT workshop and WC12 session
 - Upcoming guidance documents



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SOT Workshop: Establishing Scientific Confidence in New Approach Methods: A Modern Framework
Monday 20th March, 1:45-4:30pm