
UNITED STATES
ICCVAM
Advancing Alternatives
to Animal Testing

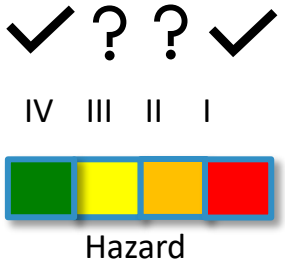
New ICCVAM Activities for 2021-2022

Warren Casey, PhD, DABT
NIEHS-NTP
September 28-29, 2021

Agency for Toxic Substances and Disease Registry • Consumer Product Safety Commission • Department of Agriculture • Department of Defense
Department of Energy • Department of the Interior • Department of Transportation • Department of Veterans Affairs Office of Research and Development
Environmental Protection Agency • Food and Drug Administration • National Cancer Institute • National Institute for Occupational Safety and Health
National Institute of Environmental Health Sciences • National Institute of Standards and Technology • National Institutes of Health
National Library of Medicine • Occupational Safety and Health Administration

Rethinking how to Establish Confidence in NAMs

- Hazard categories used by regulatory agencies have been the predominant focus in developing testing approaches
- However, the objective is ultimately the prediction of human responses
- The concept of a 1:1 alignment with the in vivo reference classification is neither feasible nor scientifically justified considering the multiple issues associated with the rabbit eye test
- Need to rethink how to assess the validity of new methods and to evaluate test methods based on which are most reliable and relevant to the human response.
- Evaluate available in vivo, in vitro and ex vivo test methods
  - Consider human ocular anatomy and physiology and mechanisms of chemically-induced ocular irritation
  - Determine relevance to predicting eye effects in humans following exposure to substances (agrochemicals and formulations)



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

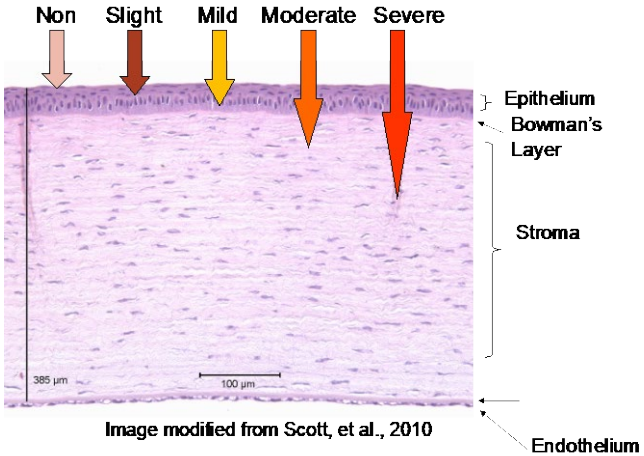


Image modified from Scott, et al., 2010

CUTANEOUS AND OCULAR TOXICOLOGY
https://doi.org/10.1080/15569527.2021.1910291
Taylor & Francis
Taylor & Francis Group
REVIEW ARTICLE
OPEN ACCESS
Check for updates

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 Y. Choksi[c], Anna J. van der Zalm[a], Nicole C. Kleinstreuer[d], João Barroso[e] and Anna B. Lowit[f]

[a]PETA Science Consortium International e.V., Stuttgart, Germany; [b]Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA; [c]Integrated Laboratory Systems, LLC, Research Triangle Park, NC, USA; [d]National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; [e]European Commission, Joint Research Centre (JRC), Ispra, VA, Italy; [f]US Environmental Protection Agency Office of Pesticide Programs, Washington, DC, USA

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.

ARTICLE HISTORY
Received 3 October 2020
Revised 21 February 2021
Accepted 22 March 2021

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

Summary

- Where discordant results exist between NAMs and the rabbit test, findings from the in vitro and ex vivo systems described herein should carry more weight than the rabbit data.
- The scientific validity of an in vitro/ex vivo method should be assessed by understanding the assay's relevance to human biology and mechanisms of eye irritation.
- Ultimately, a replacement method that provides a model grounded in human biology will be as good as or better than the currently used rabbit test at protecting human health

ICCVAM Workgroups and Expert Groups

- Acute Toxicity Workgroup
- Consideration of Alternative Methods Workgroup
- Ecotoxicology Workgroup
- IVIVE Workgroup
- Nanomaterials Workgroup
- Read Across Workgroup
- Validation Workgroup

- Developmental and Reproductive Toxicity Expert Group
- Developmental Immunotoxicity Expert Group
- FAIR Data Expert Group
- Metrics Expert Group
- Microphysiological Systems Expert Group
- Ocular and Dermal Irritation Expert Group
- Skin Sensitization Expert Group

https://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam/wg/index.html

ICCVAM Workgroups and Expert Groups

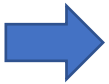
Acute Toxicity Workgroup ▲

Fostering the evaluation, promotion, and harmonization of alternatives to animal use for acute systemic toxicity testing has long been one of ICCVAM's priorities. The Acute Toxicity Workgroup evaluates the usefulness of various types of data, data analyses, and testing approaches for classifying and predicting acute oral, dermal, and inhalation systemic toxicity. The workgroup has members from six ICCVAM agencies.

Recent and ongoing activities include:

- Publishing a scoping document (Strickland et al. 2018) that identifies U.S. agency information requirements, needs, and decision contexts for acute systemic toxicity testing.
- Organizing a global project to develop in silico models of acute oral systemic toxicity that predict five specific endpoints needed by regulatory agencies (Mansouri et al. 2021).
- Analyzing variability of in vivo data used as benchmarks for evaluating alternative methods.
- Evaluating the usefulness of additivity formulas for hazard classification of formulations and mixtures (Hamm et al., 2021).
- Assessing the usefulness of available in vitro and in silico models for acute inhalation toxicity.
- Organizing a global project to develop in silico models of acute inhalation toxicity.

ICCVAM Workgroups and Expert Groups

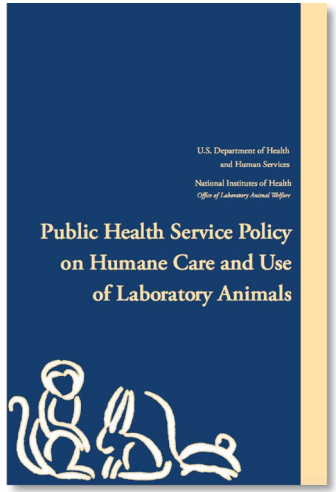


- Acute Toxicity Workgroup
- Consideration of Alternative Methods Workgroup
- Ecotoxicology Workgroup
- IVIVE Workgroup
- Nanomaterials Workgroup
- Read Across Workgroup
- Validation Workgroup

- Developmental and Reproductive Toxicity Expert Group
- Developmental Immunotoxicity Expert Group
- FAIR Data Expert Group
- Metrics Expert Group
- Microphysiological Systems Expert Group
- Ocular and Dermal Irritation Expert Group
- Skin Sensitization Expert Group

Consideration of Alternative Methods Workgroup

- ICCVAM Sponsor Agencies: USDA, NIEHS, DOD
- Work with stakeholders to publish a white paper on approaches that could potentially be used to foster the consideration and use of New Alternative Methodologies (NAMs) to replace, reduce, or refine the use of animals for testing.
- Publish a white paper on approaches that could potentially be used to foster the consideration and use of New Alternative Methodologies (NAMs) by organizations currently using animals for testing.
- Foster collaborations with authorities outside of the U.S. to share ideas and progress in order to promote greater harmonization in the requirements for considering alternatives



Helping Test Method Developers Understand Regulatory Testing Requirements

Helping Test Method Developers Understand Regulatory Testing Requirements

Guideline Number	Study Type	Food Use	Non Food Use
870.1100	Acute Oral – Rat	Required	Required
870.1200	Acute Dermal	Required	Required
870.1300	Acute Inhalation – Rat	Required	Required
870.2400	Primary Eye Irritation – Rabbit	Required	Required
870.2500	Primary Dermal Irritation – Rabbit	Required	Required
870.2600	Dermal Sensitization	Required	Required
870.6200	Acute Neurotoxicity – Rat	Required	Required
870.6100	Acute and Delayed Neurotoxicity - Hen	CR	CR
870.3100	90-Day Oral – Rodent (Rat/Mouse)	Required	CR
870.3150	90-Day Oral– Non Rodent (Dog)	Required	CR
870.3200	21/28-Day Dermal	Required	NR
870.3250	90-Day Dermal	CR	Required
870.3465	90-Day Inhalation – Rat	(CR)	CR
870.6200	90-Day Neurotoxicity - Rat	Required	Required
870.4100	Chronic Oral- Rat	Required	CR

Guideline Number	Study Type	Food Use	Non Food Use
870.4200	Carcinogenicity - Mouse	Required	CR
870.4200	Carcinogenicity –Rat	Required	CR
870.3700	Prenatal Developmental – Rat	Required	Required
870.3700	Prenatal Developmental – Rabbit	Required	Required
870.3800	Reproduction & Fertility Effects	Required	Required
870.6300	Developmental Neurotoxicity	CR	CR
870.5100	Bacterial Reverse Mutation Assay	Required	Required
870.5300/ .5375	In Vivo Mammalian cell Assay	Required	Required
870.5385/ .5395	In Vitro Cytogenetics	Required	Required
870.7485	Metabolism & Pharmacokinetics	Required	CR
870.7200	Companion Animal Safety	CR	CR
870.7600	Dermal Penetration	CR	CR
870.7800	Immunotoxicity	Required	Required

- These test are required by EPA (FIFRA) for each pesticide active ingredient
- Each repeat-dose study requires the examination of at least 45 tissue samples

ICCVAM Roadmap

ICCVAM Roadmap

Agency-Specific Workshops

Improving Consideration of NAMs

New Human-Relevant Approaches