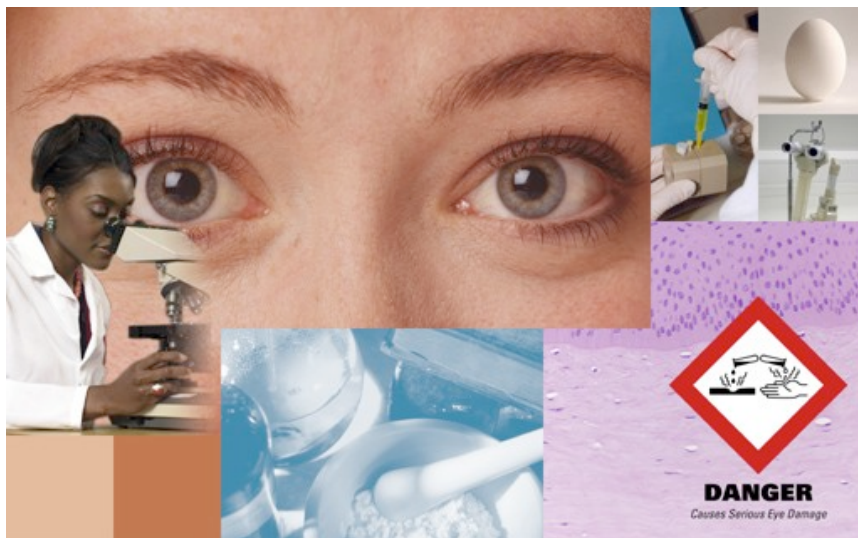


ICCVAM-NICEATM-ECVAM Ocular Toxicity Scientific Symposia



**William S. Stokes, DVM,
DACLAM**

**Captain, U.S. Public Health
Service**

Director, NICEATM

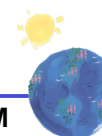
Executive Director, ICCVAM

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**National Toxicology Program Interagency
Center for the Evaluation of Alternative
Toxicological Methods**



**ICCVAM
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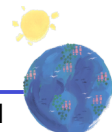
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Acknowledgements: NICEATM Staff

NIEHS

Raymond Tice, Ph.D.

Debbie McCarley

Center Support Contract (ILS, Inc.)

Jeffrey Charles, Ph.D.

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James Truax

Linda Litchfield

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Symposium on Mechanisms of Chemically-Induced Ocular Injury and Recovery



ICCVAM The Interagency Coordinating Committee on the Validation of Alternative Methods

NICEATM The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods


ECVAM European Centre for the Validation of Alternative Methods

Mechanisms of Chemically-Induced Ocular Injury and Recovery

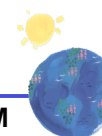
May 11-12, 2005
National Institutes of Health
Natcher Conference Center
Bethesda, Maryland

Sponsored by:
ICCVAM: The Interagency Coordinating Committee on the Validation of Alternative Methods
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COLIPA: The European Cosmetics, Toiletry and Perfumery Association

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Department of the Interior • Department of Transportation • Environmental Protection Agency • Food and Drug Administration • National Cancer Institute
National Institute of Environmental Health Sciences • National Institutes of Health, Office of the Director • National Institute for Occupational Safety and Health
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- Organized by ICCVAM, NICEATM, and ECVAM
- Sponsors
 - ICCVAM, NICEATM, ECVAM
 - European Cosmetic, Toiletry, and Perfumery Association (COLIPA)
- May 11-12, 2005
 - Bethesda, Maryland, USA
- Open to the public
 - 76 participants
 - 24 invited experts
- Discussion Panels:
 1. Mechanisms and biomarkers
 2. *In vitro* models
 3. *In vivo* endpoints and biomarkers



Symposium Goal

- **To review the state-of-the-science and understanding of the pathophysiology and mechanisms of chemically-induced ocular injury and recovery, in order to advance the development of test systems:**
 - **Necessary to meet regulatory testing requirements**
 - **That will provide for the protection of human health while reducing, refining (less pain and distress), and/or replacing the use of animals**

Symposium Invited Experts

Daniel Bagley, Ph.D.
Colgate Palmolive
Company

**Ellison Bentley, DVM,
DACVO**
University of Wisconsin

Monica Berry, Ph.D.
University of Bristol, UK

Roger Beuerman, Ph.D.
Louisiana State University

Michael Boulton, Ph.D.
Cardiff University, UK

Wiley Chambers, M.D.
US FDA

Rodger Curren, Ph.D.
Institute for In Vitro
Sciences

**George DeGeorge, Ph.D.,
DABT**
MB Research Laboratories

Odile de Silva, Ph.D.
L'Oreal/COLIPA, FR

**Henry F. Edelhauser,
Ph.D.**
Emory University

Marc Feldman, M.D.
The Cleveland Clinic

Donald A. Fox, Ph.D.
University of Houston

Larry Jackson, Ph.D.
NIOSH

James Jester, Ph.D.
Univ of California at
Irvine

**Pauline McNamee,
Ph.D.**
P&G/COLIPA, UK

Roswell Pfister, M.D.
Brookwood Medical
Center

**Donald Sawyer, D.V.M.,
Ph.D., DACVA,
HDABVP**
MINRAD, International

**Norbert Schrage, Dr.
Med.**
ACTO, GE

Janine Smith, M.D.
National Eye Institute

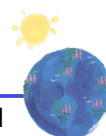
**Katherine Stitzel,
D.V.M.**
Consultant

Kirk Tarlo, Ph.D.
Allergan Inc.

**Charles S. Tressler,
M.D.**
Merck Research
Laboratories

John L. Ubels, Ph.D.
Calvin College

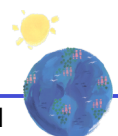
Sherry Ward, Ph.D.
Physicians Committee
for Responsible
Medicine



Panel 1: Mechanisms and Biomarkers of Ocular Injury

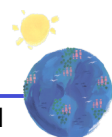
What are the currently known mechanisms and modes of action of chemically-induced ocular injury and recovery?

- Mechanisms of injury (e.g., cytotoxicity, protein coagulation, membrane saponification, disruption of extracellular matrix, inflammatory cell infiltration with release of mediators, upregulation of proteases and collagenases) are known for some chemicals and product types.
- Extent of ocular surface and depth of penetration (may) correlate with severity of lesions and recovery.
- Studies show species differences in response to the same chemical.
- Studies show dose-dependent differences in response to the same chemical (i.e., mechanisms of repair or injury at lower doses may not be relevant at higher doses).



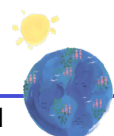
What are the current knowledge gaps in understanding of mechanisms and mode of action of chemically-induced ocular injuries and recovery?

- Further assessment of the relationship between type and severity of the initial *in vivo* damage and persistence.
- Biomarkers of injury and recovery (gene expression profiling, clustering and pathway analysis for ocular damage and repair)
- Role of tear film in ocular damage
- Translation of *in vivo* physiology to *in vitro* models
 - Role of metabolism
 - Corneal expression of specific proteins
 - Damage and recovery mechanisms



What research initiatives are needed to address current knowledge gaps and further characterize mechanisms and modes of action in order to advance the development and validation of predictive in vitro models of chemically-induced ocular injury and recovery?

- Evaluation of quantitative endpoints that could be incorporated into current *in vivo* test
 - Histopathology to correlate cellular changes with observational endpoints
 - HPLC and Mass Spec to evaluate penetration of substances
 - Depth of injury assessments
- Further evaluate species differences in response
- Further evaluate dose-dependent differences in response
- Further evaluate the role of tear film
 - Composition and role in protection
 - Consequence of tear film disruption
 - Impact on mild/moderate irritants



What research initiatives are needed to address current knowledge gaps and further characterize mechanisms and modes of action in order to advance the development and validation of predictive in vitro models of chemically-induced ocular injury and recovery?(cont)

- Early onset vs. delayed responses
- Toxicokinetics of chemicals in eye
- Role of inflammatory responses in observed damage
- Recovery mechanisms of the eye (effects on stem cells)
- Additional evaluation of *in vitro* models that may be more predictive/useful
 - Human corneal models (isolated and reconstituted)
 - Pig corneal models

What in vivo biomarkers (e.g., molecular, cellular, morphological, clinical) should be further investigated as predictive indicators of severity of lesions, reversibility vs. non-reversibility, or delayed responses)?

- Histopathology
- Quantitative endpoints obtained using standard biomicroscopy, confocal microscopy, selective staining, cytology, immunologic markers
- Gene expression profiling, clustering and pathway analysis for ocular damage and repair

Panel 2

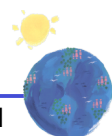
In Vitro Models of Ocular Injury and Recovery

What additional biomarkers should be considered for inclusion in in vitro test systems for ocular irritancy, or further investigated and/or developed for potential inclusion in such systems?

- Cytotoxicity - Consider as potential biomarker, but recognize advantages/limitations of current measures of cell death.
- Cytokine measurements as markers of potential inflammatory processes (e.g., edemagenic agents, chemotactic agents, PMN migration predictors); recognize that patterns of cytokine release may be cell/cell line specific.
- Depth of injury in *ex vivo* test methods.
- Histopathology

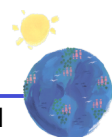
What in vitro test systems and biomarkers will be needed to adequately predict the ocular injury potential of chemicals and whether the damage would be reversible or irreversible?

- For detecting corrosives and severes, emphasis should be on *ex vivo* test systems (e.g., IRE, ICE, BCOP) as reconstructed models
 - However, at the present time, do not adequately mimic ocular anatomy/physiology
- Quantify depth of injury in *ex vivo* systems
- Histopathology
- Cell death may be useful biomarker of damage and could be correlated with *in vivo* results.
- Markers of inflammatory processes (e.g., cytokines, adhesion proteins).
- Determine if markers of endothelial cell damage (other than histopath) would be useful
- Determine if other model systems (e.g., human, pig) might be more useful
- Reversibility continues to be an issue
- A battery of *in vitro* test methods may likely be needed to better mimic the *in vivo* situation.



What are the current knowledge gaps with regard to differences in biomarker responses that occur *in vivo* and *in vitro* that should be addressed in research, development, and validation efforts?

- Ways to ensure that reconstructed *in vitro* models will adequately mimic *in vivo* ocular anatomy/physiology
- Critical inherent differences between corneas in *in vitro* and *in vivo* models
- Parameters needed to predict iridial and conjunctival damage
- Determination of whether cell death is a useful biomarker of ocular damage
- Correlation between depth of injury in *in vivo* and *ex vivo* systems.
- Biomarkers of inflammatory processes (e.g., cytokines, adhesion proteins).



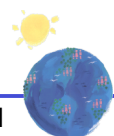
Panel 3

In Vivo Quantitative Objective Endpoints to Support Development and Validation of Predictive *In Vitro* Models

What quantitative objective endpoints/biomarkers should be considered for routine inclusion in the current *in vivo* rabbit eye test to support development and validation of predictive *in vitro* methods and improve hazard characterization and reliability?

- **Slit lamp biomicroscopy with fluorescein (or other vital dye) staining**
 - **Depth of injury assessment**
- **Pachymetry (for mild/moderate irritants only)**
 - **To assess corneal swelling**
- **Photodocumentation of injury**
 - **Gross overall eye**
 - **Slit lamp (where possible)**
- **Postmortem measures**
 - **Histopathology (various staining of preserved tissue)**
 - **Live/dead assay using fresh excised corneal tissue**

- **What quantitative objective endpoints/biomarkers should be considered for routine evaluation in human chemical injuries and ethical studies that might assist in the development and validation of predictive *in vitro* methods?**
 - **Standardized, comprehensive eye exam: fundus exam, slit lamp/fluorescein staining, visual acuity; iris, lens, IOP, anterior and posterior chamber evaluations**
 - **Pachymetry**
 - **Photodocumentation**
 - **Clinical outcome documented**
 - **A detailed description of the irritant agent and exposure conditions**
 - **Ideally, the same data should be collected in both humans and animal models!**




- **What are the current knowledge gaps with regard to potential quantitative objective endpoints/biomarkers that should be addressed in research, development, and validation efforts?**
 - **Data acquired using the following techniques:**
 - **Slit lamp biomicroscopy with fluorescein (or other vital dye) staining**
 - **Pachymetry (for mild/moderate irritants only)**
 - **Photodocumentation of injury-digitalized**
 - ✓ **Gross overall eye**
 - ✓ **Slit lamp (may not be practical)**
 - **Postmortem measures:**
 - ✓ **Histopathology (various staining of preserved tissue)**
 - ✓ **Live/dead cell assay using fresh excised tissue**
 - **The usefulness and limitations of each endpoint measurement will need to be assessed following generation of data**

Summary

- Objective quantitative endpoints and biomarkers should be used to assess the severity of chemically-induced ocular injuries in animal safety studies and human accidental exposures.
- The routine collection of objective quantitative ocular injury data can be expected to:
 - Provide insights into chemical-specific mechanisms of ocular injury and recovery
 - Support the development and validation of more predictive mechanism-based *in vitro* test models
 - Improve the accuracy and reliability of ocular hazard assessments
 - Aid in identifying predictive mechanism-based earlier humane endpoints

Symposium on Minimizing Pain and Distress in Ocular Safety Testing



ICCVAM The Interagency Coordinating Committee on the Validation of Alternative Methods

NICEATM The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods


ECVAM European Centre for the Validation of Alternative Methods

Minimizing Pain and Distress in Ocular Toxicity Testing

May 13, 2005
National Institutes of Health
Natcher Conference Center
Bethesda, Maryland

Sponsored by:
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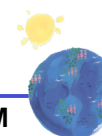
ICCVAM Agencies:
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Department of the Interior • Department of Transportation • Environmental Protection Agency • Food and Drug Administration • National Cancer Institute
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- Organized by ICCVAM, NICEATM, and ECVAM
- Sponsors
 - ICCVAM, NICEATM, ECVAM
 - European Cosmetic, Toiletry, and Perfumery Association (COLIPA)
- May 13, 2005
 - National Institutes of Health, Bethesda, MD, USA
- Open to the public
 - 56 participants
- Experts
 - Veterinary and human ophthalmology
 - Veterinary & human anesthesia
 - Toxicologists
 - Ophthalmic researchers

ICCVAM

NICEATM



Symposium Invited Experts

Ellison Bentley, D.V.M.,
DACVO
University of
Wisconsin-Madison

Roger Beuerman, Ph.D.
Louisiana State
University

Wiley Chambers, M.D.
US FDA

Marc Feldman, M.D.
The Cleveland Clinic

James Freeman, Ph.D., DABT
ExxonMobil

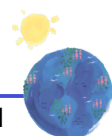
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Ph.D., DACVA, HDABVP
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ACTO,GE

Martin Stephens, Ph.D.
Humane Society of the United
States

Kirk Tarlo, Ph.D., DABT
Allergan



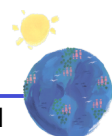
Current Ocular Testing: Sources of Pain and Distress

- Initial application of test article
- Post-application tissue injury



Focus of the Symposium

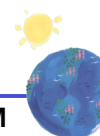
- **Can pre-application topical anesthetics be used routinely without interfering with the ocular hazard classification?**
- **Can pain and distress from induced eye injuries be routinely treated, as with human injuries, without interfering with the hazard classification?**
- **Can earlier more humane endpoints be identified to terminate studies before or at the onset of painful injuries?**



Ocular Pain:

Initial Application of Test Articles

- **Topical test article applications can be expected to usually cause more than momentary or slight pain and distress**
 - Any stimulation of the eye is sensed as some level of discomfort, irritation, or pain
 - Some level of discomfort is anticipated with the topical application of any substance
 - If it causes or would be expected to cause pain in humans, should assume that it will cause pain in animals
- **Clinical signs, lesions, and biomarkers indicative of ocular discomfort or pain**
 - Intermittent to repeated blinking and/or squinting (rabbits don't normally blink often)
 - Partial to complete closure of eye
 - Repeated pawing or rubbing of eye
 - Vocalization (although this is rarely observed)
 - Conjunctival hyperemia and chemosis
 - Increased blood pressure, respiration, heart rate

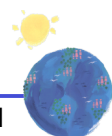


Ocular Pain: Chemically-Induced Injuries

- **What ocular lesions can be expected to be associated with more than minimal pain and discomfort?**
 - Based on human experience, any chemically-induced ocular lesion is likely to be associated with pain
 - Any scorable lesions
 - Note: Severity of the injury does not directly correlate with the severity of pain in humans

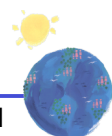
Avoiding and Minimizing Ocular Pain: Initial Test Article Applications

- **What are the optimal pre-application analgesics that should be considered?**
 - **Either topical or general anesthesia**
 - **Should be accompanied by pre-emptive systemic analgesia to minimize any treatment-related pain that might occur**
 - **Topical anesthetics**
 - **0.5% proparacaine is most common**
 - ✓ **Potential for delayed epithelial wound healing, esp. with repeated use**
 - ✓ **Single use not expected to cause effects that would alter classification categories**



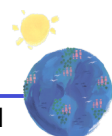
Avoiding and Minimizing Ocular Pain: Initial Test Article Applications

- **Is sufficient information available to support the routine use of pre-treatment topical anesthetics in regulatory ocular irritation/corrosivity testing?**
 - *Yes, there is sufficient understanding of their effectiveness and potential effects on toxicity testing to substantiate their routine use for testing for hazard classification purposes*
 - CPSC has routinely used pre-application topical anesthetics on all ocular toxicity studies since 1984
 - General anesthesia could also be considered as an option



Avoiding and Minimizing Ocular Pain: Initial Test Article Applications

- **What evidence is there that pre-treatment analgesics may alter the hazard classification outcome of animal ocular irritancy/corrosivity testing?**
 - Could exaggerate chemically-induced ocular injury by decreasing ocular defenses and extend the duration of injury by impairing repair processes
 - Topical anesthetics can increase ocular permeability, reduce normal blinking, reduce normal tearing, and delay wound healing, especially if used repeatedly
 - The net effect, if any, would be to a more severe hazard category
 - Topical anesthesia is used on over 2 million human cataract surgeries annually, with only rare complications from corneal healing



Avoiding and Minimizing Ocular Pain: Initial Test Article Applications

- **Research Needs**
 - Prospective use of such agents should include observations for efficacy in preventing pain from the initial application of chemical or test article

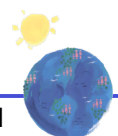
Avoiding and Minimizing Ocular Pain: Post-Application Injuries

- **Is there sufficient information available to support the routine use of anesthetics and analgesics for post-application ocular injuries in ocular safety testing?**

Systemic analgesics should be used for relieving pain associated with chemically-induced lesions

Analgesics should be routinely administered *prior to* initiation of the test

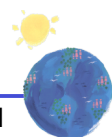
- More effective than treatment after a painful lesion has developed: Pre-emptive analgesia
- Analgesia should be maintained until the ocular lesions resolve or the study is terminated



Avoiding and Minimizing Ocular Pain: Post-Application Injuries

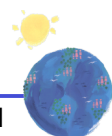
- **Would such treatment be expected to alter the hazard classification outcome of such studies?**

Systemic analgesics may alter intraocular pressure by alterations in aqueous outflow; to what extent this might affect hazard classification outcome is not known



Avoiding and Minimizing Ocular Pain: Post-Application Injuries

- **What topical or systemic agents would be the most appropriate for treatment of painful lesions, in terms of efficacy and duration?**
 - **Narcotic analgesics**
 - **Buprenorphine, longer duration of action vs. morphine**
 - **NSAIDs**
 - **Diclofenac, Indomethacin, Flurbiprofen, Ketorolac vary in potency and duration**
 - **Opiate Anxiolytics**
 - **Acepromazine, Butorphanol**
 - **Review of existing literature is necessary to identify the most appropriate agents, dosages, and dosing intervals**

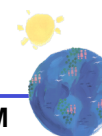


Avoiding and Minimizing Ocular Pain: Post-Application Injuries

- **Additional research needs:**
 - Evaluation of systemic analgesic agents, doses, and dosing intervals necessary to provide effective analgesia
 - Document any effects of analgesics on hazard category classification

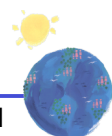
Biomarkers that Can Serve as Early Humane Endpoints for Ocular Injury

- **What current ocular lesions are sufficiently predictive of irreversible or severe effects that they should routinely be used as humane endpoints to terminate a study?**
 - Endpoints listed in current testing regulations
 - Vascularization of the corneal surface (pannus)
 - Destruction of > 75% of the limbus
 - Flourescein staining:
 - Indicative that the site of injury is not healing after 2-3 days(i.e., the stained area is not becoming smaller), and/or
 - Depth of injury is increasing in the days after the test substance is applied



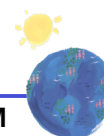
Biomarkers that Can Serve as Early Humane Endpoints for Ocular Injury

- **What other objective biomarkers (e.g., extent and depth of corneal damage) might be considered sufficiently predictive of severe/irreversible effects that they should be used as routine humane endpoints?**
 - **Lack of re-epithelialization five days after application of the test substance**
 - **The extent of the depth of injury to the cornea**
 - **Injuries where corneal ulcerations extend beyond the superficial layers of the stroma**
 - **Slit-lamp and fluorescein staining should be done routinely to evaluate**
 - **Flourescein staining of the conjunctiva**



Biomarkers that Can Serve as Early Humane Endpoints for Ocular Injury

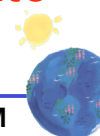
- **Are there other potentially more sensitive biomarkers that should be investigated as potential early endpoints?**
 - **Destruction of the limbus and relationship to re-epithelialization of the cornea**
 - **Impaired tear production (Schirmer's Tear Test)**
 - **Insufficient tears to prevent drying injuries to cornea**



Biomarkers that Can Serve as Early Humane Endpoints for Ocular Injury

- **What additional data are recommended for collection during future animal studies to aid in identifying earlier more humane endpoints for ocular testing?**
 - Slit lamp biomicroscopy with fluorescein staining (or other vital dye)
 - Depth of injury measurements`
 - Confocal microscopy preferred, but slit lamp estimates/photos also helpful
 - Pachymetry measurements
 - Photo -documentation of ocular injuries
 - Postmortem examinations
 - Histopathology
 - Live/Dead cell assay using fresh tissue

Guidelines with standardized procedures are needed to facilitate collection of data in a systematic fashion



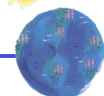
Biomarkers that Can Serve as Early Humane Endpoints for Ocular Injury

- **What are the knowledge gaps regarding predictive early humane endpoints that should be addressed in research, development, and validation efforts?**

Assessment of the usefulness of objective measurements and biomarkers as earlier humane endpoints

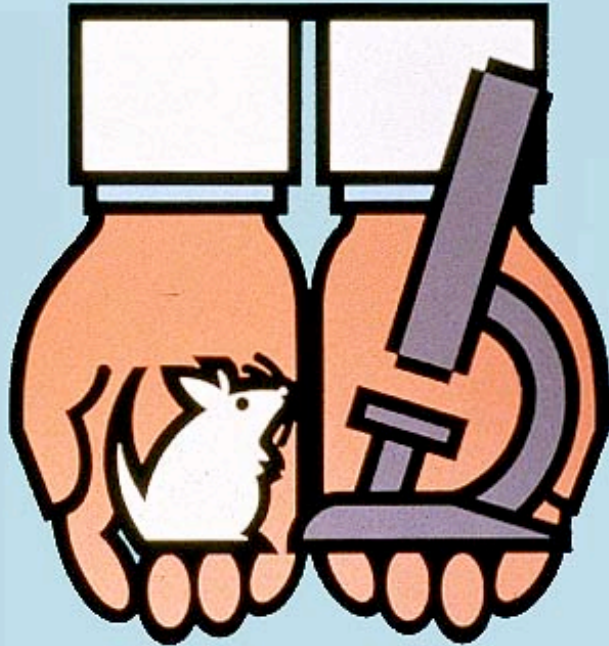
Summary: Reducing Pain and Distress in Ocular Safety Testing

- **Pre-treatment with topical (or general) anesthesia should be used routinely to avoid pain from topical application of test substances.**
- **Systemic analgesics should be administered prior to test article application and continued until injuries resolve or the study is terminated.**
- **Ocular injuries predictive of severe or irreversible ocular damage should be used as earlier humane endpoints.**
- **Objective quantitative measurements should be collected during ocular studies to assist in identifying earlier more humane endpoints**
 - **Also critical to development and validation of more predictive in vitro methods**





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A program sponsored by the
NIH Animal Research Committee
496-5424

Thank you for your
attention!

