Agenda

Ocular Toxicity Scientific Symposia

I. Mechanisms of Chemically-Induced Ocular Injury and Recovery: May 11-12, 2005

II. Minimizing Pain and Distress in Ocular Toxicity Testing: May 13, 2005

Organizers:

- The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
- The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
- The European Centre for the Validation of Alternative Methods (ECVAM)

Sponsors:

- NICEATM
- ICCVAM
- ECVAM
- The European Cosmetic, Toiletry, and Perfumery Association (COLIPA)

I. Mechanisms of Chemically-Induced Ocular Injury and Recovery: May 11-12, 2005

Symposium Goals

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

Symposium Objectives

- Review current and potential molecular, cellular, tissue (e.g., histopathology), and clinical (e.g., corneal opacity, swelling, depth of injury) biomarkers of chemical injury and recovery and their usefulness for *in vivo* and *in vitro* testing models of ocular irritancy and corrosivity.
- Identify knowledge gaps in understanding of chemically-induced ocular injury and recovery.
- Identify and prioritize future research initiatives that would address current knowledge gaps and that are considered necessary to advance the development and validation of *in vitro* models of chemically-induced ocular injury and recovery.
- Discuss and identify quantititative, objective endpoints that should be considered for inclusion in the current *in vivo* rabbit eye test and/or human clinical testing (e.g., more sensitive markers of injury and recovery) that would support development and validation of predictive *in vitro* methods and improve hazard characterization and reliability.

Symposium Agenda and Topics

Day 1 Wednesday, May 11, 2005

0800 Registration

0830 Welcome and Introduction of Symposium Objectives
- William Stokes, DVM, DACLAM (NICEATM)

Session 1 – Overview of Recent Initiatives and Regulatory Requirements for Ocular Toxicity Testing
Summary of previous workshops including major conclusions, recommendations, and initiatives
Co-Chairs: William Stokes, DVM, DACLAM (NICEATM)
Leonard Schechtman, PhD (US Food and Drug Administration)

0845 ILSI-HESI Symposium (1995): Replacing the Draize Eye Irritation Test: Scientific Background and Research Needs)
- Kathy Stitzel, DVM (Consultant)

0900 ILSI-HESI Working Group (1996): Ophthalmologic Perspectives on Eye Irritation Testing
- Wiley Chambers, MD (US Food and Drug Administration)

0915 COLIPA Workshop (1997): Mechanisms of Eye Irritation
- Pauline McNamee, PhD (Procter and Gamble, COLIPA)

0930 **Overview of Research on Chemically Induced Ocular Injury Funded by the National Eye Institute**
- Janine Smith, MD (National Eye Institute)

0945 **Regulatory Requirements and Need for *In Vivo* Rabbit Eye Testing Data**
(testing protocols, endpoints, classification and labeling)
- Debbie McCall (US Environmental Protection Agency)

1015 **Break**

Session 2 – Current Ocular Injury and Toxicity Assessments
Chair: Amy Rispin, PhD (US Environmental Protection Agency)

1030 **Extent of Human Chemically-Induced Ocular Injury**
(incidence, causes of ocular injuries)
- Larry Jackson, PhD (National Institute of Occupational Safety and Health)

1100 **Current Regulatory Assessment of Injury Responses in the Rabbit Eye**
- William Stokes, DVM, DACLAM (NICEATM)

1130 **Clinical Assessments of Chemical Eye Injuries: Injury Characterization and Quantification, Treatment, Outcomes (Permanent vs. Reversible) for Corneal, Conjunctival, and Irital Lesions**
- Roswell Pfister, MD (Brookwood Medical Center)

1215 **Lunch**

Session 3 – Mechanisms and Biomarkers of Ocular Injury and Recovery
Co-Chairs: Jill Merrill, PhD (US Food and Drug Administration)
Marianne Lewis (US Environmental Protection Agency)

1300 **Tissue and Cellular Responses to Chemical Eye Injuries: Cornea, Conjunctiva, Iris**
(extent and nature of damage to cornea, sclera, and iris alone or in combination in humans and rabbits; reversible vs irreversible damage)
- Henry Edelhauser, PhD (Emory University)

1350 **Mechanisms and Modes of Action Associated with Various Chemical Types**
- Sherry Ward, PhD (Physicians Committee for Responsible Medicine)

1405 **Histopathology of the Chemically Injured Eye; Depth of Injury as a Biomarker of Reversibility/Irreversibility**
- James Jester, PhD (University of California at Irvine)

1435 Toxicogenomic Responses in Chemically- Injured Eye Tissues
- Michael Boulton, PhD (Cardiff University)

1455 Break

1505 Role of Chemical Absorption and Metabolism in Ocular Injuries
(delayed onset of irritant/corrosive effect, how absorption and/or metabolism is related to specific chemical/substance types and/or physical states, chemical toxicokinetics and detoxification processes in the eye)
- Donald Fox, PhD (University of Houston)

1525 Measuring Chemical Effects on Tear Film and the Consequences of Tear Film Disruption on Ocular Injury
- Monica Berry, PhD (University of Bristol)

1545 Panel Discussion 1: Mechanisms and Biomarkers of Ocular Injury and Recovery
Moderator: Kathy Stitzel, DVM (Consultant)
Panelists: Monica Berry, Michael Boulton, Wiley Chambers, Henry Edelhauser, Donald Fox, James Jester, Debbie McCall, Pauline McNamee, Roswell Pfister, Janine Smith, Sherry Ward

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

1700 Close of Day 1
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1730-1900 Reception

Day 2 Thursday, May 12, 2005

0800 Registration

Session 4 – Current *In Vitro* Models of Ocular Injury and Recovery
Brief descriptions of current in vitro research and testing models and the biomarkers of injury and/or recovery assessed in each system

Co-Chairs: Karen Hamernik, PhD (US Environmental Protection Agency)
Chantra Eskes, Eng., PhD (ECVAM)

0830 Reconstructed Corneal Models
- Dan Bagley, PhD, DABT (Colgate-Palmolive)

0845 Isolated Corneal Models
- John Ubels, PhD (Calvin College)

0900 Isolated Whole Eye Models
- David Allen, PhD (ILS, Inc., NICEATM)

0915 Cellular Assays
- Chantra Eskes, Eng., PhD (ECVAM)

0930 Vascular Assays
- Neepa Choksi, PhD (ILS, Inc., NICEATM)

0945 3-Dimensional Epithelium
- Monica Berry, PhD (University of Bristol)

1000 Long-Term Culture System
- Pauline McNamee, PhD (Procter and Gamble, COLIPA)

1015 Break

1030 Panel Discussion 2: Current *In Vitro* Models of Ocular Injury and Recovery
Moderator: George DeGeorge, PhD, DABT (MB Research Laboratories)
Panelists: Monica Berry, Michael Boulton, Rodger Curren, Odile de Silva, Henry Edelhauser, James Jester, Pauline McNamee, Sherry Ward

- 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?
- 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?
- 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?

1200 Lunch

Session 5 – *In Vivo* Quantitative Objective Endpoints to Support Development and Validation of Predictive *In Vitro* Models

Discuss and identify quantitative objective endpoints that should be considered for inclusion in the current in vivo rabbit eye test, human chemical eye injury assessments, or in ethical human studies. Discuss which of these endpoints might serve as more sensitive markers of injury and recovery that would support development and validation of predictive in vitro methods and improve hazard characterization and reliability

Co-Chairs: Wiley Chambers, MD (US Food and Drug Administration)
Meta Bonner, PhD (US Environmental Protection Agency)

1300 Objective Measures for Animal Studies
(e.g., depth of injury, corneal swelling, fluorescein staining)
- Ellison Bentley, DVM, DACVO (University of Wisconsin)

1330 Objective Measures for Accidental Human Chemical Injuries
(e.g., corneal swelling, fluorescein staining)
- Roswell Pfister, MD (Brookwood Medical Center)

1400 Objective Measures for Ethical Human Studies
(e.g., indicators of non-irritation or non-classifiable minor effects)
- Charles Tressler, MD (Merck)

1430 Break

1445 Panel Discussion 3: In Vivo Quantitative Objective Endpoints to Support Development and Validation of Predictive *In Vitro* Models
Moderator: Donald Fox, PhD (University of Houston)
Panelists: Ellison Bentley, Henry Edelhauser, George DeGeorge, James Jester, Roswell Pfister, Janine Smith, Charles Tressler

- What quantitative objective endpoints/biomarkers (e.g., depth of injury, corneal swelling, fluorescein staining) should be considered for routine inclusion in the current *in vivo* rabbit eye test in order to support development and validation of predictive in vitro methods and improve hazard characterization and reliability?
- What quantitative objective endpoints/biomarkers should be considered for routine evaluation in human chemical injuries and ethical studies that might assist in development and validation of predictive in vitro methods?
- What are the current knowledge gaps with regard to potential quantitative objective endpoints/biomarkers that should be addressed in research, development, and validation efforts?

1545 Break

1600 Summary of Symposium Discussions

- **Mechanisms and Biomarkers of Ocular Injury and Recovery**
  - Jill Merrill, PhD (US Food and Drug Administration) - in conjunction with Marianne Lewis (US Food and Drug Administration) and Kathy Stitzel, DVM (Consultant)
- ***In Vitro* Models of Ocular Injury and Recovery**
  - Karen Hamernik, PhD (Us Environmental Protection Agency) - in conjunction with Chantra Eskes, Eng., PhD (ECVAM) and George DeGeorge, PhD (MB Research Laboratories)
- **Quantitative Endpoints for *In Vivo* Studies**
  - Wiley Chambers, MD (US Food and Drug Administration) - in conjunction with Meta Bonner, PhD (US Environmental Protection Agency) and Donald Fox, PhD (University of Houston)

1700 Close of Meeting

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II Symposium on Minimizing Pain and Distress in Ocular Toxicity Testing: May 13, 2005

Current regulatory testing procedures require the use of animals to determine the potential for chemicals and products to injure the eye. However, pain and distress often occur in such testing from the initial instillation of the test substance into the eye and from subsequent chemical-induced damage to ocular tissues. While testing guidelines state that pretreatment with topical anesthetics and treatment of ocular lesions can be considered, Good Laboratory Practice regulations state that such treatments should only be undertaken if there is assurance that they will not interfere with the outcome of the study. Some studies suggest that topical anesthetics may enhance or dampen ocular responses (Durham, 1992). Accordingly, current testing guidelines do not provide for the routine use of pre- and post-treatment anesthetics to prevent and/or relieve more than minimal pain and distress. This symposium will review current understanding of the sources and mechanisms of pain and distress in human eye injuries and ocular toxicity testing; identify current best practices for preventing, recognizing, and alleviating ocular pain and distress in ocular toxicity testing; and identify additional research, development, and validation studies necessary to support scientifically valid ocular testing procedures for hazard classification purposes that do not involve pain and distress.

Symposium Goal

To review current understanding of the sources and mechanisms of pain and distress in ocular toxicity testing; identify current best practices for preventing, recognizing and alleviating ocular pain and distress; and to identify additional research, development, and validation studies necessary to support scientifically valid ocular testing procedures that avoid pain and distress.

Symposium Objectives

- Review the pain resulting from accidental chemical ocular exposures and injuries to humans, and pain associated with the various types and severities of chemically-induced ocular damage; review clinical signs and lesions known to be indicative of ocular pain in animals, review clinical biomarkers of ocular pain and distress.
- Review current knowledge of the pathophysiology and mechanisms by which chemical eye injury induces pain.

- Review current understanding of the potential effects of pretreatment with topical anesthetics on hazard classification outcome of toxicity studies, and identify research and/or validation studies needed to support the routine use of pretreatment anesthetics that do not alter hazard classification outcome.
- Review the current understanding and identify knowledge gaps regarding: the effectiveness of various topical ophthalmic anesthetics and/or systemic analgesics in relieving pain associated with chemically-induced lesions; treatment regimes and frequencies necessary to alleviate pain and distress; the potential influence of such agents on the hazard classification outcome of *in vivo* ocular irritancy testing; and strategies for alleviation of pain and distress that provide for accurate hazard classification.
- Review current understanding and identify knowledge gaps regarding types and severity of lesions involving pain that are indicative of irreversible or persistent (>7≤21 days, or >21days) chemically-induced effects in humans and animals, and that could be used as routine earlier endpoints for terminating a study.

Symposium Agenda and Topics

0800 Registration

0830 Welcome and Introduction of Symposium Objectives
- William Stokes, DVM, DACLAM (NICEATM)
- Leonard Schechtman, PhD (US Food and Drug Administration)

0845 An Overview of the IRAG Workshop on Updating Eye Irritation Test Methods (1991): Use of Ophthalmic Topical Anesthetics
- Wiley Chambers, MD (US Food And Drug Administration)

Session 1 – Recognition and Sources of Pain in Ocular Injuries and Ocular Safety Testing

Co-Chairs: Abby Jacobs, PhD (US Food And Drug Administration)
Kailash Gupta, DVM, PhD (US Consumer Product Safety Commission)

0900 Human Ocular Injury and Sources of Pain
(review of the scenarios that result in pain in humans from both the initial accidental exposure to chemicals and products as well as pain that results from subsequent injury to ocular tissues, e.g,, conjunctiva, cornea, iris)
- Marc Feldman, MD (The Cleveland Clinic)

0930 **Recognition of Chemically-Induced Pain in Ocular Toxicity Studies in Animals**
(review pain and clinical signs indicative of pain and distress resulting from the initial treatment with solids, liquids, or pastes, and from subsequent lesions; review the duration and severity of pain associated with the initial topical application of various types of chemicals and products)
- Roger Beuerman, PhD (Louisiana State University)

1000 **Mechanisms and Biomarkers of Chemically-Induced Pain**
- Roger Beuerman, PhD (Louisiana State University)

1030 **An *In Vitro* Assay that is Predictive of Discomfort *In Vivo***
- Kirk Tarlo, PhD, DABT (Allergan)

1045 **Break**

1100 **Panel Discussion 4: Clinical Signs, Lesions and Other Biomarkers of Pain and Distress in Animals**
Moderator: Maggie Snyder, PhD (National Institutes of Health)
Panelists: Ellison Bentley, Roger Beuerman, Wiley Chambers, Marc Feldman, Debbie McCall, Roswell Pfister, Donald Sawyer, Norbert Schrage, Kirk Tarlo

- Pain and Distress Associated with Initial Application of Test Articles
  - What clinical signs, lesions, or other biomarkers are indicative of pain associated with initial test article application in ocular irritancy testing?
  - Do all substances cause more than minimal pain and distress when applied to the eye? If not, how can substances be identified that will ensure that they will not cause discomfort and could therefore be applied without a topical anesthetic?
  - Are there any physicochemical properties (e.g., pH, solids) that can be expected to cause more than minimal pain and distress on initial application that should always be preceded by topical anesthetics? Are there any properties that indicate that there will not be discomfort from the initial substance application?
  - How long can the pain associated with initial ocular application be expected to last?
- Pain and Distress from Chemically-Induced Ocular Injury
  - What clinical signs and ocular lesions can be expected to be associated with more than minimal pain and discomfort?
  - Are there gaps in our knowledge regarding the severity and duration of pain associated with the range and severity of ocular lesions in animals? If so, how might these be addressed?

1145 **Lunch**

Session 2 – Alleviation and Avoidance of Ocular Injury and Pain

Chair: Robert Bronaugh, PhD (US Food and Drug Administration)

1230 Options for Alleviating Ocular Pain and Distress in Humans

(review evidence of efficacy, duration of effect, and effects on repair for agents used topically, systemically and locally)

- Marc Feldman, MD (The Cleveland Clinic)

1300 Options for Avoiding/Minimizing Ocular Pain and Distress in Animals - Use of Analgesics and Anesthetics

(review evidence of efficacy, duration of effect, and known or expected effect on study outcome when used as a pre-application treatment or as a post-application treatment, using either local or systemic administration)

- Donald Sawyer, DVM, PhD, DACVA, HDABVP (MINRAD, Inc.)

1330 Panel Discussion 5: Avoiding/Minimizing Pain and Distress

Moderator: William Stokes, DVM, DACLAM (NICEATM)

Panelists: Ellison Bentley, Marc Feldman, Debbie McCall, Donald Sawyer, Norbert Schrage, Martin Stephens

- Initial Test Article Applications
  - What are the optimal pre-treatment analgesics that should be considered? What is the duration of analgesia that can be expected?
  - What evidence is there that pre-treatment analgesics may alter the hazard classification outcome of animal ocular irritancy/corrosivity testing? What alterations are known to occur, and what effect would these have on the outcome of the test with regard to current hazard classification categories, if any?
  - Is there sufficient information and data available to substantiate the routine use of pre-treatment topical anesthetics in regulatory ocular irritation/corrosivity testing? If not what additional investigations would be necessary to develop and validate strategies that would avoid pain and distress from initial test article applications?
- Post Treatment Analgesia and Topical Anesthesia
  - Is there sufficient information available to support the routine use of anesthetics and analgesics for post-treatment ocular lesions that cause or can be expected to cause pain and discomfort in ocular safety testing? Would such treatment be expected to alter the hazard classification outcome of such studies? Is there any reason why a rabbit with an eye lesion still present after the first few hours should not be given systemic pain relief?
  - Since it is possible for a corneal abrasion to get infected, and since one rabbit with a severe effect can drive the regulatory classification of a test substance, should measures be taken to prevent secondary infections since such an outcome could result in overclassification?

- What agents would be the most appropriate for treatment of painful lesions, in terms of efficacy and duration?
- What additional research would be necessary to support the development and validation of treatment strategies that would avoid pain and distress from ocular injuries during testing without altering hazard classification outcome?

1415 Break

Session 3 – Biomarkers Predictive of Severe and/or Irreversible Effects that Might Serve as Earlier Humane Endpoints for Ocular Studies

Co-Chairs: Donnie Lowther (US Food and Drug Administration)
Marilyn Wind, PhD (US Consumer Product Safety Commission)

1430 Current "Humane" Endpoints in Ocular Toxicity Testing

- William Stokes, DVM, DACLAM (NICEATM)

1445 Early Adverse Responses Predictive of Ocular Injury Outcome in Humans
(e.g., depth of injury, damage to limbal cells)

- Norbert Schrage, Prof. Dr. med. (Aachen Center for Technology Transfer in Ophthalmology)

1515 Panel Discussion 6: **Biomarkers that Can Serve as Early Humane Endpoints for Ocular Injury**

Moderator: James Freeman, PhD, DABT (ExxonMobil)
Panelists: Ellison Bentley, Roger Beuerman, Wiley Chambers, Marc Feldman, John Redden, Donald Sawyer, Norbert Schrage, Kirk Tarlo

- What current ocular lesions and severity are sufficiently predictive of irreversible or severe effects (GHS Category 1, US EPA Category I, EU R41) that they should routinely be used as humane endpoints to terminate a study as soon as they are observed?
- Are there other objective biomarkers (e.g., extent and depth of corneal damage) that are or would be considered sufficiently predictive of severe or irreversible effects that they should be used as routine humane endpoints?
- Are there other potentially more sensitive biomarkers that are indicative of severe or irreversible effects that should be investigated for their usefulness as early endpoints?
- Are there other earlier biomarkers/criteria indicative that painful lesions can be expected to fully reverse to US EPA Category II (<21 days) or III lesions (<7 days) and which could thus be used as a basis for early termination of studies?
- Are there additional data that are recommended for collection during future animal studies that might aid in identifying earlier more humane endpoints for ocular testing?
- What are the knowledge gaps regarding predictive early humane endpoints that should be addressed in research, development, and validation efforts?

1630 Summary of Symposium Discussions

- **Recognition and Sources of Pain in Ocular Injuries and Ocular Safety Testing**
  - Abby Jacobs, PhD (US Food and Drug Administration) - in conjunction with Kailash Gupta, DVM, PhD (US Consumer Product Safety Commission) and Maggie Snyder, PhD (National Institutes of Health)
- **Alleviation and Avoidance of Ocular Injury and Pain**
  - Robert Bronaugh, PhD (US Food and Drug Administration) - in conjunction with William Stokes, DVM, DACLAM (NICEATM)
- **Biomarkers that Could Serve as Early Humane Endpoints**
  - Marilyn Wind, PhD (US Consumer Product Safety Commission) - in conjunction with Donnie Lowther (US Food and Drug Administration) and James Freeman, PhD (ExxonMobil)

1700 Adjournment

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Previous Workshops/Symposia on Chemically-Induced Ocular Injury and Recovery

Related workshops and symposia that have been held in recent years are listed below. Selected related reviews are also provided. The ICCVAM/ECVAM symposium will review progress made since these meetings relevant to the Symposium objectives, and develop recommended initiatives needed to support future progress.

- **ILSI/HESI Workshop on Replacing the Draize Eye Irritation Test: Scientific Background and Research Needs, 1995** (In conjunction with Association for Research in Vision and Ophthalmology Meeting)
  - ILSI Health and Environmental Sciences Institute 1996. Replacing the Draize eye irritation test: scientific background and research needs. J. Toxicology – Cutaneous and Ocular Toxicology, 15:211-234.
  - Reviewed the current methods for predicting eye irritation
  - Discussed problems with the current *in vitro* models
  - Highlighted future research needs and how such information might facilitate development of improved alternative tests

- **ILSI/HESI Technical Committee on Alternatives to Animal Testing Expert Meeting on Eye Irritation Testing, September 29-30, 1996**
    - Nussenblatt et al. 1998. Ophthalmologic Perspectives on Eye Irritation Testing. Journal of Toxicology-Cutaneous and Ocular Toxicology 17:103-109.
    - Purpose was to provide an ophthalmologic perspective for future research to develop better nonanimal eye irritation test methods
    - Another purpose was to identify scientific approaches for generating human and animal data most likely to lead to development of alternative test methods

- **COLIPA Workshop on Mechanisms of Eye Irritation, October 5-8, 1997; Brighton, UK**.
    - Bruner et al. 1998. Report on the COLIPA Workshop on Mechanisms of Eye Irritation. ATLA 26:811-820
    - Reviewed the state of the science and identified a research program for development of alternatives based on a better understanding of eye injury and wound repair mechanisms

Selected References

Bruner et al. 1998. Report on the COLIPA Workshop on Mechanisms of Eye Irritation. ATLA 26:811-820.

ILSI Health and Environmental Sciences Institute 1996. Replacing the Draize eye irritation test: scientific background and research needs. J. Toxicology – Cutaneous and Ocular Toxicology, 15:211-234.

Nussenblatt et al. 1998. Ophthalmologic Perspectives on Eye Irritation Testing. Journal of Toxicology-Cutaneous and Ocular Toxicology 17:103-109.

Wagoner, MD. 1997. Chemical Injuries of the Eye: Current Concepts in Pathophysiology and Therapy. Survey of Ophthalmology 41:275-313.

Previous Workshop on Minimizing Pain and Distress in Ocular Toxicity Testing

One related workshop has been held on this topic. The ICCVAM/ECVAM symposium will review progress made subsequent to this workshop relevant to the symposium objectives.

- **IRAG 1991 Workshop on Updating Eye Irritation Test Methods—Proposal for Regulatory Consensus**
  - Seabaugh et al. 1993. Use of Ophthalmic Topical Anaesthetics. Food and Chemical Toxicology 31:95-98.
  - Largely focused on reduction and refinement measures, due to the insufficient development of *in vitro* test methods that could replace the in vivo rabbit eye test.
  - Further substantiated and recommended the use of 3 animals instead of 6 for the Draize eye test.
  - Recommended anesthetic pretreatment before conducting test; analgesics/anesthetics where pain induced

Selected References

Durham RA, Sawyer DC, Keller WF, Wheeler CA. 1992. Topical ocular anesthetics in ocular toxicity testing: A review. Laboratory Animal Science 42: 535-541.

Seabaugh et al. 1993. Use of Ophthalmic Topical Anaesthetics. Food and Chemical Toxicology 31:95-98.