Minimizing Pain and Distress in Ocular Toxicity Testing: Summary of an ICCVAM/NICEATM/ECVAM Scientific Symposium

May 13, 2005

Interagency Coordinating Committee on the Validation of Alternative Methods

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

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

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List of Abbreviations and Acronyms

BLS	U.S. Bureau of Labor Statistics
COLIPA	European Cosmetic, Toiletry, and Perfumery Association
CPSC	Consumer Products Safety Commission
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
EU	European Union
FDA	U.S. Food and Drug Administration
FHSA	U.S. Federal Hazardous Substances Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILS	Integrated Laboratory Systems
IRAG	Interagency Regulatory Alternatives Group
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	U.S. National Institute of Environmental Health Sciences
NSAIDs	Nonsteroidal anti-inflammatory drugs
NTP	U.S. National Toxicology Program
OECD	Organisation of Economic Cooperation and Development
OSHA	U.S. Occupational Safety and Health Authority
TSCA	Toxic Substances Control Act
USDA	United States Department of Agriculture

Acknowledgements

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Overview

The symposium “Minimizing Pain and Distress in Ocular Toxicity Testing” was organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the European Centre for the Validation of Alternative Methods (ECVAM) with support from the European Cosmetic, Toiletries and Perfumery Association (COLIPA). The symposium was held at the National Institutes of Health (NIH), Bethesda, Maryland, on May 13, 2005. The goals of the symposium were to (1) review current understanding of the sources and mechanisms of pain and distress in chemically induced ocular toxicity testing; (2) identify current best practices for preventing, recognizing, and alleviating ocular pain and distress; and (3) identify additional research, development, and validation studies to support scientifically valid ocular testing procedures that avoid pain and distress. Invited participants included human and veterinary ophthalmologists and anesthesiologists, scientific experts in ocular hazard testing, research scientists, U.S. Federal regulators, and industrial toxicologists. Implementation of recommendations from the symposium should eliminate most of the pain and distress associated with ocular safety testing in the rabbit Draize test.

1.0 Introduction

Societal concern for evaluating consumer products for ocular irritation and/or corrosion was heightened in 1933 when a 38-year-old woman went blind after her eyelashes and eyebrows were tinted with a product containing paraphenylenediamine, a chemical with the potential to cause allergic blepharitis, toxic keratoconjunctivitis, and secondary bacterial keratitis (Wilhelmus 2001). In 1938, the U.S. Congress responded to these concerns by enacting the Federal Food, Drug, and Cosmetic Act of 1938, which included extending the regulatory control of the U.S. Food and Drug Administration (FDA) to cosmetics (FDA 1938). This legislation required manufacturers to evaluate product safety before marketing their products (Wilhelmus 2001). Later, several additional legislative statutes were enacted to enable government agencies to regulate a variety of substances that could pose a risk to ocular health. **Table 1** provides a synopsis of current U.S. regulatory laws pertaining to eye irritation and corrosion.

Table 1 Summary of Current U.S. Legislation Related to Ocular Health*

Legislation (Year of Initial Enactment)	Agency	Substance
Food, Drug and Cosmetic Act (1938)	FDA	Pharmaceuticals and cosmetics
FIFRA (1947) and Federal Environmental Pesticide Control Act (1972)	EPA	Pesticides
FHSA (1964)	CPSC	Household products
FHSA (1964) and TSCA (1976)	Department of Agriculture and EPA	Agricultural and industrial chemicals
Occupational Safety and Health Act (1970)	OSHA	Occupational materials
Clean Air Act Amendments (1990)	Chemical Safety and Hazard Investigation Board and EPA	Accidentally released chemicals and air pollutants

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; EPA = U.S. Environmental Protection Agency; FDA = U.S. Food and Drug Administration; FHSA = U.S. Federal Hazardous Substances Act; FIFRA = Federal Insecticide, Fungicide and Rodenticide Act; OSHA = U.S. Occupational Safety and Health Administration; TSCA = Toxic Substances Control Act.

* Adapted from Wilhelmus (2001)

According to the Bureau of Labor Statistics (BLS), accidental eye injury is the leading cause of visual impairment in the U.S. (BLS 2003). In 2003, eye injuries from chemicals and their products (6,080) accounted for 16% of all eye injuries (36,940) reported as the cause of Days Away From Work for employees. Chemical products in general (e.g., solvents, caustics, soaps/detergents, cleaning/polishing agents, disinfectants) were responsible for approximately half of the injuries, whereas acids and alkalis accounted for 11% of the injuries.

The FDA issued requirements for ocular safety testing in response to the enacted consumer safety laws. The rabbit eye test was developed to identify and classify the ocular hazard potential of new chemicals or chemical products (Draize et al. 1944). The resulting hazard classification is then used to determine labeling requirements that will alert the public to take appropriate precautions in order to prevent ocular injury. Public concern about the use of animals in testing has resulted in significant

efforts to develop and validate alternative *in vitro* test methods for ocular hazard assessment. Despite over 25 years of effort, including several large validation studies (e.g., Balls et al. 1995; Gettings et al. 1996), there are still no validated and accepted non-animal ocular safety testing methods. Until valid alternatives are accepted as complete replacements, the animal test will continue to be required by U.S. Federal and European regulatory agencies for ocular hazard evaluation. One of the main concerns with this test method is the pain and distress that may be produced in the test animals.

Previous meetings and workshops have reviewed methods and strategies for reducing pain and distress in ocular safety testing (Seabaugh et al. 1993, Nussenblatt et al. 1988). However, current testing regulations and guidelines only suggest consideration of topical anesthetics after pain and distress is observed in the first animal tested. Routine pre-treatment with topical anesthetics is not recommended, and no mention of how to address post-application pain and distress associated with ocular damage exists. This symposium was organized to review the current understanding of ocular pain mechanisms and physiological pathways, symptoms and signs of the pain response, and methods and strategies that could be used to avoid or alleviate pain and distress, including the incorporation of earlier, more humane endpoints.

2.0 Symposium Objectives

The objectives of the symposium were to:

- Identify and better understand mechanisms of pain by reviewing the physiological pathways affected by chemically-induced ocular injury
- Review the known responses to chemical injury in humans (based on accidental exposures) and the levels of pain associated with specific ocular lesions
- Identify available approaches to:
  - Alleviate or avoid ocular pain resulting from initial test article application
    - Can pre-application topical anesthetics be used routinely without interfering with the ocular hazard classification?
  - Alleviate or avoid post-application ocular pain and distress
    - Can pain and distress from induced eye injuries be routinely treated, as with human injuries, without interfering with the hazard classification?
- Identify earlier, more humane endpoints to terminate studies before or at the onset of painful injuries

3.0 Overview of 1991 Interagency Regulatory Alternatives Group (IRAG) Workshop

In 1991, an *ad hoc* committee of the IRAG organized the workshop “Updating Eye Irritation Methods: Use of Ophthalmic Topical Anesthetics” (Seabaugh et al. 1993) to evaluate the use of anesthetics in eye irritation testing. Commonly used anesthetics, tetracaine (0.5-5%) and proparacaine (0.1-0.5%), produce an almost immediate effect lasting up to 20 minutes. These anesthetics eliminate local pain and touch sensation, but also increase ocular permeability, reduce tear volume, reduce blink frequency, and delay wound healing. The level of injury may be exaggerated by a reduction in ocular defense mechanisms (e.g., reduced tear fluid secretion), and duration of injury may be lengthened by impairment of repair processes (e.g., reduced collagen deposition). Despite these issues, and although not official policy of all U.S. Federal agencies, the use of anesthetics was considered acceptable by a consensus of those participating on the committee, since pain is at least temporarily relieved for the animal and the time and extent of injury can still be evaluated.

4.0 Symposium Sessions

Following are summaries of the information communicated by the speakers in each session of the symposium.

4.1 Recognition and Sources of Pain in Ocular Injuries and Ocular Safety Testing

Presenters for this session included Dr. Marc Feldman of the Cleveland Clinic, Dr. Roger Beuerman of Louisiana State University, and Dr. Kirk Tarlo, of Allergan, Inc.

4.1.1 Human Ocular Injury and Sources of Pain

The human pain response occurs through nociception accompanied by hypersensitivity with central and peripheral sensitization of the injured area. Nociception is an early warning sign, whereas inflammatory pain is present to reduce further injury. Nociceptive pain involves the descending track of the trigeminal nerve. Primary sensory neurons transduce the nociceptive signal, provide peripheral sensitization and produce transcriptional changes in ganglion cells. Numerous physical (e.g., heat, cold, pressure, mechanical) and chemical (e.g., capsaicin, bradykinin, cationic species) agonists are capable of activating nociceptors (e.g., acid sensing ion channels, purinergic receptors). Increased peripheral sensitization occurs from mediators released during the inflammatory process (e.g., bradykinin, prostaglandins) that induce receptor sensitization and activation. Inflammatory pain may lead to either neuropathic pain that is maladaptive and pathologic, or functional pain that limits mobility and perhaps serves as a mechanism to prevent further damage. Central sensitization from secondary hyperalgesia or tactile allodynia[1] has been reported. Disinhibition (e.g., reduced inhibitory transmission, altered modulation from brain) also may result in centrally induced hypersensitivity or late effects (e.g., diffuse pain sensitivity, sickness syndrome).

Treatment of a pain response associated with human ocular injury, therefore, should be based on knowledge of the location of its origin and the mechanism(s) involved in its production. Pain therapy should be guided toward the nociception, modulation, and sensitization components.

4.1.2 Mechanisms and Biomarkers of Chemically Induced Pain in Animals

The sensation of pain is unique and differs depending on the type of stimulation (e.g., thermal, mechanical). Pain intensity also varies with gender, age, and ethnicity, and is affected by stress and other environmental factors. In humans, pain assessment is based on verbal responses from the patient. However, an accurate assessment of chemically induced pain in animals requires an understanding of the mechanisms and biomarkers associated with pain, since the degree of pain cannot be assessed by vocalization. There are sensory nerve terminals located in the corneal epithelium and therefore, chemicals may elicit a pain response without producing noticeable damage. Numerous involuntary reflexes occur in response to painful stimuli in animals (e.g., tearing, blinking, head movement, vascular changes). The corneal pain system is linked to the neurogenic inflammatory response. Disruption of the tear film results in breakdown of the blood-conjunctiva barrier, platelet release mechanism activation, inflammatory cell infiltration, fibronectin deposition, and plasmin production. Disruption of the corneal epithelium results in intracellular calcium modulation, changes in metabolism and pH, inflammatory processes, and wound healing with maturation and repair. Various ion channels (e.g., calcium, sodium, potassium) are involved in the pain response and may be modulated to stimulate or abrogate the pain response.

Prediction of ocular discomfort also may be based on scoring blinking frequency along with the extent of conjunctival hyperemia. Discomfort is scaled using a score of 0 to 4 as normal, minimal

[1] *Allodynia* refers to pain from stimuli that are not normally painful. The pain may occur in areas other than those stimulated.

(intermittent blinking and/or squinting), mild (blinking and/or squinting with partial eye closure), moderate (repeated blinking and/or squinting; partial to complete eye closure), and severe (prolonged and complete closure of eye; repeated pawing or rubbing). Hyperemia is scored on a scale of 0 to 3 as normal, mild (flushed reddish palpebral conjunctiva with perilimbal dilation), moderate (crimson red palpebral conjunctiva with perilimbal dilation), and severe (dark beefy red palpebral conjunctiva with congestion of bulbar and palpebral conjunctiva and pronounced perilimbal dilation).

4.2 Panel Discussion on Indicators of Pain and Discomfort in Animals

With regard to initial test article application, the panel concluded that if a substance causes ocular pain in humans, pain in an animal should be anticipated. Any eye stimulation, including topical application of a test article, may be sensed as painful or irritating.

It is expected that substances with certain physicochemical properties (e.g., pH less than 6 or above 8, solids, substances that alter normal osmolarity) will cause pain. However, there are no known physicochemical properties that can be used to indicate that a test substance will not cause pain. Application of the test substance at the same temperature as the eye's surface (approximately 32°C) may reduce the pain and discomfort associated with application.

Panelists suggested that, based on human experience, it should be assumed that any chemically induced ocular lesion is associated with pain, regardless of the severity of the injury. They also recommended that a thorough list of lesions that are likely to be indicators of pain and distress should be compiled.

4.3 Alleviation and Avoidance of Ocular Injury and Pain

Presenters for this session included Dr. Marc Feldman of the Cleveland Clinic and Dr. Donald Sawyer of MINRAD International.

4.3.1 Options for Alleviating Ocular Pain in Humans

Pain can be a confounding factor that can impact study results. Treatment modalities for ocular pain in humans include local anesthetics (topical or infiltrative), topical or oral nonsteroidal anti-inflammatory drugs (NSAIDs), opiates, and general anesthetics. Topical anesthetics are generally safe, effective, and increasingly used for invasive ocular surgical procedures (e.g., cataract surgeries, glaucoma surgeries, vitrectomies, globe repairs), but are typically cytotoxic under prolonged, repeated use conditions. Side effects of topical anesthetics used preemptively may be reduced by washout. Infiltration local anesthesia requires retrobulbar block, peribulbar block, and sub-Tenon's block, and is associated with a number of risks (e.g., retrobulbar hemorrhage, diplobia, vagal syncope, ocular puncture, central apnea). Furthermore, brainstem anesthesia following a retrobulbar block could induce such adverse effects as blindness and immobility in the contralateral eye, dyspagia, hearing difficulties, hyper- or hypo-tension, or tachycardia.

NSAIDs provide the advantage of a wide safety index and are effective in preventing sensitization, but do not block nociception. However, NSAIDs at high doses produce gastrointestinal toxicity and renal impairment and some members of this class have been associated with a higher incidence of cardiovascular problems. NSAIDs are useful for pain relief of corneal abrasions and do not appear to adversely effect wound healing. Systemic opiates are commonly used perioperatively and affect modulation systems in nociception and sensitization. Adverse effects associated with opiates include respiratory depression and nausea, and tolerance also may develop during prolonged use. The partial κ-receptor agonist butorphanol and the partial μ-receptor agonist buprenorphine appear to have longer durations of action than morphine. General anesthetics (e.g., isoflurane, ketamine) primarily affect nociception and are used for some ocular surgical procedures, or in patients with dementia, claustrophobia, or movement disorders. Adverse effects include increased intraocular pressure and

incidences of nausea. Some are used in combination with anxiolytics (e.g., ketamine and the α-2 receptor agonist xylazine or a combination of morphine, acepromazine, and a topical anesthetic). Competitive depolarizing neuromuscular blocking agents (e.g., d-tubocuarine and pancuronium) should not be used as anesthetics, since they only immobilize the animals without pain relief.

4.3.2 Minimizing Ocular Pain in Animals with Analgesics/Anesthetics

Sensitivity to pain may depend on the level of innervation of the cornea and increases progressively from lowest to highest across species (canines, felines, equines, and humans, respectively). Ocular pain is managed using anesthetics (general and regional), cycloplegics, corticosteroids, NSAIDs, opioids, and alpha agonists. Topical anesthetics decrease the permeability to sodium that results from depolarization of neuronal membranes during injury in which large transient increases in sodium permeability produce the pain sensation. Onset of action is one minute and the duration is 10 to 15 minutes or longer. Proparacaine (0.5% solution) is most widely used as a topical anesthetic, but may delay wound healing, which limits its use to diagnostic procedures. Lidocaine also with an onset of five minutes and duration of 2 to 3 hours is used. Corticosteroids inhibit phospholipase A2 and prevent release of the proinflammatory mediators of arachidonic acid metabolites. Topical corticosteroids (e.g., dexamethasone acetate, prednisolone acetate) are used for anterior uveitis, but are contraindicated for corneal ulceration because they delay epithelial healing, increase collagenase activity, and depress local immunity. Systemic corticosteroids (e.g., oral prednisone) are used for orbital, posterior segment, and extensive anterior segment pathology at either anti-inflammatory or immunosuppressive dose levels. Subconjunctival triamcinolone may provide long-lasting relief (2 to 3 weeks) and is used for episcleritis, scleritis, uveitis, or noninfectious keratoconjunctivitis, but granulomas can occur at the injection site. NSAIDs (e.g., diclofenac, indomethacin, flurbiprofen, ketorolac) reduce corneal sensitivity. For surgical pain management, acepromazine or butorphanol are used as premedicaments. Parasympatholytics (e.g., reversibly bind to acetylcholine receptors) prevent ciliary spasm and are used to relieve pain of anterior uveitis and corneal ulceration. Ketoprofen is used for postoperative analgesia. Propofol is used for induction, and isoflurane for general anesthesia. Postsurgical pain is managed using the longer lasting opiate partial μ-receptor agonist buprenorphine (intravenous, subcutaneous, or bucchal) and the anxiolytics diazepam or midazolam.

Topical ocular anesthetics may be divided into those with either ester (e.g., cocaine, procaine, tetracaine, proparacaine), amide (e.g., lidocaine, bupivacaine, mepivacaine), or other linkages (e.g., benzocaine, dibucaine). These topical agents act on the inner surface of the axonal membrane sodium channels and must penetrate lipid barriers for access. Onset of action ranges from 0.5 to 3 minutes with a duration of effect of 20 minutes to 2 to 3 hours. Application frequency of these topical anesthetics increases duration, but not depth of anesthesia. As previously discussed, topical anesthetics are associated with a series of local adverse effects (e.g., delayed wound healing, production of corneal erosions and epithelial sloughing, decreased lacrimation, and tear film disruption). Furthermore, increased frequency and longer use may result in epithelial defects with corneal stromal ring infiltrates. Topical anesthetics may also interfere with test substances (e.g., increase permeability of corneal epithelium, breakdown barriers that shield toxicity) and thus confound test results. Topical anesthetics should be used for ocular pain relief in animal testing, but observations for corneal damage, decreased tearing, or increased penetration of test materials should be closely monitored for impact on test results.

4.4 Panel Discussion on Avoiding and Minimizing Ocular Pain and Distress

Optimal pretreatment analgesics to be considered to reduce pain on initial test article application include combinations of general or topical anesthesia with pre-emptive systemic analgesia for maximal efficacy in treating study-related pain. Local topical anesthetics such as proparacaine (0.5%) are recommended for short term use with the understanding that wound healing might be delayed on

long term administration, which could increase the hazard classification of a test substance. As noted with local topical anesthetics, pretreatment analgesics could increase the hazard classification of test substances by inhibition of wound healing. However, the efficacy of pretreatment with topical anesthetics for pain resolution and the known complications of their use are sufficiently understood to warrant their continued use for pain relief.

General anesthetics may be administered by injection or inhalation, and systemic analgesics (e.g., buprenorphine) may be delivered via a topical patch system. Analgesia or anesthesia depends on the specific drug used and may vary considerably within a single class.

Since 1984, the CPSC has recommended preapplication of tetracaine ophthalmic anesthetic for all rabbit eye toxicity studies. Topical anesthetics can exaggerate chemically induced ocular injury by decreasing ocular defenses (e.g., increased epithelial permeability, reduced tearing, reduced blinking) and impairing wound healing. However, documented effects of delayed wound healing are more pronounced with repeated exposure, rather than single use.

Post-treatment modalities include the use of systemic analgesics for relief of pain associated with chemically induced lesions. Repeated use of topical anesthetics could exaggerate chemically induced lesions by mechanisms previously mentioned, but pain relief should be obligatory in animals with eye lesions.

Perhaps a more appropriate approach would be to administer pre-emptive analgesics before the ocular insult, because these drugs are most effective at preventing pain, rather than as therapeutic agents after the development of a lesion. Potentially useful agents include narcotic analgesics (e.g., buprenorphine), NSAIDs (e.g., indomethacin, diclofenac, flurbiprofen, ketorolac), and anxiolytics (e.g., acepromazine). New research should focus on the evaluation of systemic analgesic agents, doses, and dose intervals to provide effective analgesia. The effects of analgesics/anesthetics on hazard category classification should be documented.

4.5 Biomarkers for Severe/Irreversible Ocular Effects as Earlier Humane Endpoints

Presenters for this session included Dr. William Stokes of the National Institute of Environmental Health Sciences and Dr. Norbert Schrage of the Aachen Center of Technology Transfer in Ophthalmology.

Public Health Service policy and U.S. Department of Agriculture (USDA) regulations on pain and distress in laboratory animals state that more than momentary or light pain and distress: 1) must be limited to that which is unavoidable for the conduct of scientifically valuable research or testing; 2) must be conducted with appropriate pain relief medication unless justified in writing by the principal investigator; and 3) will continue for only a necessary amount of time. These regulations also state that animals suffering severe or chronic pain or distress that cannot be relieved should be humanely killed after or, if appropriate, during the procedure, and finally, that Institutional Animal Care and Use Committees must ensure that the principal investigator complies with the requirements. The majority of animals reported to the USDA that experience unrelieved pain and distress are justified by regulatory testing requirements. Use of analgesics and tranquilizers for regulatory purposes requires a determination that these agents do not interfere with a study. For this reason, they are rarely used (EPA 1998, OECD 1987). Most regulatory agencies recommend euthanasia for severe pain and distress or moribund conditions.

The Organisation of Economic Co-operation and Development (OECD) has published a guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety assessment (OECD 2000). According to this document, guiding principles for humane endpoints include: 1) designing studies to minimize any pain, distress, or suffering, consistent with the scientific objective of the study, 2) sacrifice of animals at the earliest

indication of severe pain and distress or impending death, and severe pain, suffering, or death are to be avoided as endpoints, 3) termination of animal studies once study objectives are achieved or when it is realized that these objectives will not be achieved, 4) including knowledge about the test substance in the study design, 5) defining in the protocol or standard operating procedure, conditions under which interventions to alleviate pain and distress by humane killing should be made by authorized personnel. Accordingly, humane endpoints recognized and accepted by current Environmental Protection Agency (EPA 1996), European Union (EU) (EU 2001), and the Globally Harmonized System (UN 2003) regulatory guidelines for ocular hazard assessment include severe and enduring signs of pain or distress, or eye lesions considered to be irreversible.

4.6 Panel Discussion on Biomarkers for Severe/Irreversible Ocular Effects

In an attempt to identify additional biomarkers to serve as humane endpoints, panelists discussed early adverse responses predictive of ocular injury outcome in humans. Signs of minor irritation that were cited included tearing, pain, conjunctival redness, fluorescein stippling, loss of superficial wing cells (cells in the corneal epithelium with convex anterior surfaces and concave posterior surfaces) observed using confocal microscopy, and epithelial edema. Early predictive reactions include chemosis of the conjunctiva, blood vessel occlusion, epithelial erosion (cornea and conjunctiva), necrosis demarcation, limbal necrosis, or corneal edema. Intermediate reactions that are predictive of pain include conjunctival necrosis, hyperemic revascularization, persistent epithelial erosion, ulceration, limbal degeneration, conjunctival overgrowth, and corneal vascularization.

Currently, empirical ocular lesions predictive of maximal severity (severe irritant or corrosive with irreversible effects including GHS Category I [UN 2003], EU Category R41 [EU 2001], or EPA Category I [EPA 1996]) that could be used routinely as humane endpoints to terminate a study are (1) endpoints currently accepted for study termination (e.g., Draize corneal opacity score of 4); (2) vascularization of the corneal surface (i.e., pannus); (3) greater than 75% of the limbus destroyed; (4) area of fluorescein staining not diminished over time and/or depth of injury increased over time; (5) lack of re-epithelialization five days after application of the test substance; (6) extent of depth of injury to the cornea (routinely using slit-lamp and fluorescein staining) where corneal ulceration extends beyond superficial layers of the stroma.

The panel discussion suggested that additional endpoints might allow for early termination of a study. These include destruction of the limbus and the relationship to re-epithelialization of the cornea, and positive results in Shirmer's test (measures moisture content of the corneal tear film). A positive result in Shirmer's test would suggest that conjunctival redness is likely to return to normal within 21 days.

Potential biomarkers suggesting that lesions would fully reverse were also discussed. Panelists suggested that conjunctival redness present at day 7 would typically be expected to fully reverse by day 21, and that a test could be terminated if the cornea is clear and no inflammation is present at 48 hours using a slit-lamp examination.

Methods also were identified that were recommended for additional study to determine their utility in producing humane endpoints. These included (1) photodocumentation of ocular injuries (gross and slit-lamp), 2) slit-lamp biomicroscopy with fluorescein or other vital dye staining, 3) pachymetry measurements, 4) depth of injury measurements, 5) postmortem observations (e.g., histopathology, live/dead cell assays using fresh excised tissue), 6) extent and destruction of the limbus and relationship to re-epithelialization of the cornea, and 7) altered tear production and lesion persistence. The Panelists noted that standardized procedures with these methods are needed to facilitate the collection of data in a systematic fashion.

5.0 Conclusion and Recommendations

This symposium provided a forum for the presentation and discussion of: 1) known and putative mechanisms of ocular pain and distress in humans and animals; 2) treatment and prevention of pain and distress; 3) impact of these treatments on regulatory testing requirements; and 4) areas for future research. Ophthalmologists, academic scientists, federal regulators, industrial toxicologists, and experts in the development and use of alternative toxicological methods provided various perspectives on current use of specific treatments. Importantly, specific treatments to alleviate pain and distress in animal models of ocular toxicity required for the optimization and validation of alternative toxicological methods and their impact on regulatory requirements were considered.

The primary conclusions of the experts who participated in this symposium were:

- Pain relief in animals used for ocular toxicity testing should be provided as a pretreatment when there is reason to believe a painful response will be produced (e.g., test substance produces pain in humans, solution is not iso-osmotic or isotonic, pH is less than 6 or greater than 8, etc.).
- Clinical signs of pain in animals should be carefully observed (examples of some of these signs are provided in Table 2) and the study terminated if significant pain or distress is evident.
- Combinations of general or topical anesthesia with pre-emptive systemic analgesia should be used for maximal efficacy in treating study-related pain on initial test article application.
- Adverse responses likely to induce painful responses include minor reversible effects (e.g., conjunctival redness and chemosis, hyperemic revascularization), intermediate predictive effects (e.g., blood vessel occlusion, epithelial erosion or ulceration, limbal degeneration), and severe irreversible effects (e.g., pannus, significant depth of injury, corneal opacity score of 4, etc.).
- Additional biomarkers and techniques should be incorporated into in vivo ocular testing to improve the prediction of the humane endpoints (e.g., lack of re-epithelialization)

Table 2 Clinical Signs and Biomarkers Indicative of Pain

Sign/Biomarker
Intermittent to repeated blinking and/or squinting[1]
Partial to complete eye closure
Repeated pawing or eye rubbing
Vocalization[2]
Conjunctival hyperemia and chemosis
Increased blood pressure, respiration, or heart rate
Electrophysiological responses measured in trigeminal ganglia

[1] Under normal conditions, rabbits do not blink often (Wilhelmus 2001).

[2] Rarely occurs

6.0 Participants in the Symposium

6.1 ICCVAM Ocular Toxicity Working Group

Robert Bronaugh, Ph.D., U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Laurel, MD

Wiley Chambers, M.D., U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD

Kailash Gupta, D.V.M., Ph.D., U.S. Consumer Product Safety Commission, Bethesda, MD (Retired 2006)

Abigail Jacobs, Ph.D., U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD

Donnie Lowther, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD

Debbie McCall, U.S. Environmental Protection Agency, Washington, D.C.

John Redden, U.S. Environmental Protection Agency, Washington, D.C.

Leonard Schechtman, Ph.D., U.S. Food and Drug Administration, National Center for Toxicological Research, Rockville, MD (Retired 2006)

Margaret Snyder, Ph.D., National Institutes of Health, Office of Extramural Research, Bethesda, MD

Marilyn Wind, Ph.D., U.S. Consumer Product Safety Commission, Bethesda, MD

6.2 Invited Experts

Ellison Bentley, D.V.M., DACVO, University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI

Roger Beuerman, Ph.D., Louisiana State University, Health Sciences Center, School of Medicine, New Orleans, LA

Marc Feldman, M.D., The Cleveland Clinic, Cole Eye Institute, Cleveland, OH

James Freeman, Ph.D., DABT, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ

Roswell Pfister, M.D., Brookwood Medical Center, The Eye Research Laboratories, Birmingham, AL

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

Norbert Schrage, Dr. Med., ACTO Aachen Center for Transfer Technology in Ophthalmology, Aachen, Germany

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

William S. Stokes, D.V.M., DACLAM, National Institute of Environmental Health Sciences, National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, Research Triangle Park, NC

Kirk Tarlo, Ph.D., DABT, Allergan, Inc., Irvine, CA

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