ICCVAM Test Method Evaluation Report: Recommendations for Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing

Interagency Coordinating Committee on the Validation of Alternative Methods

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

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ºC	Degrees centigrade
AHT	Animal Health Technologist
BRD	Background review document
CPSC	U.S. Consumer Product Safety Commission
CV	Coefficient of variation
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
ESAC	European Centre for the Validation of Alternative Methods Scientific Advisory Committee
EU	European Union
FDA	U.S. Food and Drug Administration
FR	*Federal Register*
g	Gram
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILS	Integrated Laboratory Systems, Inc.
IS	Irritation score
JaCVAM	Japanese Center for the Validation of Alternative Methods
kg	Kilogram
LVET	Low volume eye test
MAS	Maximum average score
MeSH	Medical Subject Headings
mg	Milligram
mL	Milliliter
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NSAID	Nonsteroidal anti-inflammatory drug
NTP	U.S. National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Ocular Toxicity Working Group
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SC	Subcutaneous
SRD	Summary review document
TG	Test guideline
TSA	Test substance administration
UN	United Nations

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Preface

Eye injury is a leading cause of visual impairment in the United States with 40,000 to 50,000 new cases of impaired vision reported each year.[1] Many eye injuries occur due to contact with workplace or household products or chemicals. Accidents involving common household products (e.g., oven cleaner and bleach) cause about 125,000 eye injuries each year.[2] These products often result in chemical burns and emergency room visits.[3] Each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Although the majority of these eye injuries result from mechanical sources, chemical burns from industrial chemicals or cleaning products are common.[4]

To prevent eye injuries, regulatory agencies require testing to determine if chemicals and products may cause eye damage. This testing information is used to classify the ocular hazard and determine appropriate labeling to warn consumers and workers of the potential hazard. Appropriate labeling tells users how to avoid exposure that could damage the eye and what emergency procedures should be followed if there is accidental exposure. Nearly all ocular safety testing has been conducted using the Draize rabbit eye test, although *in vitro* methods can now be used to identify whether substances cause severe irritation or permanent eye damage. The Draize rabbit eye test (Draize et al. 1944) involves instillation of 0.1 mL of the test substance into the conjunctival sac of one eye. The other eye serves as the untreated control. The eye is examined at least daily for up to 21 days. The presence and severity of any injuries to the cornea, conjunctiva, and the iris (tissues inside the eye) are scored, and the duration that the injuries persist is recorded.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid or minimize pain and distress during ocular safety testing. As a part of this evaluation, ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) requested the submission of data and experience with topical anesthetics and systemic analgesics to alleviate pain and distress in rabbits during eye irritation testing (72 FR 26396).[5]

ICCVAM carefully compiled and assessed all available data and arranged an independent international scientific peer review. ICCVAM and the Ocular Toxicity Working Group (OTWG) solicited and considered public comments and stakeholder involvement throughout the evaluation process. As part of their ongoing collaboration with ICCVAM, scientists from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) served as liaisons to the OTWG. ICCVAM, NICEATM, and the OTWG prepared (1) a draft background review document (BRD) on the use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during ocular safety testing and (2) draft test method recommendations for their usefulness and limitations. ICCVAM released this document to the public for comment on March 31, 2009. ICCVAM also announced a meeting of the independent international scientific peer review panel (Panel) (74 FR 14556).[6]

The Panel met in public session on May 19–21, 2009, to review the ICCVAM draft BRD for completeness and accuracy. The Panel then evaluated (1) the extent to which the draft BRD addressed established validation and acceptance criteria and (2) the extent to which the draft BRD supported

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[1] Available at http://www.preventblindness.org/resources/factsheets/Eye_Injuries_FS93.pdf
[2] Available at http://www.geteyesmart.org/eyesmart/injuries/home.cfm
[3] From the CPSC NEISS database, 2007
[4] Available at http://www.cdc.gov/niosh/topics/eye/
[5] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_8898.pdf
[6] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf

ICCVAM's draft test method recommendations. Before concluding their deliberations, the Panel considered written comments and comments made at the meeting by public stakeholders. The Panel prepared a report summarizing their conclusions and recommendations.[7]

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the Topical Anesthetics/Systemic Analgesics/Humane Endpoints draft BRD and draft test method recommendations, the Panel report, and all public comments for discussion at their meeting on June 25–26, 2009, where public stakeholders were given another opportunity to comment. A detailed timeline of the evaluation is included with this report.

ICCVAM solicited and considered public comments and stakeholder involvement throughout the test method evaluation process. ICCVAM considered the SACATM comments, the conclusions of the Panel, and all public comments before finalizing the ICCVAM test method recommendations. The recommendations and the BRD, which is provided as an appendix to this report, are incorporated in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act, ICCVAM will forward its recommendations to U.S. Federal regulatory agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving the ICCVAM test method recommendations. ICCVAM recommendations are available to the public on the NICEATM–ICCVAM website, and agency responses will also be made available on the website as they are received.

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[7] Available at http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf

Executive Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid or minimize pain and distress during *in vivo* ocular safety testing. This test method evaluation report provides ICCVAM's recommendations. The report also includes (1) ICCVAM's recommended changes to the protocol for the Draize rabbit eye test and (2) a final background review document (BRD) on the use of topical anesthetics, systemic analgesics, and earlier humane endpoints in the Draize rabbit eye test.

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), ICCVAM, and ICCVAM's Ocular Toxicity Working Group prepared a draft BRD on the use of topical anesthetics, systemic analgesics, and earlier humane endpoints to minimize pain and distress in ocular safety testing. The BRD is based upon published studies and forms the basis for the draft ICCVAM test method recommendations. NICEATM provided the draft BRD and ICCVAM recommendations to an independent international scientific peer review panel (Panel) and the public for comment. A detailed timeline of the ICCVAM evaluation process is appended to this report.

The Panel met in public session on May 19–21, 2009, to discuss its review of the ICCVAM draft BRD and to provide conclusions and recommendations on these proposed changes to the Draize rabbit eye test protocol. The Panel also reviewed how well the information in the draft BRD supported ICCVAM's draft test method recommendations. In finalizing this test method evaluation report and the BRD, which is included as an appendix, ICCVAM considered (1) the conclusions and recommendations of the Panel, (2) comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods, and (3) public comments.

Routine Use of Topical Anesthetics and Systemic Analgesics in the Draize Rabbit Eye Test

Specific ICCVAM Test Method Recommendations

Balanced preemptive pain management should be provided whenever the Draize rabbit eye test is conducted for regulatory safety testing. Pain management should include (1) treating the animals with a topical anesthetic and a systemic analgesic before applying test substances; (2) following a routine schedule of systemic analgesia after applying test substances; (3) scheduled observation, monitoring, and recording of animals for clinical signs of pain and/or distress; and (4) scheduled observation, monitoring, and recording of the nature, severity, and progression of all eye injuries. ICCVAM further recommends that ocular safety testing protocols include a pain management procedure and schedule.

Changes to Ocular Safety Testing Protocol to Include the Routine Use of Topical Anesthetics and Systemic Analgesics

When required for regulatory safety assessment of potential ocular hazards (EPA 1998; OECD 2002), the current Draize rabbit eye test should be conducted with the following changes unless pain response monitoring is required (e.g., pharmaceutical tolerability testing). Alternative pain management procedures may be considered if they provide analgesia and anesthesia as good or better than the following pain management procedure:

- Sixty minutes before test substance application (TSA), provide a therapeutic level of systemic analgesia by administering 0.01 mg/kg buprenorphine by subcutaneous injection.
- Five minutes before applying the test substance, apply one or two drops of a topical ocular anesthetic (e.g., 0.5% proparacaine hydrochloride or 0.5% tetracaine

hydrochloride) to each eye. For each animal, the eye that is treated with topical anesthetics and no test substance will serve as a control. If the test substance is anticipated to cause significant pain and distress, consider applying more than one dose of topical anesthetic at 5-minute intervals before TSA. Be aware that multiple applications of topical anesthetics could increase the severity of chemically induced lesions and/or extend the time required for them to heal.

- If a test subject shows signs of pain and distress during the test interval, immediately give additional analgesia (i.e., a "rescue" dose of 0.03 mg/kg subcutaneous buprenorphine). Repeat 0.03 mg/kg buprenorphine every 8 hours (+/- 30 minutes) instead of 0.01 mg/kg subcutaneously every 12 hours. Continue meloxicam with the same dose and interval described below. If preemptive analgesia is inadequate, give the "rescue" analgesia immediately after TSA.
- Eight hours (+/- 30 minutes) after TSA, administer 0.01 mg/kg buprenorphine and 0.5 mg/kg meloxicam subcutaneously to provide a continued therapeutic level of systemic analgesia.
- If ocular lesions and/or clinical signs of pain and distress are present following the buprenorphine and meloxicam treatment that was administered 8 hours after TSA, continue to administer 0.01 mg/kg buprenorphine subcutaneously every 12 hours (+/- 30 minutes) in conjunction with 0.5 mg/kg meloxicam subcutaneously every 24 hours. If the "rescue dose" described above is needed, administer buprenorphine at 0.03 mg/kg every 8 hours instead of 0.01 mg/kg every 12 hours.

Future Studies on the Routine Use of Topical Anesthetics and Systemic Analgesics

ICCVAM recommends routinely observing and recording lesions and clinical signs during ocular safety studies in order to evaluate the effectiveness of pain management and to determine if the enhanced "rescue" analgesia procedure should be implemented. These data should be reviewed to determine whether adjustments are needed to (1) improve the effectiveness of analgesia before and after treatment and (2) optimize dosages and treatment intervals. Data should be analyzed periodically to determine the effectiveness of the pain management procedures for specific types of lesions and clinical signs of pain and distress associated with ocular safety testing.

To support the development of improved pain management strategies, ICCVAM recommends evaluating detailed animal injury and pain response data collected from animals used for regulatory safety testing. This could help gauge the adequacy of the recommended pain management procedures and help identify the need for modifications to dosages and dosing intervals for anesthetics and/or analgesics. Additionally, where possible, ICCVAM recommends that the eyes of test animals be collected for histopathology to more thoroughly evaluate depth and area of ocular damage, as well as to provide a reference against which to compare effects produced *in vitro*. ICCVAM emphasizes that new animal studies should be considered only when absolutely necessary in developing new pain management strategies for testing.

Use of Earlier Humane Endpoints — Test Method Usefulness and Limitations

ICCVAM recognizes that current ocular testing guidelines include criteria for study termination in the case of certain types of severe ocular injuries or evidence of severe pain and distress (EPA 1998; OECD 2002). There is also international guidance on general humane endpoints that can be used as the basis for ending an experiment (OECD 2000). In addition to these currently accepted endpoints, and consistent with the recommendations of the Panel, ICCVAM recommends that the following ocular lesions be used as earlier humane endpoints to terminate studies before the end of the scheduled 21-day observation period. These lesions are considered predictive of severe irritant or corrosive injuries and injuries that are not expected to fully reverse by the end of the 21-day observation period after treatment:

- Severe depth of injury (e.g., corneal ulceration extending beyond the superficial layers)
- Destruction of more than 50% of the limbus, as evidenced by blanching of the conjunctival tissue
- Severe eye infection (purulent discharge)

A combination of the following endpoints may be useful in clinical decisions on study termination. However, these endpoints cannot be used individually to justify early study termination:

- Vascularization of the cornea surface (i.e., pannus)
- Area of fluorescein staining not diminishing over time based on daily assessment
- Lack of re-epithelialization 5 days after test substance application

ICCVAM emphasizes that, once severe ocular effects have been identified, a qualified laboratory animal veterinarian should perform a clinical exam to determine if the combination of these effects warrants early study termination.

Changes to the Ocular Safety Testing Protocol to Include the Use of Humane Endpoints

The current protocol for the Draize rabbit eye test, as used for regulatory safety testing (EPA 1998; OECD 2002), should be updated to incorporate ICCVAM's recommended use of humane endpoints. ICCVAM recommends that test animals be comprehensively evaluated for the presence or absence of ocular lesions one hour after TSA, followed by at least daily evaluations. Animals should be evaluated once daily for the first 3 days, or more often if necessary, to ensure that termination decisions are made promptly. ICCVAM also recommends that test animals should be routinely evaluated for clinical signs of pain and/or distress at least twice daily with at least 6 hours between observations. Examples of relevant clinical signs include (Wright et al. 1985; NRC 2008, 2009)

- repeated pawing or rubbing of the eye
- excessive blinking
- excessive tearing

Decisions to end a study based on humane endpoints should ensure that reversal of the clinical signs is not expected or that no further useful information can be obtained from the study. A written record of all observations should be kept, including evidence of an infection and/or pain and distress. Such records can facilitate decisions on the progression or resolution of ocular lesions. ICCVAM emphasizes that fluorescein staining should be used routinely. A slit-lamp biomicroscope should also be used, when considered appropriate (e.g., assessing depth of injury when corneal ulceration is present), to help detect and measure ocular endpoints. Digital photographs should be taken to document ocular lesions and to help assess their severity, progression, and resolution.

Future Studies on the Use of Humane Endpoints

ICCVAM recommends that additional data should be collected on the use of fluorescein staining to monitor wound healing. These data should be evaluated to identify criteria that may be useful as humane endpoints to terminate studies.

ICCVAM encourages users to provide NICEATM with detailed data and observations collected in ocular safety studies that can be used to create a database to (1) further characterize the usefulness and limitations of proposed humane endpoints and (2) identify potential new endpoints. Such data submissions will contribute to efforts to find ways to further prevent and minimize pain and distress in ocular safety assessments.

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1.0 Introduction

Current U.S. Environmental Protection Agency (EPA) and Organisation for Economic Co-operation and Development (OECD) test guidelines for the Draize rabbit eye test provide for the use of topical anesthetics only when the user demonstrates that such pretreatments do not interfere with the test results (EPA 1998; OECD 2002).[8] Topical anesthetics are seldom used because a separate study would likely be necessary to meet this requirement. EPA (1998), European Union (EU 2001), and the Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) regulatory guidelines recognize and accept certain humane endpoints for ocular hazard assessment. These include (1) severe and enduring signs of pain or distress and (2) eye lesions considered to be irreversible. However, current testing guidelines underemphasize the routine use of such endpoints.

Consequently, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid or minimize pain and distress during *in vivo* ocular safety testing.

The ICCVAM Authorization Act of 2000 (Public Law 106-545, 42 United States Code 285*l*-3) charged ICCVAM with coordinating the technical evaluations of new, revised, and alternative test methods with regulatory applicability. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) administers ICCVAM and provides scientific support for ICCVAM activities. The ICCVAM Ocular Toxicity Working Group (OTWG) worked with NICEATM in evaluating alternative methods and testing strategies. Drs. João Barroso, Tom Cole, and Valerie Zuang were the European Centre for the Validation of Alternative Methods (ECVAM) liaisons, and Dr. Hajime Kojima was the Japanese Center for the Validation of Alternative Methods (JaCVAM) liaison to the OTWG.

To facilitate peer review, the OTWG and NICEATM prepared a comprehensive draft background review document (BRD). The BRD provided information and data from published and unpublished data on the use of topical anesthetics, systemic analgesics, and humane endpoints in ocular safety testing.

ICCVAM and NICEATM requested the submission of data and experience with topical anesthetics and systemic analgesics for alleviating pain and distress in rabbits during ocular safety testing (72 FR 26396).[9] One individual provided comments supporting the use of anesthetics to minimize pain and distress in rabbit eye irritation studies. No additional data were received.

On April 4, 2008, NICEATM published a *Federal Register* notice (73 FR 18535)[10] requesting relevant data and nominations of individuals to serve on an independent international scientific peer review panel (Panel). The request was also disseminated via the ICCVAM electronic mailing list and through direct requests to over 100 stakeholders. Twenty individuals were nominated as potential panelists for consideration. No additional data were received (see **Section 6.0**).

The BRD forms the basis for these ICCVAM test method recommendations. The ECVAM and JaCVAM liaisons to the OTWG provided input and contributed throughout the evaluation process. A detailed timeline of the ICCVAM evaluation is provided in **Appendix A**. The ICCVAM-recommended test method protocol and final BRD are provided in **Appendices B** and **C**, respectively.

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[8] OECD Test Guideline 405 states: "The type, concentration, and dose of a local anesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use." Similarly, EPA (1998) states that "The type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use."

[9] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_8898.pdf

[10] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-6969.pdf

On March 31, 2009, ICCVAM announced the availability of the ICCVAM draft BRD. ICCVAM also announced a public Panel meeting to review the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints in ocular safety testing (74 FR 14556). The ICCVAM draft BRD and draft test method recommendations were posted on the NICEATM–ICCVAM website (http://iccvam.niehs.nih.gov/). All of the information provided to the Panel and all public comments received before the Panel meeting were made available on the NICEATM–ICCVAM website.

The Panel met in public session from May 19–21, 2009, to review a proposal for the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints in ocular safety testing. The Panel also reviewed the completeness and accuracy of the ICCVAM draft BRD. They then evaluated (1) the extent to which the draft BRD addressed established validation and acceptance criteria and (2) the extent to which the BRD supported ICCVAM's draft test method recommendations. Public stakeholders were provided opportunities to comment at the Panel meeting. The Panel considered all comments during their deliberations. On July 13, 2009, ICCVAM posted the final report of the Panel's recommendations (**Appendix D**) on the NICEATM–ICCVAM website for public review and comment (announced in 74 FR 33444).[11]

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the draft BRD, draft test method recommendations, the Panel report, and all public comments. SACATM discussed this material at their meeting on June 25–26, 2009. Public stakeholders were given another opportunity to comment.

After the SACATM meeting, ICCVAM and the OTWG considered the SACATM comments, the Panel report, and all public comments before finalizing the ICCVAM test method evaluation report and the BRD, provided as an appendix to this report. As required by the ICCVAM Authorization Act, ICCVAM will make this test method evaluation report and the accompanying final BRD available to the public and to U.S. Federal agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving ICCVAM test method recommendations. Agency responses to the ICCVAM test method recommendations will be made available to the public on the NICEATM–ICCVAM website at *http:///www.iccvam.niehs.nih.gov* as they are received.

[11] Announcement available at http://niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E9-16388; report available at http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf

2.0 ICCVAM Recommendations for the Routine Use of Topical Anesthetics and Systemic Analgesics to Avoid or Minimize Pain and Distress in Ocular Safety Testing

2.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

ICCVAM recommends that balanced preemptive pain management should always be provided when the Draize rabbit eye test is conducted for regulatory safety testing. Pain management should include (1) pretreatment with a topical anesthetic and systemic analgesic prior to test substance administration; (2) routine post-treatment with systemic analgesics, with additional treatments as necessary; (3) scheduled observation, monitoring, and recording of animals for clinical signs of pain and/or distress; and (4) scheduled observation, monitoring, and recording of the nature, severity, and progression of all eye injuries. ICCVAM further recommends that ocular safety testing protocols include a pain management plan and schedule consistent with that outlined below.

When required for ocular safety testing, the Draize rabbit eye test protocol currently used for regulatory safety assessments of potential ocular hazards (EPA 1998; OECD 2002) should be conducted with the following modifications unless there is a requirement for monitoring the pain response (e.g., pharmaceutical tolerability testing). Alternative pain management procedures may also be considered that provide as good or better analgesia and anesthesia than the recommended pain management procedure below:

- Sixty minutes before test substance administration (TSA), buprenorphine 0.01 mg/kg is administered by subcutaneous injection (SC) to provide a therapeutic level of systemic analgesia.
- Five minutes pre-TSA, one or two drops of a topical ocular anesthetic (e.g., 0.5% proparacaine hydrochloride or 0.5% tetracaine hydrochloride) is applied to each eye. The eye of each animal that is not treated with a test article, but which is treated with topical anesthetics, serves as a control. If the test substance is anticipated to cause significant pain and distress, consideration should be given to more than one application of topical anesthetic at 5-minute intervals pre-TSA. Users should be aware that multiple applications of topical anesthetics could increase the severity and/or extend the time required for chemically induced lesions to clear.
- If a test subject shows signs of pain and distress during the test interval, additional analgesia (i.e., a "rescue" dose of 0.03 mg/kg SC buprenorphine) is given immediately and repeated every 8 hours, [12] instead of 0.01 mg/kg SC every 12 hours. Meloxicam would continue with the same dose and interval described below. The "rescue" analgesia should be given immediately after TSA if preemptive analgesia is inadequate.
- Eight hours post-TSA, buprenorphine 0.01 mg/kg SC and meloxicam 0.5 mg/kg SC are administered to provide a continued therapeutic level of systemic analgesia.
- After the initial 8-hour post-TSA treatment, if ocular lesions and/or clinical signs of pain and distress are present, buprenorphine 0.01 mg/kg SC should be administered every 12 hours (0.03 mg/kg every 8 hours if the "rescue" dose is needed), in conjunction with meloxicam 0.5 mg/kg SC every 24 hours.

Independent Peer Review Panel Conclusions and Recommendations

Following the Panel's review of the BRD and draft recommendations developed by ICCVAM, the Panel proposed an alternative preemptive pain management protocol for rabbits used for ocular safety

[12] Time intervals are +/- 30 minutes.

testing. This protocol (hereafter, the alternative protocol or the Panel's protocol) was proposed by the Panel to be applied to all *in vivo* rabbit ocular safety tests intended for regulatory safety testing, unless there is a requirement for monitoring the pain response (e.g., pharmaceutical tolerability testing). The only differences in the ICCVAM-recommended plan and the Panel's protocol are that the ICCVAM-recommended plan (1) allows for either tetracaine or proparacaine as a topical anesthetic and (2) recommends only one dose of topical anesthetic unless there is reason to believe that this will be insufficient to relieve pain and distress, at which time additional pre-TSA applications can be considered. The basis for these differences arise from previous studies showing that multiple doses of proparacaine can result in significant differences in hazard classification due to the increased severity and/or prolonged appearance of ocular lesions.

2.2 ICCVAM Recommendations: Test Method Protocol for the Routine Use of Topical Anesthetics and Systemic Analgesics

When required for ocular safety testing, the Draize rabbit eye test protocol currently used for regulatory safety assessments of potential ocular hazards (EPA 1998; OECD 2002) should be conducted with the modifications as outlined in **Section 2.1** unless pain-response monitoring is required (e.g., pharmaceutical tolerability testing).

Independent Peer Review Panel Conclusions and Recommendations

The Panel considered its proposal (**Section 2.1**) more appropriate in terms of the type and frequency of dosing for topical anesthetics and systemic analgesics.

The Panel noted that the available guidance on measuring fluorescein staining as presented in the draft ICCVAM recommendations is not adequate for laboratories to obtain consistent results, and the method of fluorescein staining will have to be standardized in order to be useful. In addition, the guidelines lack details about potential preservatives in the dye, anesthesia requirements, or physical restraint that may need to be considered.

2.3 ICCVAM Recommendations: Future Studies for the Routine Use of Topical Anesthetics and Systemic Analgesics

The routine observation and recording of lesions and clinical signs is recommended during ocular irritation safety studies to evaluate the effectiveness of pain management and to determine if the enhanced "rescue" analgesia procedure should be implemented. Furthermore, periodic retrospective reviews of these data should be performed to determine if adjustments are needed to improve the effectiveness of pretreatment and post-treatment analgesia and to optimize dosages and treatment intervals. Ideally, data collected during routine safety testing should be analyzed periodically to determine the effectiveness of the pain management plan for specific types of lesions and clinical signs of pain and distress associated with ocular irritation/corrosivity testing.

ICCVAM recommends the following studies and activities to support the development of improved pain management strategies, recognizing that some involve research that would be conducted independent of regulatory safety testing.

- New animal studies should be considered only when absolutely necessary in developing new pain management strategies for testing.
- Detailed ocular injury and pain response data should be collected from animals used for required regulatory testing and evaluated to assess the adequacy of the recommended pain management procedures. This data will help identify the need for modifications to dosages and dosing intervals for anesthetics and/or analgesics.

- Where possible, eyes should be collected for histopathology to more thoroughly evaluate depth and area of ocular damage, as well as to provide a reference against which to compare effects produced *in vitro*.
- Digital photographs of observed lesions should be collected for reference and to provide a permanent record of the extent of ocular damage.
- Studies should be conducted to determine whether the timing and dosing of systemic analgesics together with topical anesthetics might alter the ocular defense sufficient to change the classification of test substances.
- Studies should be conducted to investigate other topical anesthetics that might provide longer duration of action or other advantages.
- Studies should be conducted to evaluate the impact of using other systemic analgesics that might provide longer duration of action, improved analgesia, or other advantages.
- ICCVAM encourages users to provide data generated using the recommended pain management procedures to NICEATM to create a database that can be periodically evaluated to further characterize the usefulness and limitations of such procedures for avoiding or minimizing pain and distress in ocular safety assessments.

Independent Peer Review Panel Conclusions and Recommendations

The Panel agreed with the draft ICCVAM recommendations for future studies related to the routine use of topical anesthetics and systemic analgesics. The Panel also recommended a number of additional studies, which have been incorporated into the ICCVAM recommendations listed above.

3.0 Validation Status: Routine Use of Topical Anesthetics and Systemic Analgesics in Ocular Safety Testing

Since 1984, the U.S. Consumer Product Safety Commission has recommended preapplication of tetracaine ophthalmic anesthetic in all rabbit ocular safety studies. However, current EPA and OECD test guidelines for the Draize rabbit eye test provide for the use of topical anesthetics only when the user demonstrates that such pretreatments do not interfere with the test results (EPA 1998; OECD 2002).[13] Topical anesthetics are seldom used because a separate study would likely be necessary to provide the necessary information.

In 2005, a symposium entitled "Minimizing Pain and Distress in Ocular Toxicity Testing" evaluated the use of topical ophthalmic anesthetics and/or systemic analgesics during the conduct of the Draize rabbit eye test. ICCVAM, NICEATM, and the European Centre for the Validation of Alternative Methods (ECVAM) organized the symposium. Experts acknowledged that a single treatment with a topical anesthetic to anesthetize the surface of the cornea before application of the test article could cause slight physiologic changes. However, the consensus was that such changes in the irritant response would be slight if any. Furthermore, the predominant view was that if there were any effect on the irritant response, it would tend to slightly increase the severity of the response.

Participants recommended routine use of topical anesthetics. The anesthetics at least prevent the discomfort caused by installation of the test article on the eye. They also temporarily prevent or minimize pain and distress that might result from immediate ocular damage.

NICEATM recently evaluated the effects of pretreatment with tetracaine hydrochloride (0.5% w/v) on the ocular irritancy potential of 97 formulations. The results indicate that such pretreatments have no statistically significant impact on the hazard classification severity category of observed ocular irritation (**Annex II** of **Appendix C**). For most of the formulations tested, topical anesthetic pretreatment had little or no impact on:

- The hazard classification severity category of observed ocular irritation
- The variability in ocular irritation responses among animals treated with the same test article
- The number of days required for an ocular lesion to clear

When a difference in ocular irritation response was observed, the more severe response was usually observed in the animals pretreated with topical anesthesia. However, none of the observed differences was statistically significant. Differences included both increases and decreases in the irritancy level, which suggests that they are related to the inherent inter-individual biological variability of response rather than topical anesthetic pretreatment.

Scientific experts at the 2005 workshop also recommended (**Annex I** of **Appendix C**) that animals be routinely pretreated with topical anesthetics and systemic analgesics to prevent pain. Animals that show signs of pain or distress and those with ocular lesions associated with painful conditions should be treated with systemic analgesics. Similarly, a recently convened independent international scientific peer review panel recommended the routine use of topical anesthetics and systemic analgesics to avoid or minimize pain and distress during *in vivo* ocular safety testing. The Panel recommended a protocol that includes pretreatment with systemic analgesics in conjunction with

[13] OECD Test Guideline 405 states: "The type, concentration, and dose of a local anesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use." Similarly, EPA (1998) states that "the type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use."

topical anesthetics prior to test substance administration. The protocol also includes treatment with systemic analgesics after test substance administration.

A therapeutic analgesic protocol conducted before the onset of pain is referred to as preemptive pain management (Polomano et al. 2008). The Panel recommended a balanced preemptive pain management protocol for all animals used for ocular safety testing. For routine safety testing, the Panel considered proparacaine preferable to tetracaine because the initial application to the eye is less painful (Bartfield et al. 1994). The relative merits of proparacaine and tetracaine are detailed in **Annex III** of **Appendix C**. Multiple applications of topical anesthetics before test substance administration maximize effective penetration of the epithelial layer (Sasaki et al. 1995). A 5-minute interval between the last topical anesthetic dose and test substance administration minimizes the possibility of any volume dilution (Maurice 1995).

The Panel recommended buprenorphine as the systemic analgesic of choice. Buprenorphine is an opioid agonist–antagonist analgesic that has been effective in managing pain in rabbits and other small animals (Roughan and Flecknell 2002; Sawyer 2008). It has a wide safety margin in rabbits, causes minimal sedation, and provides a long duration of analgesia (6–12 hours) (Flecknell 1984; Flecknell and Liles 1992; Roughan and Flecknell 2002). Increasing buprenorphine dose rates in rabbits has little effect on the maximum degree of analgesia produced (Flecknell and Liles 1990). For this reason, the recommended dose range in rabbits is 0.01–0.05 mg/kg (Dobromylskyj et al. 2006; Flecknell 1984, 1995; Flecknell and Liles 1990).

The Panel recommended treatment with systemic analgesics after test substance administration to maintain the prior level of analgesia. A well-tested approach to balanced analgesia is to use an opioid (e.g., buprenorphine) in combination with a cyclooxygenase-sparing nonsteroidal anti-inflammatory drug such as meloxicam (Cooper et al. 2009; Roughan and Flecknell 2002; Sawyer 2008). Meloxicam has been used for postoperative or chronic pain in humans (Akarsu et al. 2004; Aoki et al. 2006) and dogs for over 10 years. Its effectiveness has been demonstrated in rabbits (Cooper et al. 2009; Sawyer 2008). The Panel recommended a low dose of meloxicam once daily in conjunction with the buprenorphine.

4.0 ICCVAM Recommendations for the Use of Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing

4.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

ICCVAM recognizes that current ocular testing guidelines include criteria for study termination in the case of certain types of severe ocular injuries or evidence of severe pain and distress (EPA 1998; OECD 2002). These include:

- Draize corneal opacity score of 4 that persists for 48 hours
  - Corneal score of 4 is defined as: Opaque cornea, iris not discernable through the opacity
- Corneal perforation or significant corneal ulceration including staphyloma
- Blood in the anterior chamber of the eye
- Absence of a light reflex (iridial response grade 2) that persists for 72 hours
- Ulceration of the conjunctival membrane
- Necrosis of the conjunctiva or nictitating membrane
- Sloughing (separation of necrotic tissue from the living structure)

There is also international guidance on general humane endpoints that can be used as the basis for ending an experiment (OECD 2000). In addition to these currently accepted endpoints and consistent with the recommendations of the Panel, ICCVAM recommends that the following ocular lesions also be used as earlier humane endpoints to terminate studies before the end of the scheduled 21-day observation period. These lesions are considered predictive of severe irritant or corrosive injuries and injuries that are not expected to fully reverse by the end of the 21-day observation period after treatment:

- Severe depth of injury (e.g., corneal ulceration extending beyond the superficial layers)
- Destruction of more than 50% of the limbus, as evidenced by blanching of the conjunctival tissue
- Severe eye infection (purulent discharge)

The following endpoints, in combination, may be useful in clinical decisions on early study termination:

- Vascularization of the corneal surface (i.e., pannus)
- Area of fluorescein staining not diminishing over time based on daily assessment
- Lack of re-epithelialization 5 days after test substance application

However, these endpoints cannot be used individually to justify early study termination. ICCVAM emphasizes that, once severe ocular effects have been identified, a qualified laboratory animal veterinarian should perform a clinical exam to determine if the combination of these effects warrants early study termination.

Conclusions and Recommendations of the Independent Peer Review Panel

The Panel concluded that the current and proposed humane endpoints should be used routinely as humane endpoints. The Panel considered them predictive enough of irreversible or severe effects (i.e., EPA Category I, GHS Category 1, EU R41) that a study should be terminated as soon as they are observed. To ensure that termination decisions are made promptly, the Panel recommended that test animals be examined at least daily and the presence or absence of these lesions recorded. For the first three days, test animals should be examined at least twice daily, or more often if necessary. The Panel

emphasized the need for a slit-lamp examination to ensure accurate measurement of most of the ocular endpoints.

The Panel did not consider some of the endpoints adequate for early study termination when taken individually (e.g., pannus, area of fluorescein staining, lack of re-epithelialization). They can, however, be considered together. With this in mind, the Panel emphasized that decisions to terminate a study should be based on multiple endpoints when possible. Only very severe endpoints (e.g., corneal perforation) would be adequate alone to terminate a study.

4.2 ICCVAM Recommendations: Changes to the Ocular Safety Testing Protocol to Include the Use of Humane Endpoints

Ocular safety assessment studies should be conducted using the ICCVAM-recommended modifications to the current Draize eye test protocol for regulatory safety assessments of potential ocular hazards (EPA 1998; OECD 2002). ICCVAM recommends that test animals be comprehensively evaluated for the presence or absence of ocular lesions one hour after test substance administration, followed by at least daily evaluations. Animals should be evaluated once daily for the first 3 days, or more often if necessary, to ensure that termination decisions are made in a timely manner. ICCVAM also recommends that test animals be routinely evaluated for clinical signs of pain and/or distress at least twice daily with a minimum of 6 hours between observations, or more often if necessary. Examples of relevant clinical signs include (Wright et al. 1985; NRC 2008, 2009):

- Repeated pawing or rubbing of the eye
- Excessive blinking
- Excessive tearing

Study termination based on humane endpoints should ensure that reversal is not expected and that no further useful information can be obtained from the study. A written record of all observations should be kept for determinations on the progression or resolution of ocular lesions. ICCVAM emphasizes that fluorescein staining should be used routinely to help detect and objectively measure ocular endpoints. A slit-lamp biomicroscope should be used when considered appropriate (e.g., assessing depth of injury when corneal ulceration is present). Digital photographs should be taken to document ocular lesions and help assess their severity, progression, and resolution.

4.3 ICCVAM Recommendations: Future Studies for the Use of Humane Endpoints

ICCVAM recommends that additional data should be collected on the use of fluorescein staining to monitor wound healing. These data should be evaluated to identify criteria that may be useful as humane endpoints to terminate studies. ICCVAM recommends that guidelines should be developed for (1) the frequency of fluorescein staining that can be conducted without significant impacts on wound healing that would affect classification categories and (2) the usefulness of the area, intensity, and progression/regression of fluorescein staining for identifying specific hazard classification categories.

ICCVAM also recommends the following:

- Studies should be conducted to identify earlier, more predictive endpoints such as those quantifying area and intensity of fluorescein staining.
- Data should be collected during current testing to support the identification of potential earlier endpoints and to facilitate development of a database that can be used to identify useful earlier endpoints.
- Data should be collected to further evaluate pannus as a potential earlier humane endpoint. (ICCVAM did not consider the BRD data sufficient to determine the adequacy of pannus as a recommended humane endpoint for terminating a test.)

- Improved guidance should be developed on clinical signs of pain and distress in rabbits. Pain assessment training is also an important part of an effective pain management program and should be routinely provided to relevant personnel.
- Users should provide NICEATM with detailed data and observations collected from ocular safety studies that can be used to create a database to (1) further characterize the usefulness and limitations of proposed humane endpoints and (2) identify potential new endpoints. Such data submissions will contribute to efforts to find ways to further avoid or minimize pain and distress during ocular safety assessments.

Independent Peer Review Panel Conclusions and Recommendations

The Panel agreed with the draft ICCVAM recommendations for future studies related to the routine use of humane endpoints to avoid or minimize pain and distress in ocular safety testing. The Panel also recommended a number of additional studies, which have been incorporated into the ICCVAM recommendations listed above. The Panel emphasized that Animal Health Technologist (AHT) training requirements are an important part of a successful humane endpoint program.

5.0 Validation Status of the Use of Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing

Public Health Service policy and U.S. Department of Agriculture 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. Finally, Institutional Animal Care and Use Committees must ensure that the principal investigator complies with the requirements. Of the animals reported to the Department of Agriculture as experiencing unrelieved pain and distress, the majority are justified by regulatory testing requirements.

The OECD published a guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety assessment tests (OECD 2000). According to this document, guiding principles for humane endpoints include:

- Designing studies to minimize any pain, distress, or suffering, consistent with the scientific objective of the study
- Sacrificing animals at the earliest indication of severe pain, distress, or impending death, and avoiding severe pain, suffering, or death as endpoints
- Terminating animal studies once study objectives are achieved or when it is realized that these objectives will not be achieved
- Including knowledge about the test substance in the study design
- Defining in the protocol or standard operating procedure the conditions under which authorized personnel should intervene to alleviate pain and distress by humane killing

Accordingly, humane endpoints recognized and accepted by current EPA (2003), Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007), and EU (2001) regulatory guidelines for ocular hazard assessment include severe and enduring signs of pain or distress or eye lesions considered to be irreversible.

A recent report of the National Research Council Committee on Recognition and Alleviation of Pain in Laboratory Animals emphasized the need for increased efforts to identify appropriate humane endpoints (NRC 2009).

During the 2005 symposium "Minimizing Pain and Distress in Ocular Toxicity Testing," panelists recommended early adverse responses that could serve as early humane endpoints to terminate animals on a study. Among the invited participants were human and veterinary ophthalmologists and anesthesiologists, scientific experts in ocular hazard testing, research scientists, and industrial toxicologists. The following ocular lesions are predictive of maximal severity, that of a severe irritant or corrosive with irreversible effects, including EPA Category I (2003) GHS Category 1 (UN 2007), and EU Category R41 (2001). They could be used routinely as humane endpoints to terminate a study.

- Endpoints currently accepted for study termination (OECD 2002)
  - Draize corneal opacity score of 4 that persists for 48 hours
  - Corneal perforation or significant corneal ulceration including staphyloma
  - Blood in the anterior chamber of the eye
  - Absence of light reflex that persists for 72 hours
  - Ulceration of the conjunctival membrane

  - Necrosis of the conjunctiva or nictitating membrane
  - Sloughing
- Vascularization of the corneal surface (i.e., pannus)
- Destruction of more than 75% of the limbus
- No diminishment in area of fluorescein staining and/or increase in depth of injury over time
- Lack of re-epithelialization 5 days after application of the test substance
- Depth of injury to the cornea (routinely using slit-lamp and fluorescein staining) in which corneal ulceration extends beyond superficial layers of the stroma

The Panel discussed other endpoints that might allow for early termination of a study. These included destruction of the limbus and the relationship to re-epithelialization of the cornea, and positive results in Shirmer's test. Shirmer's test measures moisture content of the corneal tear film. A positive result in Shirmer's test suggests that conjunctival redness is likely to return to normal within 21 days. After these discussions, the endpoints described above were recommended for routine use. As discussed in **Section 4.0**, the Panel also recommended many of these endpoints (see the Panel's full report at http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm).

6.0 ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process provides numerous opportunities for public stakeholder involvement, including submission of written comments and oral comments at ICCVAM independent peer review panel and SACATM meetings. **Table 6-1** lists the nine different opportunities for public comments that were provided during the ICCVAM evaluation of the validation status of alternative ocular safety testing methods and approaches. The number of public comments received in response to each of the opportunities is also indicated. Thirty-seven comments were received. Comments received in response to or related to the *Federal Register* notices are accessible on the NICEATM–ICCVAM website. The following sections, delineated by *Federal Register* notice, briefly discuss the public comments received.

Table 6-1 Opportunities for Public Comment

Opportunities for Public Comment	Date	Number of Public Comments Received
70 FR 13512: Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel	March 21, 2005	0
72 FR 26396: Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing	May 9, 2007	1
72 FR 31582: Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods	June 7, 2007	0
73 FR 18535: Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data	April 4, 2008	12
74 FR 14556: Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments	March 31, 2009	8
74 FR 19562: Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)	April 29, 2009	2
Independent Scientific Peer Review Panel Meeting: Alternative Ocular Safety Testing Methods	May 19–21, 2009	12
SACATM Meeting, Arlington Hilton, Arlington, VA	June 25–26, 2009	2
74 FR 33444: Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comments	July 13, 2009	0

6.1 Public Comments in Response to 70 FR 13512 (March 21, 2005): Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel

NICEATM requested (1) submission of data that would assist in evaluating the validation status of non-animal methods and approaches used for determining the skin and eye irritation potential of AMCP formulations to meet regulatory hazard classification and labeling purposes and (2) nominations of expert scientists to serve as members of an independent peer review panel.

No data or nominations were received in response to this *Federal Register* notice.

6.2 Public Comments in Response to 72 FR 26396 (May 9, 2007): Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

NICEATM requested submission of (1) data and information on the use of topical anesthetics and systemic analgesics for alleviating pain and distress in rabbits during eye irritation testing and (2) information about other procedures and strategies that may reduce or eliminate pain and distress associated with *in vivo* eye irritation methods.

Public Response
NICEATM received one comment in response to this *Federal Register* notice.

Comment:
The commenter supported the use of anesthetics to minimize pain and distress in rabbit eye irritation studies and offered assistance in the evaluation. However, the commenter noted that data from their studies involving the use of local anesthetics could not be shared without permission of its sponsors.

ICCVAM Response:
ICCVAM encourages users to provide data that are generated from future studies, as they could be used to further characterize the usefulness and limitations of topical anesthetics and systemic analgesics for avoiding or minimizing pain and distress in ocular safety assessments.

6.3 Public Comments in Response to 72 FR 31582 (June 7, 2007): Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods

NICEATM requested data on substances tested for ocular irritancy in humans, rabbits, and/or *in vitro* to be used to:

- Review the state of the science in regard to the availability of accurate and reliable *in vitro* test methods for assessing the range of potential ocular irritation activity, including whether ocular damage is reversible or not
- Expand NICEATM's high-quality ocular toxicity database. *In vitro* test methods for which data are sought include but are not limited to (1) the bovine corneal opacity and permeability test, (2) the isolated rabbit eye test, (3) the isolated chicken eye test, and (4) the hen's egg test–chorioallantoic membrane.

No data or information was received in response to this *Federal Register* notice.

6.4 Public Comments in Response to 73 FR 18535 (April 4, 2008): Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

NICEATM requested the following:

- Nominations of expert scientists to serve as members of an independent peer review panel
- Submission of relevant data and information on AMCPs or related substances obtained from (1) human testing or experience, including reports from accidental exposures, and (2) rabbit testing using the standard eye test or the LVET
- *In vitro* ocular safety test methods such as the bovine corneal opacity and permeability test method, the Cytosensor® Microphysiometer test method, and the EpiOcular test method, including data supporting the accuracy and reproducibility of these methods

In response to this *Federal Register* notice, NICEATM received 12 comments, including nominations of 20 potential panelists. The nominees were included in the database of experts from which the Panel was selected. No additional data were received.

6.5 Public Comments in Response to 74 FR 14556 (March 31, 2009): Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments

NICEATM requested public comments on the draft BRDs, SRDs, and draft ICCVAM test method recommendations that were provided to an independent scientific peer review panel meeting (May 19–21, 2009). These documents summarized the current validation status of several test methods and testing strategies for identifying potential ocular irritants. The test methods and testing strategies included the following:

- A testing strategy that proposes the use of three *in vitro* test methods to assess the eye irritation potential of AMCPs
- Four *in vitro* test methods for identifying moderate (EPA Category II, UN Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Category 2A) and mild (EPA Category III, GHS Category 2B) ocular irritants and substances not classified as ocular irritants (EPA Category IV, GHS Not Classified)
- The *in vivo* LVET
- A proposal for the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid and minimize pain and distress during *in vivo* ocular irritation testing

NICEATM received 20 comments in response to this *Federal Register* notice. Eight written comments were received before the Panel meeting, and 12 oral comments were provided at the Panel meeting.

No written comments were relevant to the use of topical anesthetics, systemic analgesics, or earlier humane endpoints to minimize pain and distress in ocular safety testing.

None of the 12 oral public comments provided at the Panel meeting was relevant to the use of topical anesthetics, systemic analgesics, or earlier humane endpoints to avoid or minimize pain and distress in ocular safety testing.

6.6 Public Comments in Response to 74 FR 19562 (April 29, 2009): Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

NICEATM announced the SACATM meeting (June 25–26, 2009) and requested written and public oral comments on the agenda topics.

Public Response:
NICEATM received four comments. Two written comments were received before the meeting, and two oral comments were provided at the SACATM meeting.

SACATM Response:
In general, SACATM was pleased with the Panel report. One SACATM member expressed the need for harmonization in the assessment of performance standards. Another SACATM member said the focus should be on the GHS system because it will ultimately be adopted. Another SACATM member expressed concern regarding the availability of the Cytosensor® Microphysiometer.

6.7 Public Comments in Response to 74 FR 33444 (July 13, 2009): Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comments

NICEATM requested submission of written public comments on the independent scientific peer review panel report.

No public comments were received.

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Appendix A

ICCVAM Evaluation Timeline

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ICCVAM Evaluation Timeline

May 13, 2005	The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) holds symposia on Minimizing Pain and Distress in Ocular Toxicity Testing where an expert panel recommends routine use of topical anesthetics and systemic analgesics and consideration of additional humane endpoints in ocular toxicity testing.
May 9, 2007	*Federal Register* Notice (72 FR 26396) – Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing.
April 4, 2008	*Federal Register* Notice (73 FR 18535) – Non-Animal Methods and Approaches for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data.
March 31, 2009	*Federal Register* Notice (74 FR 14556) – Announcement of an Independent Scientific Peer Review Panel Meeting on the Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Availability of Draft Background Review Documents (BRD) and Summary Review Documents (SRD); Request for Comments.
May 19-21, 2009	Independent Scientific Peer Review Panel holds a public meeting, with opportunity for public comments, at CPSC Headquarters in Bethesda, MD. The Panel was charged with reviewing the current validation status of alternative ocular safety testing methods and strategies, and commenting on the extent to which the information in the draft BRD and SRD supported the draft ICCVAM test method recommendations.
June 25-26, 2009	SACATM public meeting, SACATM and public comments on the draft Panel conclusions and recommendations.
July 13, 2009	*Federal Register* notice (74 FR 33444) – Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments.
October 29, 2009	ICCVAM endorses the Test Method Evaluation Report, which includes the final Background Review Document and Summary Review Document.

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Appendix B

ICCVAM-Recommended Protocol:

Revised OECD Test Guideline 405 (Draize Test for Acute Eye Irritation/Corrosion)

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DRAFT PROPOSED REVISIONS TO GUIDELINES FOR OCULAR SAFETY TESTING

Boldface, underlined text represents ICCVAM's draft proposed revisions to Test Guideline 405.

Acute Eye Irritation/Corrosion

INTRODUCTION

Various national and international guidelines exist for acute eye irritation and corrosion testing. These guidelines are periodically reviewed to ensure that they reflect the best available science. ICCVAM and an independent international scientific peer review panel recently reviewed the usefulness and limitations of routinely using topical anesthetics, systemic analgesics, and humane endpoints during required *in vivo* ocular irritation safety testing (15). Based on this review, ICCVAM recommends that national and international guidelines be updated to require the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid and minimize pain and distress during acute eye irritation and corrosion testing. Proposed revisions to ocular test guidelines are provided as tracked changes in this document.

Balanced preemptive pain management should always be provided when the Draize rabbit eye test is conducted for regulatory safety testing and hazard classification and labeling purposes. The pain management should include (1) routine pretreatment with a topical anesthetic (e.g., proparacaine or tetracaine) and a systemic analgesic (e.g., buprenorphine), (2) a routine post-treatment schedule with systemic analgesia and a nonsteroidal anti-inflammatory drug (e.g., meloxicam), (3) scheduled observation, monitoring, and recording of animals for clinical signs of pain and/or distress, and (4) scheduled observation, monitoring, and recording of the nature, severity, and progression of all eye injuries. Further detail is provided in the updated procedures described below.

It was also recommended that test animals be comprehensively evaluated for the presence or absence of ocular lesions one hour after test substance administration (TSA), followed by at least daily evaluations. Animals should be evaluated once daily for the first 3 days, or more often if necessary to ensure that termination decisions are made in a timely manner. ICCVAM also recommends that test animals be routinely evaluated for clinical signs of pain and/or distress (e.g., repeated pawing or rubbing of the eye, excessive blinking, excessive tearing [Wright et al. 1985; NRC 2008, 2009]) at least twice daily, with a minimum of 6 hours between observations, or more often if necessary. This is necessary to (1) adequately assess animals for evidence of pain and distress in order to make informed decisions on the need to increase the dosage of analgesics and (2) assess animals for evidence of established humane endpoints in order to make informed decisions on whether it is appropriate to humanely euthanize animals, and to ensure that such decisions are made in a timely manner (see paragraph 26). ICCVAM also recommends that fluorescein staining should be routinely used and a slit lamp biomicroscope used when considered appropriate (e.g., assessing depth of injury when corneal ulceration is present), as an aid in the detection and objective measurement of ocular endpoints, and to evaluate the extent that established criteria for humane euthanasia have been addressed.

Definitions of acute eye irritation and corrosion are set out in the Annex to the Guideline.

INITIAL CONSIDERATIONS

In the interest of both sound science and animal welfare, *in vivo* testing should not be considered until all available data relevant to the potential eye corrosivity/irritation of the substance have been

evaluated in a weight-of-the-evidence analysis. Such data will include evidence from existing studies in humans and/or laboratory animals, evidence of corrosivity/irritation of one or more structurally related substances or mixtures of such substances, data demonstrating high acidity or alkalinity of the substance (4)(5), and results from validated and accepted *in vitro* or *ex vivo* tests for skin corrosion and irritation (6)(7). The studies may have been conducted prior to, or as a result of, a weight-of-the-evidence analysis.

For certain substances, such an analysis may indicate the need for *in vivo* studies of the ocular corrosion/irritation potential of the substance. In all such cases, before considering the use of the *in vivo* eye test, preferably a study of the *in vivo* dermal effects of the substance should be conducted first and evaluated in accordance with Testing Guideline 404 (8). The application of a weight-of-the-evidence analysis and the sequential testing strategy should decrease the need for *in vivo* testing for eye corrosivity/irritation of substances for which sufficient evidence already exists from other studies. If a determination of eye corrosion or irritation potential cannot be made using the sequential testing strategy, even after the performance of an *in vivo* study of dermal corrosion and irritation, an *in vivo* eye corrosion/irritation test may be performed.

A preferred sequential testing strategy, which includes the performance of validated *in vitro* or *ex vivo* tests for corrosion/irritation, is included as a Supplement to this guideline. The strategy was developed at, and unanimously recommended by the participants of, an OECD workshop (9), and has been adopted as the recommended testing strategy in the Globally Harmonized System for the Classification of Chemical Substances (GHS) (10). It is recommended that this testing strategy be followed prior to undertaking *in vivo* testing. For new substances it is the recommended stepwise testing approach for developing scientifically sound data on the corrosivity/irritation of the substance. For existing substances with insufficient data on skin and eye corrosion/irritation, the strategy should be used to fill missing data gaps. The use of a different testing strategy or procedure, or the decision not to use a stepwise testing approach, should be justified.

PRINCIPLE OF THE *IN VIVO* TEST

Following pretreatment with a systemic analgesic and induction of appropriate topical anesthesia, the substance to be tested is applied in a single dose to one of the eyes of the experimental animal; the untreated eye serves as the control. The degree of eye irritation/corrosion is evaluated by scoring lesions of conjunctiva, cornea, and iris, at specific intervals. Other effects in the eye and adverse systemic effects are also described to provide a complete evaluation of the effects. The duration of the study should be sufficient to evaluate the reversibility or irreversibility of the effects.

Animals showing continuing signs of severe distress and/or pain at any stage of the test **or lesions consistent with the humane endpoints described in this test guideline** should be humanely killed, and the substance assessed accordingly. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document (11).

PREPARATIONS FOR THE *IN VIVO* TEST

Selection of species

The albino rabbit is the preferable laboratory animal, and healthy young adult animals are used. A rationale for using other strains or species should be provided.

Preparation of animals

Both eyes of each experimental animal provisionally selected for testing should be examined within 24 hours before testing starts. Animals showing eye irritation, ocular defects, or pre-existing corneal injury should not be used.

Housing and feeding conditions

Animals should be individually housed. The temperature of the experimental animal room should be 20°C (± 3°C) for rabbits. Although the relative humidity should be at least 30% and preferably not exceed 70%, other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unrestricted supply of drinking water.

TEST PROCEDURE

Use of topical anesthetics and systemic analgesics

The following modified Draize rabbit eye test pain management procedures are to be used to avoid or minimize pain and distress in ocular safety testing procedures. Alternate procedures that have been determined to provide as good or better avoidance or relief of pain and distress may be substituted.

Sixty minutes prior to test substance application (TSA), buprenorphine 0.01 mg/kg is administered by subcutaneous injection (SC) to provide a therapeutic level of systemic analgesia.

Five minutes pre-TSA, one or two drops of a topical ocular anesthetic (e.g., 0.5% proparacaine hydrochloride or 0.5% tetracaine hydrochloride) are applied to each eye. The eye of each animal that is not treated with a test article, but which is treated with topical anesthetics, serves as a control. If the test substance is anticipated to cause significant pain and distress, consideration should be given to additional applications of the topical anesthetic at 5-minute intervals pre-TSA. Users should be aware that multiple applications of topical anesthetics could increase the severity and/or extend the time required for lesions that are chemically induced to clear.

If a test subject shows signs of pain and distress during the test interval, additional analgesia (i.e., a "rescue" dose of 0.03 mg/kg SC buprenorphine) would be given immediately and repeated every 8 hours, instead of 0.01 mg/kg SC every 12 hours. Meloxicam would continue with the same dose and interval as described below. The "rescue" analgesia should be given immediately post-TSA if pre-emptive analgesia and topical anesthesia is inadequate.

After the initial 8 hrs post-TSA treatment, if ocular lesions and/or clinical signs of pain and distress are present, buprenorphine 0.01 mg/kg SC should be administered every 12 hours (8 hours if the "rescue" dose is needed), in conjunction with meloxicam 0.5 mg/kg SC every 24 hours.

Application of the test substance

The test substance should be placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control.

Irrigation

The eyes of the test animals should not be washed for at least 24 hours following instillation of the test substance, except for solids (see paragraph 16), and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate.

Use of a satellite group of animals to investigate the influence of washing is not recommended unless it is scientifically justified. If a satellite group is needed, two rabbits should be used. Conditions of washing should be carefully documented, e.g., time of washing; composition and temperature of wash solution; duration, volume, and velocity of application.

Dose level

(1) Testing of liquids

For testing liquids, a dose of 0.1 mL is used. Pump sprays should not be used for instilling the substance directly into the eye. The liquid spray should be expelled and collected in a container prior to instilling 0.1 mL into the eye.

(2) Testing of solids

When testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 mL or a weight of not more than 100 mg. The test material should be ground to a fine dust. The volume of solid material should be measured after gently compacting it, e.g., by tapping the measuring container. If the solid test substance has not been removed from the eye of the test animal by physiological mechanisms at the first observation time point of 1 hour after treatment, the eye may be rinsed with saline or distilled water.

(3) Testing of aerosols

It is recommended that all pump sprays and aerosols be collected prior to installation into the eye. The one exception is for substances in pressurised aerosol containers, which cannot be collected due to vaporisation. In such cases, the eye should be held open, and the test substance administered to the eye in a simple burst of about one second, from a distance of 10 cm directly in front of the eye. This distance may vary depending on the pressure of the spray and its contents. Care should be taken not to damage the eye from the pressure of the spray. In appropriate cases, there may be a need to evaluate the potential for "mechanical" damage to the eye from the force of the spray.

An estimate of the dose from an aerosol can be made by simulating the test as follows: the substance is sprayed on to weighing paper through an opening the size of a rabbit eye placed directly before the paper. The weight increase of the paper is used to approximate the amount sprayed into the eye. For volatile substances, the dose may be estimated by weighing a receiving container before and after removal of the test material.

Initial test (*in vivo* eye irritation/corrosion test using one animal)

As articulated in the sequential testing strategy (Supplement to Guideline), it is strongly recommended that the *in vivo* test be performed initially using one animal.

If the results of this test indicate the substance to be corrosive or a severe irritant to the eye using the procedure described, further testing for ocular irritancy should not be performed.

Confirmatory test (*in vivo* eye irritation test with additional animals)

If a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals. If a severe irritant effect is observed in the initial test indicating a possible strong (irreversible) effect in the confirmatory testing, it is recommended that

the confirmatory test be conducted in a sequential manner in one animal at a time, rather than exposing the two additional animals simultaneously. If the second animal reveals corrosive or severe irritant effects, the test is not continued. Additional animals may be needed to confirm weak or moderate irritant responses.

Observation period

The duration of the observation period should be sufficient to evaluate fully the magnitude and reversibility of the effects observed. However, the experiment should be terminated at any time that the animal shows continuing signs of severe pain or distress (9). To determine reversibility of effects, the animals should be observed normally for 21 days post administration of the test substance. If reversibility is seen before 21 days, the experiment should be terminated at that time.

Clinical observations and grading of eye reactions

The eyes should be **comprehensively evaluated for the presence or absence of ocular lesions one hr post-TSA, followed by at least daily evaluations. Animals should be evaluated once daily for the first 3 days, or more often if necessary, to ensure that termination decisions are made in a timely manner. Test animals should be routinely evaluated for clinical signs of pain and/or distress (e.g., repeated pawing or rubbing of the eye, excessive blinking, excessive tearing [Wright et al. 1985; NRC 2008, 2009]) at least twice daily, with a minimum of 6 hours between observations, or more often if necessary. Fluorescein staining should be routinely used and a slit lamp biomicroscope used when considered appropriate (e.g., assessing depth of injury when corneal ulceration is present) as an aid in the detection and objective measurement of ocular endpoints. Digital photographs of observed lesions should be collected for reference and to provide a permanent record of the extent of ocular damage. A written record of all observations should be made to facilitate and document decisions on the progression or resolution of such ocular lesions.** Animals should be kept on test no longer than necessary once definitive information has been obtained. Animals showing continuing severe pain or distress should be humanely killed without delay, and the substance assessed accordingly.

Animals with the following eye lesions post-instillation should be humanely killed: corneal perforation or significant corneal ulceration including staphyloma; blood in the anterior chamber of the eye; grade 4 corneal opacity which persists for 48 hours; absence of a light reflex (iridial response grade 2) which persists for 72 hours; ulceration of the conjunctival membrane; necrosis of the conjuctivae or nictitating membrane; or sloughing. This is because such lesions generally are not reversible. **Furthermore, it is recommended that the following ocular lesions should also be used as earlier humane endpoints to terminate studies before the end of the scheduled 21-day observation period. These lesions are considered predictive of severe irritant or corrosive injuries and injuries that are not expected to fully reverse by the end of the 21-day observation period after treatment: severe depth of injury (e.g., corneal ulceration extending beyond the superficial layers of the stroma), limbus destruction >50% (as evidenced by blanching of the conjunctival tissue), and severe eye infection (purulent discharge). Used in combination, vascularization of the cornea surface (i.e., pannus), area of fluorescein staining not diminishing over time based on daily assessment, and lack of re-epithelialization 5 days after test substance application should be considered as potentially useful criteria to influence the clinical decision on early study termination. However, there are insufficient data to use these endpoints individually to justify early study termination. ICCVAM emphasizes that once severe ocular effects have been identified, an attending or qualified laboratory animal veterinarian should be consulted for a clinical examination to determine if the combination of these effects warrants early study termination.**

Draize scores are obtained and recorded at 1, 24, 48, and 72 hours following test substance application. Animals that do not develop ocular lesions may be terminated not earlier than 3 days post instillation. Animals with mild to moderate lesions should be observed until the lesions clear, or for 21 days, at which time the study is terminated. Observations should be performed **and recorded daily until** 21 days in order to determine the status of the lesions, and their reversibility or irreversibility.

The grades of ocular reaction (conjunctivae, cornea and iris) should be recorded at each examination (Table I). Any other lesions in the eye (e.g. pannus, staining, **anterior chamber changes**) or adverse systemic effects should also be reported.

Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device. After recording the observations at 24 hours, the eyes may be further examined with the aid of fluorescein.

The grading of ocular responses is necessarily subjective. To promote harmonisation of grading of ocular response and to assist testing laboratories and those involved in making and interpreting the observations, the personnel performing the observations need to be adequately trained in the scoring system used.

DATA AND REPORTING

Evaluation of results

The ocular irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. The individual scores do not represent an absolute standard for the irritant properties of a material, as other effects of the test material are also evaluated. Instead, individual scores should be viewed as reference values and are only meaningful when supported by a full description and evaluation of all observations.

Test report

The test report must include the following information:

Rationale for *in vivo* testing: weight-of-the-evidence analysis of pre-existing test data, including results from sequential testing strategy:

- description of relevant data available from prior testing;
- data derived in each step of testing strategy;
- description of *in vitro* tests performed, including details of procedures, results obtained with test/reference substances;
- description of *in vivo* dermal irritation / corrosion study performed, including results obtained;
- weight-of-the-evidence analysis for performing *in vivo* study

Test substance:

- identification data (e.g. CAS number, source, purity, known impurities, lot number);
- physical nature and physicochemical properties (e.g. pH, volatility, solubility, stability, reactivity with water);
- in case of a mixture, composition and relative percentages of components;

- if local anaesthetic is used, identification, purity, type, dose, and potential interaction with test substance.

Vehicle:

- identification, concentration (where appropriate), volume used;
- justification for choice of vehicle.

Test animals:

- species/strain used, rationale for using animals other than albino rabbit;
- age of each animal at start of study;
- number of animals of each sex in test and control groups (if required);
- individual animal weights at start and conclusion of test;
- source, housing conditions, diet, etc.

Results:

- description of method used to score irritation at each observation time (e.g., hand slitlamp, biomicroscope, fluorescein);
- tabulation of irritant/corrosive response data for each animal at each observation time up to removal of each animal from the test;
- narrative description of the degree and nature of irritation or corrosion observed;
- description of any other lesions observed in the eye (e.g., vascularization, pannus formation, adhesions, staining);
- description of non-ocular local and systemic adverse effects, **record of clinical signs of pain and distress, digital photographs,** and histopathological findings, if any.

Discussion of results.

Interpretation of the results

Extrapolation of the results of eye irritation studies in laboratory animals to humans is valid only to a limited degree. In many cases the albino rabbit is more sensitive than humans to ocular irritants or corrosives.

Care should be taken in the interpretation of data to exclude irritation resulting from secondary infection.

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TABLE: GRADING OF OCULAR LESIONS

Cornea

Opacity: degree of density (readings should be taken from most dense area)*

No ulceration or opacity 0

Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible 1

Easily discernible translucent area; details of iris slightly obscured 2

Nacrous area; no details of iris visible; size of pupil barely discernible 3

Opaque cornea; iris not discernible through the opacity 4

Maximum possible: 4

* The area of corneal opacity should be noted

Iris

Normal 0

Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect 1

Hemorrhage, gross destruction, or no reaction to light 2

Maximum possible: 2

Conjunctivae

Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)

Normal 0

Some blood vessels hyperaemic (injected) 1

Diffuse,crimson colour; individual vessels not easily discernible 2

Diffuse beefy red 3

Maximum possible: 3

Chemosis

Swelling (refers to lids and/or nictating membranes)

Normal 0

Some swelling above normal 1

Obvious swelling, with partial eversion of lids 2

Swelling, with lids about half closed 3

Swelling, with lids more than half closed 4

Maximum possible: 4

ANNEX

DEFINITIONS

1. Eye irritation is the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.

2. Eye corrosion is the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.

Appendix C

Background Review Document:
Use of Topical Anesthetics, Systemic Analgesics, and Earlier Humane Endpoints to Minimize Pain and Distress in Ocular Safety Testing

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Background Review Document

Use of Topical Anesthetics, Systemic Analgesics, and Earlier Humane Endpoints to Minimize Pain and Distress in Ocular Toxicity Testing

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

2010

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List of Figures

List of Abbreviations and Acronyms

BRD	Background Review Document
Colipa	European Cosmetic, Toiletry and Perfumery Association
COX	Cyclooxygenase
CPSC	(U.S.) Consumer Product 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
FR	*Federal Register*
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
HCl	Hydrochloric acid
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IRAG	Interagency Regulatory Alternatives Group
JaCVAM	Japanese Center for the Validation of Alternative Methods
kg	Kilogram
mg	Milligram
mM	Millimole
µL	Microliter
µg	Microgram
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NRC	National Research Council
NSAID	Nonsteroidal anti-inflammatory drug
NTP	(U.S.) National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	Ocular Toxicity Working Group
UN	United Nations
w/v	Weight-to-volume ratio

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Preface

The use of pretreatment analgesia in the Draize rabbit eye test method (Draize et al. 1944), though not formal policy among all U.S. Federal agencies, is a protocol refinement that could provide a significant reduction in animal pain and distress. Since 1984, the U.S. Consumer Product Safety Commission has recommended preapplication of tetracaine ophthalmic anesthetic for all rabbit eye toxicity studies. However, current Environmental Protection Agency (EPA) and Organisation for Economic Co-operation and Development (OECD) test guidelines for the rabbit eye test state that topical anesthetics can be used only if the user demonstrates that such pretreatments do not interfere with the results of the tests. Therefore, topical anesthetics often are not used because a separate study may be necessary to provide such information.

In a 1991 workshop the Interagency Regulatory Alternatives Group (IRAG) organized a workshop entitled "Updating Eye Irritation Methods: Use of Ophthalmic Topical Anesthetics." The consensus among invited experts was that use of anesthesia is acceptable in eye irritation testing because pain is temporarily relieved, and the extent of injury can be evaluated (Seabaugh et al. 1993). In 2003, the EPA nominated four areas for evaluation by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM was asked to evaluate ways of alleviating pain and suffering that might arise from administration of mild to moderate irritants in current *in vivo* eye irritation testing.

ICCVAM, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the European Centre for the Validation of Alternative Methods organized a symposium entitled "Minimizing Pain and Distress in Ocular Toxicity Testing" in May 2005 (**Annex I**). The symposium was supported by the European Cosmetic, Toiletries and Perfumery Association. Similar to the 1991 IRAG workshop, invited experts at agreed that topical anesthesia should be routinely provided as a pretreatment to animals used for ocular toxicity testing. The invited experts added that (1) combinations of general or topical anesthesia and systemic analgesia should be routinely used to avoid pain and (2) induced lesions should be treated with continued systemic analgesia during the observation period. Specifically, the invited experts indicated that sufficient data existed for combining a topical anesthetic (e.g., tetracaine or proparacaine) with a systemic analgesic (e.g., buprenorphine) to minimize or eliminate pain during ocular toxicity testing. In addition, the invited experts indicated that it might be useful to conduct controlled studies in rabbits to confirm the efficacy of this approach. Ideally, data could be collected during routine safety testing and periodically analyzed to determine efficacy for specific lesion types and clinical signs of pain.

A review of studies reported in the literature provides conflicting results on the impact of topical ocular anesthetics on ocular irritation and physiology. Some studies indicate that topical anesthetics do not interfere with the irritation response (Arthur et al. 1986; Heywood and James 1978; Seabaugh et al. 1993; Ulsamer et al. 1977). Others state that there is a trend (although not statistically significant) of increased irritancy in eyes treated with anesthesia (Johnson 1980; Durham et al. 1992). Some have also reported that anesthetics interfere with the irritant response and yield unreliable data (Walberg 1983; Rowan and Goldberg 1985).

Participants at the 2005 symposium "Minimizing Pain and Distress in Ocular Toxicity Testing" also discussed early adverse responses predictive of ocular lesions associated with severe irritant or corrosive substances (EPA Category I [EPA 1998], GHS Category I [UN 2007], EU R41 [EU 2001], or) that could be used routinely as humane endpoints to terminate a study.

The purpose of this document is to comprehensively review all available information on the safety and efficacy (or potential efficacy) of selected anesthetics and analgesics for relieving ocular pain, as well as to identify humane endpoints that could warrant terminating a study. It also describes the

results from a joint study conducted by NICEATM and Product Safety Labs to evaluate the effect of pretreatment with the topical anesthetic tetracaine hydrochloride (0.5% w/v) on the ocular irritancy potential of 97 formulations.

We gratefully acknowledge the organizations and scientists who provided data and information for this document. We also acknowledge the efforts of those individuals who helped prepare this summary review document, including the following staff from the NICEATM support contractor, Integrated Laboratory Systems, Inc.: David Allen, Jon Hamm, Nelson Johnson, Brett Jones, Elizabeth Lipscomb, Linda Litchfield, Gregory Moyer, Catherine Sprankle, and Jim Truax. We thank the members of the ICCVAM Ocular Toxicity Working Group, co-chaired by Karen Hamernik, Ph.D. (EPA), and Jill Merrill, Ph.D. (U.S. Food and Drug Administration), as well as the ICCVAM representatives who reviewed and provided comments throughout the process leading to this draft version. We also thank Valerie Zuang, Ph.D., and Dr. Hajime Kojima, Ph.D., the Ocular Toxicity Working Group liaisons from the European Centre for the Validation of Alternative Methods and the Japanese Centre for the Validation of Alternative Methods, respectively, for their participation.

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Executive Summary

Human and veterinary medicine have provided a great deal of clinical experience with a range of topical anesthetics and systemic analgesics for the relief of ocular pain. However, the subjective nature of identifying and treating pain in animals makes it difficult to establish which therapeutic options are most effective. Few published studies relate directly to the eye. Most studies focus on the relief of pain after surgery and/or pain resulting from trauma.

Since 1984, the U.S. Consumer Product Safety Commission has recommended applying tetracaine ophthalmic anesthetic before applying test substances in all rabbit eye toxicity studies. However, current test guidelines for the rabbit eye test from the U.S. Environmental Protection Agency (EPA) and Organisation for Economic Co-operation and Development (OECD) state that topical anesthetics can be used only if the user demonstrates that such pretreatment does not interfere with the results of the tests.[1] Therefore, toxicity studies seldom use topical anesthetics because providing the necessary information would likely require a separate study.

Use of Topical Anesthetics and Systemic Analgesics

In 1991, the Interagency Regulatory Alternatives Group organized a workshop titled "Updating Eye Irritation Methods: Use of Ophthalmic Topical Anesthetics." The workshop evaluated use of topical ophthalmic anesthetics and/or systemic analgesics during the Draize rabbit eye test. A symposium titled "Minimizing Pain and Distress in Ocular Toxicity Testing" re-examined this topic in 2005. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the European Centre for the Validation of Alternative Methods organized the symposium, which was supported by the European Cosmetic, Toiletry and Perfumery Association.

Both meetings produced similar recommendations and recognition of the limitations associated with the use of topical anesthetics and systemic analgesics. Experts acknowledged that a single treatment with a topical anesthetic to anesthetize the surface of the cornea before applying the test substance could cause slight physiologic changes that might alter the response. However, most felt that such alterations would be minor, if any. The effect would likely be a slight increase in irritant response. Such topical anesthesia is used in millions of cataract surgeries annually. It is also used during routine eye exams to anesthetize the corneal surface before measuring intraocular pressure for glaucoma screening. NICEATM recently evaluated how pretreatment with tetracaine hydrochloride (0.5% w/v) affected the potential of 97 formulations to irritate the eye. The results indicate that pretreatment did not affect the hazard classification observed during the test.

Most meeting participants considered the use of topical anesthetics acceptable, because the anesthetics at least prevent discomfort caused by applying the test substance on the eye and temporarily prevent any pain and distress that might result from immediate ocular damage. Participants in both meetings recommended that combinations of general or topical anesthesia and systemic analgesia be routinely used to prevent pain. They also recommended that lesions caused by the substances be treated with continued systemic analgesia. Participants also recognized that, although many types of systemic analgesics could help alleviate pain, opioid analgesics (e.g., buprenorphine) were likely to be most effective in ocular safety testing. Because of their effects on the wound healing process, other analgesics (e.g., nonsteroidal anti-inflammatory drugs) could be expected to adversely affect results.

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[1] OECD Test Guideline 405 states, "The type, concentration, and dose of a local anesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use" (OECD 1987). Similarly, the EPA (1998) states, "The type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use."

The many studies detailing the safety and efficacy of tetracaine and proparacaine suggest that they are among the most widely used topical anesthetics. Proparacaine is relatively harmless to the corneal epithelium and provides extended anesthesia. Thus, it may be more appropriate for treating ophthalmic pain. However, the reported adverse effects of tetracaine and proparacaine on wound healing suggest that their use beyond acute pain relief may be limited. Thus, they are recommended for use only as initial anesthetics in an *in vivo* ocular toxicity test.

Workshop and symposium participants also recommended pretreatment with a systemic analgesic to relieve ocular pain that might result from any chemically induced injuries. Administering preemptive analgesia is more effective than waiting to treat the pain after it begins. Preemptive analgesia is common in veterinary medicine. Among systemic analgesics, veterinarians use the lipophilic opioid, buprenorphine, most frequently. Buprenorphine's margin of safety is well characterized in multiple species. A single dose is recommended for routine pretreatment before a Draize rabbit eye test. If no painful lesions or clinical signs of pain and distress occur, then no further doses are administered. If painful lesions or clinical signs of pain and distress are observed, then continuing systemic analgesia is recommended until these lesions and/or clinical signs are absent.

The effectiveness of buprenorphine in relieving postsurgical pain in rabbits is well documented. However, few studies have evaluated how effectively buprenorphine relieves ocular pain. Trevithick et al. (1989) found that buprenorphine injected at 5-hour intervals maintained a stable degree of analgesia for 24 hours. In addition, buprenorphine has a long history of managing postoperative pain in humans.

Based on its history as an effective analgesic for moderate to severe pain in rabbits, dosing of buprenorphine is typically administered by subcutaneous or intramuscular injection every 12 hours (0.01 to 0.05 mg/kg; Kohn et al. 2007). However, Buprederm™, a new transdermal formulation of buprenorphine, has been shown to provide sustained analgesia during the 72-hour patch application period. No local irritation appeared with repeated patch application in rabbits (Park et al. 2008). This suggests that repeated use of Buprederm™ patches might provide effective pain relief during the observation period required for ocular toxicity testing (i.e., up to 21 days).

Use of Humane Endpoints to Terminate an Ocular Toxicity Study

Public Health Service policy and U.S. Department of Agriculture regulations on pain and distress in laboratory animals state that more than momentary or light pain and distress:

- Should be limited to that which is unavoidable for the conduct of scientifically valuable research or testing
- Should be conducted with appropriate pain-relief medication unless justified in writing by the principal investigator
- Should continue for only the necessary amount of time required to attain the scientific objectives of the study
- 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. Finally, the Institutional Animal Care and Use Committees must ensure that the principal investigator complies with the requirements.

A recent report of the National Research Council Committee on Recognition and Alleviation of Pain in Laboratory Animals emphasized the need for increased efforts to identify appropriate humane endpoints (NRC 2009).

Participants at the 2005 symposium "Minimizing Pain and Distress in Ocular Toxicity Testing" also discussed early adverse responses predictive of ocular lesions associated with severe irritant or corrosive substances. Such substances are classified as EPA Category I (1998), Globally Harmonized

System of Classification and Labelling of Chemicals Category 1 (UN 2007), and/or European Union R41 (EU 2001). The adverse responses under discussion could be used routinely as humane endpoints to terminate a study.

Symposium invitees included human and veterinary ophthalmologists and anesthesiologists, scientific experts in ocular hazard testing, research scientists, and industrial toxicologists. After discussion, they recommended the following endpoints for routine use for early study termination:

- Endpoints currently accepted for study termination (OECD 2002):
  - Draize corneal opacity score of 4 that persists for 48 hours
  - Corneal perforation or significant corneal ulceration, including staphyloma
  - Blood in the anterior chamber of the eye
  - Absence of light reflex that persists for 72 hours
  - Ulceration of the conjunctival membrane
  - Necrosis of the conjunctiva or nictitating membrane
  - Sloughing
- Vascularization of the corneal surface (i.e., pannus)
- Destruction of more than 75% of the limbus
- Area of fluorescein staining not diminishing over time based on daily assessment
- Lack of re-epithelialization 5 days after application of the test substance
- Depth of injury to the cornea (routinely using slit-lamp and fluorescein staining), where ulceration extends beyond superficial layers of the stroma, or increase in the depth of injury over time

ICCVAM has considered the relevant data, information, and analyses provided in this background review document and developed draft recommendations on the use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress in ocular toxicity testing. These recommendations are provided in a separate document. The recommendations include proposed usefulness and limitations, proposed changes to the current standardized test method protocol, and proposed future studies and activities.

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1.0 Background

Draize et al. (1944) developed the rabbit eye test to test the ocular hazard potential of new chemicals or chemical products. Substances identified as potential ocular hazards could then be appropriately labeled and handled to protect humans from potential exposure. Sensitivity to animal use and concerns about the reliability of this test method have led to a search for alternative *in vitro* test methods for ocular hazard assessment (e.g., cell-based models, organotypic models, hemodynamic models). Several of these *in vitro* test systems have been evaluated in large validation studies (e.g., Balls et al. 1995; Gettings et al. 1996). However, until validated alternatives are accepted as complete replacements, the Draize rabbit eye test will continue to be required for ocular hazard evaluation by U.S. Federal and European regulatory agencies.

One of the main concerns with this test method is the possibility that pain and/or discomfort may be produced in the test animals. In spite of efforts designed to screen substances for suspected corrosive or severe ocular irritant properties (e.g., eliminating pH extremes and dermal corrosives from testing), the potential remains for discomfort from materials with unknown remains. However, it should be noted that the Public Health Service Policy on Humane Care and Use of Laboratory Animals states that "Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia unless the procedure is justified for scientific reasons in writing by the investigator" (PHS 2002). This implies that such measures should be regularly considered.

Since 1984, the U.S. Consumer Product Safety Commission (CPSC) has recommended preapplication of tetracaine ophthalmic anesthetic for all rabbit eye toxicity studies (CPSC 1984). However, current U.S. Environmental Protection Agency (EPA) and Organisation for Economic Co-operation and Development (OECD) test guidelines for the rabbit eye test state that topical anesthetics can be used only if the user demonstrates that such pretreatments do not interfere with the results of the tests (EPA 1998; OECD 1987).[2] For this reason, anesthetics are seldom used because a separate study to provide such information would often be necessary.

In 1991, an *ad hoc* committee of the Interagency Regulatory Alternatives Group (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. Two 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.

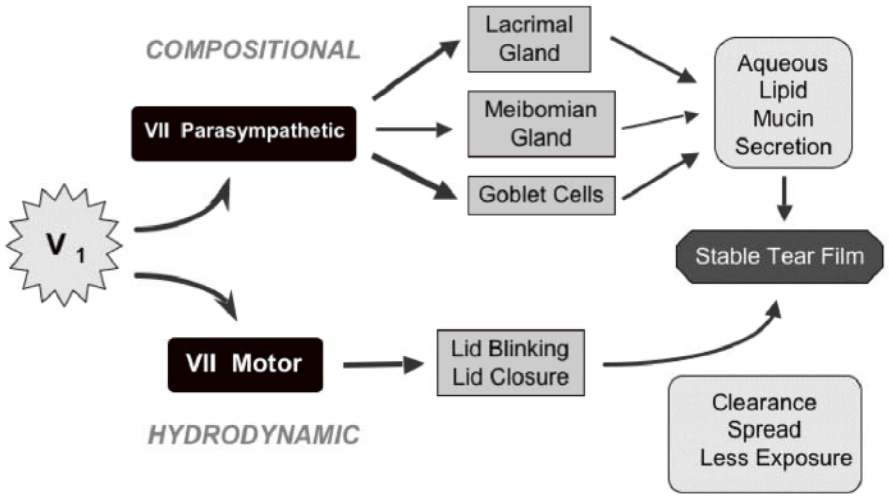
Briefly, the ocular defense is controlled by two neural reflexes via sensory input from V1 (i.e., the first branch of the trigeminal nerve) and via two separate (i.e., motor and parasympathetic) branches of the VII facial nerve. The VII facial nerve dictates the hydrodynamic and compositional elements of the external adnexae, lids and ocular surface epithelia for maintaining a stable tear film (**Figure 1-1**) (Tseng and Tsubota 1997). Therefore, the level of ocular injury may be exaggerated following topical anesthetic administration due to reduction in ocular defense mechanisms (e.g., neuronal activation of goblet cells for tear fluid secretion). Duration of injury may be lengthened by impairment of repair processes (e.g., decreased release of chemokines or reduction in level of collagen deposition). Despite these issues, and although it was not formal policy among U.S. Federal agencies, a consensus of those participating on the IRAG committee considered the use of anesthetics acceptable because such

[2] OECD Test Guideline 405 states that "The type, concentration, and dose of a local anesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use." Similarly, EPA states that "The type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use" (1998).

measures provide at least temporary pain relief for the animal, and the time and extent of injury can still be evaluated.

Despite these recommendations, there is little evidence to suggest that measures to prevent or reduce pain during the rabbit eye test are regularly employed. In order to re-examine the need for such measures, a symposium entitled "Minimizing Pain and Distress in Ocular Toxicity Testing" met at the National Institutes of Health in Bethesda, Maryland, on May 13, 2005 (**Annex I**). The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the European Centre for the Validation of Alternative Methods (ECVAM) organized the symposium.

Figure 1-1. A Stable Tear Film is Maintained by a Sound Ocular Surface Defense Governed by Neuroanatomic Integration (Tseng and Tsubota 1997)



The European Cosmetic, Toiletry and Perfumery Association provided additional funding. Invited experts included ophthalmologists, scientific experts in ocular hazard testing and method development, research scientists, U.S. Federal regulators, and industry toxicologists. This symposium was organized to better understand the mechanisms and physiological pathways of the pain response, to recognize symptoms and signs of the pain response, and to identify effective means to alleviate or prevent pain while preserving the ocular injury responses used to identify hazard potential. The experts who participated in this symposium concluded that pain relief in animals used for ocular toxicity testing should routinely be provided as a pretreatment. In addition, they recommended that combinations of general or topical anesthesia and preemptive systemic analgesia be routinely used to avoid pain on initial test article application. They also recommended the use of continued systemic analgesia treatment of any persistent lesions.

The purpose of this background review document is to comprehensively review available information on the safety and efficacy (or potential efficacy) of selected anesthetics and analgesics for relieving ocular pain, as well as to identify humane endpoints that could warrant terminating a study. It also describes the results from a joint study conducted by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods and Product Safety Labs, which

evaluated the effect of pretreatment with the topical anesthetic tetracaine hydrochloride (0.5% w/v) on the ocular irritancy potential of 97 formulations (**Annex II**).

2.0 Clinical Identification of Ocular Pain in Animals

There is no direct measure for the experience of pain, and the recognition of pain in animals has been further confounded in part by the evolutionary process (Wright et al. 1985; Hansen 1997). Ill or injured animals are typically abandoned by their companions because they may become targets for predators. In this regard, abnormal behavior is avoided at all costs to ensure survival. While domestic and laboratory animal species have largely been removed from such survival pressures, these inherited behaviors may still hinder the interpretation of animal pain (Wright et al. 1985). With that said, an animal in pain, regardless of the species in question, will likely display one or more of the following symptoms (Cramlet and Jones 1976; Wright et al. 1985):

- Increased skeletal muscle tone, blood pressure, and/or heart rate
- Attraction to the area of pain
- Pupillary dilation
- Altered respiration

Furthermore, it has been proposed that signs such as reluctance to move, scratching, and rubbing indicate ophthalmic pain specifically (Wright et al. 1985).

Pain scoring systems in humans rely on an interactive dialogue between the patient and clinician to assign a subjective approximation of intensity (e.g., Scott and Huskisson 1976). Although such an interaction with animals is not feasible, subjective pain scoring systems have been developed for companion animal species (e.g., Smith et al. 2004) that grade the extent of movement and vocalization. Comfort, appearance, and behavior are also observed and graded. These scores are then combined into a total subjective pain score that may be used to define thresholds for severe pain. Such scoring systems may not be applicable to laboratory animal species because of their behavioral differences. However, trauma eventually produces some degree of pain, and the presence of pain should be assumed following tissue injury. Therefore, it may be more important to establish whether an animal would benefit from analgesic therapy, rather than whether or not the animal is experiencing pain (Hansen 1997). Most recently, an American College of Laboratory Animal Medicine Task Force published *Guidelines for the Assessment and Management of Pain in Rodents and Rabbits* (Kohn et al. 2007), which provides methods for assessing pain and recommendations for pain management.

3.0 Options for Pain Relief in Animals

3.1 Topical Anesthetics

Local anesthesia refers to the loss of sensation in a limited area of the body (Wright et al. 1985). Topical anesthetics reduce pain by blocking sodium channels in excitable neurons, thus inhibiting the action potential generated by membrane depolarization when large, transient increases in sodium permeability are produced in response to an irritant (Catterall and Mackie 2001). However, topical anesthetics are also 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 the toxicokinetics of test substances (e.g., increase permeability of corneal epithelium, break down barriers that shield toxicity) and thus confound test results.

Topical ocular anesthetics may be divided into those with 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 after administration with a duration of 20 minutes to 2 to 3 hours. Application frequency of these topical anesthetics increases duration but not depth of anesthesia.

The two most commonly used topical ocular anesthetics are proparacaine and tetracaine (Wilson 1990, Bartfield et al. 1994). Lidocaine is also commonly used. These drugs are intended for short-term use only, because chronic use is associated with toxicity to ocular tissues that subsequently delays corneal wound healing (Zagelbaum et al. 1994; Moreira et al. 1999). They are also contraindicated in the treatment of corneal ulcers because they disrupt the tear film and retard the initial phase of re-epithelialization (Ketring 1980). Chronic use of topical anesthetics has even been associated with permanent corneal scarring and decreased vision (Rapuano 1990). However, these agents rapidly reduce the subjective signs of corneal pain, and thus can quickly differentiate between pain from superficial sources (e.g., cornea) from pain arising from deeper structures in the eye (Ketring 1980; Bartfield et al. 1994).

The presence of preservatives (e.g., benzalkonium chloride, chlorobutanol) in topical anesthetic ophthalmic formulations and their potential effect on ocular irritation classification schemes cannot be discounted either. For example, benzalkonium chloride, a Category I irritant, may cause surface epithelial damage and a complete breakdown of transcorneal electrical resistance linked to a breakdown in barrier function (Chetoni et al. 2003).

In vitro studies suggest that tetracaine is more damaging to the corneal epithelium than proparacaine (Grant and Acosta 1994; Moreira et al. 1999). In addition, clinical studies indicate that instillation of proparacaine eye drops is less painful than instillation of tetracaine (Bartfield et al. 1994). These findings suggest that proparacaine may be considered the preferred topical anesthetic for ocular studies. However, a recent evaluation by NICEATM of the effects of topical pretreatment with tetracaine hydrochloride (0.5% w/v) on the ocular irritancy potential of 97 formulations indicated that such pretreatments had no impact on (1) the hazard classification severity category of observed ocular irritation, (2) the variability in rabbit ocular irritation responses, or (3) the number of days required for an ocular lesion to clear (**Annex II**). A comparison of the relevant properties of proparacaine and tetracaine with regard to their impacts on corneal wound healing and irritant hazard classification is detailed in **Annex III.**

The rabbit has a low blink rate relative to humans and several authors have directly or indirectly studied the effect of topical anesthetics on blink rate. Maurice (1995) used fluorophores and a

noninvasive fluorometer and found that the low blink rate in rabbits would be expected to increase 3-fold the area under the curve for drug penetration in the corneal tear film relative to humans. Thus, the penetration of a drug could be underestimated on the basis of blink rate alone. However, for most drugs, the epithelial permeability is sufficiently high to permit drug penetration from the tear film into the epithelium within minutes, in which case contact time becomes irrelevant.

Schwartz et al. (1998) studied tetrodotoxin for its potential to produce long-lasting topical anesthesia in the eye of the rabbit. Anesthesia produced by topical administration of 10 mM tetrodotoxin solution produced anesthesia that lasted 8 hours compared to 1 hour or slightly longer for 0.5% proparacaine. The blink rate was reduced 67% by 10 mM tetrodotoxin compared to approximately 13% for proparacaine. Lower concentrations of tetrodotoxin, 0.1 and 1 mM, produced no anesthesia or anesthesia of shorter duration, respectively, compared to the 10 mM concentration. It should be noted that while no signs of overt systemic toxicity were observed in the study, the LD_{50} of tetrodotoxin in the rabbit is less than 10 µg/kg by intramuscular or subcutaneous routes of administration. Naase et al. (2005) studied the spontaneous eyeblink rates of human volunteers without exogenous stimuli by using the topical anesthetic, benoxinate (0.4%). The authors reported a 63% decrease in the spontaneous eyeblink rate after anesthetic treatment, but found that the patterns of the blink rates (i.e., symmetrical, J- and I-type) were unaffected by anesthetic treatment.

3.2 Systemic Analgesics

Analgesia refers to relief of pain. 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 or prolong chemically induced lesions by causing a reduction in ocular defense mechanisms (e.g., neuronal activation of goblet cells for tear fluid secretion), as previously mentioned. For this reason, administering systemic analgesics during the post-treatment observation period may be a more useful approach to relieving pain from ocular lesions.

3.2.1 Opioid Analgesics

Much of the available data on the efficacy of systemic opioid analgesics focus on peri- or postoperative uses, on which several thorough reviews are available (Flecknell 1984; Flecknell and Liles 1990; Flecknell 1991; Flecknell and Liles 1992; Flecknell 1995). Perhaps the greatest clinical concern regarding the use of these types of agents is the side effects with which they are associated. In humans, opioid administration is commonly associated with respiratory depression. However, this effect is less pronounced in animals, especially when mixed agonist/antagonist opioids (e.g., buprenorphine) are used (Flecknell 1995). In this regard, a wide safety margin for buprenorphine has been demonstrated in rabbits, where doses ranging from 0.0075 to 0.3 mg/kg produce effective analgesia without serious respiratory depression (Flecknell and Liles 1990). Reports of clinical studies in humans describe a low incidence of local and/or systemic adverse effects, a lack of immunotoxicity associated with other opioids (e.g., morphine), and maintenance of cognitive function during long-term therapy (Scott et al. 1980; Budd 2002; Budd and Collett 2003; Sorge and Sittl 2004).

Another concern regarding systemic opioid use is that many of these drugs provide only short-term analgesia, with maintenance of pain relief requiring repeated administration every 1 to 3 hours. From a practical perspective for a testing laboratory, such a regimen is clearly not feasible. One exception is buprenorphine, which has been shown in humans, pigs, rodents, and rabbits to provide effective pain relief for up to 12 hours (Cowan et al. 1977; Heel et al. 1979; Dum and Herz 1981; Hermanssen et al. 1986; Flecknell and Liles 1990; Flecknell 1996). This may be due to the fact that buprenorphine dissociates very slowly from its receptor relative to other opioids, which has been demonstrated *in vitro* (PDR 2004). Studies in multiple species have also shown that, while the intensity of analgesia induced by buprenorphine does not appear to increase with dose, the duration of analgesia is dose

dependent (Cowan et al. 1977; Hermanssen et al. 1986; Hoskin and Hanks 1987; Nolan et al. 1987; Flecknell and Liles 1990). However, the onset of action is delayed in rabbits (approximately 30 minutes after treatment), suggesting that buprenorphine treatment prior to testing a potentially irritating/corrosive substance is warranted (Flecknell and Liles 1990).

Taken together, these findings likely contribute to the fact that buprenorphine is one of the most commonly used analgesic agents in laboratory and companion animal species, as demonstrated by multiple surveys of its use in veterinary practice (Dohoo and Dohoo 1996; Hubbell and Muir 1996; Watson et al. 1996; Capner et al. 1999; Lascelles et al. 1999; Joubert 2001). However, as indicated above, many of the reported veterinary uses of buprenorphine have focused on relief of surgical pain. Based on its long history of successful veterinary use as an analgesic for moderate to severe pain in rabbits, dosing of buprenorphine is typically provided by subcutaneous or intramuscular injections every 12 hours (0.01 to 0.05 mg/kg; Kohn et al. 2007).

A limited number of studies have evaluated the efficacy of buprenorphine in the relief of ocular pain. Trevithick et al. (1989) used esthesiometry to evaluate prolonged corneal analgesia produced in rabbits by repeated intramuscular injections of buprenorphine or meperidine in the presence of short-term anesthesia induced by ketamine and xylazine. Analgesia was established based on esthesiometric measurements of the intensity of surface pressure to the cornea required to induce a blink reflex. The authors found that buprenorphine injections at 5-hour intervals were sufficient to maintain a stable degree of analgesia for the entire study period (24 hours). The dosing regimen was based on previous studies in which the maximum period of analgesia obtained was 5 hours (Trevithick et al. 1989).

3.2.1.1 Alternative Dosing Routes for Buprenorphine

Regardless of the route of administration, buprenorphine is primarily excreted in the feces, with only a small amount present in the urine. For this reason, buprenorphine is considered the safest opioid for use in cases of renal impairment (Budd and Collett 2003). Buprenorphine undergoes significant first-pass metabolism in the gastrointestinal mucosa and liver following oral administration and is therefore typically administered by intravenous, intramuscular, or subcutaneous injection. However, in an effort to reduce the pain and distress associated with parenteral delivery, alternative dosing strategies might be worthy of consideration. Because buprenorphine hydrochloride is lipophilic and has a low molecular weight, it has been recognized as an excellent candidate for sublingual and/or transdermal delivery, both of which bypass first-pass metabolism. However, sublingual delivery successfully bypasses first-pass metabolism only when the drug is not swallowed, and at least 50% of a sublingual dose may be recovered in the saliva (Mendelson et al. 1997; Hand et al. 1990; Lindhardt et al. 2001). This caveat makes the veterinary utility of such a route questionable.

In vitro skin penetration studies have demonstrated that transdermal delivery of buprenorphine can achieve a systemic analgesic effect (Roy et al. 1994). In fact, transdermal buprenophine is presently being prescribed clinically in Europe and Australia for the treatment of chronic severe disabling pain. It is also being studied in the United States for its safety and efficacy for similar indications. For transdermal delivery, buprenorphine is incorporated within an adhesive polymer matrix that provides slow, consistent release into the circulation at a predetermined rate, maintaining a relatively constant serum drug concentration over at least 72 hours (Sittl 2005).

A new transdermal formulation of buprenorphine currently under development using a proprietary hydrogel matrix technology (Buprederm™) has shown faster absorption and sustained analgesia throughout a 72-hour period. Maximum analgesic effect was obtained between 3 and 6 hours and was maintained for 24 hours after patch application (Park et al. 2008). In a multiple-dose study in which patches were applied to rabbits every 4 days (3 days attachment and 1 day detachment) for 28 days, Buprederm™ was found to provide maximum plasma buprenorphine concentration by 3 hours after administration, with this concentration being maintained for 72 hours. Over the 28 days, there was no

accumulation of buprenorphine systemically or in the local skin, and analgesia was maintained without measurable skin irritation (Park et al. 2008). Buprederm™ may therefore provide both fast-acting and long-lasting analgesia suitable for use in the rabbit eye irritation test. Investigations will be necessary to determine the impact of Buprederm™ on test results.

Intranasal delivery of buprenorphine has been studied in humans, rabbits, and sheep (Eriksen et al. 1989; Lindhardt et al. 2000; Lindhardt et al. 2001). A reported advantage of the intranasal route is the reduced mean time to maximal serum concentration (i.e., T_{max}) relative to the sublingual and transdermal routes (Lindhardt et al. 2001). This property may make intranasal buprenorphine delivery more amenable to the treatment of acute pain. However, it should be noted that this method requires specific manipulation of the animal to maximize drug delivery. The animal must be maintained in a supine position during dosing and for at least 1 minute after dosing.

Rectal gels containing buprenorphine have also been formulated with water-soluble dietary fibers, xanthan, and locust bean gums. Using these gels, rapid absorption and bioavailability of buprenorphine was achieved in rabbits without adversely affecting the rectal mucosa (Watanabe et al. 1996). These properties suggest that rectal gels, like the intranasal route, may be preferable to transdermal or sublingual buprenorphine delivery systems for the treatment of acute pain. This method also requires specific manipulation of the test animals because they must be restrained during the dosing procedure with the gel tube adhered to the anus and fastened with a clip to prevent rejection (Watanabe et al. 1996).

Hanson et al. (2001) reported that buprenorphine administered twice daily at an analgesic dose of 0.05 mg/kg had no effect on immunological evaluation of Shigella vaccine candidates in the Sereny test, a model of keratoconjunctivitis in the guinea pig. It did, however, result in a significant increase in mucopurulent discharge that required frequent cleaning of the affected eyes. The authors indicated that this effect did not appear to affect the outcome of the test results. The authors also reported significant weight loss of 5.5% to 5.8% in buprenorphine-treated animals relative to the saline control group, which gained 4% to 5% in body weight over the 5-day course of study.

3.2.2 Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs inhibit fever, pain, and inflammation by inhibiting the two isoforms of the enzyme fatty acid cyclooxygenase (COX; the constitutive COX-1 and the cytokine and inflammatory mediator-inducible COX-2) with varying degrees of selectivity (Vane et al. 1998). Inhibition of COX decreases arachidonic acid metabolism and the resulting prostaglandin and leukotriene products that induce pain, fever, and other inflammatory processes. One NSAID, acetaminophen, is an effective analgesic and antipyretic agent but is less effective as an anti-inflammatory agent because it inhibits COX activity only in the brain. Acetaminophen may therefore be less likely to interfere with wound healing.

Several published reports have examined the effect of NSAIDs on the eye wound healing process in rabbits, particularly following excimer laser keratectomy surgery (Loya et al. 1994; Nassaralla et al. 1995; Park and Kim 1996; Kaji et al. 2000). The results have been varied. Kaji et al. (2000) reported that topical administration of diclofenac significantly decreased early-phase conjunctival inflammation in rabbits but did not inhibit corneal haze formation. Similar studies have also reported that topical diclofenac administration influenced corneal and stromal wound healing in rabbits following excimer laser surgery (Nassaralla et al. 1995; Park and Kim 1996). In contrast, Loya et al. (1994) reported that diclofenac did not significantly affect corneal wound healing or epithelial migration rate when used up to eight times daily. Similarly, Hersh et al. (1990) observed that diclofenac decreased early epithelialization but had no apparent effect on corneal stromal healing. Finally, it was reported that suprofen and flurbiprofen, two alternative topical ophthalmic NSAIDs,

did not significantly inhibit corneal wound healing in rabbits either (Miller et al. 1981; Lee et al. 1985).

When employed as analgesics, NSAIDs are efficacious for pain of low to moderate intensity, such as dental pain. While they do not produce the maximal pain relief threshold of opioids, neither do they elicit the unwanted central nervous system effects such as respiratory depression and physical dependence attributed to many opioids. However, NSAIDs are associated with certain adverse effects. Common side effects of nonselective COX inhibitors include gastric ulceration and intolerance, inhibition of platelet function, alterations in renal and hepatic function, and hypersensitivity reactions. In contrast, selective COX-2 inhibitors produce less gastric irritation, do not inhibit platelet function, and are less likely to produce hypersensitivity reactions (Roberts and Morrow 2001).

With respect to ocular use, systemic Banamine® (flunixin megulamine) has been used with some success in combination with topical antibiotics to treat corneal stromal abscesses in horses (Hendrix et al. 1995). However, the authors noted that, similar to topical NSAIDs, Banamine's inhibition of the COX pathway provided by systemic NSAIDs likely delayed corneal vascularization, which in turn delayed resolution of the lesion. This implies that the use of systemic NSAIDs must strike a careful balance between reducing inflammation and retarding wound healing (Hendrix et al. 1995).

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

Public Health Service policy and U.S. Department of Agriculture 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 Department of Agriculture that experience unrelieved pain and distress are justified by regulatory testing requirements.

The Organisation for Economic Co-operation and Development (OECD) 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 the following:

- designing studies to minimize any pain, distress, or suffering, consistent with the scientific objective of the study
- sacrificing animals at the earliest indication of severe pain, distress, or impending death, and avoiding severe pain, suffering, or death as endpoints
- terminating animal studies once study objectives are achieved or when it is realized that these objectives will not be achieved
- including knowledge about the test substance in the study design
- defining in the protocol or standard operating procedure the conditions under which authorized personnel should intervene to alleviate pain and distress by humane killing.

Accordingly, humane endpoints recognized and accepted by current EPA (2003), Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) and European Union (EU 2001) regulatory guidelines for ocular hazard assessment include severe and enduring signs of pain or distress or eye lesions considered to be irreversible.

A recent report of the National Research Council Committee on Recognition and Alleviation of Pain in Laboratory Animals emphasized the need for increased efforts to identify appropriate humane endpoints (NRC 2009).

During the 2005 symposium "Minimizing Pain and Distress in Ocular Toxicity Testing," panelists discussed early adverse responses predictive of ocular injury outcome in humans. Following are ocular lesions considered predictive of maximal severity (severe irritant or corrosive with irreversible effects, including EPA Category I [EPA 2003], GHS Category I [UN 2007], and EU R41 [EU 2001]) that could be used routinely as humane endpoints to terminate a study:

- Endpoints currently accepted for study termination (i.e., Draize corneal opacity score of 4 that persists for 48 hours, corneal perforation or significant corneal ulceration including staphyloma, blood in the anterior chamber of the eye, absence of light reflex that persists for 72 hours, ulceration of the conjunctival membrane, necrosis of the conjunctiva or nictitating membrane, or sloughing [OECD 2002])
- Vascularization of the corneal surface (i.e., pannus)
- Destruction of more than 75% of the limbus
- No diminishment in area of fluorescein staining and/or increase in depth of injury increased over time

- Lack of re-epithelialization 5 days after application of the test substance
- Depth of injury to the cornea (routinely using slit-lamp and fluorescein staining) in which corneal ulceration extends beyond superficial layers of the stroma

The panel discussion also led to a discussion of other endpoints that 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, which 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.

5.0 Summary

Both human and veterinary medicine have provided a great deal of clinical experience with a range of topical anesthetics and systemic analgesics for the relief of pain. However, the subjective nature of identifying and treating pain in animals makes it difficult to establish the relative usefulness of available therapeutic options. This is particularly true in the case of ophthalmic pain. Few published studies relate directly to the eye, as the majority have focused on the relief of postsurgical pain and/or pain resulting from trauma.

Based on the large volume of studies detailing the safety and efficacy of tetracaine and proparacaine, these topical anesthetics appear to be among the most widely used in practice. Proparacaine may be considered more appropriate for treating ophthalmic pain given its relative innocuousness to the corneal epithelium and the extended duration of anesthesia it affords. However, their reported adverse effects on wound healing suggest that the utility of these agents beyond acute pain relief may be limited. Thus they are recommended for use only as initial analgesic therapy in an *in vivo* ocular toxicity test.

The most commonly used systemic analgesic among veterinarians is the lipophilic opioid buprenorphine, which has a well-characterized margin of safety in multiple species. While its usefulness in relieving postsurgical pain in rabbits is well documented, little data support its use for ophthalmic pain. However, Buprederm™, a new transdermal formulation of buprenorphine currently under development, provides sustained analgesia over the 72-hour patch application period, with no local irritation with repeated patch application. This suggests that repeated use of Buprederm™ patches may provide effective pain relief over the observation period required during ocular toxicity testing (i.e., up to 21 days).

Sufficient data suggest that combining a topical anesthetic (e.g., proparacaine) with a systemic analgesic (e.g., buprenorphine or Buprederm™ patches used repeatedly) may provide an effective therapeutic approach to minimizing or eliminating ocular pain during ocular toxicity testing. For this reason, ICCVAM proposes that topical anesthetics be routinely used prior to instillation of a test substance unless adequate scientific rationale indicates that they should not be used. In addition, in order to minimize pain and distress from ocular damage caused by corrosive or severely irritating substances, a single dose of a systemic analgesic should be used routinely before instillation of a test substance. Treatment with a systemic analgesic should continue as long as a test animal displays clinical signs of more than momentary or slight pain or distress (e.g., vocalization, pawing at the treated eye).

As an additional measure to minimize pain and distress, ICCVAM recommends that ocular lesions considered predictive of severe irritant or corrosive substances (EPA Category I [EPA 2003], GHS Category 1 [UN 2007], and EU R41 [EU 2001]) be used routinely as humane endpoints to terminate a study.

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7.0 Glossary[3]

Adnexa: Adjacent anatomical parts.

Analgesia: A deadening or absence of the sense of pain without loss of consciousness.

Anesthesia: The loss of sensation or of the response to pain stimuli that results from inhibition of nerve excitation or conduction.

Anesthetic: A drug that induces anesthesia by inhibiting nerve excitation or conduction when applied or injected locally at the site of injury or topically (e.g., on the skin, mucous membrane, or surface of the cornea).

Assay:[4] The experimental system used. Often used interchangeably with *test* and *test method*

Chemokines: Any of various cytokines produced in acute and chronic inflammation that mobilize and activate white blood cells.

Chemosis: A form of eye irritation in which the membranes that line the eyelids and surface of the eye (*conjunctiva*) become swollen.

Classification system: An arrangement of quantified results or data into groups or categories according to previously established criteria.

Conjunctiva: The mucous membrane that lines the inner surfaces of the eyelids and folds back to cover the front surface of the eyeball, except for the central clear portion of the outer eye (the cornea). The conjunctiva is composed of three sections: palpebral conjunctiva, bulbar conjunctiva, and fornix.

Cornea: The transparent part of the coat of the eyeball that covers the iris and pupil and admits light to the interior.

Corneal opacity: A subjective measurement of the extent of opaqueness of the cornea following exposure to a test substance. Increased corneal opacity is indicative of damage to the cornea.

Corrosion: Destruction of tissue at the site of contact with a substance.

Corrosive: A substance that causes irreversible tissue damage at the site of contact.

Cyclooxygenase: Either of two related enzymes (i.e., COX-1 and COX-2) that control the production of prostaglandins and are blocked by aspirin

Cytokines: Any of several regulatory proteins, such as the interleukins and lymphokines, that are released by cells of the immune system and act as intercellular mediators in the generation of an immune response.

Depth-of-injury: The level of penetration to which injury to various tissue layers of the corneal epithelium produced by a test substance (e.g., epithelium, stroma, endothelium).

Distress: To cause pain, or stress, or suffering to.

Endpoint:[4] The biological process, response, or effect assessed by a test method.

Esthesiometry: The measurement of the degree of tactile or other sensibility.

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[3] The definitions in this Glossary are restricted to their uses with respect to the Draize rabbit eye test method and in the assessment or treatment of pain and distress.

[4] Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003)

Fluorescein staining: A subjective measurement of the extent of fluorescein sodium that is retained by epithelial cells in the cornea following exposure to a test substance. Increased fluorescein retention is indicative of damage to the corneal epithelium.

Globally Harmonized System (GHS): A classification system presented by the United Nations that provides (a) a harmonized criteria for classifying substances and mixtures according to their health, environmental and physical hazards, and (b) harmonized hazard communication elements, including requirements for labeling and safety data sheets.

Hazard:[4] The potential for an adverse health or ecological effect. A hazard potential results only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

Humane endpoints: Predetermined criteria (e.g., severe opacity, perforation, ulceration, or necrosis of the cornea) used to evaluate whether a study should be discontinued early for humane or ethical reasons.

Intramuscular injection: An injection into the substance of a muscle.

Intravenous injection: An injection into a vein.

In vitro: In glass. Refers to assays that are carried out in an artificial system (e.g., in a test tube or petri dish) and typically use single-cell organisms, cultured cells, cell-free extracts, or purified cellular components.

In vivo: In the living organism. Refers to assays performed in multicellular organisms.

Iris: The contractile diaphragm perforated by the pupil and forming the colored portion of the eye.

Lacrimation: Secretion and discharge of tears.

Light reflex: Contraction of the pupil when light falls on the eye.

Limbus: The edge of the cornea where it joins the sclera.

Necrosis: Death of cells or tissues through injury or disease, especially in a localized area of the body.

NSAID: A nonsteroidal anti-inflammatory drug such as aspirin or ibuprofen.

Ocular: Of or relating to the eye.

Ocular corrosive: A substance that causes irreversible tissue damage in the eye following application to the anterior surface of the eye.

Ocular irritant: A substance that produces a reversible change in the eye following application to the anterior surface of the eye.

Ophthalmic: Of or relating to the eye; ocular.

Opioid: Any of various sedative narcotics containing opium or one or more of its natural or synthetic derivatives or a drug, hormone, or other chemical substance having sedative or narcotic effects similar to those containing opium or its derivatives: a natural brain opiate.

Organotypic: An alternative test method that uses an organ harvested from animals that have been killed for food or for other purposes (e.g. isolated chicken eye).

Pain: An unpleasant sensation occurring in varying degrees of severity as a consequence of injury, disease, or emotional disorder; suffering or distress.

Pannus: A specific type of corneal inflammation that begins within the conjunctiva, and with time spreads to the cornea. Also referred to as *chronic superficial keratitis*.

Parenteral injection: Taken into the body or administered in a manner other than through the digestive tract; intravenous or intramuscular.

pH: A measure of the acidity or alkalinity of a solution. A pH of 7.0 is neutral; higher pHs are alkaline, lower pHs are acidic.

Protocol:[4] The precise, step-by-step description of a test, including the listing of all necessary reagents, criteria and procedures for the evaluation of the test data.

Re-epithelialization: The mechanism of reparation of the epithelium involving formation of new cells in the limbus and their growth and migration to replace those cells lost in an area of tissue damage.

Refinement alternative:[4] A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.

Reliability:[4] A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and inter-laboratory reproducibility and intra-laboratory repeatability.

Replacement alternative:[4] A new or modified test method that replaces animals with non-animal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate).

Sereny test: A model of keratoconjunctivitis produced within 24 hours after inoculation of the conjunctival sac with bacteria such as *Escherichia coli* or *Listeria monocytogenes*.

Severe irritant: (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye that is not reversible within 21 days of application or causes serious physical decay of vision. (b) Substances that are classified as GHS Category 1, EPA Category I, or EU R41 ocular irritants.

Shirmer's test: A test for tear production performed by measuring the area of moisture on a piece of filter paper inserted over the conjunctival sac of the lower lid, with the end of the paper hanging down on the outside.

Slit-lamp microscope: An instrument used to directly examine the eye under the magnification of a binocular microscope by creating a stereoscopic, erect image; may also be used with a depth-measuring device to objectively measure corneal thickness.

Sloughing: To shed or cast off epithelial cells; necrotic tissue in the process of separating from viable portions of the body.

Staphyloma: Protrusion of the sclera or cornea, usually lined with uveal tissue, due to inflammation.

Subcutaneous injection: An injection into the subcutaneous layer of the skin.

Tear film: The field covering the anterior surface of the cornea composed of three layers (i.e., mucous, aqueous, lipid) produced by lacrimal fluid and secretions of the meibomian and conjunctival glands.

Test:[4] The experimental system used; used interchangeably with *test method* and *assay*.

Test method[4]**:** A process or procedure used to obtain information on the characteristics of a substance or agent. Toxicological test methods generate information regarding the ability of a substance or agent to produce a specified biological effect under specified conditions. Used interchangeably with *test* and *assay*. See also *validated test method* and *reference test*.

Ulceration: The process of forming a lesion (e.g., erosion) of the corneal epithelium that over time may be accompanied by formation of pus and necrosis of surrounding tissue, usually resulting from inflammation or ischemia.

Validated test method:[4] An accepted test method for which validation studies have been completed to determine the relevance and reliability of this method for a specific proposed use.

Validation:[4] The process by which the reliability and relevance of a procedure are established for a specific purpose.

Vascularization: The process of becoming vascular; angiogenesis.

Weight of evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.

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Annex I

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

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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

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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

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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

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6.2 Invited Experts

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Annex II

Effect of Topical Anesthetic Pretreatment on *In Vivo* Ocular Irritation Hazard Classification

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Effect of Topical Anesthetic Pretreatment on *In Vivo* Ocular Irritation Hazard Classification

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
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2010

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Executive Summary

Background

Accidental eye injury is the leading cause of visual impairment in the United States (U.S. Dept. of Labor Statistics [DOL] 2004). In 2002, injuries from chemicals and their products accounted for 16% of all eye injuries reported as the cause of days away from work f (DOL 2004). Because not all employers are required to report such injuries, these numbers may underestimate the actual number of eye injuries. Based on emergency department reports for work-related eye injuries, the National Institute of Occupational Safety and Health (NIOSH) estimated that approximately 39,200 chemical-related eye injuries occurred in 1998 (NIOSH Work-related Injury Statistics, 2004).

The ocular irritation or corrosion potential of substances to which humans may be exposed has been evaluated since 1944 using the Draize rabbit eye test (Draize et al. 1944). Due to the potential pain and distress that may occur in rabbits after application of a severely irritating or corrosive test substance, several approaches have been undertaken to revise the current *in vivo* test method protocol and testing scheme to decrease the likelihood of causing pain and distress. For example, a weight-of-evidence approach based on all available information (e.g., pH values, dermal corrosivity information, structure-activity relationship data) has been used to classify substances as severely irritating or corrosive prior to *in vivo* testing. However, despite these efforts, some substances that are tested in rabbits may cause pain and distress. Therefore, additional refinements to the *in vivo* test method have been proposed, which include the use of a topical ocular anesthetic prior to test substance administration in the rabbit eye test. This report focuses on results of an evaluation of the effects of pretreatment with the topical anesthetic tetracaine hydrochloride (0.5% w/v) on the ocular irritancy potential of 97 formulations.

Database Used for the Evaluation

Product Safety Laboratories (Dayton, NJ) provided *in vivo* rabbit eye test scores for all observation days for 97 formulations, together with information about testing conditions (e.g., concentration of formulation tested, amount tested). Due to confidentiality requirements, the compositions of the tested formulations were unknown for the purposes of this evaluation.

Test Method Protocol

The formulations were tested in either 3 or 6 rabbits. Sixteen substances were tested in 6 rabbit studies (n=96 rabbits), and 81 substances were tested in three rabbit studies (n=243 rabbits). *In vivo* testing was conducted in accordance with the U.S. Environmental Protection Agency (EPA) guideline on acute eye irritation testing (EPA 1998). Rabbits were tested sequentially, with the first tested rabbit not receiving anesthesia. If any of the subsequently tested rabbits displayed signs of pain or distress after test article application (e.g., vocalization, pawing at the treated eye), the remaining rabbits were pretreated with 0.5% (w/v) tetracaine hydrochloride ophthalmic solution. Two drops of the anesthetic were placed directly on the cornea in each rabbit eye between 30 seconds and approximately 2 minutes prior to instillation of test substance. The conduct of the remainder of the test method protocol was identical to the protocol described in the EPA guideline on acute eye irritation testing (EPA 1998).

Eyes were evaluated at predetermined intervals (e.g., 1 hour and 1, 2, 3, 7, 14, and 21 days after test substance instillation) for development of irritation and/or corrosion. If eye irritation was considered irreversible (e.g., corneal opacity and/or conjunctival irritation was considered severe), the study was terminated. The degree of irritation was scored using the Draize irritation scale. The observation period was at least 72 hours and not longer than 21 days to allow for evaluation of reversal of observed effects.

Results: Impact of Topical Anesthetic Pretreatment on Regulatory Irritancy Classification

Each formulation tested was assessed to determine if the average irritancy response for the rabbits pretreated with topical anesthesia was more severe or less severe than that observed for the rabbits not pretreated with topical anesthesia. Rabbits pretreated with topical anesthesia tended to produce more severe responses than rabbits that were not pretreated with topical anesthesia for all three regulatory hazard classification schemes. However, none of the observed differences were statistically significant.

An additional analysis was conducted to evaluate the variability among rabbit responses, within a given formulation, when topical anesthesia pretreatment was used as a criterion. For most of the formulations, there was no difference in rabbit irritancy classifications between rabbits pretreated with topical anesthesia and those that were not pretreated. For all the evaluated regulatory hazard classifications, there appeared to be better agreement in rabbit responses when rabbits that were not pretreated with anesthesia were compared to those that were pretreated with anesthesia. However, none of the observed differences were statistically significant.

Results: Impact of Topical Anesthetic on the Number of Days Required for an Ocular Lesion to Clear

Each formulation tested was assessed to determine if the number of days required for a lesion to reverse for animals pretreated with topical anesthesia was different than animals that were not pretreated with topical anesthesia. None of the differences observed in the day-to-clearing evaluation (when topically anesthetized rabbits were compared to nonanesthetized rabbits) were statistically significant. The largest observed difference was for opacity clearing day, which tended to be slightly greater in the rabbits pretreated with topical anesthesia when compared to those that were not pretreated. However, this difference (33 vs. 22) was not statistically significant. Corneal opacity was the endpoint with the largest difference in number of days until clearing. Although not statistically significant either, the time to clear for corneal lesions in rabbits pretreated with topical anesthesia was slightly longer than in rabbits that were not pretreated.

Summary

For most of the formulations tested, topical anesthetic pretreatment had no impact on (1) the hazard classification severity category of observed ocular irritation, (2) the variability in rabbit ocular irritation responses, or (3) the number of days required for an ocular lesion to clear. When a difference in ocular irritation was observed, the rabbits pretreated with topical anesthesia more frequently exhibited a more severe response than was observed for rabbits that were not pretreated. However, none of the observed differences were statistically significant. The observed differences occurred in both directions (increasing and decreasing the level of irritancy), which suggests a relation to the inherent variability of the rabbit response rather than to topical anesthetic pretreatment.

These results indicate that topical pretreatment with 0.5% (w/v) tetracaine hydrochloride ophthalmic solution had no significant impact on the variability in rabbit responses to formulations or the number of days required for an ocular lesion to clear. The topical anesthesia pretreatment also did not significantly affect the irritancy classification for the United Nations Globally Harmonized System of Classification and Labelling, EPA, and European Union classification systems.

1.0 Introduction

Accidental eye injury is the leading cause of visual impairment in the United States (U.S. Dept. of Labor [DOL] 2004). In 2002, injuries from chemicals and their products accounted for 16% of all eye injuries reported as the cause of days away from work for employees (DOL 2004). Because not all employers are required to report such injuries, these numbers may underestimate the actual number of eye injuries. Based on emergency department reports for work related eye injuries, the National Institute of Occupational Safety and Health (NIOSH) estimated that approximately 39,200 chemical-related eye injuries occurred in 1998 (NIOSH, 2004).

The ocular irritation or corrosion potential of substances to which humans may be exposed has been evaluated since 1944 using the Draize rabbit eye test (Draize et al. 1944). Several approaches have been undertaken to revise the current *in vivo* test method protocol and testing scheme to decrease the likelihood of potential pain and distress in rabbits during instillation of an irritating test substance. For example, a weight-of-evidence approach has been used to eliminate severely irritating or corrosive substances prior to *in vivo* testing. Criteria that may be used to identify and classify substances as ocular corrosives or severe irritants prior to *in vivo* testing include high or low pH values ($2 < pH < 11.5$), dermal corrosivity, and structure-activity relationship studies that indicate corrosive properties. However, despite these efforts, some substances that are tested *in vivo* are likely to cause pain and distress in the rabbit. Therefore, additional refinements to the *in vivo* test method have been proposed, including the use of a topical ocular anesthetic prior to test substance administration.

Previous studies have shown that the efficacy of topical ocular anesthetics can be dependent upon a variety of a factors including, but not limited to, the anesthetic used, the anesthetic dose used, the application procedure, and the species tested (Ulsamer et al. 1977; Heywood et al 1978; Johnson, 1980; Anonymous, 1981; Walberg, 1983; Rowan and Goldberg, 1985; Arthur et al. 1986; Durham et al. 1992; Seabaugh et al. 1993). Commonly evaluated topical anesthetics include proparacaine, tetracaine, butacaine, and amethocaine.

In 1986, the Modified Ocular Safety Testing Task Force of the Pharmacology and Toxicology Committee of the Cosmetic, Toiletry, and Fragrance Association, Inc., evaluated proparacaine and tetracaine (both tested at 0.5% (w/v)) for their potential to increase or decrease the irritancy of four test substances. Results showed that neither topical anesthetic had a significant effect on the observed irritancy of substances tested but noted a trend of increased irritancy in anesthetized eyes (Arthur et al. 1986). Heywood and James stated that 0.5% proparacaine produced no statistically significant difference between the anesthetized and nonanesthetized corneas when 10% sodium lauryl sulfate was used as the irritant.

In 1991, an *ad hoc* committee of the Interagency Regulatory Alternatives Group (IRAG) organized the workshop Updating Eye Irritation Methods: Use of Ophthalmic Topical Anesthetics to evaluate the use of anesthetics in eye irritation testing. The workshop indicated that the commonly used anesthetics tetracaine (0.5-5%) and proparacaine (0.1-0.5%) produced an almost immediate anesthetic effect lasting up to 20 minutes. These anesthetics eliminated local pain and touch sensation but increased ocular permeability, reduced tear volume, reduced blink frequency, and delayed wound healing (Seabaugh et al. 1993).

Studies by Walberg (Walberg 1983; Rowan and Goldberg 1985) suggested that use of tetracaine hydrochloride (0.5%, two drops on the eye 30 seconds before test substance application) interfered with the irritant response and yielded data that were not reliable. Comparatively, other studies indicated that two doses of tetracaine (10 minutes apart) were effective in abolishing pain and did not interfere with the irritant response (Walberg 1983; Anonymous 1981).

Ulsamer and colleagues reported that when one eye was pretreated with 0.1 mL of 2% butacaine sulfate and the other eye was not, the mean corneal opacity scores significantly differed in 14% (4/29)

of the comparisons made between eyes. In all cases, the anesthetized eye had a higher mean corneal opacity score (Ulsamer et al.1977). Johnson described an *in vivo* evaluation of 31 unidentified substances in which, if the first tested rabbit showed evidence of pain (e.g., eye closure), then the remaining rabbits were pretreated with a topical anesthetic (amethocaine hydrochloride) prior to test substance application (Johnson 1980). The results showed that the level of eye irritation for 14 substances was equivalent between anesthetized and nonanaesthetized rabbits. Of the remaining 17 test substances, the level of eye irritation was greater in anesthetized rabbits in all cases.

Studies also have shown that topical anesthetics can alter ocular physiology (Seabaugh et al. 1993; Rowan and Goldberg, 1985; Durham et al. 1992). Local effects of topical anesthetics include but are not limited to increased permeability of the corneal epithelium, corneal epithelial cell sloughing, decreased lacrimation, and alteration of tear film production. Alone or in combination, these effects may influence the irritancy classification of the tested substance.

The present evaluation focuses on the effect of topical application of 0.5% (w/v) tetracaine hydrochloride on the irritancy potential of 97 formulations. The impact of the anesthetic on irritancy scores, agreement in irritancy classifications between pretreated and untreated rabbits tested with the same formulation, and on the days-to-clearing of ocular lesions were evaluated. Irritancy classifications were assigned according to three hazard classification schemes that are used or proposed for future use in the future for regulatory hazard classification and labeling; the United Nations Globally Harmonized System for Classification and Labelling (GHS) (UN 2007), the U.S. Environmental Protection Agency (EPA 2003) classification scheme, and the European Union (EU 2001) classification scheme.

2.0 Materials and Methods

2.1 Database

Product Safety Laboratories (Dayton, NJ) provided *in vivo* rabbit eye test scores in tabular form for all observation days for 97 formulations, together with information about testing conditions (e.g., concentration of formulation tested, amount tested). Due to confidentiality requirements, the compositions of the tested formulations were unknown during this evaluation.

2.2 *In Vivo* Test Method Protocol

The formulations were tested in either 3 or 6 rabbits. Sixteen substances were tested in six rabbit studies (n=96 rabbits), and 81 substances were tested in three rabbit studies (n=243 rabbits). *In vivo* testing was conducted in accordance with the EPA guideline on acute eye irritation testing (EPA 1998). Briefly, formulations were applied in a single dose to one eye of a rabbit with the other eye serving as a control. Eyes were evaluated for development of irritation and/or corrosion at predetermined intervals (e.g., 1 hour and 1, 2, 3, 7, 14, and 21 days after test substance instillation). If eye irritation was considered irreversible (e.g., corneal opacity and/or conjunctival irritation is considered severe), the study was terminated. The degree of irritation was scored using the Draize irritation scale (Draize et al. 1944). The observation period was at least 72 hours and not longer than 21 days to allow for evaluation of reversal of observed effects.

Anesthetic pretreatment was provided to rabbits in a protocol similar to the one described by Johnson (Durham et al. 1992). Rabbits were tested sequentially, with the first tested rabbit not receiving anesthesia. If any of the subsequently tested rabbits displayed signs of pain or distress after test article application (e.g., vocalization, pawing at the treated eye), the remaining rabbits were pretreated with 0.5% (w/v) tetracaine hydrochloride ophthalmic solution (Bausch & Lomb, Tampa, FL; stored at ambient laboratory temperature and humidity). Two drops of the anesthetic were placed directly on the cornea in each rabbit eye between 30 seconds and approximately 2 minutes before instillation of test substance. The remainder of the test method protocol was conducted exactly as described in the protocol described in the EPA guideline on acute eye irritation testing (EPA 1998).

All studies were conducted in accordance with Good Laboratory Practice guidelines (EPA 2005a, 2005b; FDA 2006).

2.3 Irritancy Classification of Test Substances

As noted above, the *in vivo* rabbit eye database used to conduct this analysis included studies that were conducted in 3 or 6 rabbits. However, some of the *in vivo* classification systems used in this analysis (see below) were intended for studies using 3 or fewer rabbits. Thus, to maximize the amount of data available for the evaluation, the decision criteria for each classification system were expanded to include studies that used more than 3 rabbits.

All regulatory systems require eye lesions to be scored using the Draize scoring system (Draize et al. 1944). In order for a formulation to be included in this evaluation, the following criteria must have been fulfilled:

- A volume of 0.1 mL for liquids, solids, pastes, or particulates (with a weight of not more than 0.1 g) was tested in each rabbit.
- Observations of the eye were recorded at least 24, 48, and 72 hours after test substance application if no severe effect was observed.

- Observations of the eye were made until reversibility was assessed (i.e., lesions were cleared, as defined by the hazard classification definition) or until 21 days had passed. Results from a study terminated early were included if the rationale for the early termination was documented.

If any of the above criteria were not fulfilled, the data were not used for the analysis.

2.4 Hazard Classification Systems

Three regulatory hazard classification systems were used for evaluation of the data. The criteria required by each of these systems for ocular irritancy classification is provided below.

2.4.1 United Nations Globally Harmonized System for Classification and Labelling

The classification of substances according to the GHS classification system was conducted sequentially. Initially each rabbit tested was classified in one of four categories (Category 1, Category 2A, Category 2B, and Not Classified) based on the criteria outlined in **Table 2-1**.

Table 2-1 Criteria for Classification of Rabbits According to the GHS Classification System

GHS Category	Rabbit Criteria Used for Classification
Category 1	Group A[1]: - Effects in the cornea, iris, or conjunctiva that were not expected to reverse or did not fully reverse[2] within the observation period of 21 days, or - A corneal opacity score of 4 on the Draize scoring scale (Draize et al. 1944) at any time during the test Group B[1]: - Rabbit with mean scores (average of the scores on Days 1, 2, and 3) for opacity ≥3 and/or iritis ≥1.5
Category 2A	- Rabbit with mean scores (rabbit values are averaged across observation Days 1, 2, and 3) for one of more of the following: Iritis ≥1 but <1.5 Corneal opacity ≥1 but <3 Redness ≥2 Chemosis ≥2 and the effects fully reverse within 21 days
Category 2B	- Rabbit with mean scores (rabbit values are averaged across observation Days 1, 2, and 3) for one of more of the following: Iritis ≥1 but <1.5 Corneal opacity ≥1 but <3 Redness ≥2 Chemosis ≥2 and the effect fully reversed within 7 days
Not Classified	Rabbit mean scores fall below threshold values for Category 1, 2A, and 2B

Abbreviation: GHS = United Nations Globally Harmonized System

[1] "Group A" and "Group B" designations are internal designations used for classification purposes; they are not GHS-defined designations.

[2] Full reversal of the effects was defined as corneal opacity, iritis, redness, and chemosis = 0.

After each result was categorized, the ocular irritancy hazard classification was determined for each substance. As shown in **Table 2-2**, substance classification depended on the proportion of tests that produced the same response. If a substance was tested in more than 3 rabbits, decision criteria were modified so that the proportionality needed for classification was maintained (e.g., 1 out of 3 or 2 out of 6 rabbits were required for classification for most categories). However, in some cases, additional classification rules were necessary to include the available data (which are distinguished by italicized text in **Table 2-2**).

Table 2-2 Criteria for Classification of Substances According to the GHS Classification System, Listed in Order of Decreasing Severity

GHS Category	Criteria Necessary for Substance Classification
Category 1	At least 1 of 3 rabbits or 2 of 6 rabbits classified as Category 1, Group A[1] *One of 6 rabbits classified as Category 1, Group A and at least 1 of 6 rabbits classified as Category 1, Group B*[1] At least 2 of 3 rabbits or 4 of 6 rabbits classified as Category 1, Group B[1]
Category 2A	1. At least 2 of 3 rabbits or 4 of 6 rabbits classified as Category 2A 2. *One of 3 (2 of 6) rabbits classified as Category 2A and 1 of 3 (2 of 6) rabbits classified as Category 2B*
Category 2B	At least 2 of 3 rabbits or 4 of 6 rabbits classified as Category 2B
Not Classified	At least 2 of 3 rabbits or 4 of 6 rabbits classified as Not Classified

Abbreviations: GHS = United Nations Globally Harmonized System

Italicized text indicates rules that were developed to include additional data.

[1] "Group A" and "Group B" designations are internal designations used for classification purposes; they are not GHS-defined designations.

If an unequivocal substance classification could not be made due to the response pattern of the tested rabbits for a substance (e.g., 1 rabbit classified as Category 1, Group B; 2 rabbits classified as Category 2B; 3 rabbits classified as Not Classified), the data were excluded.

2.4.2 U.S. Environmental Protection Agency

The classification of substances according to the EPA classification system was conducted sequentially. Initially each rabbit was classified in one of four categories (Category I, II, III, or IV) (**Table 2-3**). Substance classification depended upon the most severe category observed among the tested rabbits.

Table 2-3 Criteria for Ocular Hazard Classification of Rabbits According to the EPA Classification System, Listed in Order of Decreasing Severity

EPA Category	Criteria for Rabbit Classification
Category I	- Corrosive, corneal involvement or irritation (iris or cornea score ≥1 or redness or chemosis ≥2) persisting more than 21 days or - Corneal effects that are not expected to reverse by 21 days
Category II	- Corneal involvement or irritation clearing[1] in 8 to 21 days
Category III	- Corneal involvement or irritation clearing in 7 days or less
Category IV	- Minimal or no effects clearing in less than 24 hours

Abbreviation: EPA = U.S. Environmental Protection Agency

[1] For the purposes of this analysis, clearing was defined as iritis or cornea score <1 and redness or chemosis score <2.

2.4.3 European Union

Substance classification according to the EU classification system (**Table 2-4**) was conducted sequentially. Average Draize scores were used for classification of substances in the EU system; calculations depended on the number of rabbits tested in a study. For studies therein which 3 rabbits were tested, the average Draize scores (over observation Days 1, 2, and 3) for each endpoint were calculated for each rabbit. For studies in which more than 3 rabbits were tested, the average Draize scores (over observation Days 1, 2, and 3) for each endpoint was calculated for all tested rabbits. The criteria used for substance classification are provided in **Table 2-4**.

2.5 Analysis

For each of the 97 formulations evaluated, the impact of the anesthesia was assessed based on (1) the severity of the irritancy and (2) the number of days necessary for the lesion to clear. The formulations were then classified into one of three categories: (1) anesthesia increased or worsened the observed variable, (2) anesthesia decreased or lessened the observed variable, or (3) anesthesia did not affect the observed variable. These relative frequencies of observed variables that increased/worsened and those that decreased/lessened were then compared by a sign test (Siegel and Castellan, 1956) to assess statistical significance of the anesthesia effect.

Table 2-4 Criteria for Classification of Substances According to the EU Classification System, Listed in Order of Decreasing Severity

EU Category	Three Rabbits Tested	Greater than Three Rabbits Tested
R41	1. Two or more rabbits with the following average Draize scores over Days 1, 2, and 3: Opacity ≥3 Iritis =2 2. At least 1 rabbit (on Day 21) in which the effect has not reversed[1] 3. At least 1 rabbit (when study is terminated after Day 14 and before Day 21) with Opacity ≥3 or Iritis =2 4. At least 1 rabbit with any of the following noted effects: (a) Corneal perforation or ulceration (b) Blood in the anterior chamber of the eye (c) Opacity = 4 for 48 hours (d) Absence of light reflex for 72 hours (e) Ulceration of the conjunctival membrane (f) Necrosis of the conjunctivae or nictitating membrane (g) Sloughing	1. The following overall mean rabbit Draize scores over Days 1, 2, and 3: Opacity ≥3 or Iritis >1.5 2. At least 2 rabbits (on Day 21) in which the effect has not reversed 3. At least 2 rabbits (when study is terminated after Day 14 and before Day 21) with Opacity ≥3 or Iritis =2 4. At least 1 rabbit with any of the following noted effects: (a) Corneal perforation or ulceration (b) Blood in the anterior chamber of the eye (c) Opacity = 4 for 48 hours (d) Absence of light reflex for 72 hours (e) Ulceration of the conjunctival membrane (f) Necrosis of the conjunctivae or nictitating membrane (g) Sloughing

continued

Table 2-4 Criteria for Classification of Substances According to the EU Classification System, Listed in Order of Decreasing Severity (continued)

EU Category	Three Rabbits Tested	Greater than Three Rabbits Tested
R36	Two or more rabbits with the following average Draize scores over Days 1, 2, and 3: 2 ≤ Opacity <3 1 ≤ Iritis <2 Redness ≥2.5 Chemosis ≥2	The following overall mean rabbit Draize scores over Days 1, 2, and 3: 2 ≤ Opacity <3 1 ≤ Iritis <1.5 Redness ≥2.5 Chemosis ≥2
Not Labeled	Substance cannot be classified as R41 or R36	Substance cannot be classified as R41 or R36

Abbreviations: EU = European Union.

[1] Full reversal of the effects was defined as corneal opacity, chemosis, redness, or iritis = 0.

3.0 Results

3.1 Classification of Formulations

A subset of the rabbits could not be classified based on the GHS, EPA, or EU systems because the criteria described in the Materials and Methods section were not fulfilled. Based on these criteria, 25 rabbits (8 not pretreated and 17 pretreated with anesthesia) could not be classified using the GHS classification system. For the EU and EPA classification systems, 27 rabbits (9 not pretreated and 18 pretreated with anesthesia) and 23 rabbits (6 not pretreated and 17 pretreated with anesthesia) could not be classified, respectively.

Based on the above results, a subset of formulations could not be used to compare the effects of anesthesia on irritancy classification due to insufficient animal response data (i.e., irritancy data for anesthetized and nonanesthetized rabbits treated with the same formulation were unavailable). In the present database, nine formulations were excluded from the GHS and EU classification system evaluations, and seven formulations were excluded from the EPA classification system evaluation (**see Table 3-1**).

3.2 Effect on Irritancy Classification

Each formulation tested was assessed to determine if the average irritancy response for the animals pretreated with tetracaine hydrochloride was different (i.e., more or less severe) than for the animals not pretreated with tetracaine hydrochloride.

As shown in **Table 3-1**, for all three hazard classification schemes, rabbits pretreated with anesthesia tended to produce more severe responses than rabbits that were not pretreated with anesthesia. However, none of the observed differences were statistically significant. The greatest difference was observed in the GHS classification scheme, in which 20 formulations produced a more severe average response in the pretreated rabbits, while 13 formulations produced a less severe average response in the rabbits that were pretreated with tetracaine hydrochloride.

Table 3-1 Effect of Anesthesia Pretreatment on Irritancy Classification Response

Direction of Response	GHS	EU	EPA
More severe average response in anesthetized animals	20[1]	17	22
Less severe average response in anesthetized animals	13	11	16
No difference in average response between anesthetized and nonanesthetized animals	55	60	52
Number of formulations that could not be used because there was insufficient data[2]	9	9	7
Total Number of Formulations	**97**	**97**	**97**

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = United Nations Globally Harmonized System

[1] *Number* represents the number of formulations identified with the noted criteria.

[2] Some formulations and the animals tested with that formulation could not be used for this evaluation because there was insufficient animal data with which to compare anesthetized and nonanesthetized animals.

Of the substances that elicited a more or less severe response in rabbits pretreated with tetracaine hydrochloride, only five formulations where shown differ by more than two ocular hazard

classification categories for at least one of the hazard classification systems evaluated (**Table 3-2**). There was no consistent pattern regarding whether the anesthesia played a role in this variability of response. In some cases, the animals with anesthesia clearly produced a more severe response than those animals without anesthesia, while for other chemicals an opposite trend was seen (**Table 3-2**).

Table 3-3 shows the distributions of individual rabbit responses for different severity classifications used for each regulatory hazard classification system. The results collapse data over different formulations and, therefore, preclude a formal statistical analysis. However, the data in this table support the results presented in **Table 3-1** (i.e., rabbits pretreated with anesthesia tend to produce more severe responses than rabbits that were not pretreated with anesthesia).

Table 3-2 Animal Classifications for Substances with Differences of at Least Two Hazard Classification Categories

Substance Code	Animal Number	Pretreated	Animal GHS Classification	Overall GHS Classification	Animal EU Classification	Overall EU Classification	Animal EPA Classification	Overall EPA Classification
10640	1	NO	Cat2A	Category 2A	R36	R36	Category II	Category I
10640	2	NO	Cat2A		R36		Category II	
10640	3	NO	Cat 1, Group A[1]		R41		Category I	
10640	4	YES	Cat2A		R36		Category III	
10640	5	YES	Cat2B		R36		Category III	
10640	6	YES	Not Classified		Not Labeled		Category III	
12422	1	NO	Cat2B	Category 1	R36	R41	Category III	Category I
12422	2	YES	Cat2B		R36		Category III	
12422	3	YES	Cat 1, Group A		R41		Category I	
12483	1	NO	Cat2A	Category 1	R36	R41	Category II	Category I
12483	2	NO	Cat 1, Group A		R41		Category I	
12483	3	YES	Cat2B		Not Labeled		Category III	
13375	1	NO	Cat2B	Category 1	Not Labeled	R41	Category III	Category I
13375	2	YES	Cat 1, Group A		R41		Category I	
13375	3	YES	Cat 1, Group A		R41		Category I	
13381	1	NO	Cat 1, Group A	Category 1	R41	R41	Category I	Category I
13381	2	YES	Cat2A		R36		Category II	
13381	3	YES	Cat2A		R36		Category III	

Abbreviations: Cat = category; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = United Nations Globally Harmonized System

[1] "Group A" is an internal designation used for classification purposes; it is not a GHS-defined designation (see **Table 2-4** for additional details).

Table 3-3 Distribution of Rabbits Among Hazard Classification Irritancy Categories

GHS				EU				EPA			
Classification Category	**Number of Rabbits**	**Anesthesia Pretreatment**		**Classification Category**	**Number of Rabbits**	**Anesthesia Pretreatment**		**Classification Category**	**Number of Rabbits**	**Anesthesia Pretreatment**	
		No	**Yes**			**No**	**Yes**			**No**	**Yes**
Category 1	36	13[1] (10.9%)	27 (13.8%)	R41	40	13 (11.0%)	27 (13.9%)	Category I	36	12 (9.9%)	24 (12.3%)
Category 2A	72	27 (22.7%)	45 (23.1%)	R36	101	35 (29.7%)	66 (34.0%)	Category II	63	23 (19.0%)	40 (20.5%)
Category 2B	79	31 (26.1%)	48 (24.6%)	NL	171	70 (59.3%)	101 (52.1%)	Category III	161	67 (55.4%)	94 (48.2%)
Not Classified	123	48 (40.3%)	75 (38.5%)					Category IV	56	19 (15.7%)	37 (19.0%)
Total	314	119	195	Total	312	118	194	Total	316	121	195
SCNM	25	8	17	SCNM	27	9	18	SCNM	23	6	17
Overall Total	339	127	212	Overall Total	339	127	212	Overall Total	339	127	212

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = United Nations Globally Harmonized System; NL = Not labeled; SCNM = Study criteria not met

[1] *Number* represents the number of rabbits identified with the noted severity classification. The number in parentheses represents the percentage of rabbits based on the total number of classifiable rabbits ("Total" row).

An additional analysis used anesthesia pretreatment as a criterion to evaluate the variability among animals within a given formulation. For most of the formulations, irritancy classifications for rabbits pretreated with tetracaine hydrochloride did not differ from those of rabbits not pretreated (**Table 3-4**). Interestingly, for all these classification systems (especially the EU system), the agreement in irritancy response between rabbits was better when the anesthesia pretreatments were different (EU = 18 substances) than in those in which the anesthesia pretreatments were the same, regardless of whether or not an anesthetic was used (EU =10 substances). However, none of the observed differences was statistically significant.

Table 3-4 Effect of Anesthesia Pretreatment on Agreement of Irritancy Classification Response

Agreement of Response	GHS	EU	EPA
Better agreement in irritancy response among rabbits with matching pretreatment (either anesthesia or no anesthesia)	16[1]	10	17
Better agreement in irritancy response among rabbits without matching pretreatment	17	18	20
No difference between matched and unmatched pretreatment	55	60	53
Number of formulations that could not be used because there was insufficient data[2]	9	9	7
Total Number of Formulations	**97**	**97**	**97**

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = United Nations Globally Harmonised System

[1] *Number* represents the number of formulations identified with the noted criteria.

[2] Some formulations, and the animals tested with that formulation, could not be used for this evaluation because there was insufficient animal data with which to compare anesthetized and nonanesthetized animals.

3.3 Effect on Day of Lesion Clearing

Since regulatory classifications rely in part on the day all ocular lesions reverse, we evaluated whether pretreatment with tetracaine hydrochloride lengthened or shortened the number of days required for lesion clearing. Based on the available data, when anesthetized rabbits were compared to nonanesthetized rabbits, none of the differences observed in the day-to-clearing evaluation were statistically significant (**Table 3-5**). The largest difference observed was for opacity clearing time, which tended to be slightly greater in the rabbits pretreated with tetracaine hydrochloride than in those that were not pretreated. However, this difference (33 vs. 22) was not significant using a sign test ($p < 0.10$).

Table 3-5 Effect of Anesthesia Pretreatment on Day of Clearing of Ocular Lesions

	Opacity Clearing	Iris Clearing	Redness Clearing (EPA)[1]	Redness Clearing (EU/GHS)[1]	Chemosis Clearing (EPA)[1]	Chemosis Clearing (EU/EPA)[1]
Longer clearing time, on average, for anesthetized animals versus nonanesthetized animals	33[2]	28	30	33	24	22
Shorter clearing time, on average, for anesthetized animals versus nonanesthetized animals	22	22	30	29	25	29
No difference in clearing time on average between anesthetized and nonanesthetized animals	27	37	32	24	43	39
Number of formulations that could not be used because there was insufficient data[3]	15	10	5	11	5	7
Total Number of Formulations	**97**	**97**	**97**	**97**	**97**	**97**

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = United Nations Globally Harmonized System

[1] Different analyses were conducted for the EPA classification system than for the EU and GHS classification system because the day of clearing is defined differently. Clearing for the EPA is defined as a score of 0 or 1, while clearing for the GHS and EU classification systems is defined as a score of 0.

[2] *Number* represents the number of formulations identified with the noted criteria.

[3] Some formulations, and the animals tested with that formulation, could not be used for this evaluation because there was insufficient animal data with which to compare anesthetized and nonanesthetized animals.

Table 3-6 provides a comparison of the number of animals for each clearing day evaluated for the corneal opacity endpoint. The data show that, overall, the time for corneal lesions in rabbits pretreated with tetracaine hydrochloride was slightly longer than in rabbits that were not pretreated with tetracaine hydrochloride.

Table 3-6 Distribution of Rabbits (With and Without Anesthesia Pretreatment), Based on Clearing Day for Corneal Opacity Lesions

Clearing Day for Opacity Lesion	Number of Rabbits Not Pretreated with Anesthesia	Number of Rabbits Pretreated with Anesthesia
>21[1]	11 (9.2%)	19 (9.9%)[2]
21	6 (5.0%)	5 (2.6%)
14	4 (3.3%)	19 (9.9%)
10	12 (10.0%)	18 (9.4%)
7	15 (12.5%)	25 (13.0%)
4	9 (7.5%)	13 (6.8%)
3	11 (9.2%)	22 (11.5%)
2	4 (3.3%)	9 (4.7%)
1	0 (0.0%)	2 (1.0%)
0[3]	48 (40.0%)	60 (31.3%)
No Clearing[4]	7	20
Total Number of Rabbits	**127**	**212**

[1] Lesion was present on last day of observation period (21 days).

[2] *Percentage* represents the number of animals for the noted clearing day per the total number of usable animals (192 for the number of animals pretreated with anesthesia, and 120 for the number of animals not pretreated with anesthesia).

[3] No lesions were observed at any time points evaluated.

[4] These experiments were terminated prior to clearing of lesions; therefore, the data could not be used in the evaluation.

4.0 Discussion

Efforts increasingly have focused on refining the current *in vivo* Draize rabbit eye test method protocol to reduce the level of pain and distress experienced by rabbits when test substances are placed in the eye. One area that has been reviewed extensively has been the use of topical anesthetics prior to administration of a test substance. While it is generally agreed that the application of a topical anesthetic will likely decrease the pain perceived by a rabbit in the early stages of the *in vivo* eye irritation test, there are competing concerns that topical anesthetics may alter ocular physiology and thus modify the irritation response observed.

Overall, previous studies provide conflicting results on the impact of topical ocular anesthetics on ocular irritation and physiology. While some studies indicate that topical anesthetics do not interfere with the irritation response (Ulsamer et al. 1977; Heywood and James 1978; Anonymous 1981; Arthur et al. 1986; Seabaugh et al. 1993), others state that there is a trend (although not statistically significant) of increased irritancy in anesthetized eyes (Johnson 1980; Durham et al. 1992). Still others note that anesthetics interfere with the irritant response and yielded data that were not reliable (Walberg 1983; Rowan and Goldberg 1985). Differences in efficacy of the topical ocular anesthetics evaluated in these studies could depend on a variety of a factors including but not limited to the type and dose of anesthetic used, the application procedure, and the species tested (Ulsamer et al. 1977; Heywood et al. 1978; Johnson 1980; Anonymous 1981; Walberg 1983; Rowan and Goldberg 1985; Arthur et al. 1986; Durham et al. 1992; Seabaugh et al. 1993). Due to the limited data available, however, an in-depth assessment on the impact of these different factors on the overall results has yet to be conducted.

Despite these conflicting issues and although not formal policy among all U.S. Federal agencies, the use of anesthetics was considered acceptable by a consensus of those participating in a 1991 IRAG workshop (Seabaugh et al. 1993). It was noted that because pain is relieved at least temporarily and the time and extent of injury can still be evaluated, anesthetic use should be considered on a case-by-case basis. It is noteworthy that in 1984 the U.S. Consumer Products Safety Commission (CPSC) stated that two applications of tetracaine, 10 to 15 minutes apart, should be administered prior to test substance administration during ocular irritation testing (CPSC 1984).

The present study examined topical anesthetics to assess the impact of using two drops of tetracaine hydrochloride (0.5% (w/v)), 30 to 120 seconds prior to test article application, on ocular irritancy. For a majority of the formulations evaluated no difference was observed in the severity of irritancy observed in rabbits pretreated with tetracaine and in those that were not pretreated (i.e., the irritancy classifications between treated and untreated rabbits were the same). When a difference in irritancy classifications was observed, the rabbits pretreated with anesthesia tended to produce a slightly more severe response than those without anesthesia. This is similar to results seen in previous studies (Durham et al. 1992). This trend, which was not statistically significant, was observed for all hazard classification systems evaluated. Since the formulation compositions were unknown, an assessment of whether there were similarities among formulations that were comparably affected by the anesthetic pretreatment could not be conducted.

A lack of association between severity of classification and anesthesia pretreatment also was observed when the distribution of rabbits among irritancy classification categories was evaluated. Similar to the results described above, the distribution of rabbits indicated that pretreatment with anesthesia did not increase the likelihood of producing a more severe response than those without anesthesia.

The argument could be made that, although 0.5% (w/v) tetracaine hydrochloride did not appear to affect the responses of the pretreated rabbits and those not pretreated, it could have altered the variability in the individual rabbit responses for each tested formulation. Therefore, we examined the variability among rabbit irritancy responses when anesthesia pretreatment was used as a defining

criterion. The results show that anesthesia pretreatment had no significant effect on the observed variability among rabbit responses.

Of the five formulations with which rabbit responses differed by more than two classification categories (e.g., GHS Category 2B classification for one test rabbit and GHS Category 1, Group A for another test rabbit), there was no consistent pattern in the pretreatment effect. In some cases, the rabbits pretreated with tetracaine hydrochloride produced a more severe response than those animals not pretreated with tetracaine hydrochloride, while for other formulations the opposite trend was observed. Because the observed variability occurs in both directions (increasing and decreasing the level of irritancy), the observed variability in rabbit response may be unrelated to the anesthesia but instead related to the inherent variability of the rabbit response to the tested formulations.

Because all three evaluated hazard classification systems use for irritancy classification the day of clearing of all lesions, the impact of anesthesia pretreatment on this criterion was evaluated also. Similar to the results of the previous analyses, none of the observed differences in the days-to-clearing were statistically significant. Interestingly, while pretreatment with tetracaine tended to increase the length of time needed for ocular and iridal lesions to clear, anesthesia pretreatment tended to decrease the length of time needed for conjunctival chemosis lesions to clear. The significance and the mechanisms for this observed effect are currently unknown.

Due to the lack of available comparative data, further evaluations comparing the efficacy of tetracaine versus other topical anesthetics and the optimal dosing regimen (e.g., number of drops to be administered, location of anesthetic application) could not be assessed. Thus additional studies are recommended to further evaluate these areas.

In conclusion, these results indicate that pretreatment with 0.5% (w/v) tetracaine hydrochloride ophthalmic solution had no significant impact on the irritancy classification of rabbits according to the GHS, EPA, and EU classification systems. The anesthesia pretreatment did not affect the variability in rabbit response either. Furthermore, anesthetic pretreatment had no statistically significant effect on the number of days until ocular lesions cleared. Therefore, this evaluation combined with previous studies supports the routine use of 0.5% tetracaine hydrochloride prior to testing rabbits in the *in vivo* Draize rabbit eye test.

5.0 References

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Annex III

Comparative Evaluation of Topical Anesthetics Proparacaine and Tetracaine

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Comparative Evaluation of Topical Anesthetics Proparacaine and Tetracaine

Local anesthetics produce reversible loss of sensation in a limited area of an animal's body without the loss of consciousness or alteration of central nervous system activity (Wright et al. 1985). Topical anesthetics reduce pain by blocking sodium channels in excitable neurons, thus inhibiting the action potential generated by membrane depolarization when large, transient increases in sodium permeability are produced in response to painful stimuli (Catterall and Mackie 2001). The two most commonly used topical ocular anesthetics are proparacaine and tetracaine (Wilson 1990; Bartfield et al. 1994). A comparative evaluation of the relevant properties of proparacaine and tetracaine with regards to their impacts on corneal wound healing and irritant hazard classification is detailed below in **Table 1**.

Table 1 Comparative Evaluation of Topical Anesthetics Proparacaine and Tetracaine

Characteristic	Proparacaine	Tetracaine	References
Onset of Action	Faster, based on clinical veterinary experience and observations	Slower, based on clinical veterinary experience and observations	Webb 2009
	0.25 minutes (0.5% solution), species not specified	5 minutes (0.5% solution), species not specified	Bryant 1969
	Approximately 30 seconds after instillation, based on an assessed blink reflex in a human clinical study	Approximately 30 seconds after instillation, based on an assessed blink reflex in a human clinical study	Bartfield et al. 1994
	Within 60 seconds in rabbits	NP	Schwartz et al. 1998
	6-20 seconds	Tetracaine may not produce complete anesthesia to pain even when dosed twice and onset time is 10-15 min.	CPSC Report B
Duration of Action	Shorter, based on clinical veterinary experience and observations	Longer, based on clinical veterinary experience and observations	Webb 2009
	15 minutes, species not specified	30-120 minutes, species not specified	Bryant 1969
	Approximately 60 minutes in rabbits using 40 µl of 0.5% solution	NP	Schwartz et al. 1998
	Approximately 15 minutes, based on human refractive surgery procedures	Approximately 15 minutes, based on human refractive surgery procedures	Nomura et al. 2001
	10 minutes in humans using 0.5% solution, as determined by return of corneal blink reflex evaluated every 2 min	9 minutes in humans using 0.5% solution, as determined by return of corneal blink reflex evaluated every 2 min	Bartfield et al. 1994

continued

Table 1 Comparative Evaluation of Topical Anesthetics Proparacaine and Tetracaine (continued)

Characteristic	Proparacaine	Tetracaine	References
Duration of Action (continued)	34 minutes in normal human corneas using one drop of 0.5% solution, as determined by Cochet-Bonnet measurements	NP	Weiss and Goren 1991
	5 minutes in cats (maximal anesthetic effect) using one drop of 0.5% solution, as determined by Cochet-Bonnet measurements	NP	Binder and Herring 2006
	15 minutes in dogs (maximal anesthetic effect) using one drop of 0.5% solution, as determined using a Cochet-Bonnet measurements; 25 minutes for 2-drop treatment	NP	Herring et al. 2005
	Approximately 10-20 minutes	NP	Proparacaine (OPHTHETIC®) FDA Final Labeling Requirements (2000)
Usage/Dosage Requirements	For procedures in which a topical ophthalmic anesthetic is indicated: (e.g., corneal anesthesia of short duration); Safety and effectiveness of proparacaine HCl ophthalmic solution in pediatric patients have been established; Use of proparacaine HCl is supported by evidence from adequate and well-controlled studies in adults and children over the age of twelve, and safety information in neonates and other pediatric patients Removal of foreign bodies and sutures, and for tonometry: 1 to 2 drops (in single instillations) in each eye before operating. Short corneal and conjunctival procedures: 1 drop in each eye every 5 to 10 minutes for 5 to 7 doses	CPSC Policy states "when animal testing is the only feasible method of determining if a substance is an eye irritant, the animals are treated with two applications of tetracaine ophthalmic anesthetic, 10-15 minutes apart, prior to instilling the product to the eye, in order to reduce the pain and suffering of the animals tested".	Proparacaine (OPHTHETIC®) FDA Final Labeling Requirements (2000) CPSC Policy 1984

continued

Table 1 Comparative Evaluation of Topical Anesthetics Proparacaine and Tetracaine (continued)

Characteristic	Proparacaine	Tetracaine	References
Pain of Instillation	Less painful using a validated visual-analog pain scale following 1 drop	More painful using a validated visual-analog pain scale following 1 drop	Bartfield et al. 1994
	Negligible	Significant - stinging and burning	Bartfield et al. 1994
	Occasional temporary stinging, burning and conjunctival redness	NP	Proparacaine FDA Final Labeling Requirements (2000)
Common Preservative	Benzalkonium chloride (0.01%)	Chlorobutanol (0.4%)	Proparacaine FDA Final Labeling Requirements (2000) Tetracaine Hydrochloride Ophthalmic solution (Akorn 2009)
SEM examination	No disruptive effects with single dose application of 0.5% solution to rabbit eyes	No disruptive effects with single dose application of 0.5% solution to rabbit eyes	Pfister and Burstein 1976
In Vitro Toxicity (as evaluated with primary cultures of rabbit corneal epithelial cells)	NP	Approximately 4X more toxic than proparacaine, as determined by mitochondrial reduction assay, lactate dehydrogenase leakage cytotoxicity test, and morphological changes	Grant and Acosta 1994
Penetration of sulphorhodamine B into corneas of mice (ratio provides a numerical index of toxicity to corneal epithelium)	No effect on ratio using 0.1 and 1% proparacaine	Rise in the ratio of three out of a maximum achievable rise of 30 with 0.5% preservative-free tetracaine	Maurice and Singh 1986
In Vivo Toxicity (as evaluated with cultured human keratocytes)	Exhibited toxic effects, as determined by phase-contrast microscopy, and tetrazolium salt colorimetric assay	Produced a larger decrease in cell viability than proparacaine. Exhibited toxic effects	Moreira et al. 1999
Delayed Healing of Experimental Corneal Lesions in Rats	0.5% proparacaine (11 times over 3h) caused a complete inhibition of healing in test rat eyes compared with lesions in control	0.5% tetracaine delayed healing compared to contralateral ocular lesions; Some healing noted	Marr 1957

continued

Table 1 Comparative Evaluation of Topical Anesthetics Proparacaine and Tetracaine (continued)

Characteristic	Proparacaine	Tetracaine	References
Effect on Corneal Wound Healing in Humans after PRK	NP	1% tetracaine given every 30 minutes for 24 hours did not adversely affect corneal wound healing	Verma et al. 1995
Effect on Reparative Regeneration of Corneal Epithelium	NP	0.5% tetracaine caused more delay of wound healing than 2% cocaine and 2% lidocaine	Bykov and Semenova 1972
Effect on Corneal Epithelial Permeability	NP	Not significantly increased following five instillations of one drop of solution	Ramselaar et al. 1988
Effect on Tear Dynamics in Rabbits	0.5% solution significantly reduced tear production in rabbit eyes	0.5% solution significantly reduced tear production; Reduction in lacrimal turnover dependent upon number of drops applied	Patton and Robinson 1975
Effect of anesthetic pretreatment on administration of 10% SLS in rabbits	No statistical differences between anesthetized and unanesthetized rabbit corneas with 0.5% after TSA; Some evidence that intensity of reaction was increased following anesthesia	NP	Heywood and James 1978
Effect of anesthetic pretreatment on Ocular response and recovery time	Effective in producing anesthesia; Tended to increase the severity of ocular reactions and the time of recovery	Dosing pattern (single application) not fully adequate; Two different instillations are required to produce anesthesia from tetracaine; No adverse effects were caused by the dose administered	Falahee et al. 1981
10% dishwashing detergent application in rabbits	Proparacaine pretreatment caused significant opacity, iritis, and redness and an irritant classification; Without - not an irritant.	NP	CPSC Report A
40% dishwashing or powdered detergent application in rabbits	Irritancy not affected by pre-treatment with proparacaine	NP	CPSC Report B
Study examining pre-treatment anesthetics on the application of Acetic Acid (5%), NaOH (1%), dishwashing detergent (10%), ETOH (70%)	"Some of the scores produced by [these] substances were altered by proparacaine pretreatment, but their classification as irritants under the FHSA remained the same".	Long onset of action (5-10 minutes) and effect inconsistent; Repeat application required; Preliminary data indicates it does not alter test scores in general	CPSC Report A (date unknown)

continued

Table 1. Comparative evaluation of topical anesthetics proparacaine and tetracaine (continued)

Characteristic	Proparacaine	Tetracaine	References
Collaborative study investigating the potential of anesthetics to alter the irritation response in rabbits (eight labs)	2 drops of 0.5% solution; No appreciable effect on the course or intensity of ocular responses from treatment with 20% or 100% shampoo, 80% ethyl alcohol, or 100% talc	2 drops of 0.5% solution, 2 applications; No appreciable effect on the course or intensity of ocular responses from treatment with 20% or 100% shampoo, 80% ethyl alcohol, or 100% talc	Arthur et al. 1986
Repeated Application	0.5% solution (every 4h for 6 days) delayed corneal wound closure in rabbits	NP	Peyman et al. 1994
	NP	0.05% solution delayed reepithelialization in patient after overuse	Lee and Stark 2008
	0.5% proparacaine and tetracaine caused toxic keratopathy- non-healing epithelial defect, marked stromal edema in patients	0.5% proparacaine and tetracaine caused toxic keratopathy- non-healing epithelial defect, marked stromal edema in patients	Rocha et al. 1995

Abbreviations: NP = Not provided; FDA = Food and Drug Administration; CPSC = Consumer Product Safety Commission; TSA = Test Substance Administration; FHSA = Federal Hazardous Substances Act; PRK = Photorefractive Keratotomy; SEM = Scanning Electron Microscopy

Proparacaine is a widely used ophthalmic topical anesthetic in both human and veterinary clinical practices (Webb 2009). Although a range of onset times have been reported, proparacaine typically provides fast and effective anesthesia within 30 seconds following administration of a single dose (Bryant 1969; Bartfield et al. 1994; CPSC Report B (date unknown). In contrast, tetracaine, a related ester topical anesthetic, reportedly exhibits a slower onset of action of approximately 5-10 minutes and does not produce complete anesthesia to pain even when dosed twice (Bryant 1969; CPSC Report B (date unknown); Webb 2009). For studies where both anesthetics were evaluated, tetracaine generally provided a longer duration of action than proparacaine (Bryant 1969; Nomura et al. 2001; Webb 2009). However, Bartfield et al. (1994) reported that 0.5% proparacaine conferred slightly longer anesthesia (i.e., 10 minutes) than tetracaine (i.e., 9 minutes) on human volunteers. Studies of proparacaine for which there was no corresponding tetracaine data reported maximal anesthesia for a duration range of 5-60 minutes in a variety of species (Weiss and Goren, 1991; Schwartz et al. 1998; Binder and Herring 2006; Herring et al. 2005; Proparacaine (Ophthetic®) Final Label 2000).

The specified usage/dosage requirements for proparacaine in humans for short corneal and conjunctival procedures are 1 drop of proparacaine to be instilled into the eye every 5 to 10 minutes for 5 to 7 doses (Proparacaine (Ophthetic®) Final Label 2000).

Clinical studies indicate that instillation of proparacaine eye drops is considerably less painful than instillation of tetracaine (CPSC Report A (date not provided); Falahee et al. 1981; Bartfield et al. 1994). Proparacaine contains the common preservative benzalkonium chloride (0.01%), as opposed to tetracaine, which contains chlorobutanol (0.4%) (Proparacaine (Ophthetic®) Final Label 2000; Tetracaine Hydrochloride Ophthalmic solution Akorn 2009).

Several studies have evaluated the *in vitro* and *in vivo* toxicity of topical application of proparacaine on the cornea, in addition to its impact on the ocular irritant response.

Pfister and Burstein (1976) reported that a single dose application of 0.5% proparacaine or tetracaine to rabbit eyes produced no disruptive effects when examined by scanning electron microscopy. Proparacaine exhibited lower *in vivo* toxic effects than tetracaine on cultured human keratocytes when using phase contrast microscopy (Moreira et al. 1999). Topical application of tetracaine, unlike proparacaine, was also associated with an increase in acute toxicity to the corneal epithelium in mice, as measured by the penetration of sulforhodamine (Maurice and Singh 1986). In addition, a study utilizing primary cultures of rabbit corneal epithelial cells showed that tetracaine was significantly more toxic than proparacaine, as determined by several *in vitro* assays and observed morphological changes (Grant and Acosta 1994).

Tetracaine has previously been reported not to significantly effect corneal wound healing or corneal epithelial permeability, using either limited or repeated applications (Ramselaar et al. 1988; Verma et al. 1995). However, Bykov and Semenova (1972) noted that 0.5% tetracaine delayed wound healing more than either 2% cocaine or 2% lidocaine. Comparative data on the effects of proparacaine on corneal wound healing are not available.

Heywood and James (1978) reported no statistical differences in the ocular response of anesthetized (0.5% proparacaine) and unanesthetized rabbit corneas following the administration of 10% sodium lauryl sulfate. An increase in the intensity of the reaction was noted for the anesthetized animals, however this was not sufficient to alter hazard classification. A collaborative study involving eight laboratories investigated the potential of anesthetics to alter the irritation response in rabbits (Arthur et al. 1986). It was reported that pre-treatment with two drops of 0.5% proparacaine had no appreciable effect on the course or intensity of ocular responses after administration with 20% or 100% shampoo, 80% ethyl alcohol or 100% talc. CPSC Report B (date not provided) also found that ocular irritancy after application of 40% dishwashing or powdered detergent in rabbits was not affected by pre-treatment with proparacaine. In contrast, proparacaine pretreatment caused significant opacity, iritis and redness resulting in an irritant classification following the application of 10% dishwashing detergent in rabbits, where unanesthetized animals produced no response (CPSC Report A date not provided).

In summary, these findings indicate that there are advantages to using either proparacaine or tetracaine as the preferred topical anesthetic for ocular irritation studies. Both of these drugs have a long history of safe and effective use for relieving pain for either human or veterinary clinical practice.

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Appendix D

Independent Scientific Peer Review Panel Assessment

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Appendix D1

Summary Minutes from the Peer Review Panel Meeting on May 19-21, 2009

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Summary Minutes

Independent Scientific Peer Review Panel Meeting

Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

Consumer Product Safety Commission Headquarters
Fourth Floor Hearing Room
Bethesda Towers Building
Bethesda, MD

May 19 - 21, 2009

Peer Review Panel Members:

A. Wallace Hayes, Ph.D., DABT, FATS, ERT (Peer Review Panel Chair)	Visiting Scientist (Harvard), Harvard School of Public Health, Andover, MA; Principal Advisor, Spherix Incorporated, Bethesda, MD
Hongshik Ahn, Ph.D.	Professor, Stony Brook University, Stony Brook, NY
Paul Bailey, Ph.D.	Bailey & Associates Consulting, Neshanic Station, NJ
Richard Dubielzig, D.V.M.	Professor, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI
Henry Edelhauser, Ph.D.[1]	Professor of Ophthalmology and Director of Ophthalmic Research, Emory University School of Medicine, Atlanta, GA
Mark Evans, D.V.M., Ph.D., DACVP	Pathology Lead for Ophthalmology Therapeutic Area, Pfizer Global Research and Development at La Jolla Drug Safety Research and Development, San Diego, CA
James Jester, Ph.D.	Professor of Ophthalmology and Biomedical Engineering, Endowed Chair, University of California-Irving, Orange, CA

[1] Unable to attend the Panel meeting, but participated in the review of all materials.

Peer Review Panel Members:

Tadashi Kosaka, D.V.M., Ph.D.	Associate Director, Chief, Laboratory of Immunotoxicology and Acute Toxicology, Toxicology Division, The Institute of Environmental Toxicology, Ibaraki, Japan
Alison McLaughlin, M.Sc., DABT	Health Canada, Environmental Impact Initiative, Office of Science and Risk Management, Health Products and Food Branch, Ottawa, Ontario, Canada
J. Lynn Palmer, Ph.D.	Associate Professor, Department of Palliative Care and Rehabilitation Medicine, University of Texas, MD Anderson Cancer Center, Houston, TX
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Denise Rodeheaver, Ph.D., DABT	Assistant Director, Alcon Research Ltd., Department of Toxicology, Fort Worth, TX
Donald Sawyer, D.V.M., Ph.D., DACVA	Professor Emeritus, Retired, College of Veterinary Medicine, Michigan State University, East Lansing, MI
Kirk Tarlo, Ph.D., DABT	Scientific Director, Comparative Biology and Safety Sciences, Amgen, Inc., Thousand Oaks, CA
Daryl Thake, D.V.M., Dipl. ACVP[1]	Midwest ToxPath Sciences, Inc., Chesterfield, MO
Scheffer Tseng, M.D., Ph.D.[1]	Director, Ocular Surface (OS) Center, Medical Director OS Research & Education Foundation, Directory R&D Department, Tissue Tech, Inc., Ocular Surface Center, P.A., Miami, FL
Jan van der Valk, Ph.D.	Senior Scientist, Departments of Animals, Science and Society, Faculty of Veterinary Medicine, Utrecht University, Netherlands Centre Alternatives to Animal Use (NCA), Utrecht, Netherlands
Philippe Vanparys, Ph.D., DABT	Managing Director, CARDAM (VITO), Mol, Belgium
Maria Pilar Vinardell, Ph.D.	Director, Department of Physiology, Professor of Physiology and Pathology, Department Fisologia, Facultat de Farmacia, Universitat de Barcelona, Barcelona, Spain
Sherry Ward, Ph.D., M.B.A.	In Vitro Toxicology Consultant, BioTred Solutions, Science Advisor, International Foundation for Ethical Research, New Market, MD

Peer Review Panel Members:

Daniel Wilson, Ph.D., DABT	Mammalian Toxicology Consultant, Toxicology and Environmental Research Consulting, The Dow Chemical Company, Midland, MI
Fu-Shin Yu, Ph.D.	Director of Research, Department of Ophthalmology & Anatomy, School of Medicine, Wayne State University, Detroit, MI

ICCVAM and ICCVAM Ocular Toxicity Working Group Members:

Meta Bonner, Ph.D.	EPA, OPP, Washington, DC
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Pertti Hakkinen	NLM, Bethesda, MD
Masih Hashim, D.V.M., Ph.D.	EPA, OPP, Washington, DC
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John Redden	EPA, OPP, Crystal City, VA
RADM William Stokes, D.V.M., DACLAM (Director, NICEATM)	NIEHS, Research Triangle Park, NC
Marilyn Wind, Ph.D., (ICCVAM Chair)	CPSC, Bethesda, MD

Invited Experts:

Rodger Curren, Ph.D.	Institute for In Vitro Sciences (IIVS), Gaithersburg, MD
Arnhild Schrage, Ph.D.	Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany

European Centre for the Validation of Alternative Methods, ICCVAM OTWG Liaison:

João Barroso, Ph.D.	European Centre for the Validation of Alternative Methods, Ispra, Italy

Public Attendees:

Attendee	**Affiliation**	**Day Attended**		
		1	**2**	**3**
Odelle Alexander	Syngenta Crop Protection, Greensboro, NC	√	√	√
Ian Blackwell	EPA, Antimicrobials Division, Arlington, VA	√	√	-
Krishna Deb	EPA, Antimicrobials Division, Arlington, VA	√	√	-
Noe Galvan	Clorox Services Co., Pleasanton, CA	√	√	√
Earl Goad	EPA, Antimicrobials Division, Arlington, VA	√	√	√
John Harbell	Mary Kay Inc., Addison, TX	√	√	√
Leon Johnson	EPA, Antimicrobials Division, Crystal City, VA	√	-	-
Eli Kumekpor	Invitrogen, Frederick, MD	√	-	√
Pauline McNamee	The Procter & Gamble Co., Egham, Surrey, U.K.	√	√	√
Michelle Piehl	MB Research Laboratories, Spinnerstown, PA	√	-	-
Patrick Quinn	Accord Group, Washington, DC	-	-	√
Hans Raabe	Institute for In Vitro Sciences, Gaithersburg, MD	-	√	√
Mary Richardson	Bausch & Lomb, Rochester, NY	√	√	√
Michael Rohovsky	Johnson & Johnson, New Brunswick, NJ	√	√	√
Kristie Sullivan	Physicians Committee for Responsible Medicine, Oakland, CA	-	-	√
Neil Wilcox	Consultant/FDA, College Park, MD	√	√	-

NICEATM:

RADM William Stokes, D.V.M., DACLAM	Director
Debbie McCarley	Special Assistant to the Director

Support Contract Staff— Integrated Laboratory Systems, Inc.:

David Allen, Ph.D.	Elizabeth Lipscomb, Ph.D.
Jonathan Hamm, Ph.D.	Linda Litchfield
Nelson Johnson	Greg Moyer, M.B.A.
Brett Jones, Ph.D.	James Truax, M.A.

Abbreviations used in participants' affiliations:

CDER = Center for Drug Evaluation and Research

CFSAN = Center for Food Safety and Applied Nutrition

CPSC = U.S. Consumer Product Safety Commission

ECVAM = European Centre for the Validation of Alternative Methods

EPA = U.S. Environmental Protection Agency

FDA = U.S. Food and Drug Administration

ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods

ILS = Integrated Laboratory Systems, Inc.

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

NIEHS = National Institute of Environmental Health Sciences

NLM = National Library of Medicine

OPP = Office of Pesticide Products

OTWG = Ocular Toxicity Working Group

USDA = U.S. Department of Agriculture

TUESDAY, MAY 19, 2009

Call to Order and Introductions

Dr. Hayes (Peer Review Panel Chair) called the meeting to order at 8:30 a.m. and introduced himself. He then asked all Peer Review Panel (Panel) members to introduce themselves and to state their name and affiliation for the record. He then asked all the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) staff, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) members, the ICCVAM Ocular Toxicity Working Group (OTWG) members, the European Centre for the Validation of Alternative Methods (ECVAM) staff person, and members of the public to introduce themselves. Dr. Hayes stated that there would be opportunities for public comments during the discussions associated with each of the ten test method topics. He asked that those individuals interested in making a comment register at the registration table and provide a written copy of their comments, if available, to NICEATM staff. Dr. Hayes emphasized that the comments would be limited to seven minutes per individual per public comment session, and that, while an individual would be welcome to make comments during each commenting period, repeating the same comments at each comment period would be inappropriate. He further stated that the meeting was being recorded and that Panel members should speak directly into the microphone.

Welcome from the ICCVAM Chair

Dr. Wind, U.S. Consumer Product Safety Commission (CPSC) and Chair of ICCVAM, welcomed everyone to CPSC and to the Panel meeting. Dr. Wind stressed the importance of this Panel's efforts, especially considering the public health importance of ocular safety testing and hazard labeling. Dr. Wind noted that approximately 125,000 home eye injuries occur each year and over 2,000 workers suffer eye injuries each day, many of which are caused by accidental exposure to chemicals or chemical products. Dr. Wind also reviewed the statutes and regulations requiring ocular testing.

Dr. Wind thanked the Panel members for giving their expertise, time, and effort and acknowledged their important role in the ICCVAM test method evaluation process. Dr. Wind also emphasized the importance of public comments that are considered by the Panel in this process and the Panel's role in the development of ICCVAM final test method recommendations.

Welcome from the Director of NICEATM, and Conflict-of-Interest Statements

Dr. Stokes, Director of NICEATM, stated the Panel meeting was being convened as a National Institutes of Health (NIH) Special Emphasis Panel and was being held in accordance with applicable U.S. Federal Advisory Committee Act regulations. As such, Dr. Stokes indicated that he would serve as the Designated Federal Official for this public meeting. He reminded the Panelists that, when they were originally selected, they had signed conflict-of-interest statements in which they identified any potential conflicts of interest. He then read the conflict-of-interest statement and again asked members of the Panel to identify any potential conflicts for the record. Dr. Hayes asked the Panel members to declare any direct or indirect conflicts based on Dr. Stokes' statements and to recuse themselves from voting on any aspect of the meeting where these conflicts were relevant.

Dr. Sawyer declared a potential conflict-of-interest regarding his employment with Minrad Inc., a company that manufactures inhalation anesthetics. Dr. Ward declared a potential conflict-of-interest regarding her consulting relationship with a company that manufactures antimicrobial cleaning products. Dr. Rodeheaver indicated that she worked for Alcon, a manufacturer of the topical anesthetics proparacaine and tetracaine. Dr. Vanparys declared a potential conflict-of-interest regarding his company's involvement in the conduct of the Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) test method.

Overview of the ICCVAM Test Method Evaluation Process

Dr. Stokes opened his presentation by thanking the Panel members for their significant commitment of time and effort preparing for and attending the meeting. He noted that this is an international Panel, made up of 22 different scientists from six different countries (Belgium, Canada, The Netherlands, Japan, Spain, and the United States). He explained that the purpose of the Panel was to conduct an independent scientific peer review of the information provided on several proposed alternative ocular safety test methods, a testing strategy, and proposed refinements to the *in vivo* rabbit eye test method. This assessment is to include an evaluation of the extent that each of the established ICCVAM criteria for validation and regulatory acceptance has been appropriately addressed for each test method or testing strategy. The Panel is then asked to comment on the extent that the available information and test method performance in terms of accuracy and reliability supports the ICCVAM draft recommendations. Dr. Stokes noted that the first ICCVAM Ocular Peer Review Panel met in 2005 to evaluate the validation status of four alternative test methods (Bovine Corneal Opacity and Permeability [BCOP], Isolated Chicken Eye [ICE], Isolated Rabbit Eye [IRE], and the HET-CAM) for their ability to identify ocular corrosives or severe irritants. The Panel recommended two of these test methods (BCOP and ICE) on a case-by-case basis for use in a tiered-testing strategy with test method-specific applicability domain restrictions. ICCVAM and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) endorsed the Panel's recommended use for these test methods. The Panel also recommended that, while the IRE and HET-CAM test methods were potentially useful in a tiered-testing strategy with appropriate restrictions, additional data were needed to fully assess their usefulness and limitations for regulatory testing. ICCVAM prepared a test method evaluation report (TMER) and provided a transmittal package (i.e., Panel report, SACATM and public comments, TMER and associated materials) to the ICCVAM Federal agencies for their response as required by the ICCVAM Authorization Act of 2000 (ICCVAM 2000). All Federal agencies with ocular testing requirements endorsed the BCOP and ICE test method recommendations. Dr. Stokes noted that five Panel members from the 2005 review are on the current Panel (i.e., Drs. Henry Edelhauser, A. Wallace Hayes, Robert Peiffer, Scheffer Tseng, and Philippe Vanparys).

Dr. Stokes then provided a brief overview of ICCVAM and NICEATM, and identified the 15 Federal agencies that comprise ICCVAM. He summarized the purpose and duties of ICCVAM (as described in the ICCVAM Authorization Act of 2000[2]), noting that ICCVAM, as an interagency committee, does not carry out research and development or validation studies. Instead, ICCVAM, in conjunction with NICEATM, carries out critical scientific evaluations of the results of validation studies for proposed test methods to assess their usefulness and limitations for regulatory testing, and then makes formal recommendations to ICCVAM agencies.

Dr. Stokes then described the ICCVAM test method evaluation process, emphasizing the many opportunities for stakeholder input during numerous public comment periods.

As part of this process, a working group of Federal scientists designated for the relevant toxicity testing area (e.g., the OTWG) and NICEATM prepare a draft background review document (BRD) that provides a comprehensive review of all available data and information. ICCVAM considers all of this available data and information and then develops draft test method recommendations on the proposed usefulness and limitations of the test methods, test method protocol, performance standards, and future studies. The draft BRD and the ICCVAM draft test method recommendations are made available to the Panel and the public for review and comment. The Panel reviews the draft BRD and evaluates the extent to which the established ICCVAM validation and regulatory acceptance criteria have been adequately addressed and the extent that the demonstrated accuracy and reliability support the ICCVAM draft test method recommendations. A Panel report is published and then considered, along with public and SACATM comments, by ICCVAM in developing final recommendations.

[2] http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf

ICCVAM forwards these final recommendations to the ICCVAM member agencies for their consideration and possible incorporation into relevant testing guidelines.

He concluded by summarizing the timeline for 2009 for the ICCVAM evaluation and peer review of the ocular test methods and approaches, including a *Federal Register* notice in March announcing the Panel meeting, the projected publication of the Panel report in July, and transmittal of ICCVAM final recommendations to Federal agencies in November.

ICCVAM Charge to the Panel

Dr. Stokes reviewed the charge to the Panel:

(1) Review the ICCVAM draft BRDs for completeness and identify any errors or omissions (e.g., other relevant publications or available data).

(2) Evaluate the information in the draft BRDs to determine the extent to which each of the applicable ICCVAM criteria for validation and regulatory acceptance of toxicological test methods have been appropriately addressed.

(3) Consider the ICCVAM draft test method recommendations for the following and comment on the extent to which they are supported by the information provided in the BRDs: proposed test method usefulness and limitations, proposed recommended standardized protocols, proposed test method performance standards, and proposed future studies.

Dr. Stokes thanked the OTWG and ICCVAM for their contributions to this project and acknowledged the contributions from the participating liaisons from ECVAM, the Japanese Center for the Validation of Alternative Methods (JaCVAM), and Health Canada. He also acknowledged the NICEATM staff for their support and assistance in organizing the Panel meeting and preparing the review materials.

Overview of the Agenda

Dr. Hayes outlined the process for reviewing each of the topics. First, the test method developer or other expert will describe the test method protocol and procedures, followed by a presentation summarizing the test method validation database and test method performance for each draft BRD or summary review document (SRD) given by a member of the NICEATM staff. An ICCVAM OTWG member will then present the ICCVAM draft test method recommendations. Following presentations, the Evaluation Group Chair responsible for the topic under consideration will present the Evaluation Group's draft recommendations and conclusions followed by Panel discussion. Public comments will then be presented followed by the opportunity for questions to the public commenters and additional Panel discussion. After consideration of the public comments, the Panel will then vote to accept the Panel consensus, with any minority opinions being so noted with a rationale for the minority opinion provided.

Draize Rabbit Eye Test and Current Ocular Regulatory Testing Requirements and Hazard Classification Schemes

Ms. McCall of the U.S. Environmental Protection Agency (EPA) presented the relevant U.S. and international statutes and regulations for ocular safety testing (e.g., EPA, CPSC, Food and Drug Administration [FDA], Occupational Safety and Health Administration [OSHA], European Union [EU], and Organisation for Economic Co-operation and Development [OECD]). She summarized the Draize scoring system for corneal, iridal, and conjunctival lesions in the rabbit, using representative photographs for reference. She also discussed optional but potentially useful assessments of ocular injury (e.g., fluorescein staining, corneal thickness, depth of corneal injury, photographic documentation, and histopathology) that are not routinely included in the Draize eye test. Ms. McCall then provided an overview of the various U.S. and international hazard classification schemes for ocular corrosivity and irritation (i.e., EPA, EU, Globally Harmonized System of Classification and

Labelling of Chemicals [GHS], and Federal Hazardous Substances Act [FHSA]). She noted that, based on the recently adopted European Union Regulation on the Classification, Labelling and Packaging of Substances and Mixtures (i.e., the CLP Regulation), the EU will move to the GHS system after December 1, 2010, for substances and after June 1, 2015, for mixtures. Ms. McCall also identified the required signal words for labeling based on each regulatory classification.

Use of Topical Anesthetics and Systemic Analgesics to Avoid or Minimize Pain and Distress in Ocular Toxicity Testing

On behalf of NICEATM, Dr. Allen reviewed the relevant sections of the draft BRD on the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing.

Dr. Merrill then presented the ICCVAM draft recommendations for the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing and ICCVAM draft test method recommendations. Dr. Sawyer indicated that anesthetic requirements vary enormously among species. For instance, cats require approximately 40% more anesthetic than humans to achieve a similar level of anesthesia. Therefore, any protocol designed to minimize or eliminate pain needs to be individualized to the target species. The Evaluation Group proposed an alternative to the ICCVAM anesthetic/analgesic protocol to be used during all *in vivo* rabbit ocular irritation testing. Dr. Sawyer outlined the Evaluation Group's proposed protocol, which is divided into pretreatment and posttreatment regimens as follows:

Pretreatment Analgesia:
Buprenorphine 0.01 mg/kg subcutaneous (SC) (60 minutes before test substance application [TSA]). Dr. Sawyer noted that buprenorphine is classified as an opioid agonist-antagonist analgesic with a wide margin of safety in rabbits, minimal sedation, and relatively long duration. It has been found to be effective in managing pain in small animals, and is given before application of the test substance because the most effective method of managing pain and distress is to administer the analgesic preemptively to prevent establishment of central sensitization.

One or two drops of 0.5% proparacaine hydrochloride, applied to the eye three times at 5-minute intervals starting 15 minutes pre-TSA. Last application would be five minutes pre-TSA. Anticipated duration of action: 30 - 60 minutes. Dr. Sawyer stated that proparacaine is preferred because application to the eye would be less painful and the suggested application sequence is to assure effective penetration of the epithelial layer.

Eight hours post-TSA:
Buprenorphine 0.01 mg/kg SC and meloxicam 0.5 mg/kg SC. Dr. Sawyer noted that the timing is to reinforce the initial level of analgesia to carry over until the next morning (the duration of analgesia is expected to be at least 12 hours for buprenorphine and at least 24 hours for meloxicam). The combination of an opioid and a nonsteroidal anti-inflammatory drug (NSAID) such as meloxicam is a well-tested approach to balanced analgesia. Used for post-operative or chronic pain in dogs since 1997, meloxicam has been found to have effective application in rabbits.

Day two through day seven post-TSA:
Buprenorphine 0.01 mg/kg SC every 12 hours and meloxicam 0.5 mg/kg SC every 24 hours. Dr. Sawyer noted that buprenorphine and meloxicam should be continued for seven days post-TSA unless signs of ocular injury sufficient to cause pain and discomfort appear. If so, this systemic analgesic protocol would continue until the test is completed.

Rescue Analgesia:
Dr. Sawyer also outlined a procedure where, if a test subject shows signs of physical pain or discomfort during the test interval using the above protocol, a rescue dose of buprenorphine at 0.03 mg/kg SC could be given as needed every eight hours instead of 0.01 mg/kg SC every 12 hours. Meloxicam would continue with the same dose and interval.

Dr. Sawyer pointed out that buprenorphine and meloxicam were synergistic and have an excellent safety profile in clinical practice. A question was raised concerning the interval of dosing throughout the test period and the burden that it would impose on the testing laboratory. The Panel agreed that a ±30-minute interval is appropriate for the administration of the systemic analgesics.

Dr. Dubielzig indicated that the impact of the NSAID on inflammatory aspects of the Draize rabbit eye test is unknown, but the Panel did not consider such affects to be limited and therefore not likely to be a problem. Dr. Jester questioned the need to continue analgesic treatment through day seven when Category III or IV substances would have cleared by day three. He suggested an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approach where treatment is continued through day four. Dr. Peiffer suggested that the temporal aspect be removed and that treatment be continued only if there are signs of discomfort. **The Panel agreed that treatment should be stopped after day four (instead of day 7, as suggested above) if there are no signs of discomfort**. The Panel agreed that pain assessment should be made and recorded daily.

Dr. Jester raised a concern that the use of preservatives in the topical anesthetics may interfere with the irritation response. The Panel agreed that the use of preservative-free proparacaine should be required. Dr. Stokes asked how long after the administration of the systemic analgesics a rescue dose can be administered. Dr. Sawyer indicated that, due to the wide margin of safety, the rescue dose can be given immediately afterward if necessary.

Dr. Jester expressed concern that dilution of the test substance could occur if a significant amount of liquid anesthetic remained in the eye. Dr. Peiffer indicated that, in his experience, the 5-minute interval is reasonable and should not pose a problem for test substance dilution.

In response to the evaluation guidance question specific to testing situations where the use of topical anesthetics would be considered inappropriate, the Panel indicated that drugs to be used for ocular effects, such as eye drops, need to be tested by other means. However, the focus of this evaluation is eye irritation hazard classification; therefore, the proposal would be relevant to all such testing. The Panel did not know of additional systemic analgesics that might have greater efficacy in relieving ophthalmic pain associated with chemically-induced injuries. The Panel also agreed that there were no additional pain-related chemically-induced injuries to the eye that the proposed alternate analgesic proposal would not adequately address.

The Panel expressed general concern about the use of transdermal patches to deliver anesthetics due to the need for shaving prior to patch application and the possibility of skin irritation. In addition, with multiple applications, the availability of irritation-free skin sites may pose a problem. Most importantly, analgesic patches have proven to be unreliable in clinical practice with significant animal-to-animal variation as well as species-to-species variation when comparing effectiveness and duration of effect. The Panel also indicated a greater concern about self-mutilation due to severe pain during eye irritation testing than about the potential for the systemic analgesics to alter the ocular injury response. Dr. Jester indicated that there was insufficient information in the BRD to make this assessment.

The majority of the Panel agreed that the tetracaine information provided in the ICCVAM BRD could be applied to other topical anesthetics such as proparacaine. Dr. Ward indicated that additional studies on cell proliferation, migration, and cytotoxicity could be done with topical anesthetics to provide some assurance that they behave in a manner similar to tetracaine. Although it was previously noted

that anesthetic/analgesic use was for all *in vivo* eye irritation tests, the Panel indicated that administration of post-application analgesics is not a concern if a standard dosing regimen is used throughout and not adjusted for each animal to avoid overdosing side effects.

The Panel also agreed that the clinical signs of post-application pain and distress are adequately described and that no other clinical signs should be added. In the event of an eye infection, the Panel agreed that secondary treatment should be considered, the signs and symptoms of the eye infection should be documented, and the animal should be immediately removed from the study. Finally, the Panel agreed that all relevant data had been adequately considered in the BRD.

The Panel considered its proposal to be more appropriate than the ICCVAM-proposed recommendations in terms of the type and frequency of dosing for topical anesthetics and systemic analgesics. The Panel agreed with the ICCVAM draft recommendations for future studies. Therefore, it recommended refinement of the current *in vivo* test system to evaluate ocular irritation utilizing contemporary/novel technologies to address both concerns. The Panel recommended the following:

- New animal studies should only be considered when absolutely necessary in developing new strategies for testing.
- Products that are overpredicted when anesthetic and analgesic pretreatment is used should be identified.
- Animal responses should be collected in tests currently being conducted to determine whether refinements are warranted in the dosing and timing of anesthetic, analgesic, and antibiotic treatments.
- Rabbit ocular specimens should be submitted for histopathological evaluation to develop an archive of specimens.
- Digital photographs of lesions/observations should be collected.
- Analysis of the variability in rabbit wound-healing responses would help determine whether or not it is due to variability in the ocular defense linking to the neuroanatomic integration.
- Studies should be conducted to determine whether the timing and dosing of systemic analgesics with topical anesthetics might alter the ocular defense enough to change the classification of test substances.
- Cytology samples from the surface of the eye should be collected.
- Studies should be conducted to investigate the appropriateness of using proparacaine instead of tetracaine.
- Studies should be conducted to evaluate the impact of using the NSAID meloxicam with buprenorphine.
- New technologies (e.g., new imaging modalities and quantitative/mechanistic endpoints) should be incorporated into the Draize rabbit eye test, refining/changing it to make it a more humane test that is also more reliable.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Rodeheaver, who cited a potential conflict-of-interest due to her employment by a manufacturer of anesthetic products.

Use of Humane Endpoints in *In Vivo* Ocular Irritation Testing

On behalf of NICEATM, Dr. Allen reviewed the relevant sections of the draft BRD on the use of humane endpoints in *in vivo* ocular irritation testing for the Panel.

Dr. Merrill then presented the ICCVAM draft recommendations for the use of humane endpoints in *in vivo* ocular irritation testing for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the use of humane endpoints in *in vivo* ocular irritation testing and ICCVAM draft test method recommendations. The Panel agreed that each of the current and proposed humane endpoints detailed in the BRD are sufficiently predictive of irreversible or severe effects (i.e., GHS Category 1, U.S. EPA Category I, EU R41) that they should be used routinely as humane endpoints to terminate a study as soon as they are observed. The Panel also agreed that animals should be observed at least once per day (at least twice daily for the first three days) to ensure that termination decisions are made in a timely manner. The Panel agreed that there was insufficient data in the BRD to determine the adequacy of pannus as a recommended humane endpoint. The Panel also agreed that the use of fluorescein staining was an appropriate technique for evaluating eye injury; however, the technique needs to be better described before a reasonable conclusion regarding its value can be made. Dr. Jester suggested that the use of fluorescein staining had not been adequately discussed in this BRD.

The Panel emphasized that, in some cases, decisions to terminate a study should be based on more than one endpoint. Very severe endpoints (e.g., corneal perforation) would be adequate alone to terminate a study. Other biomarkers considered useful by the Panel as routine humane endpoints included extent of epithelial loss, limbal ischemia, and/or stromal loss, and depth of corneal damage.

In response to the question regarding other earlier biomarkers/criteria indicative that painful lesions can be expected to fully reverse, the Panel indicated eyes with conjunctival scores without corneal/iris scores would be expected to recover. The Panel indicated that the destruction of 50% of the limbus will result in pannus in rabbits and, therefore, the ICCVAM draft recommendation requiring 75% for early termination may be excessive. In addition, the Panel indicated that the humane endpoints described in the BRD were sufficient to ensure that the lesions would not reverse. The Panel did agree that the available data and information supported the ICCVAM draft recommendations on humane endpoints. The Panel recommended that studies be developed to identify better and earlier endpoints, such as those seen with fluorescein staining, and that these endpoints should be incorporated into current testing guidelines.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Adjournment

Dr. Hayes adjourned the Panel for the day at 5:45 p.m., to reconvene at 8:30 a.m. on Wednesday, May 20, 2009.

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Dr. Hayes called the meeting to order at 8:28 a.m. and asked Dr. Stokes to discuss the conflict-of-interest for the day's planned topics. Dr. Stokes read the conflict-of-interest statement and Dr. Hayes asked the Panel to declare any conflicts-of-interest. The conflicts-of-interest declared by Panel members on day one of the meeting were repeated.

Dr. Hayes then asked for introductions from the Panel, NICEATM staff, members of ICCVAM and the OTWG, and those in attendance for the public session.

HET-CAM Test Method

Dr. Schrage reviewed the various HET-CAM test method protocols (i.e., IS[A], IS[B], S-Score, Q-Score, and IT) and BASF experience with the test method. Dr. Schrage stressed the need for harmonization of HET-CAM protocols, endpoints, and scoring methods. BASF has conducted a retrospective review of 145 test substances, including a broad variety of chemicals and formulations, which revealed that overall accuracy, false positive rates, and false negative rates were not acceptable. The specificity and sensitivity were especially affected by solubility in both water and oil. These data were submitted to the journal Alternatives to Laboratory Animals in April 2009. Dr. Schrage said she would be willing to share the HET-CAM data on these 145 substances with NICEATM following publication.

Dr. Vanparys said that he would be willing to provide NICEATM with HET-CAM data using the IS(B) analysis method to determine if conversion to the IS(A) method was feasible. He added that, in his experience, the HET-CAM test method can be sensitive for the identification of substances not labeled as irritants.

On behalf of NICEATM, Dr. Allen reviewed the HET-CAM draft BRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the HET-CAM test method for the Panel to consider.

Panel Evaluation

Dr. Wilson (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the HET-CAM test method and ICCVAM draft test method recommendations. He noted that HET-CAM classified four EPA Category III substances incorrectly as Category IV (i.e., they were false negative in HET-CAM). However, he said that regulators would be more concerned if the false negative substances were EPA Category I or Category II. Some Panelists did not consider these substances likely to be a significant risk. Dr. Stokes suggested adding a statement defining an acceptable rate for false positives and false negatives. Dr. Wilson expressed concern that, while three of the four animals had an EPA Category III classification that cleared in seven days, one animal had a conjunctival redness score of two that cleared to one in seven days but required 14 days to completely resolve (i.e., return to a score of zero). Such lesions would not be considered inconsequential.

The Panel discussed the low number of mild and moderate substances used in the performance analyses, and that additional substances in these categories would be needed before a conclusion on the usefulness of HET-CAM could definitively be reached. The Panel also recognized that the validation database does not include substances currently regulated by EPA and that collection of additional data is needed. Therefore, given the limited data for mild and moderate substances, the Panel did not support the ICCVAM draft test method recommendation for use of the HET-CAM to identify substances not labeled as irritants from all other classes.

Dr. Peiffer said that he was concerned with the recommendation to test increasing concentrations of test substances. He stated that while dose-response curves are preferred for scientific studies, they are

not practical for regulatory testing. Dr. Sawyer agreed that increasing concentrations should not be a requirement. Ms. McLaughlin argued that use of different concentrations allows the investigator to see if increasing the concentration affects the outcome. She stated that poor predictivity might result from use of a concentration that produces an ineffectual or weak response, whereas the comparative effect of a higher concentration would provide useful information. The Panel agreed to remove the concentration requirement from the test method protocol but to include it as a general recommendation for additional research.

Ms. McLaughlin offered a minority opinion with respect to the Panel's recommendation on the use of the HET-CAM test method to identify substances not labeled as irritants from all other classes. Ms. McLaughlin stressed that personal care products are not regulated in the U.S. as they are in Europe and Canada. Ms. McLaughlin stated that the HET-CAM test method could be used as an alternative to the Draize rabbit eye test to evaluate personal care products in situations where they are regulated. Dr. Hayes asked Ms. McLaughlin to write a short paragraph to note the rationale for her opposition to the majority view for inclusion in the Panel report. Ms. McLaughlin drafted the following text:

Based on the demonstrated performance as outlined in the ICCVAM draft recommendations, HET-CAM can be used to screen not labeled as irritants from other irritant categories for the restricted applicability domain (surfactant-based formulations and oil/water emulsions). The rationale for this dissenting view is based on the fact that there were 60 substances in the overall database. The hazard category distribution was: 25 Category I; 2 Category II; 18 Category III; and 15 Category IV, The sensitivity of HET-CAM is 91% (41/45), resulting in a false negative rate of 9% (4/45). Among the four false negatives for the EPA system, 100% (4/4, all oil/water emulsion cosmetic formulations) were EPA Category III substances based on conjunctival redness score of two that required at least three days to resolve. The lesions noted *in vivo* indicated mild ocular irritation and are unlikely to represent a significant hazard. As such, the HET-CAM could be considered useful as a screening test for EPA Category IV substances not labeled as irritants from all other categories for the restricted applicability domain of surfactant-based formulations and oil/water emulsions. The sensitivity for GHS and EU was high enough for each system to warrant HET-CAM test method use (i.e., 100% sensitivity; 31/31 and 26/26, respectively for GHS and EU [from the ICCVAM draft BRD, Tables 6-2 and 6-12]) also with domain restriction. This performance demonstrates that HET-CAM could be used to screen EU or GHS hazard not labeled as irritant classifications from other irritant categories for the restricted applicability domain of surfactant-based formulations and oil/water emulsions. It should be noted that, for regulatory purposes, sensitivity (the proportion of all positive substances that are classified as positive) is most important from a public health perspective and the HET-CAM performed well in this regard.

The Panel discussed the ICCVAM draft recommended protocol for the HET-CAM test method. Dr. Vinardell said that she would like to see a statement added to the protocol to wash out any leftover solids after 30 seconds (as currently recommended in the EU Annex V). Dr. Hayes asked Dr. Vinardell to provide a statement for Dr. Wilson to include in the Panel report.

The Panel discussed the HET-CAM test method performance. One Panelist suggested that a Chi-square analysis should be included to ensure that differences in classification were statistically significant. Dr. Ahn was asked if a power analysis could be used to determine if the number of substances in the mild and moderate classification was adequate to differentiate the irritant classifications. Dr. Ahn said that there should be at least three substances in each classification category to conduct a power analysis.

The Panel discussed the need for Good Laboratory Practice (GLP) studies. Dr. Hayes emphasized that a study is either GLP compliant or it is not. He said that the phrase "spirit of GLP" should not be used in the Panel report. He also said that the term "original data" should be used rather than "raw data."

The Panel agreed that data from studies not conducted under GLP guidelines could be used to increase knowledge about the applicability domain of a test method but that laboratories should provide sufficient detail about the conduct of the study to understand any deviations from GLP guidelines.

The Panel discussed additional sources of HET-CAM data to expand the applicability domain and the number of mild and moderate substances tested. Dr. Allen noted that Dr. Debbasch, a principal contact for data acquisition, had left L'Oreal. Dr. Hayes said that *cosmeceuticals* represented a gray zone between cosmetics and personal-care formulations, and this class of products should be considered. Ms. McLaughlin said that the inclusion of a single ingredient (e.g., a UV-blocking material) could change the regulatory requirements for a formulation from an unregulated personal care product to a regulated material in Canada. She said that the applicability domain and database used in the ICCVAM draft BRD should be adequate to warrant use of the HET-CAM test method for personal care products that are not labeled as irritants. The Panel did not support the use of additional studies to identify the full range of irritation but supported additional studies to identify substances not labeled as irritants from all other classifications.

Public Comments

Dr. Barroso from ECVAM commented that the false negatives using the EPA classification system, which are substances not labeled as irritants using the GHS classification system, result because the EPA classification system categorizes substances based upon the most severe category observed among the test rabbits (i.e., not based on the majority classification among rabbits tested). Dr. Barroso also said that because the types of formulations regulated by EPA are not present in the database that the EPA classification system should not be given too much weight.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted to approve the recommendations as revised during the discussion with one minority opinion, Ms. McLaughlin, and one abstention, Dr. Vanparys, who cited a potential conflict-of-interest with the HET-CAM test method, which he had worked on at Johnson & Johnson.

Isolated Chicken Eye Test Method

On behalf of NICEATM, Dr. Allen presented an overview of the ICE test method protocol and reviewed the ICE draft BRD. One Panelist asked why the test method was limited to three eyes. Dr. Allen explained that the incubation apparatus contained 10 chambers, sufficient for three groups of three eyes and a negative control. However, the ICCVAM ICE test method protocol, upon which the recently submitted OECD Test Guideline is based, includes both positive and negative controls.

Dr. Jester said that the term fluorescein *staining* should be used rather than *retention*. He also asked how the EPA classification categories were determined using the ICE test method. Dr. Allen replied that the four-tiered EPA classification system was considered equivalent to the four-tiered GHS system and used the same ICE test method decision criteria (e.g., EPA Category I – GHS Category 1, EPA Category II = GHS Category 2A, EPA Category III = GHS Category 2B, EPA Category IV = GHS Category Not labeled).

Dr. Yu asked if the evaluation of the eyes was subjective and whether photographs were taken. Dr. Allen said that the evaluation of the eyes for corneal lesions was subjective, except for the measurement of corneal swelling, which is measured quantitatively using a pachymeter. He said that photographs were not typically taken but were recommended by the previous ocular Panel.

Dr. Merrill then presented the ICCVAM draft recommendations for the ICE test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the ICE test method and ICCVAM draft test method recommendations. The Panel agreed that the available data and test method performance supported the ICCVAM draft recommendations that the ICE test method is not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems. The Panel further agreed that the ICE test method is not recommended as a screening test to identify substances not labeled as irritants from all other hazard classifications defined by GHS, EPA, and EU, because one of the false negatives included a GHS Category 1 substance. The Panel agreed with the ICCVAM draft recommendation that the ICE test method should not be used as a screening test to identify GHS substances not labeled as irritants. Dr. van der Valk noted that the ICE test method is used by the Netherlands Organisation for Applied Scientific Research (TNO) to obtain good results, but the results obtained by other laboratories using the ICE test method in the validation study were variable. Dr. Vanparys recommended that the source of the variability be noted in the appropriate text.

The Panel agreed that the available data supported the ICCVAM draft recommendations that the proposed standardized protocol appeared acceptable. However, the Panel suggested that the protocol could be improved by adding objective endpoints for corneal opacity and fluorescein staining. The Panel also added that inclusion of a histopathological evaluation might improve ICE test method performance.

The Panel agreed with the ICCVAM draft recommendations for the ICE test method in terms of the proposed future studies that additional optimization studies would be required to validate the test method for the identification of all ocular irritancy hazard categories. The use of histopathology evaluation might add to the accuracy and determination of the test. The Panel also agreed with ICCVAM that the ICE test method performance standards are not warranted at this time.

Public Comments

Dr. Barroso said that variability of the ICE test method was similar to that of the Draize rabbit eye test because of the subjective assessments. He stated that the ICE test method should not be held to a higher standard than the Draize test. He also noted that the concordance among laboratories was reasonable.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Isolated Rabbit Eye (IRE) Test Method

On behalf of NICEATM, Dr. Allen presented an overview of the IRE test method and reviewed the IRE draft BRD. Dr. Hayes asked whether the rabbits used by GlaxoSmithKline (GSK) were from PelFreeze Biologicals or if fresh eyes were used for each test. Dr. Allen replied that at least some of the rabbits were obtained from other GSK laboratories and had been used as negative controls from other acute safety testing. Dr. Ward noted that PelFreeze ships rabbit eyes from its facility in Rogers, Arkansas, adding that their rabbits are used for multiple purposes. She was not aware of a formal study to determine the acceptability of eyes shipped from the U.S. to Europe. Dr. Peiffer suggested

that shipped eyes should be carefully examined prior to use. Dr. Jester said that his laboratory has compared eyes obtained from an abattoir to fresh eyes and found no significant differences.

Dr. Merrill then presented the ICCVAM draft recommendations for the IRE test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the IRE test method and ICCVAM draft test method recommendations. The Panel agreed with ICCVAM that additional optimization and validation studies using a protocol that includes all four recommended endpoints are needed to further evaluate the relevance and reliability of the IRE test method and to develop more definitive recommendations.

The Panel recommended that the planned validation study with GSK/SafePharm include an evaluation of fresh versus shipped eyes. In general, the Panel felt there should be rigid criteria on the handling and storage of the eyes. Finally, the Panel recommended that criteria on test article administration/washout (e.g., viscous substances) were warranted.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Bovine Corneal Opacity and Permeability Test Method (BCOP)

Dr. Curren, Institute for In Vitro Sciences, provided an overview of the BCOP test method. He noted that Pierre Gautheron and his colleagues initially developed the test method for occupational safety. Dr. Curren said that as many as 30% of bovine eyes are rejected upon inspection because of scratches and other defects, and emphasized the importance of including concurrent positive and negative controls in each study. With respect to histopathology evaluation, he said that it was important to carefully choose a qualified laboratory because of the impact of quality on the evaluation.

Dr. Vanparys pointed out that the 15x OD_{490} value in the *In Vitro* Score calculation was chosen to equate the data to *in vivo* data. One Panel member asked if there was an equilibration period, and Dr. Curren indicated that the bovine corneas were equilibrated for one hour before dosing.

Dr. Bailey asked if there was an example for when histopathology evaluation should be recommended based on effects associated with a particular chemical class. Dr. Curren cited as an example oxidizers, which may not produce opacity or permeability changes, but still produce substantive corneal damage that is observable only by histopathology. A Panel member asked why corneal thickness was not measured to provide a quantitative endpoint. Dr. Curren said that corneal thickness has been evaluated, but is less reliable than the opacity and permeability measurements and therefore is not measured in the current protocol.

Dr. Peiffer asked how the BCOP decision criteria for histopathology evaluation are applied to the EPA categorization scheme. Dr. Curren replied that a substance labeled as EPA Category IV would not penetrate further than the superficial corneal epithelium, whereas a Category III substance would penetrate to the basal layer, a Category II substance into the top third of the stroma, and a Category I substance into the bottom third of the stroma or to the endothelium. Minimal damage to the epithelium heals quickly, moderate damage heals more slowly, and significant damage (e.g., deep stromal or endothelial penetration) may be irreversible.

On behalf of NICEATM, Dr. Hamm reviewed the BCOP draft BRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the BCOP test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the BCOP test method and ICCVAM draft test method recommendations. With respect to the substances used in the validation studies, the Panel requested additional chemical classes be added as data becomes available to provide a more significant statistical inference. The Panel requested that Drs. Ahn and Palmer conduct a power analysis to determine the number of substances needed in each hazard classification to provide statistical significance.

The Panel discussed the performance of the BCOP test method to identify the intended range of classification categories. The Panel indicated that the available data and analyses were adequate for the intended purpose. The Panel indicated that all available and relevant data had been used in the ICCVAM BCOP test method analyses.

The Panel agreed with ICCVAM that the test method performance supported the ICCVAM draft recommendations. Accordingly, the BCOP test method was not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems. However, the BCOP test method can be used as a screening test to distinguish substances not labeled as irritants from all other hazard categories when results are to be used for EU or GHS hazard classifications. Because of the significant lesions associated with 50% (4/8) of the EPA Category III substances that tested as false negatives, the BCOP test method cannot be recommended as a screening test to identify EPA Category IV substances.

The Panel agreed with the ICCVAM draft recommendation that the BCOP test method could be used to distinguish substances not labeled as irritants from all other irritant classes, because the false negative rate for the EU and GHS systems was 0% (0/54 or 0/97, respectively). By comparison, the false negative rate was 6% (8/141) for the EPA system. Among the eight false negatives for the EPA system, 100% (8/8) were EPA Category III substances based on Draize rabbit eye test data.

The Panel said that, while the BCOP test method is unable to identify all irritant classifications, further test method development and refinement in future studies was encouraged.

The Panel recommended that performance standards should be developed, because the BCOP test method is now being considered as a screening test for both ocular corrosives/severe irritants and for the identification of substances not labeled as irritants.

Public Comments

Dr. Curren said that, based on his experience with the BCOP test method, performance of the BCOP for the four hazard classification systems was unlikely to improve based on the lack of Draize rabbit eye test reproducibility in the mild and moderate categories. He said that results from Weil and Scala (1971) show that the extremes are reproducible, but the mild and moderate levels of ocular irritation are highly variable. He referenced the antimicrobial cleaning products (AMCP) BRD that includes an analysis of the impact on the ocular hazard category when the results of a six-rabbit Draize test are randomly sampled for a three-rabbit test.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Harbell, Mary Kay Inc., said that his laboratories have used over 30,000 bovine eyes that were kept cold at 4ºC. He added that damaged eyes are quickly removed and excluded from the test. He pointed out that Gautheron et al. (1992) used both fresh eyes and eyes maintained at 4ºC and found no differences in their test method results. Dr. Harbell emphasized the utility of the BCOP in comparison to the other methods being considered given its focus on quantitative measurements.

Dr. Harbell also asked the Panel to consider how histopathology evaluation might contribute to the BCOP test method performance. He said that the experts at the 2005 ICCVAM workshop considered the depth of injury to be an important consideration in the assessment of ocular injury. The purpose of including histopathology evaluation is to evaluate the depth of injury that may not be visible to the naked eye. Dr. Harbell cited the example of oxidizing chemicals that may not affect the opacity or permeability of bovine eyes but do still damage the corneal tissue. Therefore, for these substances, depth-of-injury analysis may be important to differentiate corrosives or severe irritants from moderate irritants. Dr. Harbell said he would like to see histopathology evaluation reconsidered. Dr. Ward asked if he was recommending histopathology evaluation for all classes. Dr. Harbell said that he was but that it would be used primarily for EPA Categories I and II.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Barroso commented on what he referred to as the "top-down" (i.e., screening for corrosives/severe irritants) and "bottom-up" (i.e., screening for substances not labeled as irritants) approaches using the ICE and BCOP test methods. ECVAM is developing a paper to recommend the use of these proposed testing strategies for both ICE and BCOP, where substances could be tested in the BCOP or ICE test methods in order to identify corrosives/severe irritants or substances not labeled as irritants without using an animal test.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion (pending the results of a power analysis by Dr. Ahn) with one abstention, Dr. Vanparys, who cited a potential conflict-of-interest with the BCOP test method, which he had worked on at Johnson & Johnson.

Adjournment

After the discussion, Dr. Hayes adjourned the Panel for the day at 7:25 p.m., to reconvene at 8:30 a.m. on Thursday, May 21, 2009.

THURSDAY, MAY 21, 2009

Dr. Hayes convened the Panel at 8:30 a.m. and asked Dr. Stokes to discuss the conflict-of-interest for the day's planned topics. Dr. Stokes read the conflict-of-interest statement and Dr. Hayes asked the Panel to declare any conflicts-of-interest. The conflicts-of-interest declared by Panel members on day one of the meeting were repeated.

Dr. Hayes then asked for introductions from the Panel, NICEATM staff, members of ICCVAM and the OTWG, and those in attendance for the public session.

The first order of business was to address issues from the preceding day.

BCOP Power Calculation

Dr. Ahn reported on the power calculation requested on Wednesday May 20, 2009, for the BCOP test method. He determined that, for each of the four hazard classification systems, a sample size of 13 substances in each chemical class represented (i.e., 13 x 4 for each chemical class for a four-category hazard classification system) is required to achieve 80% power using a two-group normal approximation test for proportions with a one-sided 0.05 significance level. This is necessary to reject the null hypothesis that the BCOP test is inferior to the Draize rabbit eye test (the accuracy of the BCOP test is more than 0.1 less than that of the Draize test) in favor of the alternative hypothesis that the accuracies in the two groups are equivalent. Dr. Ahn also noted that his analysis included the assumption that the expected accuracy of the BCOP test is 0.6 and the expected accuracy of the Draize rabbit eye test is 0.9.

The Panel voted unanimously to include the recommendation that a sample size of 13 be used for each chemical class in each of the four hazard classifications to achieve statistical significance.

ICE Test Method False Negative Substances

Dr. Vanparys commented on the ability of the ICE test method to identify GHS substances not labeled as irritants. Dr. Vanparys indicated that the false negative substances listed in the ICCVAM BRD were either paints that stick to the cornea or solids, which are known to give inaccurate results with the ICE test method. Dr. Vanparys suggested that the ICE test method is capable of identifying GHS substances not labeled as irritants with the exception of solids and substances that stick to the cornea. The overall Panel recommendations, as stated the previous day, remained unchanged.

Low Volume Eye Test (LVET) Test Method

On behalf of NICEATM, Dr. Allen provided a brief overview of the LVET test method and reviewed the LVET draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the LVET for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the LVET and ICCVAM draft test method recommendations. The Panel noted that the LVET has been used on a wide range of substances and that it does detect the full range of ocular irritancy, but recognized that the majority of the LVET database was for surfactants and surfactant-containing products. The Panel identified several references that should be added to the SRD and noted the need to review the ECVAM BRD. If any additional historical data were obtained, there might be sufficient data to determine the performance of the LVET on several other chemical classes.

The Panel indicated that pain associated with direct application of the test substance to the cornea should not be an issue in light of the recommendations for topical anesthetic and systemic analgesic use.

When discussing the performance of the LVET compared to the Draize test, the Panel indicated that the evaluation was adequate, noting that the LVET appeared to overpredict the human response to a lesser degree than the Draize rabbit eye test. They also recommended that the full range of irritation categories are represented in the LVET validation database.

In considering whether all available data had been made available, the Panel indicated that all data had not been evaluated. Additional published sources should be considered as well as the ECVAM BRD, on which the Panel was unable to comment during this meeting. The Panel stated that in the absence of all existing data, including a background review document prepared by the European Centre for the Validation of Alternative Methods, it could not make definitive conclusions or recommendations on the validation status of the LVET. Nonetheless, the Panel did consider the limited data that are available for the LVET to support the use of historical LVET data as acceptable *in vivo* reference data on which to base comparisons to *in vitro* study results.

Public Comments

Dr. Harbell commented that eye irritation testing is done to protect the public and that accidental exposure data should be included in the evaluation. Dr. Harbell also commented on Dr. Merrill's presentation that outlined the ICCVAM draft recommendations. He stated that the suggestion in the ICCVAM draft recommendations that severe substances should be tested in humans is terrifying. (Note: This comment was in response to a misinterpretation by the commenter, which was clarified by Dr. Merrill who stated that the ICCVAM draft recommendations do not recommend human testing to be conducted [see below]).

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Curren commented that the LVET is being discussed because it was used as an *in vivo* reference test method for some of the data provided for the antimicrobial cleaning product (AMCP) testing strategy. He stated that only biologic or LVET data exist for many of the AMCPs, and these data were used to determine the prediction model to support registration of these AMCPs. The LVET test method is no longer used, but there is historical data that can and should be used. Dr. Curren stated that the question is whether we are putting people at risk based upon the cut-off points suggested in the AMCP BRD.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. McNamee (Procter & Gamble) reiterated the comments by Dr. Curren regarding the LVET and noted that 30 years of human experience data with a chemical substance are sufficient for licensing in the United Kingdom.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Merrill responded to the comment by Dr. Harbell regarding human testing. Dr. Merrill clarified that the ICCVAM draft recommendation states that if an organization or sponsor desires to more adequately characterize the usefulness and limitations of the LVET, ICCVAM recommends that a comprehensive set of substances be tested and compared with the Draize rabbit eye test results. She stated that there was no recommendation for human testing to be conducted, but that existing accidental human injury data and ethical human study data should always be considered.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention,

Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that conducts the LVET.

Cytosensor® Microphysiometer Test Method

Dr. Curren provided an overview of the Cytosensor Microphysiometer (CM) test method protocol.

On behalf of NICEATM, Dr. Lipscomb reviewed the CM test method performance as detailed in the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the CM test method for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the CM test method and ICCVAM draft test method recommendations. The Panel indicated that the test method protocol was sufficiently detailed; however, it was unlikely to be widely used because the CM instrument has been discontinued and a new instrument would require revalidation.

The Panel recommended the use of relevant positive controls in any future validation studies and, because surfactants form micelles that can influence response, surfactant concentrations should be included. The Panel recommended that an evaluation of the different classes of surfactants (i.e., nonionic, anionic, cationic, and zwitterionic) be conducted to determine if restrictions should be imposed on use of the CM test method.

The Panel agreed that, based on the database of surfactants and surfactant-based formulations, LVET data could be used to support the validity of the CM test method in the proposed AMCP testing strategy.

The Panel also agreed that the additional data on the surfactants and surfactant-containing formulations in the ECVAM BRD provided sufficient support for the use of the CM test method as a screening test to identify water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations but not pesticide formulations) as either severe or corrosive irritants or substances not labeled as irritants in a tiered-testing strategy, as part of a weight-of-evidence approach. The Panel also agreed that the intra- and interlaboratory reproducibility of the CM test method had been adequately evaluated, although for a limited range of substances as previously discussed. The Panel again noted that the instrument has been discontinued and is currently not supported by the manufacturer, making its use difficult. However, if the CM instrument were redesigned, the remanufactured instrument would require "catch-up" validation (i.e., not a full validation study).

Based upon the lesions noted for one false negative substance in the EPA classification system, the Panel expressed concern with the ability of the CM test method to identify EPA Category IV substances. The Panel noted that the rabbit data indicated that this substance would be classified as a Category III and, therefore, may cause irritation in a human. The Panel noted that further CM studies are needed, in particular for EPA Categories III and IV substances.

The Panel also expressed concern with the high false positive rate of the CM test method when identifying all four hazard categories.

Public Comments

Dr. Curren noted a correction to his presentation where he did not specifically state that the CM test method is limited to water-soluble substances. He questioned the need for performance standards for the CM test method, given that the Panel did not recommend performance standards for the BCOP

and ICE test methods. Dr. Curren commented that the surfactants referred to as *personal care products* are really detergents.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

EpiOcular Test Method

Dr. Curren provided an overview of the EpiOcular (EO) test method protocol.

On behalf of NICEATM, Dr. Lipscomb reviewed the EO test method performance as detailed in the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the EO test method for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the EO test method and ICCVAM draft test method recommendations. The Panel agreed that the EO test method protocol is adequately detailed but emphasized that the manufacturer should provide a "certificate of quality" for each batch of EO. The Panel also agreed that the critical aspects of the protocol had been justified and described in the BRD; however, in order to use the EO test method in a testing strategy to identify mild irritants and substances not labeled as irritants, positive controls that represent these hazard categories should be included in any future validation studies. The Panel noted that the EO test method cannot distinguish Category III from Category IV substances.

The Panel commented that the performance of the EO test method had not been adequately evaluated and compared to the Draize test for the types of substances included in the AMCP database. The Panel noted that the total number of products and their distribution across hazard categories were not sufficient. The Panel commented that the intralaboratory variability was not adequately assessed, although interlaboratory variability was considered to be adequate.

Public Comments

Dr. Curren indicated that he felt that it was appropriate to include EO data that used a different protocol as a measure of test method reproducibility.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that conducts the EO test method.

Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products (AMCPs) Using *In Vitro* Alternative Test Methods

Dr. Curren provided an overview of the AMCP testing strategy.

On behalf of NICEATM, Dr. Lipscomb reviewed the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the AMCP testing strategies for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the AMCP testing strategies and ICCVAM draft test method recommendations. The Panel also suggested adding more discussion of the cells used in the CM and EO test methods.

Regarding the BCOP test method, the Panel reflected on its previous discussions of the BCOP test method for the total database. The Panel indicated that use of the BCOP test method in a testing strategy to identify severe irritants (Category I) and moderate irritants (Category II), should include positive controls that represent these hazard categories in any future validation studies. The Panel noted that histopathology evaluation, as it is proposed at this time as an additional endpoint for the BCOP test method, does not justify its use for hazard classification of AMCPs. However, histopathology evaluation may prove to be a useful endpoint and, as such, collection of histopathology data and further efforts to optimize its use are encouraged.

The Panel agreed with the ICCVAM draft recommendations that there is insufficient data to support the testing strategy in terms of the proposed test method usefulness and limitations (i.e., the classification of substances in all four ocular hazard categories). There were also insufficient available data on which to base definitive recommendations on the proposed alternate testing strategy for classifying substances in all four ocular hazard categories. In discussing the validity of retrospective evaluations, the Panel stated that a retrospective evaluation of results could be considered adequate if the studies were performed with GLP compliance, coded samples, and pre-established evaluation criteria. The Panel commented that any definitive recommendations on a testing strategy should be based on prospective testing of a list of reference substances in each of the proposed *in vitro* test methods.

The Panel concurred with the ICCVAM draft recommendations in terms of the proposed test method standardized protocols. The Panel stated that routine fixation of tissue from the BCOP test method for possible histopathology evaluation should be continued. The Panel emphasized that no single *in vitro* test method alone was applicable to all types of test materials, and therefore suggested several future studies that could potentially expand the usefulness of AMCP test strategies.

Finally, the Panel commented that the development of performance standards for the AMCP testing strategy was not currently warranted and that a new approach needed to be defined for comparing testing strategies.

Public Comments

Dr. Barroso commented that ECVAM is working on a guideline for the detection of severe irritants with the BCOP test method. He indicated that they see a small change in classification when the cut-off is changed from 55 to 75. ECVAM considers 55 the best cut-off for their intended purpose.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Curren commented that concern regarding the limited number of AMCPs is misplaced due to the intended narrow applicability domain. He stated that industrial-strength cleaners are mostly severe irritants and that household cleaners are mostly mild irritants. Very few, if any, substances are in the moderate range. Dr. Curren expressed concern with the recommendation by the Panel that substances need to be tested by each test method in the testing strategy. He noted that histopathology evaluation with the BCOP test method was included in the testing strategy to provide additional safety, and clarified that most of the histopathology evaluation was performed by a certified veterinary

pathologist. He also questioned the Panel's suggested use of a transformed ocular cell line rather than a normal epidermal cell line.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that manufactures AMCPs.

Concluding Remarks

Dr. Hayes, on behalf of the Panel, thanked Dr. Stokes and the NICEATM staff for their continued assistance during the review process and Panel meeting. He also thanked Dr. Wind, ICCVAM Chair, and the members of ICCVAM and the OTWG for their contributions to the project. Finally, Dr. Hayes thanked the Panel and the Evaluation Group Chairs.

Drs. Wind and Stokes thanked the Panel again for their hard work, thoughtful and objective deliberations, and advice. Dr. Stokes further thanked public attendees for their participation and the invited test method developers for their excellent test method summaries. Dr. Stokes concluded by saying he looked forward to working further with Panel members to complete the Panel report.

Adjournment

Dr. Hayes adjourned the Panel at 7:40 p.m., concluding the meeting.

William S. Stokes, D.V.M., D.A.C.L.A.M.
NIEHS
P.O. Box 12233
Mail Stop: K2-16
Research Triangle Park, NC 27709

Dear Dr. Stokes,

The Meeting Summary Minutes, Independent Scientific Peer Review Panel Meeting, Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches, accurately summarizes the Peer Review Panel Meeting on May 19-21, 2009, in Bethesda, MD.

Sincerely,

/s/ A. Wallace Hayes 8/28/09

Signature | Printed Name | Date

Appendix D2

Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

This document is available at:
https://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/ocularprprept2009.pdf

The document is also available on request from NICEATM:

NICEATM
National Institute of Environmental Health Sciences
P.O. Box 1233, MD K2-16
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Appendix E

Federal Register Notices and Public Comments

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Appendix E1

Federal Register Notices

All Federal Register notices are available at https://www.federalregister.gov/

70 FR 13512 (March 21, 2005)
Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for

Nominations for an Independent Expert Panel

72 FR 26396 (May 9, 2007)
Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

72 FR 31582 (June 7, 2007)
Request for Ocular Irritancy Test Data from Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods

73 FR 18535 (April 4, 2008)
Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

74 FR 14556 (March 31, 2009)
Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRDs); Request for Comments

74 FR 19562 (April 29, 2009)
Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

74 FR 33444 (July 13, 2009)
Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments

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Appendix E2

Public Comments Received in Response to *Federal Register* Notices

Public comments are available on request from NICEATM

70 FR 13512 (March 21, 2005)
Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel

- No responses received.

72 FR 26396 (May 9, 2007)
Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

- Robert Guest (Safepharm Laboratories, Ltd.)

72 FR 31582 (June 7, 2007)
Request for Ocular Irritancy Test Data from Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods

- No responses received.

73 FR 18535 (April 4, 2008)
Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

- No responses received.

74 FR 14556 (March 31, 2009)
Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRDs); Request for Comments

- Dr. Raymond David (BASF Corporation)
- Dr. John Harbell
- MatTek Corporation

- Dr. Wolfgang Pape (R&D Brands)
- Dr. Ruud Woutersen and Mr. Menk Prinsen (TNO)
- Dr. Robert Rapaport (The Procter & Gamble Company)
- Dr. Gerald Renner (Colipa, the European Cosmetics Association)
- Dr. Sherry Ward

74 FR 19562 (April 29, 2009)
Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

- Mr. Troy Seidle, Ms. Sara Amundson, and Dr. Martin Stephens (HSUS), Dr. Kate Willet (PETA), and Dr. Chad Sandusky (PCRM)
- Dr. Catherine Willet (PETA)

74 FR 33444 (July 13, 2009)
Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments

- No responses received.

Appendix E3

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments

SACATM Meeting on June 25-26, 2009

Past SACATM meeting minutes are available online at:
https://ntp.niehs.nih.gov/events/past/index.html?type=SACATM

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Appendix F

Relevant U.S. Federal and International Ocular Toxicity Regulations, Labeling, and Test Guidelines

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Appendix F1

Table of Relevant U.S. Federal and International Ocular Testing Regulations for Hazard Classification and Labeling

Note to the Reader:
Regulations may be updated in the future. It is recommended that users review the most current version of all regulations identified.

Electronic versions of United States Code (U.S.C.) can be obtained at:
http://www.gpoaccess.gov/uscode/index.html

Electronic versions of the Code of Federal Regulations (CFR) can be obtained at:
http://www.gpoaccess.gov/cfr/index.html

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Eye Irritation/Corrosion Testing: Relevant U.S. Federal Laws, Regulations, Guidelines, and Recommendations				
Agency, Center, or Office	**Regulated Products**	**Statutory Requirements**	**Regulations (Applications)**	**Guidelines and Recommendations**
CPSC	Consumer Products	Federal Hazardous Substances Act (U.S.C. Title 15, Chapter 47)	16 CFR 1500.3 (Definitions) 16 CFR 1500.42 (Test for Eye Irritants) 16 CFR 1500.121 (Labeling)	Animal Testing Policy (1984)
EPA/OPPTS	Chemicals as defined by the Toxic Substances Control Act Pesticides	Toxic Substances Control Act (U.S.C. Title 15, Chapter 53) Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6)	40 CFR 716 (Safety Data) 40 CFR 717 (Adverse Reactions) 40 CFR 720 (Premanufacture Notification) 40 CFR 156 (Labeling) 40 CFR 158 (Pesticide Data)	OPPTS 870.2400 (1998)[1] Label Review Manual (2003)[2]

continued

[1] See **Appendix F2.**
[2] Available at: http://www.epa.gov/oppfead1/labeling/lrm/.

Eye Irritation/Corrosion Testing: Relevant U.S. Federal Laws, Regulations, Guidelines, and Recommendations (continued)				
Agency, Center, or Office	**Regulated Products**	**Statutory Requirements**	**Regulations (Applications)**	**Guidelines and Recommendations**
FDA/CFSAN FDA/CDER	Cosmetics[3] Pharmaceuticals	Federal Food, Drug, and Cosmetic Act (U.S.C. Title 21, Chapter 9) Public Health Service Act (U.S.C. Title 42, Chapter 6A)	21 CFR 70 (Color additives in food, medical devices, and cosmetics) 21 CFR 312 (IND Application) 21 CFR 314 (IND Approval) 21 CFR 701 (Cosmetic Labeling) 21 CFR 740 (Cosmetic Warning Statement)	No Specific Guidelines or Recommendations on Eye Irritation/Corrosion Testing Are Provided.
OSHA	Chemicals	Occupational Safety and Health Act of 1970 (U.S.C. Title 29, Chapter 15)	29 CFR 1910.1200 (Hazard Communication Standard) 16 CFR 1500.42 (Test for Eye Irritants)	No Specific Guidelines or Recommendations on Eye Irritation/Corrosion Testing Are Provided.

[3] FDA does not have authority for pre-market approval of cosmetics or cosmetic ingredients with the exception of color additives. However, the FDA may enforce action against products or ingredients that are in violation of Federal labeling laws, including provision of adequate safety information.

Relevant Ocular Testing Regulations for Hazard Classification and Labeling: European Union	
Regulated Products	**Regulations and Directives**
Substances and Mixtures	Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 (CLP, Classification Labelling and Packaging), amending and repealing Directives 67/548/EEC (DSD, Dangerous Substances Directive) and 1999/45/EC (DPD, Dangerous Preparations Directive), and amending Regulation (EC) No 1907/2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 (REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals)
Plant Protection Products	Council Directive 91/414/EEC of 15 July 1991 as amended
Cosmetics	Council Directive 76/768/EEC of 27 July 1976 as amended
Biocidal Products	Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 as amended

Relevant Ocular Testing Regulations for Hazard Classification and Labeling: United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS)	
Scope	**Legal Instruments and Recommendations**
Chemicals (Substances and Mixtures)	Globally Harmonized System of Classification and Labelling of Chemicals (UN 2007), Part 3, Chapter 3.2.4 (Serious eye damage/eye irritation)

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Appendix F2

EPA OPPTS Guidance Document 870.2400 (August 1998)

EPA OPPTS Health Effects Test Guidelines are available at https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines

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Appendix F3

EPA Office of Pesticide Programs Label Review Manual (August 2003)

Electronic versions of the EPA LRM can be obtained at:
http://www.epa.gov/oppfead1/labeling/lrm/

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Appendix F4

Organisation for Economic Co-operation and Development (OECD)

Test Guideline 405 (Adopted April 2002)

OECD Test Guideline 405 is available at
https://www.oecd-ilibrary.org/environment/test-no-405-acute-eye-irritation-corrosion_9789264185333-en

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