

**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**

**National Institute of Environmental Health Sciences
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List of Abbreviations and Acronyms

°C	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	<i>Federal Register</i>
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

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 2851-3) 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.

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 <i>In Vivo</i> Eye Irritation Testing	May 9, 2007	1
72 FR 31582: Request for Ocular Irritancy Test Data From Human, Rabbit, and <i>In Vitro</i> 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.

7.0 References

- Akarsu T, Karaman S, Akercan F, Kazandi M, Yucebilgin MS, Firat V. 2004. Preemptive meloxicam for postoperative pain relief after abdominal hysterectomy. *Clin Exp Obstet Gynecol* 31:133–136.
- Aoki T, Yamaguchi H, Naito H, Shiiki K, Izawa K, Ota Y, et al. 2006. Premedication with cyclooxygenase-2 inhibitor meloxicam reduced postoperative pain in patients after oral surgery. *Int J Oral Maxillofac Surg* 35:613–617.
- Bartfield J, Holmes TJ, Raccio-Robak N. 1994. A comparison of proparacaine and tetracaine eye anesthetics. *Acad Emerg Med* 1:364–367.
- Cooper CS, Metcalf-Pate KA, Barat CE, Cook JA, Scorpio DG. 2009. Comparison of side effects between buprenorphine and meloxicam used postoperatively in Dutch belted rabbits (*Oryctolagus cuniculus*). *J Am Assoc Lab Anim Sci* 48:279–285.
- Dobromylskyj P, Flecknell PA, Lascelles BDX et al. 2006. Management of postoperative pain and other acute pain. In: *Pain Management in Animals* (Flecknell PA, Waterman-Pearson A, eds). London:WB Saunders, 81–145.
- Draize J, Woodard G, Calvery H. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J Pharmacol Exp Ther* 82:377–390.
- EPA. 1998. Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. EPA 712- C-98-195. Washington, DC:U.S. Environmental Protection Agency.
- EPA. 2003. Label Review Manual. 3rd ed. EPA735-B-03-001. Office of Prevention, Pesticides and Toxic Substances. Washington, DC:U.S. Environmental Protection Agency.
- EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Official Journal of the European Communities* L255:1–333.
- Flecknell PA. 1984. Relief of pain in laboratory animals. *Lab Anim* 18:147–160.
- Flecknell PA. 1995. Anaesthesia and analgesia for rodents and rabbits. In: *Handbook of Rodent and Rabbit Medicine* (Laber-Laird K, Swindle MM, Flecknell PA, eds). Newton, MA:Pergammon Press, 219–237.
- Flecknell PA, Liles JH. 1990. Assessment of the analgesic action of opioid agonist-antagonists in the rabbit. *J Assoc Vet Anaesthetists* 17:24–29.
- Flecknell PA, Liles JH. 1992. Evaluation of locomotor activity and food and water consumption as a method of assessing postoperative pain in rodents. In: *Animal Pain* (Short CE, Van Poznak A, eds). New York:Churchill Livingstone, 482–488.
- Maurice D. 1995. The effect of the low blink rate in rabbits on topical drug penetration. *J Ocul Pharm Ther* 11:297–304.
- National Research Council (NRC). 2008. *Recognition and Alleviation of Distress in Laboratory Animals*. Washington, DC:The National Academies Press.
- National Research Council (NRC). 2009. *Recognition and Alleviation of Pain in Laboratory Animals*. Washington, DC:The National Academies Press.
- OECD. 2000. *OECD Environmental Health and Safety Publications Number 19: Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation*. Paris:Organisation for Economic Co-operation and Development.

- OECD. 2002. OECD Guideline for the Testing of Chemicals 405: Acute Eye Irritation/Corrosion. Paris:Organisation for Economic Co-operation and Development.
- Polomano RC, Rathmell JP, Krenzischek DA, Dunwoody CJ. 2008. Emerging trends and new approaches to acute pain management [review]. *J Perianesth Nurs* S43–53.
- Roughan JV, Flecknell PA. 2002. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating post-operative pain in animals. *Lab Anim* 36:322–343.
- Sasaki H, Nagano T, Yamamura K, Nishida K, Nakamura J. 1995. Ophthalmic preservatives as absorption promoters for ocular drug delivery. *J Pharm Pharmacol* 47:703–707.
- Sawyer DC. 2008. *The Practice of Veterinary Anesthesia: Small Animals, Birds, Fish and Reptiles*. Jackson, WY:Teton New Media.
- UN. 2007. *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*. 2nd ed. New York:United Nations Publications.
- Wright EM, Marcella KL, Woodson JF. 1985. Animal pain: evaluation and control. *Lab Anim* 19:20-36.