ICCVAM Test Method Evaluation Report: Recommendation to Discontinue Use of the Low Volume Eye Test for 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

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
U.S.	United States

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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.

More recently, Griffith et al. (1980) developed the low volume eye test (LVET) with the intention that it would more accurately reflect the human response, since the traditional Draize rabbit eye test was considered to consistently overpredict the human ocular hazard potential. The LVET differs from the Draize rabbit eye test in that only 10% of the volume used in the Draize is applied to the eye (10 µL vs. 100 µL), and the test substance is applied directly on the center of the cornea instead of in the conjunctival sac.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently reviewed the validity of the LVET as a replacement for the Draize rabbit eye test. This was necessary because LVET data were used to support the validity of a proposed non-animal *in vitro* testing strategy for antimicrobial cleaning products. 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 information on substances tested in rabbits using the LVET protocol (73 FR 18535).[5]

ICCVAM carefully compiled and assessed all available data and arranged an independent 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 a draft summary review document (SRD) describing the validation status of the LVET, including its reliability and accuracy, and draft test method recommendations for its usefulness and limitations. ICCVAM released this document to the public for comment on March 31, 2009. ICCVAM also

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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-E8-6969.pdf

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 SRD for completeness and accuracy. The Panel then evaluated (1) the extent to which the draft SRD addressed established validation and acceptance criteria and (2) the extent to which the draft SRD supported ICCVAM's draft test method recommendations. Before concluding their deliberations, the Panel considered written comments and comments made at the meeting by public stakeholders.

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the LVET draft SRD and draft test method recommendations, a summary of the conclusions and recommendations from the Panel meeting, 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 SRD, 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 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.[7] Agency responses will also be made available on the website as they are received.

We gratefully acknowledge the many individuals who contributed to the preparation, review, and revision of this report. We especially recognize the Panel members for their thoughtful evaluations and generous contributions of time and effort. Special thanks are extended to Dr. A. Wallace Hayes for serving as the Panel Chair and to Dr. Paul Bailey, Dr. Donald Sawyer, Dr. Kirk Tarlo, and Dr. Daniel Wilson for their service as Evaluation Group Chairs. We thank the OTWG for assuring a meaningful and comprehensive review. We especially thank Dr. Jill Merrill (U.S. Food and Drug Administration Center for Drug Evaluation and Research) and Dr. Karen Hamernik (U.S. Environmental Protection Agency, until April 2009) for serving as Co-Chairs of the OTWG. Integrated Laboratory Systems, Inc., the NICEATM support contractor, provided excellent scientific support, for which we thank Dr. David Allen, Dr. Jonathan Hamm, Nelson Johnson, Dr. Brett Jones, Dr. Elizabeth Lipscomb, and James Truax. Finally, we thank the European Centre for the Validation of Alternative Methods liaisons Dr. João Barroso, Dr. Thomas Cole, and Dr. Valerie Zuang and the Japanese Center for the Validation of Alternative Methods liaison Dr. Hajime Kojima for their participation and contributions.

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6 Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf
7 Available at http:// iccvam.niehs.nih.gov/methods/ocutox/AMCP.htm

Executive Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the validation status of the *in vivo* low volume eye test (LVET). This test method evaluation report provides ICCVAM's recommendations on the usefulness and limitations of the LVET as an alternative to the Draize rabbit eye test (Draize et al. 1944) for assessing substances' ocular irritation potential.

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, ICCVAM, and its Ocular Toxicity Working Group prepared a summary review document (SRD). The SRD, which summarizes the current validation status of the LVET, is based on published studies and forms the basis for draft ICCVAM test method recommendations. The draft SRD and ICCVAM recommendations were provided to an independent international scientific peer review panel (Panel) and to 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 peer review of the ICCVAM draft SRD. The Panel members discussed how well the information contained in the draft SRD supported ICCVAM's draft test method recommendations. In finalizing this test method evaluation report and the SRD, 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.

Specific ICCVAM Test Method Recommendations

Test Method Usefulness and Limitations

ICCVAM does not consider the LVET a valid replacement for the Draize rabbit eye test. Accordingly, ICCVAM does not recommend the LVET for prospective ocular safety testing. If animals must be used for ocular safety testing, ICCVAM recommends using the modified Draize rabbit eye test protocol that incorporates the recommended topical anesthetics, systemic analgesics, and humane endpoints. However, ICCVAM concluded that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards provided that the validity of each type of evidence used for such assessments is adequately characterized.[8]

ICCVAM recommends using Draize data to select reference chemicals for all future validation studies of new, revised, and alternative test methods for ocular safety testing. Priority should be given to chemicals for which there are both Draize data and human data (e.g., from accidental exposures or standardized ethical human studies).

Test Method Protocol

As indicated above, ICCVAM does not recommend any future testing using the LVET and therefore does not recommend a test method protocol.

Future Studies

ICCVAM recommends that additional requests be made for available historical data that participating companies may have on the LVET (e.g., in-house or external studies they have supported, or research and testing studies). Where such data are available, efforts should be made to determine (1) which could be used in a weight-of-evidence approach and (2) how they might be considered.

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[8] The ECVAM Scientific Advisory Committee (ESAC) does not consider the LVET a valid replacement for the Draize rabbit eye test. ESAC also concludes that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards (ESAC 2009; **Appendix D**).

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1.0 Introduction

The low volume eye test (LVET) is an *in vivo* rabbit eye test that, like the Draize test, was designed to determine the extent of potential ocular hazard of a test substance. Both tests evaluate the ocular irritation response when a single dose of a test substance is applied to the eye of a rabbit. Developed by Griffith et al. (1980), the LVET differs from the Draize rabbit eye test primarily by applying 10 μL of a test substance directly on the cornea instead of 100 μL in the conjunctival sac. Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that in the Draize rabbit eye test.

To date, the LVET has not been demonstrated as an adequately valid *in vivo* reference test method. It has not been formally accepted by any regulatory agency as a stand-alone test for ocular safety testing. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently reviewed the usefulness and limitations of the LVET as a proposed replacement for ocular safety testing, because LVET data were used to support the validity of an *in vitro* testing strategy for antimicrobial cleaning products.

The ICCVAM Authorization Act of 2000 (Public Law 106-545, 42 United States Code 285*l*-3) charged ICCVAM with coordinating the technical evaluation of new, revised, and alternative test methods that have 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.

NICEATM works with the ICCVAM Ocular Toxicity Working Group (OTWG) to evaluate alternative methods and testing strategies. Drs. João Barroso, Tom Cole, and Valerie Zuang represented the European Centre for the Validation of Alternative Methods (ECVAM), and Dr. Hajime Kojima was the liaison from the Japanese Center for the Validation of Alternative Methods (JaCVAM) to the OTWG.

To facilitate the peer review, the OTWG and NICEATM prepared a draft summary review document (SRD) on the use of the LVET in ocular toxicity testing. The document provided information and data from published and unpublished data. A background review document for the LVET was originally submitted to ECVAM. However, the companies that provided unpublished data for the document would not agree to its release. Therefore, the data included in the ECVAM background review document are not considered here.

In April 2008, NICEATM and ICCVAM published a *Federal Register* notice requesting the submission of data and information on substances tested in rabbits using the LVET protocol (73 FR 18535).[1] The notice also requested nominations for an independent expert peer review panel (Panel). These requests were also disseminated via the ICCVAM electronic mailing list and through direct requests to over 100 stakeholders. No data were received in response to the request; however, 12 individuals or organizations submitted comments. Twenty potential panelists were nominated for consideration (see **Section 4.0**).

The SRD forms the basis for the ICCVAM test method recommendations described in this test method evaluation report. The ECVAM and JaCVAM liaisons to the OTWG provided input and contributed throughout the evaluation process. Detailed timelines of the ICCVAM evaluation and the development of the final SRD for the LVET method are provided as **Appendices A** and **B**, respectively.

On March 31, 2009, ICCVAM announced the availability of the ICCVAM draft documents. The *Federal Register* notice also announced a public Panel meeting (74 FR 14556[2]) to review the

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[1] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-6969.pdf
[2] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf

validation status of the LVET test method and several other proposed alternatives for ocular safety testing, The ICCVAM draft SRD and draft test method recommendations were provided to the Panel and posted on the NICEATM–ICCVAM website, along with all public comments received before the Panel meeting.

The Panel met in public session from May 19–21, 2009, to review the completeness and accuracy of the ICCVAM draft SRD. The Panel then evaluated (1) the extent to which the draft SRD addressed established validation and acceptance criteria and (2) the extent to which the draft SRD supported ICCVAM's draft test method recommendations. Interested stakeholders from the public commented at the Panel meeting. The Panel considered all comments before concluding their deliberations. On July 12, 2009, ICCVAM posted the final report of the Panel's recommendations (see **Appendix C**) on the NICEATM–ICCVAM website for public review and comment (announced in 74 FR 33444).[3]

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

ICCVAM and the OTWG considered the SACATM comments, the Panel report, and all public comments when finalizing this test method evaluation report and the accompanying SRD (**Appendix B**). As required by the ICCVAM Authorization Act, ICCVAM will make this test method evaluation report and the final LVET SRD 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 will be posted on the NICEATM–ICCVAM website as they are received.

[3] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-16388.pdf

2.0 ICCVAM Recommendations for the LVET Test Method

2.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

ICCVAM does not consider the LVET a complete replacement for the Draize rabbit eye test and therefore does not recommend the LVET for prospective ocular safety testing. If animals must be used in ocular safety testing, ICCVAM recommends that the Draize rabbit eye test be used as recommended with topical anesthetics, systemic analgesics, and humane endpoints (ICCVAM 2010). However, ICCVAM concluded that retrospective LVET data can be used in a weight-of-evidence approach to identify potential ocular irritants.[4] ICCVAM also recommends that the selection of reference chemicals for validation of alternative ocular toxicity test methods be based on Draize data, not on LVET data.

Independent Peer Review Panel Conclusions and Recommendations

The Panel concluded that, in the absence of all available data, including a background review document (BRD) prepared by ECVAM, they could not make definitive conclusions or recommendations on the validation status of the LVET.

2.2 ICCVAM Recommendations: Test Method Protocol for the LVET Test Method

As indicated above, ICCVAM does not recommend prospective testing with the LVET and therefore does not recommend a specific test method protocol.

Independent Peer Review Panel Conclusions and Recommendations

As noted above, the Panel could not make definitive conclusions and recommendations on the LVET test method.

2.3 ICCVAM Recommendations: Future Studies for the LVET Test Method

ICCVAM recommends that further inquires be made about the existence of any additional historical data that participating companies have on the LVET (e.g., research and testing studies, or in-house or external studies they have supported). Where such data are available, efforts should be made to determine which data could be used in a weight-of-evidence approach and how it might be considered.

Independent Peer Review Panel Conclusions and Recommendations

The Panel emphasized the need to further inquire about the existence of any additional historical data the participating companies have on the LVET (e.g., in-house or external studies they have supported).

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[4] The ECVAM Scientific Advisory Committee (ESAC) does not consider the LVET a valid replacement for the Draize rabbit eye test. ESAC also concludes that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards (ESAC 2009; **Appendix D**).

3.0 Validation Status of the LVET Test Method

ICCVAM reviewed the validity of the LVET because LVET data is used to support the validity of one of the *in vitro* test methods proposed in the *in vitro* testing strategy for antimicrobial cleaning products. The accuracy of the LVET was compared to that of the Draize rabbit eye test and to available human data and experience. A BRD for the LVET was originally submitted to ECVAM, but the companies that provided unpublished data for the document would not agree to its release. In addition, the ECVAM BRD does not include additional reference data for severe irritants tested in both the LVET and the Draize test. Consequently, it provides no additional data to evaluate the accuracy of the LVET compared to the Draize rabbit eye test for severe irritants. Therefore, the data included in the ECVAM background review document are not considered here.

The LVET is an *in vivo* rabbit eye test developed by Griffith et al. (1980). Like the Draize rabbit eye test, the LVET was designed to determine the extent of a test substance's potential ocular hazard. It evaluates the irritation response when a single dose of the test substance is administered to the eye of a rabbit. The LVET differs from the Draize rabbit eye test primarily by applying 10 μL of a test substance directly on the cornea instead of 100 μL applied in the conjunctival sac. Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that in the Draize rabbit eye test.

Most publicly available LVET data represent only limited types (i.e., surfactant-containing personal care and household cleaning products) and numbers of substances. The same is true for traditional Draize rabbit data with which to compare and evaluate the accuracy of the LVET. Available human data (clinical studies and accidental exposures) proposed to support the accuracy of the LVET are largely with mild irritants or nonirritating substances, as are the corresponding LVET data. These substances are predominantly surfactant-containing cosmetic and personal care product formulations.

Ethical considerations have limited the types of substances that can be tested in human clinical studies. As a result, LVET comparisons to human clinical study data are based on tests with mild irritants or substances not labeled as irritants. Such data provide little assurance to the regulatory agencies charged with protecting public health that the LVET can provide adequate protection from substances that may cause moderate or severe ocular injuries in humans.

Accidental exposures are generally not considered a reliable source of information on true ocular hazard potential. Eyes are likely flushed with large volumes of water immediately after accidental exposure. They may not represent the most severe lesion that might be produced by such an exposure. Accidental exposures do not allow definitive quantitative measures of amount and time of exposure needed for human reference data. Some consumer products (e.g., bleach) that cause corrosive ocular lesions in humans at certain concentrations have not been tested in the LVET at comparable concentrations. The LVET is proposed as more likely to approximate the volume of a substance that could enter the human eye experimentally; however, there are limited data to indicate whether it can accurately identify the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries.

In contrast, there are no documented instances in which a substance that produced a severe irritant/corrosive response in humans was not also classified as a severe irritant/corrosive in the Draize rabbit eye test.

4.0 ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process provides numerous opportunities for stakeholder involvement. The public may submit written comments and provide oral comments at ICCVAM independent peer review panel meetings and SACATM meetings. **Table 4-1** lists the nine opportunities for public comments 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 submitted. Comments received in response to or related to the *Federal Register* notices are accessible on the NICEATM–ICCVAM website.[5] The following sections, delineated by *Federal Register* notice, briefly discuss the public comments received.

Table 4-1 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

[5] Available at http://ntp-apps.niehs.nih.gov.iccvambp/searchPubCom.cfm

4.1 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.

4.2 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.

NICEATM received no public comments relevant to the LVET test method.

4.3 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.

4.4 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 irritation 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.

4.5 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; 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 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.

Public Responses, Written
Two written comments were relevant to the LVET test method.

Comment:
One commenter provided additional information and references for the use of LVET data as *in vivo* reference data. The commenter's main points were that (1) personal care and surfactant-based cleaning products do not result in eye injuries observed in people, (2) accidental human exposure data should be included in the assessment of eye irritation, and (3) both the sensitivity and specificity of the LVET should be evaluated. The commenter also provided additional data on the performance of known human corrosives in the LVET and comments on the analysis of data in Gettings et al. (1996, 1998).
ICCVAM Response:
The additional data and references were provided to the Panel before its public meeting and are included in the LVET final summary review document (**Appendix B**). ICCVAM considers human experience data to be important for consideration in a weight-of-evidence approach to hazard categorization.

Comment:
One commenter provided additional information and references on the historical LVET database to support use of the LVET as an *in vivo* reference test method. The commenter's main points follow:

- The historical LVET database includes known human ocular corrosives and a range of substances from different chemical classes and hazard categories.
- Several historical parallel LVET–Draize datasets are available and include a range of substances from different hazard categories.
- The Draize test is subject to inherent variability.
- Both the LVET and the Draize overpredict the human response, but the LVET is more representative of the human response than the Draize test.
- Human experience data are an important source of data that should be considered in a weight-of-evidence approach.
- The choice of 10 μL as the dose volume for LVET is supported by anatomical/physiological considerations between rabbits and humans.

ICCVAM Response:
ICCVAM does not consider the LVET a valid replacement for the Draize rabbit eye test. ICCVAM does not recommend the LVET for prospective ocular safety testing. ICCVAM also concluded that retrospective LVET data can be used in a weight-of-evidence approach to identify potential ocular irritants, provided that there is adequate characterization of the validity of each type of evidence used for such weight-of-evidence assessments.[6]

<u>Public Responses, Oral</u>
Twelve oral public comments were provided at the Panel meeting. Three comments remarked specifically on the LVET test method.

Comment:
One commenter stated that eye irritation testing is done to protect the public and that accidental exposure data should be included in the evaluation.
ICCVAM Response:
While it is important to consider accidental exposure data in a weight-of-evidence approach to hazard categorization, accidental exposures are generally not considered a reliable source of information on true ocular hazard potential because of the uncertain concentration and volume of the substance.

Comment:
Two commenters indicated 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 AMCP testing strategy. The commenters stated that only 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 are historical data that can and should be used.
ICCVAM Response:
Most publicly available LVET data represent only limited types and numbers of substances (i.e., surfactant-containing personal care and household cleaning products). The same is true for traditional Draize rabbit data with which to compare and evaluate the accuracy of the LVET. The available comparative LVET and human (clinical studies and accidental exposures) data proposed to support its accuracy are largely with substances that are mild irritants or nonirritating. These substances are predominantly surfactant-containing cosmetic and personal care product formulations.

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[6] ESAC does not consider the LVET a valid replacement for the Draize rabbit eye test. ESAC also concludes that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards (ESAC 2009; **Appendix D**).

4.6 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 in response. Two written comments were received before the meeting, and two oral comments were provided at the SACATM meeting.

NICEATM received no public comments relevant to the LVET test method.

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.

4.7 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 Comment

NICEATM requested submission of written public comments on the independent scientific peer review panel report. No public comments were received.

5.0 References

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Griffith J, Nixon G, Bruce R, Reer P, Bannan E. 1980. Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. Toxic Appl Pharmacol 55:501–513.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report on Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing. NIH Publication No. 10-7514. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

Appendix A

ICCVAM Evaluation Timeline

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ICCVAM Evaluation Timeline

December 27, 2007	Background Review Document titled In Vitro Approach for EPA Toxicity Labeling of AMCPs received from the Institute for In Vitro Science, Inc. (IIVS).
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 Summary Review Document:
The Low Volume Eye Test

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ICCVAM Summary Review Document:

The Low Volume Eye Test

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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Table of Contents

List of Tables

List of Abbreviations and Acronyms

A.I.S.E.	International Association for Soaps, Detergents and Maintenance Products
ATSDR	Agency for Toxic Substances and Disease Registry
BRD	Background review document
CASRN	Chemical Abstracts Service Registry Number
Conj	Conjunctiva
CPSC	(U.S.) Consumer Product Safety Commission
CR	Conjunctival redness
DER	Data Evaluation Report
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC/HO	European Commission/British Home Office
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Community
EI	Extremely irritating
EPA	(U.S.) Environmental Protection Agency
EU	European Union
FDA	(U.S.) Food and Drug Administration
FR	*Federal Register*
FHSA	U.S. Federal Hazardous Substances Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practices
hr	Hour
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
JaCVAM	Japanese Center for the Validation of Alternative Methods
LVET	Low volume eye test
µL	Microliter
MAS	Maximum average score
MeSH	(National Library of Medicine) Medical Subject Headings
MI	Maximally irritating
Minim	Minimally irritating
MMAS	Mean maximum average score
MSDS	Material Safety Data Sheet
NA	Not applicable
NI	Nonirritating
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute of Occupational Safety and Health
NP	Not provided
NT	Not tested
NTP	(U.S.) National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
OSHA	Occupational Safety and Health Administration
OTWG	Ocular Toxicity Working Group
PNI	Practically nonirritating
PSB	Product Safety Branch
scs	Test substance dosed on the superior conjunctival sac
SD	Standard deviation
TSCA	Toxic Substances Control Act
TG	Test Guideline
UN	United Nations

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Preface

Accidental contact with hazardous chemicals frequently causes eye injury and visual impairment. United States and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals. The U.S. Consumer Product Safety Commission, U.S. Environmental Protection Agency, U.S. Food and Drug Administration, and U.S. Occupational Health and Safety Administration have testing regulations and/or guidelines and recommendations for assessing the ocular irritation potential of substances such as pesticides, household products, pharmaceuticals, cosmetics, and agricultural and industrial chemicals.

Although ocular safety assessment has clearly helped to protect consumers and workers, concerns have been raised about the humane aspects of the Draize rabbit eye test. Regulatory authorities have adopted various modifications that reduce the number of animals used and the potential pain and distress associated with the procedure. Significant progress has been made during the last decade. Now only one to three rabbits are required per test, compared to six rabbits in the original protocol. Provisions have been added that allow for animals with severe lesions or discomfort to be humanely euthanized.

The low volume eye test (LVET) was developed by Griffith et al. (1980) with the intent of refining the Draize rabbit eye test to reduce overlabeling of commercial products and more closely predict the human accidental response to ocular hazard. The Draize test was refined by applying the test substance to the corneal surface rather than to the conjunctival sac and by reducing the volume of exposure from 100 µL to 10 µL. However, the hypothesis that the LVET more closely predicts the human response than the Draize test for a wide applicability domain of test substances has not been clearly demonstrated yet. Thus the LVET has yet to be adopted as a reference test method by any regulatory agency.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) reviewed the validity of the LVET because LVET data was used to support the validity of a test method described in the *ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed* In Vitro *Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products* (ICCVAM 2010). The ICCVAM Ocular Toxicity Working Group and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) have prepared this draft summary review document to summarize the current validation status of the LVET based on available information and data obtained by NICEATM. This draft summary review document forms the basis for draft ICCVAM test method recommendations, which are provided in a separate document.

An independent international scientific peer review panel met in public forum on May 19–21, 2009, to develop conclusions and recommendations for the LVET. The Panel included expert scientists nominated by the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods. We anticipate that these organizations will be able to use the Panel's independent report for their deliberations and development of test method recommendations. The Panel considered this summary review document and evaluated the extent to which the available information supported the draft ICCVAM test method recommendations. ICCVAM considered the conclusions and recommendations of the Panel, along with comments received from the public and the Scientific Advisory Committee on Alternative Toxicological Methods, before finalizing the summary review document and test method recommendations. These will be forwarded to Federal agencies for their consideration and acceptance decisions where appropriate.

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, Elizabeth Lipscomb, Brett Jones, Linda Litchfield, Gregory Moyer, Catherine Sprankle, and James Truax. We also thank the members of the ICCVAM Ocular Toxicity Working Group, chaired by Karen Hamernik, Ph.D. (EPA), and Jill Merrill, Ph.D. (U.S. Food and Drug Administration), and ICCVAM representatives who reviewed and provided comments throughout the process leading to this draft version. We also want to 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 Center for the Validation of Alternative Methods, respectively, for their participation.

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Executive Summary

Accidental eye injury due to contact with hazardous chemicals is a major cause of visual impairment. United States and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals. In the Draize rabbit eye test, 100 µL of the test substance is introduced into the conjunctival sac of each animal's eye. Alternatives to the Draize test have been explored to reduce the possibility of pain and distress during the test procedure.

Griffith et al. (1980) developed the low volume eye test (LVET) to both refine the rabbit eye test and more closely predict the human response to ocular hazard. In the LVET, the test substance is applied to the corneal surface rather than the conjunctival sac. The volume of exposure is decreased from 100 µL to 10 µL. However, the LVET has not been shown to predict the human response more closely than the Draize test for a wide array of test substances. Thus, the LVET has not yet been adopted as a reference test method by any regulatory agency. This report reviews available scientific literature and summarizes the usefulness and limitations of the LVET as an acceptable *in vivo* reference test method.

Most available LVET data were generated with surfactant-based mixtures or products, which produce only a mild ocular irritant response or no response. Gettings et al. (1996a) evaluated 25 surfactant formulations and their hazard classifications by the Environmental Protection Agency and Globally Harmonized System of Classification and Labelling of Chemicals. The authors reported several instances in which the LVET underpredicted an ocular corrosive or severe irritant response identified in the Draize test. While some claim that these data show the Draize test to be excessively overpredictive, there is limited information on the performance of known human corrosives in the LVET.

Freeberg et al. (1984) conducted both the LVET and the Draize test on 29 household cleaning products for which human accidental exposure data are available. The authors concluded that the LVET more accurately predicts the human accidental response to such substances. Similarly, Freeberg et al. (1986b) tested 14 cleaning products with both the LVET and Draize tests and compared the responses to human accidental eye exposures. They concluded that the LVET response corresponds more closely to the human experience than does the Draize rabbit eye test.

Ghassemi et al. (1993) and Roggeband et al. (2000) concluded that the smaller volume used in the LVET (10 µL) is more appropriate when compared directly with human clinical data. However, the lack of available Draize test data in these studies precludes any direct comparison with the LVET.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) reviewed the validity of the LVET because LVET data was used to support the validity of a test method described in the *ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed* In Vitro *Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products* (ICCVAM 2010). LVET data are available for only limited types and numbers of substances (i.e., surfactant-containing personal and household cleaning products), precluding comprehensive evaluation of LVET performance.

Comparative human data from clinical studies and accidental exposures have been proposed to support the accuracy of the LVET. However, these data are primarily for mild or nonirritating substances. Ethical considerations have limited the severity of substances that can be tested in human clinical studies. As a result, LVET comparisons to human clinical study data are based on tests with mild irritants or substances not labeled as irritants. Regulatory agencies charged with protecting public health cannot be assured that the LVET can adequately protect against substances that may cause moderate or severe ocular injuries in humans.

The LVET may approximate experimentally the volume of a substance that could enter the human eye accidentally, but there are limited data to indicate whether it can accurately identify the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries. In contrast, there are no documented instances in which a substance that produced a severe irritant/corrosive response in humans was not also classified as a severe irritant/corrosive in the Draize rabbit eye test.

1.0 Background on Ocular Safety Testing

Accidental eye injury is a leading cause of visual impairment in the United States. Many of these injuries occur due to contact with workplace or household chemicals. According to the National Institute of Occupational Safety and Health (NIOSH), each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Additional eye injuries occur in the home, with about 125,000 eye injuries a year caused by accidents involving common household products such as oven cleaner and bleach (source, American Academy of Ophthalmology). U.S. regulatory agencies such as the Consumer Product Safety Commission (CPSC), Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and Occupational Safety and Health Administration (OSHA) have testing regulations and/or guidelines and recommendations to assess the hazard potential of substances that may come in contact with human eyes.

These testing requirements have effectively protected consumers and workers from potential eye injury (Wagoner 1997; Chiou 1999; McGwin et al. 2006). The primary method currently accepted by U.S. and international regulatory agencies for assessing ocular safety hazards is the Draize rabbit eye test (Draize et al. 1944). Testing guidelines describing the procedure have been published (EPA OPPTS 870.2400 [EPA 1998]), Organisation for Economic Co-operation and Development Test Guideline 405 [OECD 2002]) and several legislative statutes have been enacted that enable government agencies to regulate a variety of substances with the potential to pose a risk to ocular health and safety (see **Table 1-1**).

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

Legislation (Year of Initial Enactment)	Agency	Substance
Food, Drug, and Cosmetic Act (1938)	Food and Drug Administration	Pharmaceuticals and cosmetics
Federal Insecticide, Fungicide, and Rodenticide Act (1947) and Federal Environmental Pesticide Control Act (1972)	Environmental Protection Agency	Pesticides
Federal Hazardous Substances Act (1964)	Consumer Product Safety Commission	Household products
Federal Hazardous Substances Act (1964) and Toxic Substances Control Act (1976)	Department of Agriculture and Environmental Protection Agency	Agricultural and industrial chemicals
Occupational Safety and Health Act (1970)	Occupational Safety and Health Administration	Occupational materials
Clean Air Act Amendments (1990)	Chemical Safety and Hazard Investigation Board and Environmental Protection Agency	Accidentally released chemicals and air pollutants

Adapted from Wilhelmus (2001).

2.0 Regulatory Testing Requirements for Ocular Hazards

The classification of irritant responses evaluated by each regulatory agency varies depending on their legislative mandate and specific goals for protecting human health (**Table 2-1**). The EPA ocular irritation classification regulation and testing guidelines (EPA 1998, 2003) are based on the most severe response in one animal in a group of three or more animals. This classification system takes into consideration the kinds of ocular effects produced, as well as the reversibility and severity of the effects. The EPA classifies substances in ocular irritant Categories I through IV (EPA 2003). Category I substances are defined as corrosive or severe irritants, while classification from II to IV is based on decreasing severity of irritation and time required for irritation to clear. Irritation that clears in 8 to 21 days is classified as Category II, while irritation that clears within 7 days is classified as Category III. For Category IV substances, irritation clears within 24 hours.

The U.S. Federal Hazardous Substances Act (FHSA) guideline for ocular irritation classification (CPSC 1995) categorizes a test substance as corrosive, irritant, or substance not labeled as irritant. A corrosive, according to the FHSA, is a substance that causes visible destruction or irreversible alterations in the tissue at the site of contact (CPSC 1995). FHSA classification depends on the number of test animals that exhibit a positive ocular response within 72 hours after application of the test substance in the conjunctival sac.

For the purpose of harmonizing the classification of ocular irritants internationally, the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) includes two harmonized categories. One designates irreversible effects on the eye/serious damage to the eye (Category 1), and one designates reversible effects on the eye (Category 2). *Reversible effects* are further classified based on the duration of persistence. Category 2A (irritating to eyes) reverses within 21 days, and Category 2B (mildly irritating to eyes) reverses within 7 days. The GHS categories are based on severity of the lesions and/or the duration of persistence.

Hazard classification of ocular irritants in the European Union is characterized by two risk phrases: (1) R36 denotes “irritating to eyes”; (2) R41 denotes “risk of serious damage to the eyes” (EU 2001). These risk phrases are based on whether the levels of damage, averaged across the 24-, 48- and 72-hour observation times for each ocular lesion, fall within or above certain ranges of scores.

Table 2-1 Ocular Toxicity Classification Systems

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
U.S. CPSC (Federal Hazardous Substances Act) OSHA (Occupational Safety and Health Act)	6 (12, 18 possible)	1, 2, 3	No	Opacity or Iritis ≥1 or Redness or Chemosis ≥2 for any animal on any day	<u>1st Tier:</u> 4 or more positive animals = Irritant 2–3 positive animals = Go to 2nd Tier <u>2nd Tier</u> 3 or more positive animals = Irritant 1–2 positive animals = Go to 3rd Tier <u>3rd Tier :</u> 1 positive animal = Irritant
U.S. EPA (FIFRA, Federal Environmental Pesticide Control Act, and TSCA)	At least 3	1 hr, 1, 2, 3, 7, 21	No	–Maximum score in an animal used for classification –Opacity or Iritis ≥1 or Redness or Chemosis ≥2	– One or more positive animals needed for classification in categories below. Category: I = Corrosive, corneal involvement, or irritation persisting more than 21 days II = Corneal involvement or irritation clearing in 8–21 days III = Corneal involvement or irritation clearing in 7 days or less IV = Minimal effects clearing in less than 24 hours **Definition of Full Reversal:** Opacity and Iritis scores = 0 and Redness and Chemosis scores ≤1

continued

Table 2-1 Ocular Toxicity Classification Systems (continued)

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
European Union	1 if severe effects are suspected or 3 if no severe effects are suspected	1, 2, 3 (observation until Day 21)	Yes	Mean study values (scores of all animals in study averaged over Days 1, 2, and 3) of: Opacity or Chemosis ≥2, Redness ≥2.5, or Iritis ≥ 1 **OR** Individual animal mean values (scores for each endpoint are averaged for each animal over Days 1, 2, and 3) of: Opacity or Chemosis ≥2, Redness ≥ 2.5, or Iritis ≥ 1	**R36 Classification** (3) Mean study value where: 2 ≤ Opacity < 3 or 1 ≤ Iritis < 1.5 or Redness ≥2.5 or Chemosis ≥2 (2) If 2/3 tested animals have individual animal mean values that falls into one of the following categories: 2 ≤ Opacity <3 1 ≤ Iritis <2 Redness ≥2.5 Chemosis ≥2 **R41 Classification** (3)Mean study value where: Opacity ≥3 or Iritis >1.5 (2) If 2/3 tested animals have individual animal mean values that fall into one of the following categories: Opacity ≥3 Iritis = 2 (3) At least one animal (at the end of the observation period, typically Day 21) where Opacity or Chemosis ≥2, Redness ≥2.5 or Iritis ≥1
GHS: Irreversible Eye Effects	3	1, 2, 3 (observation until Day 21)	Yes	Mean animal values (over Days 1, 2, and 3) of: Opacity ≥3 and/or Iritis ≥1.5	–At least 2 positive response animals = Eye Irritant Category 1 –At least 1 animal with an Opacity, Iritis, Redness, or Chemosis score >0 on Day 21 = Eye Irritant Category 1 **Definition of Full Reversal:** Opacity, Iritis, Redness, and Chemosis scores = 0

continued

Table 2-1 Ocular Toxicity Classification Systems (continued)

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
GHS: Reversible Eye Effects	3	1, 2, 3 (observation until Day 21)	Yes	Mean animal values (over Days 1, 2, and 3) of: Opacity or Iritis ≥1 or Redness or Chemosis ≥2 and the effect fully reverses in 7 or 21 days	–At least 2 positive response animals and the effect fully reverses in 21 days = Eye Irritant Category 2A –At least 2 positive response animals and effect fully reverses in 7 days = Eye Irritant Category 2B **Definition of Full Reversal:** Opacity, Iritis, Redness, and Chemosis scores = 0

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; EPA = U.S. Environmental Protection Agency; FDA = U.S. Food and Drug Administration; FIFRA = Federal Insecticide, Fungicide, and Rodenticide Act; GHS = United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals; OSHA = U.S. Occupational Safety and Health Administration; TSCA = Toxic Substances Control Act.

3.0 Principle of the Low Volume Eye Test

The low volume eye test (LVET) is an *in vivo* rabbit eye test that, like the Draize test, was designed to determine the extent of potential ocular hazard of a test substance. The tests evaluate the ocular irritation response when a test substance is administered as a single dose to the eye of a rabbit. Developed by Griffith et al. (1980), the LVET differs from the Draize rabbit eye test primarily by applying 10 μL (instead of 100 μL) of a test substance directly on the cornea (instead of the conjunctival sac) (**Table 3-1**). Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that of the Draize rabbit eye test (**Table 3-2**).

Table 3-1 Comparison of LVET and Draize Rabbit Eye Test Protocols

	LVET	**Draize**
Dose volume	10 μL	100 μL
Dose location	Applied directly onto the cornea	Applied into the lower conjunctival sac
Eyelid closure	No forced eyelid closure	Eyelids held closed for one second
Scale for scoring ocular lesions	Draize	Draize

Abbreviation: LVET = low volume eye test

To date, the LVET has not been demonstrated as an adequately valid *in vivo* reference test method. It has not been formally adopted by any regulatory agency. For this reason, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is reviewing the validity of the LVET as an acceptable *in vivo* reference test method. In February 2007, the International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) submitted a background review document to the European Centre for the Validation of Alternative Methods (ECVAM) for an independent peer review by their Scientific Advisory Committee. The A.I.S.E. background review document provides a comprehensive summary of available data and information with which to evaluate the usefulness and limitations of the LVET.

Since its original development, proponents of the LVET have suggested that it is a more appropriate *in vivo* reference test method for comparisons to *in vitro* data than is the Draize rabbit eye test. This is based primarily on the assertion that the LVET is more representative of the human response to a potential ocular hazard than the Draize rabbit eye test, given that the site (corneal surface) and volume of exposure used in the LVET more closely resemble that of accidental human exposure. As a result, a reported advantage of the LVET is that it underpredicts the Draize test and is thereby less overpredictive of the human response than the Draize test. However, definitive data to support this claim are not available.

Table 3-2 Scale of Weighted Scores for Grading the Severity of Ocular Lesions

Cornea	
Lesion	**Score[1]**
A. Opacity – Degree of density (area which is most dense is taken for reading)	
Scattered or diffuse area – details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
B. Area of cornea involved	
One quarter (or less) but not zero	1
Greater than one quarter but less than one half	2
Greater than one half but less than three quarters	3
Greater than three quarters up to whole area	4
Score equals A x B x 5	Total maximum = 80
Iris	
Lesion	**Score[1]**
A. Values	
Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage; gross destruction (any one or all of these)	2
Score equals A x 5	Total possible maximum = 10
Conjunctiva	
Lesion	**Score[1]**
A. Redness (refers to palpebral conjunctiva only)	
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
B. Chemosis	
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of the lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
C. Discharge	
Any amount different from normal (does not include small amount observed in inner canthus of normal animals	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3
Score equals (A + B + C) x 2	Total maximum = 20

From Draize et al. (1944).

[1] The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctiva. Scores of 0 are assigned for each parameter if the cornea, iris, or conjunctiva is normal.

4.0 Performance of the Low Volume Eye Test vs. the Draize Rabbit Eye Test

In general, most of the original data generated with the LVET were from surfactant-based mixtures or surfactant-based products (Freeberg et al. 1984; Gettings et al. 1996a, 1998). A comparison of the substances that have been classified by the Draize rabbit eye test as ocular corrosives or severe irritants that have also been tested in the LVET indicates that the LVET routinely underpredicts the ocular corrosive or severe irritant response in the Draize, in many cases by more than one hazard category. Gettings et al. (1996a, 1998) illustrate this in their evaluation of 25 surfactant-containing formulations and the resulting hazard classifications according to the EPA and GHS classification systems (EPA 2003; UN 2007) (**Tables 4-1** and **4-2**).

Table 4-1 Performance of the LVET in Identifying Ocular Hazard Classification According to the EPA Classification System When Compared to Draize Rabbit Eye Test Results

EPA Category[1]		LVET Classification				
		I	II	III	IV	Totals
Draize	I	3	1	6	0	10
	II	0	0	0	0	0
	III	0	0	9	2	11
	IV	0	0	0	4	4
	Totals	3	1	15	6	25

From Gettings et. al. 1996a and 1998.

Abbreviations: EPA = Environmental Protection Agency; LVET = low volume eye test

[1] EPA classification system (EPA 2003).

Table 4-2 Performance of the LVET in Identifying Ocular Hazard Classification According to the GHS Classification System When Compared to Draize Rabbit Eye Test Results

GHS Category[1]		LVET Classification				
		1	2A	2B	Not Labeled	Totals
Draize	1	0	0	4	4	8
	2A	0	0	0	0	0
	2B	0	0	0	1	1
	Not Labeled	0	0	0	16	16
	Totals	0	0	4	21	25

From Gettings et. al. 1996a and 1998.

Abbreviations: GHS = Globally Harmonized System; LVET = low volume eye test

[1] GHS classification system (UN 2007).

Tables 4-1 and **4-2** show multiple instances of underprediction of an ocular corrosive or severe irritant response in the Draize rabbit eye test by the LVET. When the EPA hazard classification system (EPA 2003) was used, the LVET underpredicted 60% (6/10) of Draize Category I substances as Category III (mild irritant) (**Table 4-3**). When the GHS hazard classification system (UN 2007) was used, the LVET underpredicted all eight of the Draize Category 1 substances: 50% (4/8) as

Category 2B (mild irritant) and 50% (4/8) as Not Labeled (not labeled as an irritant) (**Table 4-4**). These data raise concern about the ability of the LVET to reliably detect ocular corrosives or severe irritants (i.e., EPA Category I, EU Category R41, GHS Category 1).

Table 4-3 Extent of Underprediction of LVET vs. Draize Rabbit Eye Test Results According to the EPA Classification System[1]

EPA Category	LVET Category	Product
Category I	Category II	HZY (Antidandruff shampoo)
Category I	Category III	HZA (Shampoo #7)
Category I	Category III	HZE (Gel cleanser)
Category I	Category III	HZF (Baby shampoo #2)
Category I	Category III	HZL (Foam bath)
Category I	Category III	HZR (Facial cleaning foam)
Category I	Category III	HZX (Shampoo #2)

Abbreviations: EPA = Environmental Protection Agency; LVET = low volume eye test
[1] EPA classification system (EPA 2003).

Table 4-4 Extent of Underprediction of LVET vs. Draize Rabbit Eye Test Results According to the GHS Classification System[1]

GHS Category	LVET Category	Product
Category 1	Category 2B	HZI (Skin cleanser)
Category 1	Category 2B	HZK (Bubble bath)
Category 1	Category 2B	HZS (Shower gel)
Category 1	Category 2B	HZY (Antidandruff shampoo)
Category 1	Not Classified	HZL (Foam bath)
Category 1	Not Classified	HZF (Baby shampoo #2)
Category 1	Not Classified	HZX (Shampoo #2)
Category 1	Not Classified	HZA (Shampoo #7)

Abbreviations: GHS = United Nations Globally Harmonized System; LVET = low volume eye test
[1]GHS classification system (UN 2007).

Gettings et al. (1996b) published another study investigating the relationship between the LVET and Draize eye irritation test data for 10 representative hydroalcoholic personal-care formulations. **Table 4-5** provides the eye irritation profile for each of the 10 substances tested. A range of irritancy classification was demonstrated for the LVET; however, only one of the test substances was considered moderately irritating and none severely irritating according to the criteria developed by Kay and Calandra (1962). A further comparison of the LVET using the classification scheme of Bruner et al. (1992) revealed a range of responses from nonirritating to moderately irritating. The Bruner et al. (1992) LVET classification appeared to be more consistent with the Kay and Calandra irritancy classification as determined by the Draize rabbit eye test (**Table 4-5**).

Table 4-5 Summary of Available Rabbit LVET and Draize Data from Gettings et al. (1996b)

Ethanol (%)	Rabbit LVET			Rabbit Draize	
	MAS	Category[1]	Category[2]	MAS	Category[1]
5	2.2	PNI	I	7.7	Mild
10	1.3	PNI	I	3.0	Minim
15	0.7	PNI	I	0.7	PNI
20	0.7	PNI	I	0.7	PNI
33	4.3	Minim	I	14.3	Mild
40	15.5	Mild	III	38.7	Moderate
55	14.3	Mild	II	36.7	Moderate
65	22.5	Mild	III	28.3	Moderate
83	22.5	Mild	III	36.0	Moderate
90	26.0	Moderate	III	45.7	Moderate

Modified from Gettings et al. (1996b).

Abbreviations: LVET = low volume eye test; MAS = maximum average score.

[1] Kay and Calandra (1962): PNI = practically nonirritating; Minim = minimally irritating; Mild = mildly irritating; Moderate = moderately irritating.

[2] Bruner et al. (1992): I = none to inconsequential irritation (LVET-MAS = 0–5); II = slight irritation (LVET-MAS > 5–15); III = moderate to severe irritation (LVET-MAS > 15–50); IV = severe irritation (LVET-MAS > 50–65); V = extremely irritating to corrosive (LVET-MAS > 65–110).

The authors noted a similarity between the irritant responses observed in the Draize rabbit eye test and the LVET, with both tests ranking the substances in a similar order. In addition, the observed irritation for both tests significantly increased when ethanol levels exceeded 33%. Indeed, the LVET consistently underpredicted ethanol solutions above this range when compared to the Draize rabbit eye test data (**Table 4-5**).

Maurer et al. (2001a, 2001b) used pathology to evaluate the relationship of the ocular irritation response to the extent of initial injury for several nonsurfactant materials using the LVET. In these studies, they reported maximum average score (MAS) data for the LVET and irritation classifications based on Kay and Calandra (1962) as shown in **Table 4-6**. These LVET data are compared to available Draize data obtained from the database of the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC 1998) in **Table 4-7**. Maurer et al. (2001a, 2001b) applied test substances directly to the cornea and performed macroscopic assessments for irritation 3 hours after dosing and periodically thereafter up to 35 days. The alcohols, cyclohexanol and parafluoroaniline, were moderate to severe irritants in the LVET. Only cyclohexanol was tested in the Draize test, and it was a severe irritant/corrosive. Of the aldehydes, acetone was a mild irritant in the LVET and a moderate irritant in the Draize test. Formaldehyde (37%; w/v) was a severe irritant in the LVET but was not tested in the Draize test.

Four bleaches, sodium perborate monohydrate ($NaBO_3$), sodium hypochlorite (NaOCl), 10% hydrogen peroxide (H_2O_2), and 15% H_2O_2, were evaluated in the LVET, but no corresponding Draize data were available. $NaBO_3$ and NaOCl were classified as mild and minimal irritants in LVET respectively, with corneal injuries being limited to the epithelium and superficial stroma, as determined using *in vivo* confocal microscopy. It should be noted that some Material Safety Data Sheets (MSDS) from various manufacturers label NaOCl as moderately irritating or a severe irritant/corrosive in humans at or above 5.25%, while label it corrosive in humans above 14%. The

15% H_2O_2 solution would be classified as a severe irritant based on LVET data. Both concentrations affected the epithelium and deep stroma, as determined using *in vivo* confocal microscopy. In undiluted form, H_2O_2 is a known human ocular corrosive/severe irritant.

Table 4-6 Summary of MAS Categorization Data

MAS Score	Ocular Irritation Rating
0–0.5	Nonirritating— NI
0.5–2.5	Practically nonirritating— PNI
2.5–15	Minimally irritating— Minim
15–25	Mildly irritating— Mild
25–50	Moderately irritating— Moderate
50–80	Severely irritating— Severe
80–100	Extremely irritating— EI
100–110	Maximally irritating— MI

From Kay and Calandra (1962).

Abbreviation: MAS = maximum average score.

Table 4-7 Summary of Available Rabbit LVET Data

Chemical Class	Eye Data			
	Rabbit LVET		Rabbit Draize	
	MAS	Category[1]	MMAS	Category[2]
Alcohols	-	-	-	-
Cyclohexanol	50.8	Moderate/Severe	79.8	1/I/R41
Parafluoroaniline	55.0	Moderate/Severe	69.8	
Aldehydes	-	-	-	-
Acetone	19.1	Mild	65.8	2A/II/R36
Formaldehyde, 37% (w/v)	80.0	Severe		
Bleaching Agents	-	-	-	-
Sodium Perborate Monohydrate	23 ± 31.2	Mild	-	-
Sodium Hypochlorite (2.4%)	11 ± 3.6	Minim	-	-
10% Hydrogen Peroxide	16 ± 7.5	Mild	-	-
15% Hydrogen Peroxide	58.3 ± 26.1	Severe	-	-

Data from Maurer et al. (2001a, 2001b).

Abbreviations: ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; LVET = low volume eye test; MAS = maximum average score; MMAS = mean maximum average score;

1 MAS categorization data compiled from classification table of Kay and Calandra (1962): PNI = practically nonirritating; Minim = minimally irritating; Mild = mildly irritating; Moderate = moderately irritating; Severe = severely irritating. Eye irritancy classification scores based on *in vivo* confocal microscopy and light microscopy also available in Jester (2006).

2 Data obtained from ECETOC database (ECETOC 1998). Hazard classifications based on the Globally Harmonized System (UN 2007)/EPA (EPA 2003)/European Union (EU 2001) were determined by NICEATM based on available ECETOC Draize data.

Maurer et al. (2001a, 2001b) concluded that results obtained on these nonsurfactant materials support their hypothesis that ocular irritation is principally defined by the extent of initial injury, despite clear differences in the means by which irritants cause tissue damage.

Jester (2006) used the LVET to investigate the ocular irritancy of 22 substances varying in type (i.e., surfactant, acid, alkali, bleach, alcohol, aldehyde, and acetone) and severity (**Table 4-8**). Jester evaluated the extent of ocular irritation using light microscopy, *in vivo* confocal microscopy, and laser scanning confocal microscopy. Of the 22 substances, five produced slight irritation, nine produced mild irritation, three produced moderate/severe irritation, and five produced severe irritation. However, of the three substances for which Draize data were identified (i.e., 10% acetic acid, cyclohexanol, and acetone), the LVET underpredicted Draize results.

Table 4-8 Summary of Available Rabbit LVET Data

Chemical Class	Eye Data					
	Human[2]		Rabbit LVET		Rabbit Draize[1]	
			MAS	Category[3]	MMAS	Category[4]
Surfactant	-	-	-	-	-	-
Nonionic	-	-	-	-	-	-
Polyoxyethylene glycol monoalkylether	-	-	0.0	NI	-	-
Polyoxyethelenesorbitan	-	-	0.0	NI	-	-
Alkyl E ethoxylate	-	-	33.0	Moderate	-	-
Anionic	-	-	-	-	-	-
Sodium lauryl sulfate, 5%	-	-	4.8	Minim	-	-
Sodium linear alkylbenzene sulfonate	-	-	49.3	Moderate	-	-
Sodium alkyl ethoxylate sulfate	-	-	31.2	Moderate	-	-
Cationic	-	-	-	-	-	-
Cetyltrimethylammonium chloride, 50%	-	-	76.3	Severe	-	-
3-Isotridecyloxypropyl-bis(polyoxyethylene) ammonium chloride	-	-	7.7	Minim	-	-
3-Decyloxypropyl-bis(polyoxyethylene amine, 5%	-	-	40.0	Moderate	-	-
Alkylbenyldimethylammonium chloride, 10%		-	70.6	Severe	-	-
Acid	-	-	-	-	-	-
3% Acetic Acid	-	-	5.0	Minim	-	-
10% Acetic Acid	-	-	9.5	Minim	68	1/I/R41
Base	-	-	-	-	-	-
2% Sodium Hydroxide	-	-	5.0	Minim	-	-
8% Sodium Hydroxide	-	-	50.8	Severe	-	-

continued

Table 4-8 Summary of Available Rabbit LVET Data (continued)

Chemical Class	Eye Data					
	Human[2]		Rabbit LVET		Rabbit Draize[1]	
			MAS	Category[3]	MMAS	Category[4]
Aldehyde	-	-	-	-	-	-
Acetone	-	-	3.8	Minim	65.8	2A/II/R36
Formaldehyde, 37%	-	-	79.7	Severe	-	-
Alcohol	-	-	-	-	-	-
Parafluoroaniline	-	-	43.3	Moderate	-	-
Cyclohexanol	-	-	45.8	Moderate	79.8	1/I/R41
Bleach	-	-	-	-	-	-
Sodium Perborate Monohydrate	-	-	8.3	Minim	-	-
Sodium Hypochlorite (2.4%)	Severe[5]	-	11.8	Minim	-	-
10% Hydrogen Peroxide	-	-	30.3	Moderate	-	-
15% Hydrogen Peroxide	-	-	68.3	Severe	-	-

Data from Jester (2006).

Abbreviations: ATSDR = Agency for Toxic Substances and Disease Registry; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; LVET = low volume eye test; MAS = maximum average score; MMAS = mean maximum average score; MSDS = material safety data sheet.

1 Data obtained from ECETOC database (ECETOC 1998). Hazard classifications based on EPA (EPA 2003), Globally Harmonized System (UN 2007), and European Union (EU 2001) were determined by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods based on ECETOC Draize data.

2 Data compiled from accidental exposures (ATSDR database).

3 MAS categorization data compiled from classification table of Kay and Calandra, (1962) (see Table 4-8). Eye irritancy classification scores based on *in vivo* confocal microscopy and light microscopy also available in Jester (2006).

4 Category classification– EPA/GHS/EU.

5 Labeled as moderately irritating or severe irritant/corrosive in humans at or above 5.25% based on some MSDS reports, while labeled as corrosive in humans above 14%.

5.0 Performance of the Low Volume Eye Test vs. the Draize Rabbit Eye Test Considering Human Study Data and Experience

Human data on potential ocular hazards are available either from accidental exposures or from clinical studies. Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential because such exposures are likely immediately followed by flushing the eyes with large volumes of water. Thus they may not represent the most severe lesion that might be produced by such an exposure. Griffith et al. (1980) conducted a series of rabbit eye test studies using either 10 or 100 μL of substances "recognized as slightly irritating, moderately irritating, or severely irritating/corrosive to humans."

The ocular corrosive or severe irritant substances included the following:

- Acetic acid (10%), which is referenced as a severe irritant based on splashes of vinegar (containing 4% to 10% acetic acid) reported to cause pain, conjunctival hyperemia, and occasionally permanent opacity of the human cornea
- Calcium hydroxide (hydrated lime), which is referenced as one of the most common causes of severe chemical burns of the eye (McLaughlin 1946; Grant and Schuman 1993)
- Formaldehyde (38%), which is referenced for the range of injuries caused by splashes in the human eye from minor transient discomfort to severe, permanent corneal opacities (Grant and Schuman 1993)

Although detailed animal data are not available, the summary data provided by Griffith et al. (1980) indicate that the lesions induced by either 10 or 100 μL of these substances were not reversible within 21 days. However, such accidental exposures as human reference data make definitive quantitative measures of amount and time of exposure impossible to obtain. Ethical considerations and results based largely on the Draize rabbit eye test have limited the severity of substances that can be tested in human clinical studies. As a result, comparisons to human data are based on clinical study tests with mild irritants or substances not labeled as irritants. Such data provide little assurance to the regulatory agencies charged with protecting public health that the LVET can provide adequate protection from substances that may cause moderate or severe ocular injuries.

The fact that seemingly innocuous commercial consumer products were identified as ocular corrosives or severe irritants by the Draize eye test could be seen as supporting the contention that the Draize eye test is excessively overpredictive of the actual hazard to humans. However, because of the paucity of information on the performance of known human corrosives in the LVET, these data cannot simply be dismissed.

Several studies have published supporting data for the demonstrated usefulness of the LVET (Ghassemi et al. 1993; Roggeband et al. 2000; Freeberg et al. 1984, 1986a, 1986b).

5.1 Ghassemi et al. (1993)

Ghassemi et al. (1993) provides an evaluation of *a single product*, a liquid household cleaner (pH 3) reportedly containing the following qualitative formula: nonionic surfactant, amphoteric surfactant, hydrotrope, solvent, and water. This study directly compares LVET results to human clinical data (using either 10 or 100 μL doses) for the same test substance. No Draize rabbit eye test data had been reported; therefore, LVET results could not be compared to those of the standard eye test. The ocular lesions that were produced in this study and their subsequent time to clear suggest that this product is a mild ocular irritant (**Table 5-1**). The authors conclude that because the direct application to the human eye using either 10 or 100 μL doses produced similar results, the smaller volume for testing is more appropriate anatomically and physiologically based on eye volume capacity and subsequent tear volume.

Table 5-1 Summary of Rabbit and Human Responses to an Undiluted Liquid Household Cleaner

Species	Ocular Tissues Involved	Number of Eyes Affected			Mean CR at 24 hr	Eyes Cleared/ Time to Clear	Max Time to Clear
		Cornea	Iris	Conj			
Rabbit LVET	Cornea Iris Conj	3/3	2/3	3/3	2	2/4 days 1/7 days	7 days
Human (10 μL)	Conj	0/10	0/10	10/10	0.1	1/1hr; 4/2hr; 6/4hr; 10/24hr	48 hr
Human (100 μL)	Conj	0/10	0/10	10/10	0.2	1/1hr; 2/2hr; 9/24hr; 2/46hr	70hr

Data from Ghassemi et al. (1993).

Abbreviations: Conj = conjunctiva; CR = conjunctival redness; hr = hour; LVET = low volume eye test (10 μL dose volume).

5.2 Roggeband et al. (2000)

Roggeband et al. (2000) evaluates two products, a dishwashing liquid (pH 8, contains anionic surfactant, nonionic surfactant, soap, ethanol, water) and a liquid laundry detergent (pH 7, contains anionic surfactant, nonionic surfactant, ethanol, water). This study directly compares modified LVET results to those of a human clinical study. Both rabbits and humans were dosed with either 3 μL (dishwashing detergent) or 1 μL (liquid laundry detergent) of the test products. There are no corresponding Draize rabbit eye test data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products are mild ocular irritants (**Table 5-2**). The authors conclude that these data support the notion that (1) an accidental exposure would be approximately 10 μL or less and (2) a volume of 10 μL provides a suitable margin of safety. This is based on (1) knowledge of the anatomical and physiological characteristics of the eye and (2) the fact that study participants in Roggeband et al. (2000) could "only be exposed to 1 μL of dishwashing liquid and 3 μL of liquid laundry detergent before predetermined 'cut-off' ocular responses were observed above which it would have been ethically unacceptable to proceed" (Roggeband et al. 2000).

Table 5-2 Human and Rabbit Eye Responses to a Liquid Laundry Detergent (1 μL)

Human Volunteer	Human				Animal Number	Rabbit LVET[1]			
	1 hour		24 hours			1 hour		24 hours	
	Cornea	Conj	Cornea	Conj		Cornea	Conj	Cornea	Conj
5	0	1/1	0	0/0	28 (c)	0/0	1/1/0	1/2	2/1/1
6	0	1/0	0	0/0	29 (c)	0/0	1/1/0	1/2	2/1/1
21	0	1/0	0	0/0	30 (c)	0/0	1/1/0	0/0	2/1/1
23	1/2	1/0	0	1/0	31 (scs)	0/0	1/1/0	1/4	2/1/0
25	1/1	1/0	0	0/0	32 (scs)	0/0	1/1/0	1/3	2/1/1
27	0	1/0	0	1/0	33 (scs)	0/0	1/1/0	1/4	2/1/1
28	0	1/0	0	0/0					
30	0	0/0	0	0/0					
32	0	1/0	0	0/0					
34	0	1/0	0	0/0					

Data from Roggeband et al. (2000).

Abbreviations: (c) = test substance dosed on the central cornea; Conj = conjunctiva; LVET = low volume eye test; (scs) = test substance dosed on the superior conjunctival sac.

[1]Low volume eye test was modified to use 1 μL instead of 10 μL.

5.3 Freeberg et al. (1984)

A series of studies by Freeberg et al. (1984) compare data from LVET, Draize rabbit eye test, and human studies or experience. Freeberg et al. (1984) compares LVET and Draize rabbit eye test data for 29 cleaning products (laundry products, household cleaning products, and dishwashing products) to human experience data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products are either mild ocular irritants or substances not labeled as irritants (**Table 5-3**). The human data were obtained from medical records of factory and consumer accidental eye exposures (515 reports over a 2-year period). The results indicate that both rabbit LVET and Draize eye tests overpredicted (based on time to clear of ocular lesions) the human response based on accidental eye exposure to the cleaning products. The time to clear was longer in the Draize eye test than in the LVET for the same product, forming the basis for the conclusion that the LVET more closely predicts the human response.

Table 5-3 Summary of Rabbit and Human Accidental Exposure Data from Freeberg et al. (1984)

Species	Test Method	Number of Products	Average ± SD Mean Time to Clear (Day Range)	Average ± SD Median Time to Clear) (Day Range)	Average ± SD Number of Incidents (Range)
Rabbit	LVET	17	7.3 ± 7.2 (1.3–28.8)	6.2 ± 8.8 (0.7–35)	Not Applicable
Rabbit	Draize	26	20.4 ± 7.2 (3.1–33.5)	20.2 ± 12.3 (1.4–35)	Not Applicable
Human	Experience data[1]	29	2.4 ± 2.1 (0.2–9.5)	1.5 ± 1.5 (0.1–1.8)	16.2 ± 8.4 (3–68)

Data from Freeberg et al. (1984).

Abbreviations: LVET = low volume eye test; SD = standard deviation.

[1]Experience data = combined manufacturing and consumer accidental exposures.

5.4 Freeberg et al. (1986a)

Freeberg et al. (1986a) compared rabbit eye test results (both LVET and Draize) with those of human studies (both 10 µL and 100 µL dose volumes) for four cleaning products (a liquid fabric softener, liquid shampoo, liquid hand soap, and liquid laundry detergent). The results indicate that the LVET overpredicted the human response to 10 µL and 100 µL of the same product. The ocular lesions in the Draize rabbit eye test (100 µL) were more severe (both type and longevity) than in the human test using the same volume. While the majority of effects in humans were conjunctival, the corneal effects in humans were minimal and transient. The corneal effects in rabbits were more severe and recovered less quickly. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products would be classified as mild ocular irritants based on the Draize rabbit eye test results, the LVET, and human results (**Table 5-4**).

Table 5-4 Human Clinical Study and Rabbit Data

Test Product	Concentration (% in water)	Time to Clear (hr) Dosing Volume			
		Rabbit 10 µL	Human 10 µL	Human 100 µL	Rabbit 100 µL
Liquid fabric Softener	60	45	18.9	24.9	45
	80	66	12.6	33.6	93
	100	27	13.2	12.5	84
Liquid shampoo	4	5	1.5	2.5	NT
	16	19.8	1.9	2.6	36.5
	20	33	7.5	7.9	63
Liquid hand soap	8	24	1.5	31.5	63
	10	42	10.5	9.1	66
	12	42	1.7	NT	NT
Liquid laundry detergent	2	8.8	2	24.1	27.8
	3	19.8	4.7	1.8	60
	4	39.8	4.8	19.8	75

Data from Freeberg et al. (1986a).
Abbreviation: NT = not tested.

5.5 Freeberg et al. (1986b)

Freeberg et al. (1986b) compares LVET and Draize rabbit eye test data for 14 cleaning products (liquid and solid laundry products, liquid and solid household cleaning products, liquid and solid dishwashing products, and liquid shampoos) to human experience data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products would be classified as moderate to severe ocular irritants based on the Draize rabbit eye test results. Most would be classified as mild ocular irritants by the LVET (**Table 5-5**). The human data were obtained from medical records of factory and consumer accidental eye exposures (218 reports over an 18-month period). Similar to Freeberg et al. (1986a), rabbit LVET and Draize tests both overpredicted the human response due to accidental eye exposure (based on time to clear). Because the time to clear was longer for substances tested in the Draize rabbit eye test than in the LVET, the authors concluded that the LVET outcome more closely relates to the human experience than the Draize rabbit eye test does.

Table 5-5 Human Accidental Exposure and Rabbit Data

Product	Mean Time to Clear (Days)		
	Human	Rabbit LVET	Rabbit Draize
Liquid Laundry Product #1	1.92	26.6	35.0
Liquid Dishwashing Product #1	0.77	8.2	25.7
Solid Dishwashing Product #1	0.59	4.6	18.3
Liquid Dishwashing Product #2	0.43	7.7	11.7
Liquid Household Cleaning Product #1	0.38	-	11.1
Liquid Dishwashing Product #3	0.30	3.9	22.2
Liquid Household Cleaning Product #2	0.23	4.0	15.2
Solid Household Cleaning Product #1	0.19	1.3	29.2
Solid Dishwashing Product #1	0.08	2.1	13.8
Solid Dishwashing Product #1	0.06	2.9	15.1

Data from Freeberg et al. (1986b).
Abbreviation: LVET = low volume eye test.

6.0 Summary

Because studies conducted with the LVET have been limited to tests of surfactant-containing personal and household cleaning products, the applicability domain for which the LVET can be considered is necessarily restricted to these product types. As summarized in **Table 6-1**, LVET data have previously been used by one personal-care product company to support submission of data to the EPA for the registration of at least five antimicrobial cleaning products. The results were used by EPA reviewers in a weight-of-evidence approach, in conjunction with either consumer incidence data (i.e., commercial products for which there is an opportunity for adverse events to be reported by the consumer) and/or Draize data for similar, structurally related substances. Each study was considered on a case-by-case basis and several submissions were deemed unacceptable by the EPA because either the LVET study was not considered an acceptable fulfillment of the eye irritation data requirement and/or the further confirmatory information provided by the submitter was insufficient (**Table 6-1**). Based on the data provided to NICEATM in the Data Evaluation Reports (DERs), it appears that a final EPA ocular hazard classification was not assigned for any product using LVET data alone.

As indicated in the studies summarized above, human data on potential ocular hazards are available either from accidental exposures or from clinical studies. Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential because such exposures are likely immediately followed by flushing the eyes with large volumes of water. Such accidents make definitive quantitative measures of amount and time of exposure impossible to obtain. Although the Draize eye test is reported to be excessively overpredictive of the human response, ethical considerations based largely on results from the Draize rabbit eye test are used to limit the types of substances that can be tested in human clinical studies. As a result, comparisons to human clinical study data are based on tests of mild irritants or substances not labeled as irritants. Such data provide little assurance to the regulatory agencies charged with protecting public health that the LVET can provide adequate protection from more severe ocular injuries.

Thus, while the LVET is proposed as more likely to approximate the volume of a substance that could enter the human eye accidently, there are limited data to indicate whether it can accurately identify the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries. In contrast, there are no documented instances in which a substance with a hazard category determined in the Draize eye test produced a more severe hazard category response in humans following accidental exposures or ethical human studies.

Table 6-1 Summary of Ocular Hazard Classifications for EPA Registered Antimicrobial Cleaning Products: Consideration of LVET Data and EPA Determinations[1]

EPA Registration Number or Submission Code	Submission Date	Animal Data from LVET Study	EPA Hazard Category Based on LVET Data	Additional Submission Information	Final EPA Classification Provided in DER
3573-AO	Jul 20, 2000	No corneal opacity, iritis, or conjunctival irritation (n=6).	Category IV	Consumer incidence data	Study unacceptable[2]
	Jun 6, 2001	Same as for Jul 20, 2000	Consumer incidence data; LVET and Draize data for similar substances		Category III

continued

Table 6-1 Summary of Ocular Hazard Classifications for EPA Registered Antimicrobial Cleaning Products: Consideration of LVET Data and EPA Determinations[1] (continued)

EPA Registration Number or Submission Code	Submission Date	Animal Data from LVET Study	EPA Hazard Category Based on LVET Data	Additional Submission Information	Final EPA Classification Provided in DER
3573-TE	Aug 9, 2000	No corneal opacity, iritis, redness, or chemosis at day 1 (n=3).	Category IV	None	Study unacceptable[2]
	Feb 7, 2001	Repeat submission from Aug 9, 2000		Animal data for similar substances	Category IV
3573-72	Jun 6, 2001	NP	Category III	Consumer incidence data; LVET and Draize data for similar substances	Category III
3573-AI	Jun 6, 2001	NP	NP	NP	Category II
S596273	Jun 27, 2001	No corneal effects or iritis observed. Conjunctivitis resolved by 72 hr (n=3).	Category III	None	Study unacceptable[2]
3573-TG	Jul 25, 2001	NP	Category III	Consumer incidence data; Animal skin irritation study-Category I (severe irritant)	Study unacceptable[3]

Abbreviations: DER = Data Evaluation Reports; EPA = U.S. Environmental Protection Agency; LVET = low volume eye test; NP = not provided (i.e., information not contained in and/or not provided to NICEATM in DERs).

1 Data source: Obtained from a Freedom of Information Act request submitted to EPA for LVET data used to support the submission of data for the registration of antimicrobial cleaning products.

2 "The EPA does not consider the LVET study to be an acceptable fulfillment of the eye irritation data requirement."

3 "It is now the Product Safety Branch's (PSB) policy to take a weight of the evidence approach to the situation by considering individual LVET studies for possible acceptance on a case by case basis if they are significantly supplemented by further, confirmatory information. In the present case, that confirmatory further information is not sufficient."

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8.0 Glossary[1]

Assay:[2] The experimental system used. Often used interchangeably with *test* and *test method.*

Canthus: The angle formed by the meeting of the upper and lower eyelids at either side of the eye.

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.

Confocal microscopy: An optical imaging technique that increases the contrast of micrographs. It can used to reconstruct three-dimensional images by use of a spatial pinhole to eliminate out-of-focus light or flare in specimens that are thicker than the focal plane.

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.

Conjunctival sac: The space located between the eyelid and the conjunctiva-covered eyeball. Substances are instilled into the sac to conduct an *in vivo* eye test.

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.

Corneal stroma: The substantia propia: a tough, fibrous, transparent layer consisting of plates of collagen fibrils (lamellae) produced by keratocytes that make up 10% of the stroma. The fibrils run parallel to each other, but are positioned at right angles to adjacent lamellae.

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.

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

Endpoint:[2] The biological process, response, or effect assessed by a test method.

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:[2] 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.

Hyperemia: An increase in blood flow to a tissue (e.g., cornea).

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.

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1 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.

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

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.

Not Labeled: (a) A substance the produces no changes in the eye following application to the anterior surface of the eye. (b) Substances that are not classified as GHS Category 1, 2A, or 2B; or EU R41 or R36 ocular irritants.

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.

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

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:[2] 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.

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.

Test:[2] The experimental system used; used interchangeably with *test method* and *assay*.

Test method:[2] 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*.

Validated test method:[2] 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:[2] The process by which the reliability and relevance of a procedure are established for a specific purpose.

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.

Appendix C

Independent Scientific Peer Review Panel Assessment

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Appendix C1

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
Robert Peiffer, Jr., D.V.M., Ph.D., DACVO	Senior Investigator, Merck Research Laboratories, Safety Assessment Toxicology, West Point, PA
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
Robert Bronaugh, Ph.D.	FDA, CFSAN, College Park, MD
Pertti Hakkinen	NLM, Bethesda, MD
Masih Hashim, D.V.M., Ph.D.	EPA, OPP, Washington, DC
Jodie Kulpa-Eddy, D.V.M. (ICCVAM Vice-Chair)	USDA, Riverdale, MD
Donnie Lowther	FDA, CFSAN, College Park, MD
Deborah McCall	EPA, OPP, Washington, DC
Jill Merrill, Ph.D. (OTWG Chair)	FDA, CDER, Silver Spring, MD
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 <u>all</u> *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.

WEDNESDAY, MAY 20, 2009

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 C2

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
Research Triangle Park, NC 27709 USA
Telephone: 984-287-3118
E-mail: niceatm@niehs.nih.gov

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Appendix D

ECVAM Scientific Advisory Committee (ESAC)

Statement on the Use of Existing Low Volume Eye Test (LVET) Data for Weight of Evidence Decisions on Classification and Labelling of Cleaning Products and Their Main Ingredients

Available at: https://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/lvet/appd-esac.pdf

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Appendix E

Federal Register Notices and Public Comments

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Appendix E1

Federal Register Notices

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

SACATM meeting minutes are available 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 Health Effects Test Guidelines are avaialble 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)

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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