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 The document provided information and data from published and unpublished data.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 However, the companies that provided unpublished data for the document would not agree to its release.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

[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

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.

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.

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).

[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

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.

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.

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.

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.

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]

Public Responses, Oral
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.

[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**).

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.

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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