1.0 INTRODUCTION

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is charged by the ICCVAM Authorization Act of 2000 (42 U.S.C. § 2851-2, 2851-5 [2000]; available at http://iccvam.niehs.nih.gov/about/PL106545.pdf) to evaluate the scientific validity of new, revised, and alternative toxicological test methods applicable to U.S. Federal agency safety testing requirements. Following such evaluations, ICCVAM is required to provide recommendations to U.S. Federal agencies regarding the usefulness and limitations of such methods.

In August 2003, the ICCVAM Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) recommended that ICCVAM give high priority to reviewing the validation status of existing *in vitro* test methods proposed for identifying ocular corrosives and severe irritants. In October 2003, the U.S. Environmental Protection Agency (EPA) formally nominated four *in vitro* ocular irritation test methods and related activities for evaluation by ICCVAM. This included review of the current validation status of four *in vitro* test methods proposed for identifying potential ocular corrosives and severe irritants in a tiered-testing strategy, since validation[5] of a test method is a prerequisite for it to be considered for regulatory acceptance (ICCVAM 1997, 2003). The four test methods were the Bovine Corneal Opacity and Permeability (BCOP) assay, the Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) assay, the Isolated Chicken Eye (ICE) assay, and the Isolated Rabbit Eye (IRE) assay. Within Europe, the European Commission has concluded that positive results from these four methods can be used to classify and label substances as severe ocular irritants and corrosives (EU 2004). However, the policy specifically states:

> "These tests are not yet validated, and therefore not included in Annex V. Positive results can be used to consider a substance a severe irritant and R41 applied with no further testing. Where a negative result is obtained, an in vivo test should subsequently be required, as the in vitro tests have not been shown to adequately discriminate between eye irritants and non-irritants."

ICCVAM unanimously agreed that the four nominated *in vitro* test methods should have a high priority for evaluation. An ICCVAM Ocular Toxicity Working Group (OTWG) was established to work with the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to carry out these evaluations. ICCVAM and NICEATM also collaborate closely with the European Centre for the Validation of Alternative Methods (ECVAM), a component of the European Commission's Joint Research Centre. Accordingly, an ECVAM liaison was designated for the ICCVAM OTWG to ensure input and contributions during the evaluation and review process.

[5]Validation is the process by which the reliability and relevance of a test method are established for a specific purpose (ICCVAM 1997, 2003).

NICEATM, in conjunction with the OTWG, subsequently prepared four comprehensive background review documents (BRDs) reviewing the available data and information for each of the four *in vitro* test methods. Each BRD described the current validation status of the *in vitro* test method, including what is known about its reliability and accuracy, the scope of the substances tested, and the availability of a standardized protocol.

The BRDs were based on published studies using the respective test method, and other data and information submitted in response to a 2004 public call for information, which was published in a *Federal Register* (*FR*) notice (*FR* Vol. 69, No. 57, pp. 13859-61; available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm). On November 3, 2004, the availability of the draft BRDs was announced in an *FR* notice (Vol. 69, No. 212, pp. 64081-2; available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm). The BRDs were made available in electronic format on the ICCVAM/NICEATM website and from NICEATM on request.

ICCVAM convened an international independent Expert Panel on January 11-12, 2005, to assess the validation status of these four *in vitro* test methods for identifying ocular corrosives or severe irritants. Comments from the public and scientific community on the BRDs were provided to the Expert Panel and made available on the ICCVAM/NICEATM website (http://iccvam.niehs.nih.gov/methods/ocudocs/ocucomm.htm). Public comments at the meeting revealed that additional relevant data was available that had not yet been provided in response to earlier requests for data. The Expert Panel recommended that the additional data be requested and that a reanalysis of the accuracy and reliability of each test method be conducted, where appropriate. On March 21, 2005, the availability of *The ICCVAM Expert Panel Evaluation of the Current Validation Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants* was announced via an *FR* notice (Vol. 70, No. 53, pp. 13513-4; available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm).

In response to the Expert Panel's recommendation, an *FR* notice was published on February 28, 2005 (Vol. 70, No. 38, pp. 9661-2; available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm). The notice requested all available *in vitro* data on these four *in vitro* ocular irritancy test methods and corresponding *in vivo* rabbit eye test method data, as well as any human exposure data (either via ethical human studies or accidental exposure). A request for relevant data was re-sent directly to the primary developers or users of each test method. In response to these requests, additional *in vitro* test method data and corresponding *in vivo* rabbit eye test results were submitted for the BCOP, HET-CAM, and ICE test methods, which were used for reanalysis of test method performance.

Further clarification of hazard classification rules for severe irritants also was obtained subsequent to the release of the four draft BRDs. This change resulted in a small number of substances previously classified as nonsevere irritants now being classified as severe irritants, and necessitated a reanalysis of the accuracy and reliability of all four test methods.

The original draft BRDs also provided an evaluation of the accuracy of each test method by chemical class. The chemical classes assigned to each test substance were revised based on a chemical classification system consistent with the U.S. National Library of Medicine's

Medical Subject Headings (MeSH; available at http://www.nlm.nih.gov/mesh), an internationally recognized standardized classification scheme. This scheme was used to ensure consistency in classifying substances by chemical class among all the *in vitro* ocular test methods under consideration, and resulted in some chemicals being re-classified into different chemical classes. As a result, the accuracy of each test method by chemical class was reanalyzed.

Finally, an additional accuracy analysis was conducted. In this analysis, the accuracy of each *in vitro* ocular irritancy test method for detecting ocular corrosives or severe irritants, depending on whether the *in vivo* rabbit classification was based on the severity of the response and/or its persistence to day 21 post-treatment, was determined.

A list of proposed reference substances for validation of *in vitro* tests to detect ocular corrosives and severe irritants was included in the draft BRDs released on November 3, 2004. A revised list of proposed reference substances was prepared after consideration of the following:

- Recommendations of the Expert Panel that resulted from their deliberations on January 11-12, 2005
- Submission of additional Draize rabbit eye test results for approximately 300 substances
- Clarification regarding the United Nations (UN) Globally Harmonized System (GHS) rules for classification of severe irritants (UN 2003) that resulted in the reclassification of two proposed reference substances from nonsevere to severe irritants
- Reassignment of the candidate reference substances to chemical classes using MeSH (NLM 2005)

The accuracy and reliability reanalyses and the revised reference substances list for validation of *in vitro* tests to detect ocular corrosives and severe irritants were presented in a BRD Addendum that was released on July 26, 2005, with notification of its release through the ICCVAM electronic mailing list and via an *FR* notice (Vol. 70, No. 142, p. 43149; available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm). The BRD Addendum was made available in electronic format on the ICCVAM/NICEATM website and from NICEATM on request.

The Expert Panel was subsequently reconvened via teleconference on September 19, 2005 to discuss the BRD Addendum. Prior to this meeting, public comments on the Addendum were received from three organizations and provided to the Expert Panel for their consideration (http://iccvam.niehs.nih.gov/methods/ocudocs/addendcomm.htm). The Expert Panel provided formal comment on each of the four *in vitro* test methods, as well as the proposed list of reference substances. In addition, the public were provided time at the public meeting to comment (although no public comments were provided). The Expert Panel then provided final endorsement regarding the impact, if any, of the information in the BRD Addendum on their original evaluation from the January 11-12, 2005 meeting. The availability of *The ICCVAM Expert Panel Evaluation of the Draft Background Review Document for In Vitro Test Methods For Identifying Ocular Corrosives and Severe Irritants - Addendum* was

announced via an *FR* notice (Vol. 70, No. 211, p. 66451; available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm) on November 2, 2005.

Subsequently, the draft BRDs and the draft BRD Addendum, the Expert Panel report and its addendum, and all public comments were made available to SACATM for their consideration at their meeting on December 12, 2005. SACATM agreed with the conclusions of the Expert Panel.

The ICCVAM and OTWG considered the Expert Panel report and its addendum (**Appendix A**), the revised accuracy and reliability analyses (see **Appendix B** for accuracy analyses results), all public comments, and the comments of SACATM in preparing the final test method recommendations that are provided in this report. This report will be made available to the public and provided to U.S. Federal agencies for consideration, in accordance with the ICCVAM Authorization Act of 2000 (42 U.S.C. § 2851-2, 2851-5 [2000]; available at http://iccvam.niehs.nih.gov/about/PL106545.pdf). Agencies with applicable testing regulations and guidelines (**Appendix C**) must respond to ICCVAM within 180 days of receiving the ICCVAM recommendations. These responses will be made available to the public on the ICCVAM website (http://iccvam.niehs.nih.gov) as they are received.