PREFACE

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) with evaluating 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 October 2003, the U.S. Environmental Protection Agency (EPA) formally nominated several ocular toxicity test method activities to ICCVAM. ICCVAM determined that four *in vitro* test methods proposed for identifying potential ocular corrosives and severe irritants in a tiered-testing strategy should have the highest priority for evaluation. This was based on the availability of existing validation data for all four methods and the fact that determining the adequacy of validation[1] is a prerequisite for test methods 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.

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 the test method evaluations. ICCVAM and NICEATM also collaborated closely with the European Centre for the Validation of Alternative Methods (ECVAM) in conducting the evaluations, with Dr. Chantra Eskes serving as ECVAM liaison to the OTWG.

NICEATM, in conjunction with the OTWG, 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 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. The draft BRDs were made available to the public for comment on November 1, 2004, and a public, independent expert panel meeting also was announced.

ICCVAM organized an international, independent Expert Panel meeting on January 11-12, 2005, to assess the validation status of these four *in vitro* test methods for identifying ocular corrosives or severe irritants. While a comprehensive review was conducted, public comments at the meeting revealed that additional relevant data were available that had not yet been provided in response to earlier requests for data. Accordingly, the Expert Panel recommended that if such data could be obtained, a reanalysis of each test method should be

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

performed. Availability of the Expert Panel's independent report was announced on March 21, 2005.

In response to the Expert Panel's recommendation, a second public request for *in vitro* data was published on February 28, 2005. In response to this request, 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. The additional data, together with clarified rules for hazard classification and reclassification of the chemical classes of the test substances necessitated a reanalysis of the accuracy and reliability of all four test methods. The accuracy and reliability reanalyses and a revised reference substances list for validation of *in vitro* tests to detect ocular corrosives and severe irritants were provided in a BRD Addendum released on July 26, 2005.

The Expert Panel was subsequently reconvened via teleconference on September 19, 2005 to discuss the BRD Addendum. The Expert Panel provided final conclusions regarding the effects of the information in the BRD Addendum on their original evaluation from the January 11-12, 2005 meeting. The report of this meeting also was published and public comments requested.

The draft BRDs, draft BRD Addendum, Expert Panel report and addendum, and all public comments were subsequently made available to the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) for comment at their meeting on December 12, 2005. The SACATM concurred with the consensus conclusions of the Expert Panel.

ICCVAM and OTWG considered the Expert Panel report and addendum, the revised accuracy and reliability analyses, all public comments, and the comments of SACATM in preparing the final ICCVAM test method recommendations 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/or guidelines must respond to ICCVAM within 180 days after 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.

In this Test Method Evaluation Report, ICCVAM states that there are sufficient data to substantiate the use of the BCOP or the ICE test methods, with certain limitations, as screening tests to identify substances as ocular corrosives and severe irritants in a tiered-testing strategy, using a weight-of-evidence approach, for regulatory hazard classification purposes. When used in this manner, these methods should reduce the number of animals needed for ocular toxicity testing and refine animal use by avoiding the pain and distress associated with testing severely irritating and corrosive substances. Since ocular irritancy testing may involve more than slight or momentary pain or distress, available alternative test methods must be considered prior to the use of animals, as required by U.S. Federal animal welfare regulations and policies. Accordingly, *in vitro* alternative test methods should be considered prior to *in vivo* ocular testing and used where determined appropriate for a

specific testing situation. Consistent with the mission of ICCVAM, appropriate use of these methods will support improved animal welfare while ensuring the continued protection of human health.

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