Expert Panel Report:
Evaluation of the Current Validation Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants – *Addendum*

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Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
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IN VITRO OCULAR TEST METHOD EXPERT PANEL ROSTER

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PREFACE

On November 1, 2004, The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) made available draft Background Review Documents (BRDs) that provided information and data about the current validation status of four *in vitro* test methods for detecting ocular corrosives and severe irritants. 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. These draft BRDs were based on published studies using the identified test methods, and other data and information submitted in response to a 2004 *Federal Register* (*FR*) request.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) convened an Expert Panel meeting on January 11-12, 2005, to independently assess the validation status of these four *in vitro* test methods for identifying ocular corrosives or severe irritants, as determined by the rabbit response. 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. 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.

In response to this recommendation, a *FR* notice was published on February 28, 2005. 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 the reanalyses presented in this BRD Addendum.

In addition to the additional test method data, clarification of European Union (EU 2001) and United Nations (UN) Globally Harmonized System (GHS) (UN 2003) ocular hazard classification rules for severe irritants was obtained subsequent to the release of the four draft BRDs. This change resulted in 10 to 15 substances being reclassified based on their *in vivo* data from nonsevere to severe irritants, depending on which *in vitro* ocular irritancy test method and ocular hazard classification system was used.

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), 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; the results of each reanalysis are also provided in this BRD Addendum.

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 1, 2004. The BRD Addendum provides a revised list of proposed reference chemicals, which 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 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 BRD Addendum was released on July 26, 2005, with notification of its release via an *FR* notice and notification through the ICCVAM electronic mailing list. The 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 Panel for their consideration. The 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 Panel then provided final endorsement regarding the effects, if any, of the information in the BRD Addendum on their original evaluation from the January 11-12, 2005 meeting.

EXECUTIVE SUMMARY

This report describes the conclusions and recommendations of the Expert Panel ("Panel") made during the September 19, 2005 teleconference on the utility of four *in vitro* ocular toxicity test methods for identifying ocular corrosives and severe irritants (i.e., 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). This second Panel report is a supplement to the March 2005 report entitled, "Expert Panel Report: Evaluation of the Current Validation Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants." Unless indicated, all conclusions and recommendations made by the Panel in their March 2005 report remain unchanged.

For each test method, the Panel was asked to determine if the information provided in the Addendum to the November 2004 Background Review Documents (BRD) were appropriate for inclusion in the accuracy and reliability re-analyses, and then if any changes to the original recommendations established at the January 11-12, 2005 meeting (http://iccvam.niehs.nih.gov/methods/ocudocs/EPreport/ocureport.htm) were warranted based on the updated information detailed in the BRD Addendum. The Panel agreed that, for all four test methods, the information in the BRD Addendum was appropriate for inclusion, and that no errors or omissions were present. For three of the four methods (i.e., BCOP, ICE and IRE), the Panel agreed that there was no basis for changing the original conclusions and recommendations established at the January 11-12, 2005 meeting. However, the Panel concluded that, given the increases in both false positive and false negative rates based on the reanalysis, the HET-CAM IS(B) analysis method, using the decision criteria of Leupke, 1985, may have limited utility for the identification of severe ocular irritants and/or corrosives. In contrast, during the January 11-12, 2005 meeting, the Panel concluded that, for the purpose of detecting severe eye irritants in the tiered-testing strategy, the HET-CAM test has been shown to be useful for the identification of severe or corrosive ocular irritants.

The Panel was also asked to consider the adequacy of the proposed list of reference substances, which was updated (in part) based on comments received from the Panel at the January 11-12, 2005 meeting. The Panel reaffirmed the comments stated in the original Panel report and still considered the list too large if the list is intended to be the minimum number of substances required for validation of a new test method. The Panel also recommended that substances of the highest purity available from major suppliers be used.

During the deliberations of the Panel, the question was raised as to how closely the performance of an *in vitro* test must match the performance of an *in vivo* test before the *in vitro* test is considered a sufficiently accurate measure of the risk to humans. It was acknowledged that this was an appropriate and important question to bring to ICCVAM, but one that was beyond the scope of the charge to this expert panel.

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I. The Isolated Rabbit Eye Test Method

On January 11-12, 2005, the Panel concluded that the IRE BRD proposed version of the IRE test method appears to be capable of identifying ocular corrosives/severe irritants in a tiered-testing strategy with the caveat that the accuracy of this test method be corroborated using a larger number of substances and that reliability analyses be conducted when additional data become available. This recommendation was based on the relatively small number of substances (n=36) tested using the proposed IRE test method version and because only one laboratory (SafePharm, Derby, United Kingdom) had experience using this test method protocol.

During the September 19, 2005 Panel meeting, three questions were addressed with regard to the IRE BRD Addendum as follows:

Is the information provided in the Addendum to the November 2004 Background Review Document (BRD) appropriate for inclusion in the accuracy and reliability analyses?

The Panel concluded that the information was appropriate.

Are there any errors or omissions that should be corrected?

The Panel agreed that there were no errors or omissions. The Panel recognized and supported the rationale for excluding some substances from the evaluation based on lack of adequate *in vivo* rabbit eye test data (i.e., severe ocular irritancy/corrosivity classification based solely on skin corrosivity, pH extremes, etc., or no classification feasible based on eye test data provided to NICEATM). While the pH and/or dermal corrosive effects of a test substance are utilized as substitutes for animal eye irritation data for the purposes of ocular hazard classification, the goal of this evaluation was to determine whether the four *in vitro* test methods can be used to predict the outcome of the *in vivo* rabbit eye test for the same test substance. Therefore, including data on pH extremes and/or dermal corrosivity (in the absence of *in vivo* rabbit eye test data) was judged to be inappropriate due to the uncertainty of its performance in predicting the *in vivo* rabbit eye test outcome. In addition, the Panel recommended that text be included in the final BRD to underscore the fact that, where such information was available, data derived from scientifically acceptable *in vivo* rabbit eye tests terminated based on humane endpoints (e.g., severe discomfort) were included in the accuracy and reliability analysis.

Based on the revised accuracy and reliability analyses for the IRE test method for identifying ocular corrosives and severe irritants, does this new information provide the basis for any changes in the Panel's conclusions and recommendations made at the January 11-12, 2005 meeting?

The Panel agreed that there was no basis for changes to the original conclusions and recommendations.

II. The Isolated Chicken Eye Test Method

At the January 11-12, 2005 Expert Panel meeting, the Panel concluded that the ICCVAM criteria for validation (ICCVAM 2003) have not been fully met for the ICE test method. Cited deficiencies included:

- The intralaboratory reliability of the ICE test method has not been adequately evaluated
- The raw data from the three ICE studies included in this evaluation were not available for review
- Detailed drawings/diagrams of the superfusion apparatus have not been made available to allow for transferability of the experimental setup

However, the Panel concluded that the ICE test method can be used in the identification of ocular corrosives/severe irritants in a tiered testing strategy, with specific limitations. Specifically, the Panel noted that alcohols tend to be overpredicted, while surfactants tend to be underpredicted. The Panel also recognized that solids and insoluble substances may be problematic in the ICE test method, since they may not come in adequate contact with the corneal surface, resulting in underprediction. Therefore, the Panel concluded that the low overall false positive rate (8% to 10%, depending on the regulatory classification scheme evaluated) indicates that the ICE test can be used at present to screen for severe eye irritants/corrosives. However, given the high false positive rates calculated for a small number of alcohols (50% [5/10]), the Panel noted that caution should be observed when evaluating ICE test results with this class of substances.

The Panel previously recommended that, given the limited amount of ICE reliability data, additional studies using the recommended ICE test method protocol were suggested to better characterize the repeatability and the intra-and inter-laboratory reproducibility of the test method. Subsequent to the January 11-12, 2005 meeting, additional data were received that allowed for such analyses to be conducted.

During the September 19, 2005 Panel meeting, three questions were addressed with regard to the ICE BRD Addendum as follows:

Is the information provided in the Addendum to the November 2004 BRD appropriate for inclusion in the accuracy and reliability analyses?

The Panel concluded that the information was appropriate.

Are there any errors or omissions that should be corrected?

The Panel agreed that there were no errors or omissions. The Panel recognized and supported the rationale for excluding some substances from the evaluation based on lack of adequate *in vivo* rabbit eye test data (i.e., severe ocular irritancy/corrosivity classification based solely on skin corrosivity, pH extremes, etc., or no classification feasible based on eye test data provided to NICEATM). While the pH and/or dermal corrosive effects of a test substance are utilized as substitutes for animal eye irritation data for the purposes of ocular hazard classification, the goal

of this evaluation was to determine whether the four *in vitro* test methods can be used to predict the outcome of the *in vivo* rabbit eye test for the same test substance. Therefore, including data on pH extremes and/or dermal corrosivity (in the absence of *in vivo* rabbit eye test data) was judged to be inappropriate due to the uncertainty of its performance in predicting the *in vivo* rabbit eye test outcome. In addition, the Panel recommended that text be included in the final BRD to underscore the fact that, where such information was available, data derived from scientifically acceptable *in vivo* rabbit eye tests terminated based on humane endpoints (e.g., severe discomfort) were included in the accuracy and reliability analysis.

Based on the revised accuracy and reliability analyses for the ICE test method for identifying ocular corrosives and severe irritants, does this new information provide the basis for any changes in the Panel's conclusions and recommendations made at the January 11-12, 2005 meeting?

The Panel agreed that there was no basis for changes to the original conclusions and recommendations. The Panel added that the reanalysis using the new information and the GHS classification scheme showed that the test performance was essentially unchanged (1-2% difference) or directionally poorer (Table ES-1 in the Addendum).

III. The Bovine Corneal Opacity and Permeability Test Method

At the January 11-12, 2005 Expert Panel meeting, the Panel concluded that the BCOP BRD proposed version of the test method has been shown to have adequate accuracy and reliability for detecting corrosive or severe eye irritants in the tiered testing scheme outlined in the BCOP BRD, with the following caveats:

- The test should not be used to identify corrosive or severely irritating ketones, alcohols, and solids. Further optimization and validation are necessary before these classes of materials can be assessed with this test.
- It needs to be confirmed that the BCOP test method can identify, as well as or better than the Draize test, those substances known to cause serious eye injury in humans. It appears from the list of chemicals tested that at least some of these substances have been tested in BCOP (e.g., floor strippers and heavy duty cleaners).
- A histopathological examination should be added to the test unless the test substance is from a class of materials known to be accurately predicted using only opacity and permeability in the BCOP assay.

While the Panel believed these modifications were important, the Panel concluded that the data presented in the BCOP BRD support use of the BCOP assay in its current form for identifying ocular corrosives and severe irritants other than alcohols, ketones, and solids in a tiered testing strategy for regulatory hazard classification and labeling purposes.

During the September 19, 2005 Panel meeting, three questions were addressed with regard to the BCOP BRD Addendum as follows:

Is the information provided in the Addendum to the November 2004 BRD appropriate for inclusion in the accuracy and reliability analyses?

The Panel concluded that the information was appropriate.

Are there any errors or omissions that should be corrected?

The Panel agreed that there were no errors or omissions. The Panel recognized and supported the rationale for excluding some substances from the evaluation based on lack of adequate *in vivo* rabbit eye test data (i.e., severe ocular irritancy/corrosivity classification based solely on skin corrosivity, pH extremes, etc., or no classification feasible based on eye test data provided to NICEATM). While the pH and/or dermal corrosive effects of a test substance are utilized as substitutes for animal eye irritation data for the purposes of ocular hazard classification, the goal of this evaluation was to determine whether the four *in vitro* test methods can be used to predict the outcome of the *in vivo* rabbit eye test for the same test substance. Therefore, including data on pH extremes and/or dermal corrosivity (in the absence of *in vivo* rabbit eye test data) was judged to be inappropriate due to the uncertainty of its performance in predicting the *in vivo* rabbit eye test outcome. In addition, the Panel recommended that text be included in the final BRD to underscore the fact that, where such information was available, data derived from

scientifically acceptable *in vivo* rabbit eye tests terminated based on humane endpoints (e.g., severe discomfort) were included in the accuracy and reliability analysis.

Based on the revised accuracy and reliability analyses for the BCOP test method for identifying ocular corrosives and severe irritants, does this new information provide the basis for any changes in the Panel's conclusions and recommendations made at the January 11-12, 2005 meeting?

The Panel agreed that there was no basis for changes to the original conclusions and recommendations.

IV. The Hen's Egg Test - Chorioallantoic Membrane Test Method

At the January 11-12, 2005 Expert Panel meeting, the Panel concluded that, for the purpose of detecting severe eye irritants in the tiered-testing strategy outlined in the HET-CAM BRD, the HET-CAM test has been shown to be useful for identification of severe or corrosive ocular irritants. The Panel further stated that the high false positive rate was a limitation of the HET-CAM test method. It was proposed that positive results from the HET-CAM test method could be re-tested in a modified HET-CAM test method (e.g. using a lower concentration of test substance) to confirm the results. Alternatively, substances producing a positive result could be tested in a different *in vitro* test method (e.g., ICE, IRE, BCOP). Substances producing negative results (e.g., HET-CAM score defined as nonirritant, mild irritant, or moderate irritant) would follow the tiered-testing strategy.

Subsequent to the January 11-12, 2005 meeting, additional data were received and the full data set was reanalyzed. During the September 19, 2005 Panel meeting, three questions were addressed with regard to the HET-CAM BRD Addendum as follows:

Is the information provided in the Addendum to the November 2004 BRD appropriate for inclusion in the accuracy and reliability analyses?

The Panel concluded that the information was appropriate.

Are there any errors or omissions that should be corrected?

The Panel agreed that there were no errors or omissions. The Panel recognized and supported the rationale for excluding some substances from the evaluation based on lack of adequate *in vivo* rabbit eye test data (i.e., severe ocular irritancy/corrosivity classification based solely on skin corrosivity, pH extremes, etc., or no classification feasible based on eye test data provided to NICEATM). While the pH and/or dermal corrosive effects of a test substance are utilized as substitutes for animal eye irritation data for the purposes of ocular hazard classification, the goal of this evaluation was to determine whether the four *in vitro* test methods can be used to predict the outcome of the *in vivo* rabbit eye test for the same test substance. Therefore, including data on pH extremes and/or dermal corrosivity (in the absence of *in vivo* rabbit eye test data) was judged to be inappropriate due to the uncertainty of its performance in predicting the *in vivo* rabbit eye test outcome. In addition, the Panel recommended that text be included in the final BRD to underscore the fact that, where such information was available, data derived from scientifically acceptable *in vivo* rabbit eye tests terminated based on humane endpoints (e.g., severe discomfort) were included in the accuracy and reliability analysis.

Based on the revised accuracy and reliability analyses for the HET-CAM test method for identifying ocular corrosives and severe irritants, does this new information provide the basis for any changes in the Panel's conclusions and recommendations made at the January 11-12, 2005 meeting?

As indicated above, at the January 11-12, 2005 meeting, the Panel concluded that, for the purpose of detecting severe eye irritants in the tiered-testing strategy outlined in the HET-CAM

BRD, the HET-CAM test has been shown to be useful for identification of severe or corrosive ocular irritants. However, at the September 19, 2005 meeting, the Panel concluded that, given the increases in both false positive and false negative rates, the HET-CAM IS(B) analysis method, using the decision criteria of Leupke, 1985, may have limited utility for the identification of severe ocular irritants and/or corrosives, although it may be useful for the identification of mild to moderate irritants. As stated in the Panel's March 2005 Report, a retrospective analysis should be conducted to determine if different decision criteria might enhance the accuracy and/or reliability of the test method for the detection of ocular corrosives and severe irritants, as defined by the EU (2001), GHS (UN 2003), and EPA (1996) classification systems.

V. Proposed for Optimization or Validation Studies and to Use in Establishing Performance Standards

At the January 11-12, 2005 Expert Panel meeting, the Panel reviewed the adequacy and completeness of the proposed list of reference substances and concluded that the list of proposed substances is comprehensive, the substances appear to be readily available and in acceptably pure form, and the range of possible ocular toxicity responses in terms of severity and types of lesions appears to be adequately represented. However, the Panel concluded that: 1) the current list has too many substances; 2) surfactants are over-represented; 3) more inorganic substances should be added; and 4) substances known to induce severe ocular lesions in humans should be included in the list, even in the absence of rabbit data.

The Panel noted that the number of inorganic substances in the revised list of proposed reference substances was increased from 2 to 11; that the current list includes 10 substances that are known human ocular corrosives or severe irritants, even in the absence of *in vivo* rabbit data; that all formulations were removed; and that the number of surfactants were decreased from 12 to 7. However, the total number of proposed reference substances was increased from 89 to 122. ICCVAM justifies this increase because, for the detection of ocular corrosives and severe irritants, the list of substances needs to include substances that:

- Induce very severe responses within a relatively short period, as well as those where the response is delayed
- Adversely affect the cornea, iris, and/or conjunctiva
- Induce persistent and non-persistent lesions
- Represent a diverse population of chemical classes and physicochemical properties

During the September 19, 2005 Panel meeting, one question was addressed with regard to the recommended list of reference substances included in the BRD Addendum as follows:

Is the revised list of proposed reference substances, selected from the list of available candidate substances, sufficiently adequate and complete for validation studies to evaluate the usefulness and limitations of in vitro test methods proposed for identifying ocular severe irritants and corrosives?

The Panel reaffirmed the comments stated in the original Panel report (e.g., providing the list as a reference from which to generate a subset of substances to be used for evaluating *in vitro* ocular toxicity test methods on a scientifically sound, case-by-case basis.) The Panel still considered the list too large if the list is intended, as stated in the BRD Addendum, to be the minimum number of substances that should be used for validation of a new test method. A focus on mechanism of action may reduce the number of substances that need to be used to evaluate the relevance and reliability of a proposed test method. The Panel recommended that the highest purity level available from major suppliers for each substance be used and ideally, information on major impurities provided.