# APPENDIX A4
# SUMMARY MINUTES FROM EXPERT PANEL TELECONFERENCE ON SEPTEMBER 19, 2005

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**Department of Health and Human Services**
**National Institutes of Health**
**National Institute of Environmental Health Sciences**
**Interagency Coordinating Committee on the Validation of Alterative Methods (ICCVAM)**
**Expert Panel Teleconference**

Summary Minutes of the Expert Panel Teleconference to Evaluate Revised Analyses and Proposed Reference Substances

## Introduction

A public teleconference of an independent Expert Panel was convened on September 19, 2005 to evaluate several i*n vitro* ocular irritation test methods. The purpose of this meeting was to evaluate (1) revised accuracy and reliability analyses of four *in vitro* test methods proposed for detecting ocular corrosives and severe irritants, and (2) a revised list of proposed reference substances for validation studies on *in vitro* test methods for identifying ocular corrosives and severe irritants. The four *in vitro* test methods under consideration were the (1) Bovine Corneal Opacity and Permeability (BCOP) assay, (2) Hen's Egg Test--Chorioallantoic Membrane (HET-CAM), (3) Isolated Rabbit Eye (IRE) assay, and (4) Isolated Chicken Eye (ICE) assay. The teleconference was organized by ICCVAM and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and sponsored by the National Institute of Environmental Health Sciences (NIEHS) and the NTP.

The following scientists served on the Expert Panel:

- Robert Scala, Ph.D., (Panel Chair), Tucson, Arizona, United States
- Sally S. Atherton, Ph.D., Professor, Medical College of Georgia, Augusta, Georgia, United States
- Roger Beuerman, Ph.D., Professor, Louisiana State University, New Orleans, Louisiana, United States
- June Bradlaw, Ph.D., International Foundation for Ethical Research, Rockville, Maryland, United States
- Ih Chu, Ph.D., Health Canada, Ottawa, Canada
- Henry Edelhauser, Ph.D., Professor, Emory University, Atlanta, Georgia, United States
- Donald Fox, Ph.D., Professor, University of Houston, Houston, Texas, United States
- James Freeman, Ph.D., Lab Director, ExxonMobil Biomedical Sciences, Inc., Annandale, New Jersey, United States
- Sidney Green, Ph.D., A.T.S., Graduate Professor, Howard University, Washington, DC, United States
- Frederick Guerriero, M.S., Senior Occupational Toxicologist, GlaxoSmithKline, King of Prussia, Pennsylvania, United States

- A.Wallace Hayes, Ph.D., D.A.B.T., F.A.T.S., F.I.Biol., F.A.C.F.E., E.R.T., Scientist, Harvard School of Public Health, Andover, Massachusetts, United States
- Hiroshi Itagaki, Ph.D., Deputy Director of JSAAE, Manager of Alternative Section, Shiseido Co., Ltd., Japan
- David Lovell, Ph.D., Reader in Medical Statistics, University of Surrey, United Kingdom
- Yasuo Ohno, Ph.D., D.J.S.T.S., Director of JSAAE, National Institute of Health, Japan
- Robert Peiffer, D.V.M., D.A.C.V.O., Senior Investigator, Merck Research Laboratories, West Point, Ohio, United States
- Lionel Rubin, V.M.D., D.A.C.V.O., Emeritus Professor of Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania, United States
- Horst Spielmann, Dr. Med., Director and Professor, ZEBET at the BfR, Germany
- Martin Stephens, Ph.D., Vice President for Animal Research, Humane Society of the United States, Washington, DC, United States
- Katherine Stitzel, D.V.M., Consultant, West Chester, Ohio, United States
- Peter Theran, V.M.D., D.A.C.V.I.M., Vice President Animal Science, Massachusetts Society for the Prevention of Cruelty to Animals, Novato, California, United States
- Scheffer Tseng, M.D., Ph.D., Director, Ocular Surface Research and Education Foundation, Miami, Florida, United States
- Philippe Vanparys, Ph.D., Senior Research Fellow, Johnson and Johnson, Belgium

The following ICCVAM agency representatives participated in the teleconference:

- Dr. Robert Bronaugh, (Ocular Toxicity Working Group - OTWG), U.S. Food and Drug Administration
- Dr. Karen Hamernik, (OTWG), U.S. Environmental Protection Agency
- Dr. Abigail Jacobs, (OTWG), U.S. Food and Drug Administration

The following additional members of the ICCVAM Ocular Toxicity Working Group (OTWG) participated in the teleconference:

- Ms. Donnie Lowther, U.S. Environmental Protection Agency
- Dr. Jill Merrill, U.S. Food and Drug Administration

The following members of the NICEATM Staff participated in the teleconference:

- Dr. David Allen, Integrated Laboratory Systems, Inc.
- Mr. Bradley Blackard, Integrated Laboratory Systems, Inc.
- Mr. Thomas Burns, Integrated Laboratory Systems, Inc.
- Dr. Jeffrey Charles, Integrated Laboratory Systems, Inc.
- Dr. Neepa Choksi, Integrated Laboratory Systems, Inc.

- Ms. Linda Litchfield, Integrated Laboratory Systems, Inc.
- Ms. Debbie McCarley, National Institute of Environmental Health Sciences
- Dr. Raymond Tice, National Institute of Environmental Health Sciences
- Mr. James Truax, Integrated Laboratory Systems, Inc.

The following members of the public participated in the teleconference:

- Dr. Rodger D. Curren, Institute for *In Vitro* Sciences, Inc.
- Dr. Jean Domoradzki, Dow Chemicals
- Dr. John Harbell, Institute for *In Vitro* Sciences, Inc.
- Dr. Pauline M. McNamee, The European Cosmetic, Toiletry, and Perfumary Association (COLIPA)
- Dr. Brooke McManus, Rosenhect
- Dr. Pat Phibbs, Bureau of National Affairs News
- Dr. Kristina Thayer, National Institute for Environmental Health Sciences
- Dr. Sherry L. Ward, Physicians Committee for Responsible Medicine

The purpose of this meeting was to evaluate (1) revised accuracy and reliability analyses of the four *in vitro* test methods proposed for detecting ocular corrosives and severe irritants and (2) a revised list of proposed reference substances for validation studies on *in vitro* test methods for identifying ocular corrosives and severe irritants. The Expert Panel was asked to evaluate an addendum to these four ocular draft background review documents (BRDs) prepared by NICEATM (Available: http://iccvam.niehs.nih.gov).

**Call to Order and Introductions**

Dr. Robert Scala, Panel Chair, called the teleconference of the Expert Panel (Panel) to order at 9:10 a.m. He asked Mr. Blackard to take attendance of the panel members. NICEATM staff members were then asked to introduce themselves. The operator then provided the names and affiliations of the members of the public that were participating. Dr. Scala stated that there were no requests made to make a public comment during the teleconference.

Dr. Raymond Tice, Designated Federal Official, read the Statement of Conflict of Interest and explained policies and procedures regarding confidentiality and avoidance of conflict of interest, as follows: *"As a Special Emphasis Panel, the members of the ocular expert panel serve as individual scientists and not as representatives of any organization. Each member is to exercise judgment prior to any meeting as to whether a potential conflict of interest might exist relative to agenda topics or concepts for discussion by the Expert Panel due to his or her occupational affiliation, professional activity or financial interest. Should there be a potential conflict of interest, they will be handled in accordance with departmental policies and requirements."*

Dr. Scala asked if any member of the Panel had any potential conflicts of interest. None of the Panel members declared a conflict of interest.

**Overview of the Performance Reanalysis**

Dr. Tice (Deputy Director, NICEATM, NIEHS) provided a brief overview of the process that led to the public teleconference of the Expert Panel. On November 1, 2004, NICEATM made available four BRDs that provided information and data about the current validation status of four *in vitro* test methods for the ability to detect ocular corrosives and severe irritants. The four test methods evaluated in the BRDs were the BCOP assay, the HET-CAM assay, the IRE assay, and the ICE assay. The analyses in the BRDs were based on published literature and data submitted in response to a 2004 *Federal Register* (*FR*) notice. An Expert Panel was convened on January 11-12, 2005 to assess the validation status of these four *in vitro* test methods to identify ocular corrosives and severe irritants. Public comments at the meeting indicated that additional data were available that had not been provided in response to earlier *FR* notices. The Expert Panel recommended that the additional data be requested and that a reanalysis of the performance of each *in vitro* test method be conducted, where appropriate.

Dr. Tice stated that in response to this recommendation a *FR* notice was published on February 28, 2005 requesting *in vitro* ocular toxicity and corresponding *in vivo* ocular toxicity data be submitted to NICEATM for inclusion in the reanalysis. In response to this notice, data was received for the BCOP, HET-CAM, and ICE test methods. Dr. Tice discussed other additional changes and analyses that were conducted and incorporated in the reanalysis of test method performance.

Dr. Tice stated that the proposed reference list that was included in each draft BRD was revised based on:

- recommendations from the Panel
- additional *in vivo* data received for approximately 300 substances
- reclassification of substances based on clarification of ocular toxicity classification rules
- reclassification of the chemical class of a substance based on Medical Subject Headings (MeSH) chemical classes

The BRD addendum was released on July 26, 2005 for public review and comment.

Dr. Tice stated that the purpose of the teleconference was for the Expert Panel to address the following questions:

- For each test method, is the information provided in the addendum appropriate for inclusion in the accuracy and reliability analysis? Are there any errors or omissions that should be corrected?
- Based on the revised accuracy and reliability analysis, does the new information provide the basis for any change in the Panel's conclusions and recommendations made at the January 11-12, 2005 meeting?
- Is the revised list of proposed reference substances, sufficiently valid and complete for use in *in vitro* test methods to evaluate ocular corrosives and severe irritants?

Dr. Tice stated that the Panel's recommendations and public comments would be considered by ICCVAM when making their final recommendations. These recommendations would be provided to the public and U.S. Federal agencies for consideration.

**Organization of the Panel Review**

The remainder of the teleconference was devoted to Panel discussion and answering the three questions stated by Dr. Tice.

Prior to the presentations and discussions by each of the four groups, a NICEATM staff member provided a brief summary of the information contained in the test method specific BRD reanalysis addendum, including updated accuracy and reliability analyses for the test method.

Each Panel group discussed its draft response for each of the questions. After each presentation, the entire Panel discussed the draft positions and offered additional comments and suggestions. The Panel Chair summarized the discussion for each question and sought consensus from the Panel on the topic.

# I. IRE TEST METHOD EVALUATION

*Primary reviewers: Drs. James Freeman (Group Chair), Sally Atherton, David Lovell, Yasuo Ohno, Horst Spielmann, Peter Theran*

## Summary of the IRE Reanalysis

Dr. Neepa Choksi (NICEATM) stated that Mr. Jim Truax (NICEATM) had conducted the re-analysis on the IRE test method. No additional information and/or *in vitro* and comparative *in vivo* data were received in response to the *FR* notice. However, an additional analysis was conducted (based on a recommendation by the Panel at the January 11-12, 2006 meeting) where a positive score in studies that did not use all four endpoints used by Guerriero et al. (2004) were combined with the results from Guerriero et al. This new database was referred to as the "Expanded Dataset".

Dr. Choksi noted that, for comparative purposes, the addendum contained the results presented in the IRE BRD as well as the results from the reanalysis. For the United Nations Globally Harmonized System (GHS; UN 2003), the accuracy of the IRE test method based on the 38 substances tested by Guerriero et al. (2004) was 79%, with a false positive rate of 30% and false negative rate of 0%. For the Expanded Dataset, the accuracy was 68%, with a false positive rate of 68% and a false negative rate of 0%. Dr. Choksi noted that for the Expanded Dataset, only substances classified as positive based on their response in the IRE test method from the other studies were included into the analysis. This potential bias should be considered when reviewing the data.

Dr. Choksi then reviewed the accuracy analyses conducted for various subgroups of the data (based on chemical class, properties of interest, pH, and irritancy subcategories). Limiting the chemical class evaluation to those with five or more substances per chemical class, the classes with the highest rate of overprediction were ketones, esters, and alcohols. In addition, liquids tended to have a higher false positive rate than solids.

Dr. Choksi stated that, as in the draft IRE BRD, analyses on intralaboratory repeatability and intralaboratory reproducibility could not be conducted due to the lack of data. Dr. Choksi then reviewed the change to the qualitative interlaboratory reproducibility analysis, for the GHS classification system, noting that changes in the U.S. Environmental Protection Agency (EPA 1996) and European Union (EU 2001) classification are similar.

## Proposed Panel Recommendation for the IRE Test Method

Dr. Freeman reviewed the questions provided to the Panel and stated what the draft Expert Panel comments were.

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

The Panel stated that the answer was yes. There was no dissention or disagreement noted.

***Are there any errors or omissions that should be corrected?***

Dr. Freeman stated that the draft response was as follows:

> 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.

Panel discussion followed in the appropriateness of excluding chemicals that were classified as severe ocular irritants or corrosives on the basis of dermal corrosivity and/or pH extremes from the accuracy and reliability reanalysis. The Panel concurred with the decision to limit the evaluation to substances where appropriate *in vivo* ocular data were available.

***Based on the revised accuracy and reliability analysis 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[1]. There were no dissenting opinions from the Panel.

**Panel Vote on the IRE Report**

The Panel Chair asked the Panel members if there were any dissenting opinions. No dissenting opinions were indicated.

[1] *Editor's Note:* At the Expert Panel meeting in January 2005, the Panel recommended that the current version of the IRE test method appeared "to be capable of identifying ocular corrosives/severe irritants in a tiered-testing strategy." However, they noted that the available data were "too limited to allow for an adequate judgment of its accuracy and reliability."

A Panel member asked if it was acceptable to compare rabbit eye data to *in vitro* data given concerns with the reliability of the *in vivo* test. The Panel Chair stated that such a question, while an excellent and important point, was currently outside the charge of the Panel. The Panel Chair noted that evaluation of the question and efforts related to such an evaluation were important. However, these are future efforts as the work on evaluation of alternative ocular test method progress. The Panel Chair stated that a statement would be incorporated recommending such activities into the final report.

Dr. Scala concluded this discussion with a vote among the Panel members. He noted that everyone on the Panel was in agreement with the conclusions and recommendations for the IRE test method.

## II. ICE TEST METHOD EVALUATION

*Primary Reviewers: Drs. Robert Scala, Roger Beuerman, June Bradlaw, Wallace Hayes, Robert Peiffer, Nancy Flournoy*

### Summary of ICE Reanalysis

Dr. David Allen (NICEATM) stated that additional ICE and *in vivo* data were received. The total database for evaluating ICE performance increased from 92 to 144 substances.

Dr. Allen noted that the overall accuracy, when compared to the GHS classification system, increased from 82% to 83%. The false positive rate decreased from 10% to 8% and the false negative rate increased from 40% to 50%. The numbers observed for the GHS classification system were comparable to those obtained for the EPA and EU classification system.

Dr. Allen then reviewed the accuracy analyses conducted for various subgroups of the data (based on chemical class, properties of interest, pH, and irritancy subcategories). Limiting the chemical class evaluation to those with five or more substances per chemical class, the class with the highest rate of overprediction was alcohols. Surfactants and solids had the highest rate of underprediction. An analysis based on the pH indicated that basic substances tended to be underpredicted. Furthermore, substances that produce persistent lesions (lesions that last at least 21 days) also tend to be underpredicted by the ICE test method.

Dr. Allen stated that, based on newly received data, assessments of intralaboratory repeatability and reproducibility could be conducted. An intralaboratory repeatability coefficient of variation (CV) analysis evaluation, on each ICE endpoint, indicated that corneal thickness was repeatable (CV ranged from 1% to 6%). An intralaboratory reproducibility CV analysis also indicated that the corneal thickness measurement was generally reproducible (CV < 7%). Dr. Allen reviewed the changes to the qualitative interlaboratory reproducibility analysis, for the GHS classification system; he then reviewed the historical negative and positive control results received.

### Proposed Panel Recommendations for the ICE Test Method

The Panel Chair opened the discussion on the questions.

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

The Panel stated that the answer was yes.

***Are there any errors or omissions that should be corrected?***

Dr. Scala stated that the language developed for the IRE test methods was appropriate for the ICE test method. The draft response was as follows:

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 analysis 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[2]. There were no dissenting opinions from the Panel on the topic.

**Panel Vote on the ICE Report**

Dr. Scala concluded this discussion with a vote among the Panel members. He noted that everyone on the Panel was in agreement with the conclusions and recommendations for the ICE test method.

[2] *Editor's Note:* At the Expert Panel meeting in January 2005, the Panel recommended that the current version of the ICE test method could be used for identifying ocular corrosives/severe irritants in a tiered-testing strategy with the following limitations: (a) alcohols tend to be overpredicted, (b) surfactants tend to be underpredicted, (c) solids and insoluble substances may be problematic since they may not come in adequate contact with the corneal surface.

## III. BCOP TEST METHOD EVALUATION

*Primary reviewers: Drs. Kathy Stitzel (Group Chair), Ih Chu, Henry Edelhauser, Hiroshi Itagaki, Lionel Rubin, Scheffer Tseng, David Lovell*

### Summary of BCOP Reanalysis

Dr. Allen stated that additional *in vivo* data was received. The total database for evaluating BCOP performance increased from 120 to 147 substances.

Dr. Allen noted that the overall accuracy, when compared to the GHS classification system, increased from 79% to 81%. The false positive rate increased from 19% to 20% and the false negative rate decreased from 24% to 16%.

Dr. Allen then reviewed the accuracy analyses conducted for various subgroups of the data (based on chemical class, properties of interest, pH, and irritancy subcategories). Limiting the chemical class evaluation to those with five or more substances per chemical class, the classes with the highest rate of overprediction were ketones and alcohols. Solids had the highest rate of underprediction. Removal of ketones, solids, and alcohols from the database increased the accuracy to 92%, decreased the overall false negative rate to 0% and the overall false positive rate to 12%. Furthermore, substances that produce persistent lesions (lesions that last at least 21 days) also tend to be underpredicted by the BCOP test method.

Dr. Allen stated that intralaboratory repeatability and reproducibility and quantitative interlaboratory analyses were not affected by the new data received. Dr. Allen reviewed the changes to the qualitative interlaboratory reproducibility analysis, for the GHS classification system, using three different studies. Dr. Allen noted that the results from the analyses were similar to those previously presented in the BCOP BRD.

### Proposed Panel Recommendations for the BCOP Test Method

The Panel Chair opened the discussion on the questions.

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

The Panel stated that the answer was yes.

***Are there any errors or omissions that should be corrected?***

Dr. Stitzel stated that the language developed for the IRE test method was appropriate for the BCOP test method. The draft response was as follows:

> 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 analysis 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[3]. There were no dissenting opinions from the Panel on the topic.

**Panel Vote on the BCOP Report**

Dr. Scala concluded this discussion with a vote among the Panel members. He noted that everyone on the Panel was in agreement with the conclusions and recommendations for the BCOP test method.

[3] *Editor's Note:* At the Expert Panel meeting in January 2005, the Panel recommended that the current version of the BCOP test method appeared to be capable of identifying ocular corrosives/severe irritants in a tiered-testing strategy with the limitation that further optimization and validation are necessary before alcohols, ketones, and solids can be assessed with this method.

## IV. HET-CAM TEST METHOD EVALUATION

*Primary reviewers: Drs. Shayne Gad, Donald Fox, Martin Stephens, Frederick Guerriero, Sidney Green, Philippe Vanparys, Nancy Flournoy*

### Summary of HET-CAM Reanalysis

Dr. Choksi stated that additional *in vivo* data was received relating to Gilleron et al. (1996, 1997) and Spielmann et al. (1996). Dr. Choksi stated that due to the additional data received, several additional analyses were conducted and presented in the addendum.

Dr. Choksi noted that the overall accuracy, when compared to the GHS classification system, for the various IS(B) analysis methods (IS(B)-10 and IS(B)-100; using the decision criteria of Luepke [1985]) ranged from 53% to 68%. The false positive rates ranged from 33% to 61% and the false negative rates ranged from 15% to 33%. Dr. Choksi stated the results for each analysis method were comparable for all three regulatory hazard classification systems. Dr. Choksi then reviewed the accuracy analyses conducted for various subgroups of the data (based on chemical class, properties of interest, pH, and irritancy subcategories).

Dr. Choksi stated that assessments of intralaboratory repeatability and reproducibility could be conducted, using the additional data. An intralaboratory repeatability and interlaboratory CV analysis on each HET-CAM endpoint evaluation indicated that the coagulation endpoint was the lowest of the three endpoints evaluated. Dr. Choksi reviewed the changes to the qualitative and quantitative interlaboratory reproducibility analyses, for the GHS classification system. Dr. Choksi then reviewed the historical negative and positive control results received.

### Proposed Panel Recommendations for the HET-CAM Test Method

The Panel Chair opened the discussion on the questions.

***Is the information provided in the Addendum to the November 2004 HET-CAM BRD appropriate for inclusion in the accuracy and reliability analysis?***

The Panel stated that the answer was yes.

***Are there any errors or omissions that should be corrected?***

Dr. Gad stated that the language developed for the IRE test method was appropriate for the HET-CAM test method. The draft response was as follows:

> 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 analysis 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?***

The Panel agreed that the IS(B) analysis method (using the decision criteria of Luepke 1985) was not sufficiently predictable to use for identifying ocular corrosives and severe irritants. This conclusion was different from the Panel's conclusions and recommendations made at the January 11-12, 2005 meeting, where the test method was proposed to be sufficiently predictable to use for identifying ocular corrosives and severe irritants. There were no dissenting opinions from the Panel on the topic.

**Panel Vote on the HET-CAM Report**

Dr. Scala concluded this discussion with a vote among the Panel members. He noted that everyone on the Panel was in agreement with the conclusions and recommendations for the HET-CAM test method.

## V. REFERENCE SUBSTANCES FOR USE IN VALIDATION STUDIES

### Summary of the Recommended Reference Substances

Dr. Tice presented a summary of the list of reference substances included in the BRD Addendum. He summarized the selection criteria for reference substances were intended to:

- represent a range of ocular responses
- represent a range of chemical/product classes
- represent a range of known or anticipated mechanisms or modes of action
- high quality *in vivo* rabbit eye test method studies exist for these substances
- have a well-defined chemical composition
- have been tested at a defined concentration and purity
- be readily available

Based on the recommendation from the Expert Panel meeting in January, several changes were made to the original list. Overall:

- The number of inorganics on the list was increased from 2 to 11
- The list now included 10 human ocular corrosives/irritants, despite the lack of corresponding individual rabbit eye test data
- Formulations were removed from the list
- The number of surfactants on the list were decreased from 12 to 7

The Panel also recommended that the total number of proposed reference substances be decreased from 89 substances. Dr. Tice noted that the number of substances needed to evaluate the accuracy of an alternative test method depends on several factors including (1) the range of possible responses that the test method is expected to be able to measure, (2) the diversity of the known or anticipated mechanisms or modes of action that are involved in producing a toxic response, and (3) the number of chemical/physical classes and physicochemical properties that the test method is expected to be able to evaluate. Dr. Tice stated that a preliminary statistical evaluation indicates that several hundred substances could potentially be required to evaluate the accuracy of a test method with a high level of confidence

Dr. Tice went on to state that 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/or non-persistent lesions
- represent a diverse population of chemical classes and physicochemical properties

Dr. Tice stated that to meet these needs and to address the recommendations of the Panel, the list was increased from 89 substances to 122 substances. The proposed list includes 79 GHS Category 1 substances, 10 substances classified based on human data, 28 GHS Category 2

substances, and 15 GHS nonirritant substances. There were 34 chemical classes and 29 product classes represented on the list.

**Proposed Panel Recommendations on Reference Substances**

The Panel Chair opened the discussion to the Panel.

Panel discussion followed regarding assurance that substances with the same purity and quality as those on the proposed reference list would be evaluated by all testing laboratories. The Panel stated that purity of the test substance should be given and that impurities, to the extent possible, should be noted and quantified.

The Panel discussion also indicated that the list of proposed reference substances was too large if the list is intended to be the minimum number of substances that should be used for validation of a new test method. Panel discussion then followed on the proposal to revise the proposed reference substances list so that mechanisms of toxic action would be represented instead of chemical classes. Therefore, chemical classes with similar mechanisms of action could be combined into a single class to decrease the number of substances on the proposed reference substances list.

**Adjournment 11:30**

**Literature Citations**

EPA. 1996. Label Review Manual: 2nd Ed. EPA737-B-96-001. Washington, DC: U.S. Environmental Protection Agency.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255:1-333.

Gilleron L, Coecke S, Sysmans M, Hansen E, van Oproy S, Marzin D, van Cauteren H, Vanparys P. 1997. Evaluation of the HET-CAM-TSA method as an alternative to the Draize eye irritation test. Toxic In Vitro 11:641-644.

Gilleron L, Coecke S, Sysmans M, Hansen E, van Oproy S, Marzin D, van Cauteren H, Vanparys P 1996. Evaluation of a modified HET-CAM assay as a screening test for eye irritancy. Toxic In Vitro 10:431-446.

Guerriero FJ, Seaman CW, Olsen MJ, Guest R, Whittingham A. 2004. Retrospective assessment of the rabbit enucleated eye test (REET) as a screen to refine worker safety studies. [Abstract No. 1282]. Toxicol Sci (The Toxicologist Supplement). 78(1-S).

Luepke N. 1985. Hen's egg chorioallantoic membrane test for irritation potential. Food Chem Toxic 23:287-291.

Spielmann H, Liebsch M, Kalweit S, Moldenhauer F, Wirnsberger T, Holzhütter H, Schneider B, Glaser S, Gerner I, Pape WJW, Kreiling R, Krauser K, Miltenburger HG, Steiling W, Luepke NP Müller N, Kreuzer H, Mürmann P, Spengler J, Bertram-Neis, E, Siegemund B, Wiebel F. 1996. Results of a validation study in Germany on two in vitro alternatives to the Draize eye irritation test, HET-CAM test and the 3T3 NRU cytotoxicity test. ATLA 24:741-858.

UN. 2003. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). New York & Geneva: United Nations Publications. Available: http://www.unece.org/trans/danger/publi/ghs/officialtext.html. [accessed 26 August 2004].

**September 19, 2005**
**Expert Panel Teleconference to Assess the Current Validation Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability (BCOP), Hen's Egg Test – Chorioallantoic Membrane (HET-CAM), Isolated Chicken Eye (ICE) and Isolated Rabbit Eye (IRE)**

"These Summary Minutes have been read and approved by the Chair of the Expert Panel Teleconference on the Evaluation of the Validation Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants, as certified below."

_____ _____
Dr. Robert Scala Date
Panel Chair

_____ _____
Dr. Raymond Tice Date
Designated Federal Official