APPENDIX I2
ICCVAM CONSIDERATION OF PUBLIC COMMENTS RECEIVED IN RESPONSE TO *FEDERAL REGISTER* NOTICES

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In response to six *Federal Register* (*FR*) notices that were released between March 2004 and July 2005, 149 public comments were received. Comments received in response to the *FR* notices and/or were related to those *FR* notices can be obtained on CD ROM upon request to NICEATM by mail, fax, or email (NICEATM, NIEHS, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709, (phone) 919-541-2384, (fax) 919-541-0947, (email) niceatm@niehs.nih.gov). The following sections, delineated by *FR* notice, provide a brief discussion of the public comments received.

1.0 Public Comments Received in Response to *FR* Notice Released on March 24, 2004 (Volume 69, Number 57; pages 13859-13861)

NICEATM, in an *FR* notice (69 *FR* 57:13859-13861, March 24, 2004) requested (1) public comment on four test methods for ocular toxicity and related activities nominated to the ICCVAM by the EPA, (2) public comment on ICCVAM's recommended actions for the nomination, and (3) data from completed studies on chemicals and products tested for ocular irritancy using *in vitro* and/or *in vivo* test methods.

While no comments were received in response to this *FR* notice, on the four test methods or on ICCVAM's recommended actions for the nomination, NICEATM did receive submissions of data from:

- Access Business Group
- The Cosmetic, Toiletry, and Fragrance Association (CTFA)
- The European Centre for the Validation of Alternative Methods (ECVAM)
- ExxonMobil Biomedical Sciences, Inc.
- The German Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET)
- GlaxoSmithKline
- The Institute for In Vitro Sciences, Inc.
- The Japanese National Institute of Health Sciences
- L'Oréal
- Merck & Co., Inc.
- The Netherlands Organization for Applied Scientific Research (TNO) Nutrition and Food Institute
- The Procter & Gamble Company
- S.C. Johnson & Son, Inc./Johnson Diversey, Inc.
- The U.S. Food and Drug Administration

2.0 Public Comments Received in Response to *FR* Notices Released on November 3, 2004 (Volume 69, Number 212; pages 64081-64082) and December 3, 2004 (Volume 69, Number 232; pages 70268-70269)

In response to these *FR* notices, 61 comments were received on the four test method BRDs and the HET-CAM test method addendum.

All comments related to errors in the text and/or omissions of data were addressed in the final BRD for each test method. Additional information related to test method protocol

components and data analyses also was incorporated into the appropriate BRDs. Comments related to test method rationale, benefits and limitations of an *in vitro* test method and/or the *in vivo* rabbit eye test, proposed literature references for inclusion in the BRDs, and use of test methods protocols, decision criteria, and analyses in laboratories were fully reviewed and incorporated, as appropriate, into the final BRDs.

Of the comments received, 51 comments were related to requests that ICCVAM accept these test methods as replacements for the *in vivo* rabbit test and that there be no call for confirmatory testing (similar to the EU). ICCVAM agrees that some countries in the EU do accept a positive response in these test methods for classification purposes. However, ICCVAM notes that the European Chemicals Bureau has stated in July 2004:

> Although these tests [IRE, ICE, BCOP, HET-CAM] <u>are not yet validated</u> (and therefore not included in Annex V) it has been agreed that available evidence is sufficient to conclude that the methods are able to detect severe eye irritants. … 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. (emphasis added)

ICCVAM also appreciates the comments regarding acceptance of the test methods. It should be noted that it does not determine whether a test method or classification scheme is acceptable for use by U.S. Federal agencies or the international regulatory community. ICCVAM develops and forwards recommendations on the usefulness and limitations of the proposed test methods to each U.S. Federal agency for its review. Based on their specific statutory mandates, each U.S. Federal agency will consider ICCVAM's recommendations and then make a determination as to the acceptability of the test methods.

With respect to the issue of further validation efforts and the need for *in vivo* animal data, ICCVAM encourages the use of historical data, when available, to avoid further animal use.

Several comments focused on submitted data that were not used for the BRD analysis. ICCVAM and NICEATM appreciate all efforts made to provide data for the evaluation. However, specific criteria were established for inclusion of data in the BRD and/or the analysis conducted therein. When submitted data did not meet these criteria, they were excluded from the analysis. Also, the current evaluation did not focus on replacing the current method; rather, it focused on determining whether these methods could be used as part of a tiered-testing strategy to identify severe irritants <u>before</u> the test substance is evaluated in a rabbit.

Several comments related to the data collection and opportunities to increase data submission to NICEATM and ICCVAM were submitted. ICCVAM appreciates comments related to these processes and continuously strives to improve the process used in reviewing new alternative test methods. These constructive comments will be incorporated into this ongoing process as appropriate.

ICCVAM received comments concerning the replacement of the word *relevance* with the word *accuracy* in the definition of *validation* used in the BRDs. After reviewing these comments, ICCVAM acknowledges that the definition of *validation* contained in the *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods (2003)* is in error and that this definition will be corrected in the final BRDs.

ICCVAM received comments that took issue with the approach to evaluate each test method individually rather than as part of a test battery, along with comments proposing additional methods. The initial nomination of the four evaluated test methods requests that the four test methods be evaluated individually. ICCVAM appreciates the proposal to evaluate the methods as a part of a battery and future evaluation of all or some of these methods as part of an ocular toxicity test battery may be considered.

ICCVAM received a comment that the protocol for the BCOP test outlined in the BRD cannot be validated using the recommended holder for the corneas, and proposed that an alternate holder be employed. ICCVAM also received comments that outlined how the use of histology as an additional endpoint in the BCOP test might improve its accuracy. ICCVAM appreciates any suggestions related to the optimization of test method protocols, and all comments were taken under consideration in the development of the final BRDs.

3.0 Public Comments Received in Response to *FR* Notice Released on February 28, 2005 (Volume 70, Number 38; pages 9661-9662)

NICEATM, in a *FR* notice (70 *FR* 38:9661-9662,February 28, 2005), made a second request for data from completed studies on chemicals and products tested for ocular irritancy using *in vitro* and/or *in vivo* test methods.

In response to this *FR* notice, NICEATM received three submissions of data. Data were received from:

- The TNO Nutrition and Food Institute
- Johnson & Johnson Pharmaceutical Research & Development, L.L.C.
- ZEBET

ICCVAM and NICEATM gratefully acknowledge all the efforts made in obtaining and providing these data for this evaluation.

4.0 Responses to *FR* Notice released on March 21, 2005 (Volume 70, Number 53; pages 13513-13514)

NICEATM, in an *FR* notice (70 *FR* 53:13513-13514, March 21, 2005), requested submission of written comments on the report entitled "The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Expert Panel Evaluation of the Current Validation Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants."

In response to the *FR* notice, 85 comments were received on the report. All comments related to errors in the text and/or omissions of data were addressed in the final BRD of each test method. Additional background information related to test method protocol components; data sources; test method rationale; benefits and limitations of an *in vitro* test method and/or the *in vivo* rabbit eye test; proposed literature references for inclusion in the BRDs; proposed sources of additional *in vivo* and human ocular toxicity data; and use of test methods protocols, decision criteria, and analyses in individual laboratories were fully reviewed and incorporated, as appropriate, into the final BRDs.

Eighty-one comments focused on requesting that U.S. Federal agencies begin accepting alternative test methods that are found to be useful and requested that recommendations made by the Expert Panel directed at improving alternative test these methods not delay the acceptance of their current forms. ICCVAM appreciates the comments regarding acceptance of the test methods. ICCVAM notes that each U.S. Federal Agency makes the ultimate determination on whether a test method is acceptable for use. However, ICCVAM will develop and forward recommendations on the usefulness and limitations of the proposed test methods to each U.S. Federal agency for their review. Based on their specific statutory mandates, each Federal agency will then make a determination on the acceptability of the test methods.

Several comments were either (1) general responses to comments made by the Expert Panel in the report or (2) questions posed in response to the Expert Panel Report. All such comments were reviewed by ICCVAM and NICEATM. ICCVAM appreciates these comments and they were considered during the development of the ICCVAM recommendations.

Several comments related to the data collection and Expert Panel processes were submitted. Comments agreed with the Minority Opinions presented in Section 12.2 of the BCOP Expert Panel report. Comments also proposed additional methods and proposed opportunities to increase data submission to NICEATM and ICCVAM (e.g., provide corporate confidentiality). Comments related to these processes were appreciated as ICCVAM continuously strives to improve the process used in reviewing new alternative test methods. These constructive comments will be incorporated into this ongoing process, as appropriate.

One comment stated that the BRD should address the public comments that are submitted, including those provided by the supplier of the data cited. The comments proposed that inaccuracies and/or confusions about data sets should be clarified prior to finalization of the BRD for use by the Expert Panel. As a point of clarification, the BRDs used by the Expert Panel during their review of the documents were in draft, and not final, form. Additionally, all public comments on the BRDs were provided to the Expert Panel for their review and information prior to the Expert Panel meeting on January 11-12, 2005. This allowed the Expert Panel to review and request additional information, as needed, from NICEATM.

Several comments agreed with the Expert Panel's conclusion that additional information and discussion were needed in the BRDs about the accuracy and reliability of the *in vivo* test method and that variability of the *in vivo* test should be considered when comparing *in*

vivo/*in vitro* data. Additional discussion on this topic has been incorporated into Section 4.0 of each BRD.

Several comments focused on submitted data that was not used by NICEATM for the BRD analysis or was repeatedly used "incorrectly". ICCVAM and NICEATM appreciate all efforts made to provide data for the evaluation. However, it is noted that in order to conduct the evaluation (i.e., assess the ability of the nominated test methods to identify severe irritants and ocular corrosives, as classified by regulatory classification systems), specific criteria must be met prior to inclusion of the data in the BRD and/or the analysis conducted therein (see Section 4.0 of each BRD). Therefore, some data received (e.g., *in vitro* data compared to benchmark controls, *in vivo* MAS data) could not be included in the accuracy analyses because it did not meet these minimum requirements. Data provided by individuals and companies that were not used in the BRD analyses were discussed in Section 9.0 of the relevant BRD or as Appendices to the BRDs. For those data that were used "incorrectly", all errors and omissions have been corrected in the final BRD.

ICCVAM received comments concerning the replacement of the word *relevance* with the word *accuracy* in the definition of *validation* used in the BRDs. As previously noted, after reviewing these comments, ICCVAM acknowledges that the definition of *validation* contained in the *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods (2003)* is in error and that references to this definition will be corrected in the final BRDs.

Comments were received related to the potential inclusion of histopathology as an endpoint for some of the organotypic test methods. As previously indicated in public forum, ICCVAM would be very interested in identifying formal decision criteria for this endpoint that would allow for its standardized use, and would welcome any available histopathology data that are generated during studies with any of these test methods.

A comment was submitted stating that Low Volume Eye Test (LVET) more highly correlated to the human response than the *in vivo* rabbit eye test. The comment stated that the *in vivo* rabbit eye test has been documented to overpredict the human response, but the degree of overprediction was unknown. The comment stated that overprediction did not equal scientific credibility, a feature being strived for in replacing the current whole animal method. ICCVAM appreciates the comments provided, but notes that the current evaluation was not focused on replacing the current method. This evaluation was focused on determining whether these methods could be used, as part of a tiered-testing strategy, to identify severe irritants before the test substance is evaluated in a rabbit. ICCVAM also notes that LVET data was used in the accuracy evaluation, if there was corresponding *in vitro* data, and when a severe response was observed in the rabbit.

Some comments were submitted that focused on the comparison made in the BRDs and by the Expert Panel on the cost of the *in vitro* and *in vivo* studies. The comments suggest that such a comparison, as well as a comparison of time needed to conduct the studies, is complicated. ICCVAM appreciates the concerns raised by the commentors. However, it is noted that an evaluation of the practical considerations of the test method (e.g., transferability

of the test method, cost of the method) is incorporated into the ICCVAM evaluation process. While ICCVAM does not use the cost and time to complete the *in vitro* test, when compared to the reference *in vivo* test, as the main factor in evaluating alternative test methods, these considerations are evaluated together with the accuracy and the reliability when establishing the regulatory utility of an alternative method.

5.0 Public Comments Received in Response to *FR* Notice Released on July 26, 2005 (Volume 70, Number 142; page 43149)

In response to this *FR* notice, three comments were received on the revised analyses for the four test method BRDs. All comments related to errors in the text and/or omissions were incorporated into the final BRD. All comments related to proposed analyses have been reviewed and incorporated, where appropriate and practical.

One comment submitted was the concern of correlating *in vitro* results with the EPA ocular hazard classification system, given that a single animal can lead to a severe classification regardless of the results in any other tested animals. Until the GHS classification system is formally adopted, all relevant hazard classification systems (i.e., those used by the U.S. EPA, the European Union [EU], and the GHS) must be considered when determining the utility of an *in vitro* test method for hazard identification. Therefore, ICCVAM believes that an evaluation against the EPA hazard classification system is appropriate.

A comment also noted that there are instances in which different hazard classifications are assigned to the same substance depending on the ocular hazard classification system used (i.e., EPA, EU, GHS), due to the differences in weighting assigned to the same data among the different classification systems. However, the analyses demonstrate that the accuracy of an individual *in vitro* test method is largely independent of which classification system is considered, suggesting that these differences are small.

Two comments were submitted relating to the criteria for acceptance of hazard classification information for test substances evaluated in the *in vitro* test methods. These comments focused on the exclusion of substances from the analyses, based on a lack of adequate *in vivo* rabbit data. While *in vivo* dermal corrosive effects (or extremes of pH) are utilized in ocular hazard classification systems as substitutes for *in vivo* rabbit eye test results for the purposes of ocular hazard classification, the goal of this assessment is to evaluate the accuracy of four *in vitro* test methods for identifying ocular corrosives and severe irritants, as determined by the *in vivo* rabbit eye test. Therefore, substances that lacked *in vivo* rabbit eye test results were excluded from the evaluation. However, to the extent such studies could be identified, data derived from scientifically acceptable *in vivo* rabbit eye tests terminated early based on humane endpoints were included in the accuracy and reliability analysis.