PLANNED FUTURE STUDIES/ACTIVITIES FOR *IN VITRO* TEST METHODS FOR IDENTIFYING OCULAR CORROSIVES AND SEVERE IRRITANTS

1.0 BACKGROUND/INTRODUCTION

In October 2003, the U.S. Environmental Protection Agency (EPA) nominated four *in vitro* test methods proposed for identifying potential ocular corrosives and severe irritants in a tiered-testing strategy for review of their current validation status. The test methods were the Bovine Corneal Opacity and Permeability (BCOP), the Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) assay, Isolated Chicken Eye (ICE), and Isolated Rabbit Eye (IRE). The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), in conjunction with the Ocular Toxicity Working Group (OTWG), prepared a background review document (BRD) with all available data and information supporting the current validity of each test method. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) convened an Expert Panel to assess the validation status of these *in vitro* test methods. The Expert Panel Report (ICCVAM 2005) concluded that:

- Histopathological examination should be included in the BCOP study protocol unless the test substance is from a class of materials known to be accurately predicted using only opacity and permeability.
- Histopathological examination should be considered when the current ICE test method endpoints (i.e., corneal opacity, swelling, flourescein retention) and IRE test method endpoints (i.e., corneal opacity, swelling, fluorescein retention, epithelial integrity) produce borderline results.
- NICEATM/ICCVAM should facilitate the development of a histopathological scoring system for corneal damage (with visual aids).
- Modifications to the proposed BCOP test method protocol (i.e., use of a larger holder suggested by Ubels et al. (2002, 2004) may improve the accuracy and reliability of this test method.

The draft BRDs and the Expert Panel report were made available to the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) for their consideration at their

meeting on December 12, 2005. SACATM agreed with the conclusions of the Expert Panel. ICCVAM subsequently prepared final test method recommendations based on the Expert Panel report and SACATM comments, which will be made publicly available and provided to U.S. Federal agencies (ICCVAM 2006).

ICCVAM also convened a symposium, *Mechanisms of Chemically-Induced Ocular Injury and Recovery*, to review the state-of-the-science and understanding of the pathophysiology and mechanisms of chemically-induced ocular injury and recovery (reversibility vs. irreversibility) (http://iccvam.niehs.nih.gov/methods/ocudocs/ocumeet/sympinfo.htm). At that symposium, histological evaluation was recommended as a useful, additional endpoint for the BCOP, ICE, and IRE test methods (Summaries of the outcomes of the symposium are located in **Appendix A**; previously presented at SACATM meeting in December 2005).

Based on the conclusions in the Expert Panel Report and the recommendations at the symposium, ICCVAM concluded that histopathological evaluation of the corneal tissue, using a standardized scoring system, should be included when the BCOP, IRE, or ICE test methods are conducted. In addition, ICCVAM concluded that studies should be conducted to evaluate the impact of using a corneal holder that maintains normal curvature (e.g., the corneal mounting system designed by Ubels et al. 2002) on accuracy and/or reliability of the BCOP test method (ICCVAM 2006).

The following sections provide additional details regarding these proposed studies/activities, with an overall description of each project and the associated primary objectives. Because the final activity listed above (i.e., studies to evaluate the optimal corneal holder for the BCOP test method) entails a future validation study, it will be addressed in a separate document as an ICCVAM nomination for future study.

2.0 PLANNED FUTURE STUDIES/ACTIVITIES TO BE CONDUCTED BY NICEATM

2.1 Creation of a Reference Atlas for the Histopathology of Chemically-Induced Ocular Lesions

2.1.1 Description of Project

The proposed activity is the creation of a detailed reference atlas for histopathology of chemically-induced ocular lesions. This atlas will contain:

- a standardized protocol for the histopathological evaluation of excised corneas and enucleated eyes (e.g., preparation of ocular tissue for histology, including fixing and staining protocols; microscopic examination procedures; etc.) used in an *in vitro* ocular toxicity test method (rabbit, chicken, pig, bovine), as well as for eyes from rabbits used in *in vivo* tests
- visual aids for lesions (i.e., reference micrographs of chemically-induced ocular lesions)
- a written description of each chemically-induced lesion using standardized nomenclature

2.1.2 Objective

To create a detailed reference atlas for histopathology of chemically-induced lesions in excised corneas and enucleated eyes used in an *in vitro* ocular toxicity test method (rabbit, chicken, pig, bovine), as well as for eyes from rabbits used in *in vivo* tests.

2.1.3 Method/Proposed Activities

- NICEATM, working in partnership with the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM), will create an international working group to facilitate the collection of reference micrographs of chemically-induced ocular lesions in excised corneas and enucleated eyes used in an *in vitro* ocular toxicity test method (rabbit, chicken, pig, bovine) and from eyes of rabbits used in *in vivo* tests.

- The international working group developed by NICEATM, ECVAM, and JaCVAM will review the collection of reference micrographs of chemically-induced ocular lesions to ensure that they represent different hazard classifications, chemical classes, mechanisms of action, and other physico-chemical properties.
- These micrographs will be annotated with a standard ocular histological nomenclature and organized into an atlas, which can be used as a reference for histological evaluation of samples taken during *in vitro* and *in vivo* ocular toxicity tests.
- Data gaps in hazard classifications and pre-defined physico-chemical properties of tested chemicals, if any, will be identified during the collection of the reference micrographs.
- A strategy to fill the data gaps will be created and implemented. This could include solicitation of additional samples of chemically-induced ocular lesions from industry and private organizations.

2.1.4 Draft ICCVAM Recommended Priority: High

2.2 Creation of a Standardized Histological Scoring System and Revised Hazard Classification Decision Criteria for *In Vivo* and *In Vitro* Test Methods

2.2.1 Description of Project

The proposed activity is the creation of a standardized histological scoring system for chemically-induced ocular lesions in species used in *in vivo* (rabbit) and *in vitro* (rabbit, chicken, pig, bovine) ocular toxicity test methods. The proposed activity also includes development of revised hazard classification decision criteria, which includes the use of histopathological data, for the BCOP, ICE, and IRE test methods.

2.2.2 Objective

To create a standardized scoring system for the histopathological evaluation of chemically-induced lesions *in vivo* and in excised corneas and enucleated eyes used in *in vitro* ocular toxicity test methods, and establish revised hazard classification decision criteria for *in vivo*

tests and the BCOP, ICE, and IRE test methods, which would include consideration of histopathological results.

2.2.3 Method/Proposed Activity

- NICEATM will work in partnership with an international working group created in partnership with ECVAM and JaCVAM. NICEATM will use a detailed reference atlas of chemically-induced ocular lesions described with standard histopathological nomenclature (see **Section 2.1**), in animal species used in *in vivo* (rabbit) and *in vitro* ocular toxicity test methods (rabbit, chicken, pig, bovine), to create a standardized scoring system for the evaluation of these lesions.
- The ocular lesions in the reference atlas described in **Section 2.1** will be scored using the standardized scoring system.
- Decision criteria for the BCOP, ICE, and IRE test methods will be revised to utilize histological endpoints as a component for hazard classification.

2.2.4 Draft ICCVAM Recommended Priority: High

3.0 ADDITIONAL ACTIVITY TO BE CONSIDERED

3.1 Development of a Targeted Research Grants Program

3.1.1 Additional Background:

The *Mechanisms of Chemically-Induced Ocular Injury and Recovery* Symposium (http://iccvam.niehs.nih.gov/methods/ocudocs/ocumeet/sympinfo.htm) also focused on advancing the development of *in vitro* test systems that would meet regulatory testing requirements; provide for protection of human health; and reduce, refine, and/or replace animal use.

Objectives of the meeting included:

- Review current and potential molecular, cellular, tissue (e.g., histopathology), and clinical (e.g., corneal opacity, swelling, depth of injury) biomarkers of

chemical injury and recovery and their usefulness for *in vivo* and *in vitro* testing models of ocular irritancy and corrosivity.

- Identify knowledge gaps in understanding of chemically-induced ocular injury and recovery.
- Identify and prioritize future research initiatives that would address current knowledge gaps and that are considered necessary to advance the development and validation of *in vitro* models of chemically-induced ocular injury and recovery.
- Discuss and identify quantitative, objective endpoints that should be considered for inclusion in the current *in vivo* rabbit eye test and/or human clinical testing (e.g., more sensitive markers of injury and recovery) that would support development and validation of predictive *in vitro* methods and improve hazard characterization and reliability.

During the course of the symposium, several knowledge gaps that warranted further research and development were identified. These included the following potential quantitative objective endpoints or biomarkers:

- *in vivo* biomarkers (e.g., molecular, cellular, morphological, clinical) that might be predictive indicators of lesion severity
- *in vivo* biomarkers (e.g., molecular, cellular, morphological, clinical) that might be predictive indicators of reversibility, non-reversibility, or delayed responses
- modifications to current *in vitro* systems necessary for the methods to adequately predict the ocular injury potential of chemicals.

3.1.2 Description of Project:

The proposed activity is the development of a targeted research grants program, supported by interested stakeholders (e.g., government, industry, animal welfare organizations), to fund innovative research into high priority areas identified by experts at recent public workshops and meetings sponsored by ICCVAM, NICEATM, and ECVAM. The research grants

program would include, but would not necessarily be limited to, Request for Applications (RFA) and Small Business Innovative Research (SBIR) grants.

3.1.3 Objective:

Development of a research grants program, which includes the use of targeted RFAs and SBIRs, to focus research and product commercialization, respectively, for areas identified as high priority at the *Mechanisms of Chemically-Induced Ocular Injury and Recovery* Symposium. Research areas include, but are not limited to:

- Assessment of other *in vitro* test models (e.g., human, pig) as more predictive models of *in vivo* human responses to ocular injuries
- Assessment of endothelial cell damage markers as predictors of ocular corrosion and/or irritation
- Evaluation of inflammatory responses (e.g., cytokines, adhesion proteins, etc.) as markers of ocular injury
- Quantification of depth of injury in *ex vivo* models and correlation with *in vivo* responses
- Evaluation of cell death as a biomarker of damage and correlation with *in vivo* ocular injury
- Evaluation of alternative histopathological staining techniques to assess ocular injury
- Impact of using quantitative endpoints (e.g., slit lamp biomicroscopy with fluorescein staining, photodocumentation of injury, histopathology) on the predictive capacity and variability in response for *in vivo* studies
- Evaluation of gene expression profiling, clustering, and pathway analysis for ocular damage and repair

Areas of product development and commercialization might include, but not necessarily be limited to, the commercialization of alternative corneal holders for the BCOP test method, and more sensitive and predictive biomarkers of ocular injury severity and reversibility.

3.1.4 Method/Proposed Activity:

- Establish a list of high priority research areas and commercialization areas for evaluation and potential funding
- Establish a group of interested stakeholders to develop RFAs and SBIRs for the areas proposed for evaluation and commercialization, respectively
- Identify funding support for the RFA and SBIR
- Solicit and evaluate applications

3.1.5 Draft ICCVAM Recommended Priority: High

4.0 REFERENCES

ICCVAM Test Method Evaluation Report: In Vitro Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives (Available: http://iccvam.niehs.nih.gov/).

ICCVAM. 2005. EXPERT PANEL FINAL REPORT: Evaluation of the Current Validation Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants (Available: http://iccvam.niehs.nih.gov/methods/ocudocs/EPreport/ocuEPrpt.pdf).

Stokes WS, Tice RR, Allen DG, Choksi NY, Truax JF. The ICCVAM/NICEATM/ECVAM symposia on mechanisms of chemically induced ocular injury and recovery (http://iccvam.niehs.nih.gov/methods/ocudocs/ocumeet/sympinfo.htm).

Ubels JL, Ditlev JA, Clousing DP, Casterton PL. 2004. Corneal permeability in a redesigned corneal holder for the bovine cornea opacity and permeability assay. Toxicol In Vitro 18:853-857.

Ubels JL, Paauw JD, Casterton PL, Kool DJ. 2002. A redesigned corneal holder for the bovine cornea opacity and permeability assay that maintains normal corneal morphology. Toxicol In Vitro 16:621-628.