Overview of Histopathology in Ocular Safety Testing

ICCVAM Five-Year Implementation Plan

An important goal in the area of ocular safety testing that is outlined in the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Five-Year Implementation Plan (ICCVAM 2009) (available at: http://iccvam.niehs.nih.gov/docs/5yearplan.htm) is to identify and promote research, development, translation, and validation activities for alternative test methods that can partially or fully replace the rabbit eye test for identification of substances that are potential ocular hazards. The current major obstacle to replacing animals for eye hazard testing is the lack of *in vitro* methods that can accurately identify chemicals and products that are capable of causing severe or permanent eye injuries. One of the proposed implementation activities was a workshop on the use of histopathology in ocular safety testing to determine if histopathology might help to significantly improve the *in vitro* prediction of severe and irreversible injuries. However at this time, a workshop does not appear warranted given that several recent evaluations and publications have failed to demonstrate the usefulness of histopathology as an additional endpoint for the bovine corneal and permeability (BCOP) (OECD 2009a) or isolated chicken eye (ICE) (OECD 2009b) test methods.

Summary of Histopathology as an Additional Endpoint for *In Vitro* Ocular Safety Test Methods

BCOP Test Method

As part of the ICCVAM evaluation of a proposed *in vitro* testing strategy for identifying the ocular hazard potential of antimicrobial cleaning products (AMCP) (ICCVAM 2010a), a comparison of BCOP only and BCOP with histopathology data were assessed for 17 substances. Histopathology classifications were based primarily on depth on injury (Jester et al. 1998, 2001). The overall accuracy for the U.S. Environmental Protection Agency (EPA) classification system was slightly reduced from 41% (7/17) to 35% (6/17) with histopathology. Using histopathology with BCOP removed one false negative, but added three false positives. Based on this performance,

the addition of histopathology as an additional endpoint for BCOP was not considered useful for inclusion in the proposed testing strategy.

In a larger study by Schrage et al. (2011) that tested 52 substances, similar results were reported for the use of histopathology with BCOP when the analysis was restricted to nonsevere irritants (i.e., IVIS ≤55). Histopathology scores (using a grading system of severity with 1 = minimal and 5 = massive) were combined with depth of injury to assign a classification. Using histopathology with BCOP improved the classification for 4/6 false negatives, but added five false positives. Thus, inclusion of histopathology increased sensitivity from 63% (10/16) to 87% (13/15), but reduced specificity from 78% (28/36) to 62% (23/37). For ocular corrosive or severe irritants (i.e., IVIS >55), histopathology improved the classification of three false positives identified as severe irritants in BCOP, but also underpredicted two substances. The authors concluded that use of histopathology to reduce the number of false negatives with BCOP "did not completely provide the expected improvements to the test method".

The Korean Center for the Validation of Alternative Methods (KoCVAM) has also conducted a study with 14 substances exploring the usefulness of histopathological and histomorphometric evaluation as an additional endpoint for BCOP. The publication of this study is pending.

ICE Test Method

Two published studies have compared results from histopathology and ICE. Schutte et al. (2009) evaluated 15 common cleaning products and five raw materials and Prinsen et al. (2011) examined four substances ranging from not labeled as an eye irritant to corrosive/severe irritant. These authors concluded that histopathology confirms the ICE results and they propose that the use of histopathology could add to the weight-of-evidence analysis, especially in borderline cases. However, no attempt was made to quantitatively assess the reported histopathology observations or to evaluate its performance for identifying or classifying these substances.

Need for Additional Histopathology Data and Consideration of Other Approaches

To encourage the use of histopathology as an additional endpoint for *in vitro* ocular safety test methods and expand the existing data available for evaluation, the NTP

Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and ICCVAM submitted an Organisation for Economic Co-operation and Development (OECD) Guidance Document (GD) for collecting ocular tissues for histopathology to supplement the BCOP and ICE Test Guidelines. In April 2011, the GD was approved by the Working Group of National Coordinators of the Test Guidelines Programme. The primary purposes of the GD are: i) to promote the collection of tissues that could be used to further evaluate the usefulness of histopathology as an additional endpoint for *in vitro* ocular safety test methods; ii) to provide specific guidance on using BCOP and ICE to generate data that can be evaluated to determine and optimize their usefulness for identifying additional hazard categories (ICCVAM 2010b); iii) for those substances that test negative in BCOP or ICE and are tested *in vivo*, to provide procedures for enucleating, fixing, and processing eyes from *in vivo* rabbit eye studies for histopathology. The GD describes the general procedures for the collection, preservation, and preparation of ocular tissues for use in performing histopathology. However, if differences exist, laboratories that routinely perform histopathology of ocular tissue can employ their existing procedures. Importantly, the GD does not provide guidance on the evaluation or interpretation of histopathology data or the associated decision criteria to be used for ocular hazard classification. However, when such information becomes available, the GD will be updated.

A less subjective technique that may warrant additional consideration is the measurement of fluorescent biomarkers of live/dead cells using standard microscopy to assess the extent and depth of eye injury (Jester et al. 2009). Alternatively, central corneal thickness and/or anterior chamber depth have been measured using optical coherence tomography, ultrasonic pachymetry, or scanning-slit tomography in rabbits or humans (Reiser et al. 2005; Fukuda et al. 2009; Li et al. 2010; Modis et al. 2011). While these approaches have the potential to provide quantitative measurements, some have limitations due to equipment cost and the need for specialized technical expertise.

Summary

While the currently available histopathology data have not convincingly demonstrated the usefulness of this technique as an additional endpoint for *in vitro* ocular

safety test methods, the continued collection and analysis of additional histopathology data and/or data from other quantitative approaches will help further elucidate whether such methods will be useful in improving the accuracy of *in vitro* predictions of eye hazards for classification and labeling purposes.

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