



Summary of the Independent Scientific Peer Review Panel Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

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¹ Could not attend the May 2009 Panel meeting, but agreed to participate in the review of all materials



Peer Panel Meeting – May 19-21, 2009

- Convened in public session to evaluate the current validation status of alternative ocular safety testing methods and approaches.
- These are abbreviated highlights of the Independent Scientific Peer Review Panel deliberations.
 - The final report should be consulted for a detailed description of the Panel's conclusions and recommendations.
 - Available after July 8th at:
http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf



ICCVAM Charges to the Peer Panel

- Review the ICCVAM draft BRDs for completeness and identify any errors or omissions (other relevant publications or available data, etc.)
- Evaluate the information in the draft BRDs and determine the extent to which each of the applicable ICCVAM criteria for validation and acceptance have been appropriately addressed
- Consider the ICCVAM draft test method recommendations for the following and comment on the extent to which they are supported by the information provided in the BRDs:
 - Proposed test method usefulness and limitations
 - Proposed recommended standardized protocols
 - Proposed test method performance standards
 - Proposed future studies



Alternative Ocular Safety Testing Methods and Approaches Evaluated by the Panel

- Routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during *in vivo* ocular irritation testing
- Validation status of four *in vitro* test methods for identifying mild/moderate ocular irritants and substances not labeled as irritants
 - Isolated chicken eye (ICE)
 - Bovine corneal opacity and permeability (BCOP)
 - Hen's egg test – chorioallantoic membrane (HET-CAM)
 - Isolated rabbit eye (IRE)
- Validation status of the *in vivo* low volume eye test (LVET)
- Validation status of the individual test methods and testing strategies to assess eye irritation potential of antimicrobial cleaning products (AMCPs)
 - Cytosensor Microphysiometer® (CM)



Routine Use of Topical Anesthetics and Systemic Analgesics (1)

- The Panel proposed an alternative preemptive pain management protocol that should be used for **all** *in vivo* rabbit eye irritation tests intended for regulatory safety testing, unless there is requirement for monitoring the pain response (e.g., pharmaceutical tolerability testing).
- The alternative protocol consists of:
 - Pre-test substance administration (TSA):
 - 60 minutes pre-TSA: Buprenorphine 0.01 mg/kg by subcutaneous injection (SC)
 - Starting 15 minutes pre-TSA: One or two drops of 0.5% Proparacaine hydrochloride (preservative free) applied to the eye 3 times at 5 minute intervals. The last application would be 5 minutes pre-TSA.
 - Post-TSA:
 - Day 1: Buprenorphine 0.01 mg/kg SC and Meloxicam 0.5 mg/kg SC given 8 hours post-TSA
 - Days 2-4: Buprenorphine 0.01 mg/kg SC every 12 hours and Meloxicam 0.5 mg/kg SC every 24 hours
 - If signs of ocular injury sufficient to cause pain and discomfort are evident, this systemic analgesic protocol would continue until the test is completed.
 - Rescue: In the event of signs of persistent pain or discomfort
 - Buprenorphine 0.03 mg/kg SC given as needed every 8 hours instead of 0.01 mg/kg SC every 12 hours. Meloxicam continued at the same dose and interval.



Routine Use of Topical Anesthetics and Systemic Analgesics (2)

- The Panel also recommended that pain assessments should be made immediately after test substance application and recorded daily (i.e., at least twice daily, or more often as necessary).



Use of Humane Endpoints (1)

- The Panel concluded that, based on the available data and information some humane endpoints as recommended by ICCVAM are adequate to terminate a study.
 - Endpoints currently accepted for study termination (OECD 2002):
 - Draize corneal opacity score of 4 that persists for 48 hours
 - Corneal perforation or significant corneal ulceration including staphyloma
 - Blood in the anterior chamber of the eye
 - Absence of light reflex that persists for 72 hours
 - Ulceration of the conjunctival membrane
 - Necrosis of the conjunctiva or nictitating membrane
 - Sloughing
 - Additional humane endpoints (to be used in combination) endorsed by the Panel:
 - Destruction of more than 50% of the limbus (as evidenced by blanching of the conjunctival tissue)
 - Severe depth of injury (e.g., corneal ulceration extending beyond the superficial layers) (ICCVAM Workshop, 2005)
 - Lack of re-epithelialization (requires fluorescein staining)
 - The occurrence of a severe eye infection (i.e., purulent discharge) as a single criterion for study termination
 - Endpoints not endorsed by the Panel:
 - Vascularization of the corneal surface (i.e., pannus)
 - Area of fluorescein staining



Use of Humane Endpoints (2)

- The Panel agreed that the current and proposed humane endpoints are predictive enough of irreversible or severe effects (GHS Category 1, US EPA Category I, EU R41) that they should routinely be used as humane endpoints to terminate a study as soon as they are observed.
- However, the Panel emphasized that, while very severe endpoints (i.e., corneal perforation) would be adequate alone to terminate a study, determinations to terminate a study should typically be based on more than one endpoint.
- The Panel also emphasized a slit-lamp examination is necessary to ensure accurate measurement of most of the proposed endpoints.



Isolated Chicken Eye (ICE) Test Method (1)

- The Panel supported the ICCVAM draft recommendation that the available data and ICE test method performance (accuracy and reliability) does not support its use to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems.
 - The overall correct classifications for the ICE test method ranged from 59% (83/141) to 77% (118/153) depending on the hazard classification system evaluated when using the entire database.
 - The overall correct classifications for the ICE test method ranged from 64% (49/77) to 80% (66/82) depending on the hazard classification system evaluated when discordant classes (i.e., alcohols, surfactants, and solids) are removed.



Isolated Chicken Eye (ICE) Test Method (2)

- The Panel agreed with the ICCVAM draft recommendation that the the available data and ICE test method performance (accuracy and reliability) does not support its use as a screening test to identify substances not labeled as irritants from all other hazard categories as defined by GHS, EPA, and EU classification systems.
 - The overall accuracy for identification of substances not labeled as irritants from all other categories ranged from 78% (110/141) to 85% (130/153) depending on the hazard classification system used.
 - The lowest false negative rate (6% [4/62]) was noted for the GHS system, followed by 14% (11/81) for the EPA system, and 22% (13/60) for the EU system.
 - However, among these false negatives, at least one substance was classified as an ocular corrosive/severe irritant based on Draize data (n = 1 each for the EPA and GHS systems, and n = 6 for the EU system). Considering the public health impact of misclassifying a corrosive substance as Not Labeled, these false negative results cannot be minimized.



The BCOP Test Method (1)

- The Panel supported the ICCVAM draft recommendation that the available data and BCOP test method performance (accuracy and reliability) does not support its use to identify substances from **all** hazard categories as defined by GHS, EPA, and EU classification systems.
 - The overall correct classifications ranged from 49% (91/187) to 54% (101/186) depending on the hazard classification system evaluated when using the entire database.
 - The overall correct classifications ranged from 47% (31/66) to 54% (35/65) depending on the hazard classification system evaluated when discordant classes (i.e., alcohols, ketones, and solids) are removed.
 - Using alternative decision criteria for the identification of corrosive/severe ocular irritants (i.e., IVIS \geq 75 as the cutoff to define such substances instead of IVIS \geq 55.1 as the cutoff to define such substances) does not improve test method performance.



The BCOP Test Method (2)

- The Panel agreed with the ICCVAM draft recommendation that the available data and ICE test method performance (accuracy and reliability) support its use as a screening test to identify substances not labeled as irritants when results are to be used for EU or GHS hazard classifications.
 - The overall accuracy ranged from 64% (76/118) to 83% (154/186) depending on the hazard classification system used.
 - The false negative rate was 0% (0/54 or 0/97) for the EU and GHS systems, respectively.
- The Panel concluded that the BCOP test method cannot be used as a screening test to identify EPA Category IV substances.
 - The false negative rate was 6% (8/141) for EPA the system.
 - Among these eight false negatives for the EPA system, 100% (8/8) were EPA Category III substances based on Draize data.
 - However, due to the severity of lesions (i.e., conjunctival redness not cleared until Day 7) associated with 50% (4/8) of the EPA Category III substances that were false negative in the BCOP test method.



The Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method (1)

- The Panel supported the ICCVAM draft recommendation that the available data and HET-CAM test method performance (accuracy and reliability) does not support its use to identify substances from **all** hazard categories as defined by GHS, EPA, and EU classification systems.
 - The overall correct classifications ranged from 40% (23/58) to 41% (24/59) depending on the hazard classification system.



The Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method (2)

- The Panel did not support the ICCVAM draft recommendation¹ that the available data and HET-CAM test method performance (accuracy and reliability) support its use as a screening test to identify substances not labeled as irritants when results are to be used for EU or GHS hazard classifications.
 - The overall accuracy ranged from 58% (36/58) to 60% (47/60) depending on the hazard classification system used.
 - The false negative rate was 0% (0/26 or 0/31) for the EU and GHS systems, respectively.
- The Panel's conclusion was based on:
 - Too few surfactants or oil/water emulsions in the mild to moderate irritant categories to have sufficient confidence in the ability of the test to distinguish them from the not labeled as irritants category.

¹One minority opinion agreed with the ICCVAM draft recommendation that HET-CAM can be used to screen substances not labeled as irritants from other irritant categories for the restricted applicability domain (i.e., surfactant-based formulations and oil/water emulsions).



Isolated Rabbit Eye (IRE) Test Method (1)

- The Panel concluded that additional optimization and validation studies that include the four recommended endpoints (i.e., corneal opacity with or without area of opacity evaluated, corneal swelling, epithelial integrity, and fluorescein retention or penetration) are needed before definitive recommendations on the relevance and reliability of the IRE test method can be made.
 - GlaxoSmithKline, in conjunction with SafePharm Laboratories, is currently designing a validation study composed of several phases.
 - The initial phase will focus on test method improvement including exposure time optimization and additional endpoints.
 - The second phase will test the optimized protocol with a small set of reference Substances.
 - The final phase will encompass a full validation study.
 - NICEATM-ICCVAM has been asked to provide comment on the study design, etc. once it is available.



Isolated Rabbit Eye (IRE) Test Method (2)

- The Panel also recommended a validation study to compare the utility of shipped rabbit eyes versus freshly collected rabbit eyes, development of appropriate inclusion/exclusion criteria for eyes, and development of criteria on test article administration/washout.



The Low Volume Eye Test

- The Panel concluded that in the absence of all data, including the ECVAM BRD, they could not make definitive conclusions or recommendations on the validation status of the LVET.



The Cytosensor (CM) Test Method

- The Panel agreed with the ICCVAM draft recommendation that the CM test method can be used as a screening test to identify both ocular corrosive/severe irritants and substances not labeled as irritants in a tiered-testing strategy, as part of a weight-of-evidence approach specifically for:
 - Water-soluble surfactant chemicals and specific types of surfactant-containing formulations (e.g., cosmetics and personal care products, but not pesticide formulations).
- Major concerns expressed by the Panel included:
 - The continued availability of the instrument used to conduct the CM test method.
 - What new manufacturing processes, including the subsequent required revalidation, might mean to already existing CM test method data.



Antimicrobial Cleaning Products (AMCPs) Testing Strategies (1)

- The Panel agreed with the ICCVAM draft recommendation that there were insufficient data to support the use of the AMCPs testing strategy (i.e., using the BCOP, CM, and EO test methods) for classification of substances in all four ocular hazard categories.
 - None of the 228 AMCPs included in the validation database have been tested in all three *in vitro* test methods.



Antimicrobial Cleaning Products (AMCPs) Testing Strategies (2)

- The Panel agreed with the ICCVAM draft recommendation that there were insufficient available data on which to base definitive recommendations on the proposed alternate testing strategy (i.e., using the BCOP and EO test methods) for classifying substances in all four ocular hazard categories.
 - There were 28 AMCPs tested in both the BCOP and the EO for which Draize reference data were available.
 - Of these, there is only one EPA Category II substance and only four EPA Category III substances (based on Draize eye test results).



Antimicrobial Cleaning Products (AMCPs) Testing Strategies (3)

- The Panel recognized that the use of histopathological evaluation as an additional endpoint did not improve the accuracy and predictability of the BCOP test method for the limited database (n=17) of currently tested AMCPs.
 - The overall accuracy was 71% (12/17) for BCOP only and 59% (10/17) for BCOP with histopathological evaluation.
- However, histopathological evaluation may prove to be a useful endpoint and as such, collection of ocular tissue and further efforts to optimize histopathological evaluation is strongly encouraged.



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SACATM Discussion Questions (1)

- Please comment on the Panel's conclusions and recommendations on the use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid or minimize pain and distress in ocular toxicity testing
- Please comment on the Panel's conclusions and recommendations for the four draft BRDs on the validation status of the *in vitro* test methods for identifying mild/moderate ocular irritants and substances not labeled as irritants:
 - ICE
 - BCOP
 - HET-CAM
 - IRE



SACATM Discussion Questions (2)

- Please comment on the Panel's conclusions and recommendations for the draft BRD on the validation status of the LVET
- Please comment on the Panel's conclusions and recommendations for the draft BRD and appendices on the validation status of the individual test methods and the testing strategies to assess eye irritation potential of AMCPs

