UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
WASHINGTON, DC 20207

**BALLOT VOTE SHEET**

**DATE:** JAN 2 2 2008

**TO:** The Commission
Todd A. Stevenson, Secretary

**THRU:** Patricia Semple, Executive Director P. S.

**FROM:** Lowell F. Martin, Acting General Counsel L. F. Martin

**SUBJECT:** Staff Recommendation on Response to ICCVAM on Four *In Vitro* Ocular Toxicity Test Methods

**Ballot Vote Due:** JAN 2 9, 2008

The attached memorandum from the Health Sciences Directorate summarizes the recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for the use of four *in vitro* ocular toxicity test methods. The staff recommends that the Commission accept the ICCVAM recommendations and instruct staff to so inform ICCVAM by letter.

Please indicate your vote.

I. Accept the ICCVAM recommendations and instruct the staff to so inform ICCVAM by letter.

_____ _____
(Signature) (Date)

II. Reject the ICCVAM recommendations and instruct the staff to so inform ICCVAM by letter.

_____ _____
(Signature) (Date)

Attachment - *Staff Response to the ICCVAM Recommendations on Four InVitro Ocular Toxicity Test Methods for Determining Ocular Corrosives and Severe Irritants*, memorandum from Cassandra Prioleau, Ph.D., Directorate for Health Sciences, to the Commission, January 2008.

CPSA [illegible] 11/24/08
MC [illegible]
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EXCEPTED BY: PETITION RULEMAKING ADMIN. PROG
WITH PORTIONS REMOVED:



NOTE: This document has not been reviewed or accepted by the Commission.
Initial [illegible] Date 1/22/08

UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
WASHINGTON, DC 20207

**Memorandum**

Date: JAN 2 2 2008

TO : The Commission
Todd A. Stevenson, Secretary

THROUGH: Patricia M. Semple, Executive Director
Lowell F. Martin, Acting General Counsel

FROM : Robert J. Howell, Acting Assistant Executive Director
Office of Hazard Identification and Reduction
Cassandra Prioleau, Ph.D., Pharmacologist
Directorate for Health Sciences

SUBJECT : Staff Response to the ICCVAM Recommendations on Four *In Vitro* Ocular Toxicity Test Methods for Determining Ocular Corrosives and Severe Irritants

This memorandum discusses the recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for the use of four *in vitro*[1] ocular toxicity test methods: 1) the isolated rabbit eye (IRE) test, 2) the isolated chicken eye (ICE) test, 3) the bovine corneal opacity and permeability (BCOP) test, and 4) the hen's egg test – chorioallantoic (HET-CAM). In addition, information is provided on whether these alternative methods are acceptable in the regulatory context for the purpose of classification for labeling under the Federal Hazardous Substances Act (FHSA) (15 U.S.C. 1261-1278).

## I. Introduction

### A. Background

The National Institutes of Health Revitalization Act of 1993 directed the National Institute of Environmental Health Science (NIEHS) to establish a method and criteria for the validation and regulatory acceptance of alternative testing methods (Public Law No. 103-43, Section 1301). To accomplish these goals, NIEHS created an ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) which was made permanent by the ICCVAM Authorization Act of 2000 (Public Law 106-545). The duties of ICCVAM are to review, optimize, and validate new, revised, or alternative test methods that encourage the reduction, refinement, or replacement of the use of animals in testing. In addition, ICCVAM is to provide test recommendations to Federal agencies and other stakeholders to facilitate appropriate interagency and international harmonization of toxicological test protocols. In 1998, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) was established to assist ICCVAM in performing the activities necessary for the validation and regulatory acceptance of alternative test methods.

[1] *in vitro* – in a test tube (i.e., non-animal)

ICCVAM submits test recommendations along with regulatory guidelines, recommendations, and regulations for a test method to Federal agencies that require or recommend acute or chronic toxicological testing. According to Public Law 106-545, these agencies should promote and encourage the development and use of alternatives to animal test methods for regulatory purposes, and ensure that any new or revised acute or chronic toxicity test method is valid for its proposed use under the mandate of the ICCVAM Authorization Act of 2000. Federal agencies have 180 days to identify any relevant test methods for which the ICCVAM test recommendations may be added or substituted, review such test recommendations, and notify ICCVAM if they will adopt the ICCVAM test recommendations.

ICCVAM forwarded four test recommendations to the Commission for action: 1) the IRE, 2) the ICE, 3) the BCOP, and 4) the HET-CAM. CPSC needs to determine if any of the proposed alternative methods (the IRE, ICE, BCOP, or HET-CAM) would be acceptable as an alternative to the *in vivo*[2] test method, the Draize test, which uses animals for testing chemicals and mixtures. The Commission needs to respond back to ICCVAM by April 28, 2008.

B. Validation of Alternative Methods

Validation of alternative methods is required before regulatory acceptance and utilization by Federal agencies. In general, for an alternative method to be considered valid it must be reliable (i.e., the toxicity predictions of test substances are repeatable within the same laboratory and reproducible across/among different laboratories) and relevant (i.e., the alternative test method is useful for measuring the biological effect of interest such as ocular injury).

The reliability and relevancy of an alternative test method can be assessed from the statistical analysis of data. The relevance of an alternative test method can be determined by comparing the performance of the alternative test to the test that it is designed to replace. Performance is typically evaluated by calculating the accuracy[3], false positive rate[4], false negative rate[5], sensitivity[6], or specificity[7] of the alternative test method. The reliability of the alternative test method can be determined from the reproducibility or variability (e.g., coefficient of variation (CV), % agreement among laboratories, etc.) of test method results within and among laboratories.

C. Federal Hazardous Substances Act Requirements

Precautionary labeling of hazardous household substances is mandated by the Federal Hazardous Substances Act (FHSA, the Act), 15 U.S.C. § 1261-1275. Under the FHSA, to be a hazardous substance, a product must present one or more of the hazards enumerated in the statute and it must have the potential to cause substantial personal injury or substantial illness during or as a result of any customary or reasonably foreseeable handling or use. A brief description of the test method used to aid in the classification of substances as hazardous substances is provided in the FHSA.

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[2] *In vivo* – in a living body
[3] Accuracy - proportion of correct outcomes
[4] False positive rate - proportion of all negative substances that are falsely identified as positive
[5] False negative rate - proportion of all positive substances that are falsely identified as negative
[6] Sensitivity – the proportion of all positive substances that are classified as positive
[7] Specificity – the proportion of all negative substances that are classified as negative

Under the FHSA, an "eye irritant means a substance that human experience data indicates is an irritant to the eye and/or means for which a positive test is obtained when tested by the method described in 16 CFR § 1500.42". To perform the eye irritancy testing, six animals are tested. A test substance is placed directly into the eye of a rabbit and after a specified period of time the eyes are evaluated for injury. If the test substance produces any signs[8] of eye injury, the animal is scored as exhibiting a positive reaction. The substance is regarded as an eye irritant if four or more of the animals exhibit a positive reaction and negative if only one rabbit exhibits a positive reaction. If only two or three animals exhibit a positive reaction, the test is repeated in a new group of six rabbits. If three or more of the rabbits in the second group of rabbits exhibit a positive reaction, the substance is regarded as an eye irritant. If only one or two animals in the second test exhibit a positive reaction, the test should be repeated for a third time in a third group of six rabbits. If any rabbit in the third group exhibits a positive response the substance is regarded as an eye irritant.

In 1984, the Commission adopted a policy to reduce the number of animals tested and minimize the pain and suffering associated with testing (49 FR 22522). Under the 1984 policy, eye irritancy testing is not performed if a product is known to be a primary skin irritant. In addition, the utilization of laboratory animals is recommended in a tiered and sequential approach to testing. In a tiered-testing strategy, the test substance is tested *in vivo* if the appropriate hazard determination cannot be made from physicochemical characteristics, expert opinion, prior human experience or animal testing. For example, if a test substance can be classified as an ocular irritant or corrosive based on its alkalinity ( in part, based on a pH greater than 11.5) or acidity (in part, based on a pH less than 2.5), then no testing in animals is needed (Young et al., 1987). The Commission also advised that topical anesthetics be applied to the eyes of test animals prior to *in vivo* testing to reduce the pain associated with testing.

Under the FHSA, additional requirements should be considered when determining whether a consumer product is a hazardous substance. The Act states that human experience takes precedence over animal data if human results differ from the results for animals (16 CFR § 1500.4). In addition, when determining if a consumer product, which is composed of a mixture of substances, is a hazardous substance, the mixture should be tested and not the individual components of the mixture because synergistic or antagonistic reactions may lead to erroneous determinations concerning the toxic, irritant, corrosive, etc. properties of the substance (16 CFR § 1500.5).

D. Current Eye Irritancy or Corrositivity Testing

Currently, if little or no hazard information is known about a consumer product, the primary method utilized to assess the potential of the product to cause eye injury is based on the method developed by Draize (Draize et al., 1944). In the Draize eye test, six rabbits are tested by placing the test substance directly into the eyes of the rabbits. The extent of eye irritancy is determined by evaluating the eyes for injury.

[8] Signs of eye injury include ulceration of the cornea, opacity of the cornea, inflammation of the iris, or if such substance produces in the conjunctivae an obvious swelling with partial eversion (state of being inside out) of the lids or a diffuse crimson-red with individual blood vessels not easily discernible.

For regulatory purposes, the Draize method allows for the categorization of substances as corrosive, mild, moderate, or severe irritants. In addition, it can identify substances that cause reversible or irreversible eye damage. The protocol developed by Draize mandated the use of at least six animals. In 1981, the Organisation for Economic Co-operation and Development[9] (OECD) adopted guidelines, Test Guideline (TG) 405, for the testing of chemicals for acute eye irritation or corrosion that are based on the Draize test method protocol, but it reduced the recommended number of rabbits from six to three (although more may be used on a case by case basis to confirm inconclusive results). TG 405 was revised in 1987 and again in 2002 to include the use of a weight-of-evidence analysis before testing in rabbits, and recommended that if testing in rabbits is necessary, it be performed in a tiered and sequential manner.

## II. Alternative *In Vitro* Tests for Eye Irritancy or Corrositivity

In 2003, the Environmental Protection Agency (EPA) nominated for evaluation by ICCVAM, four *in vitro* alternative tests to be utilized to identify potential ocular corrosives and severe irritants. After initially reviewing several *in vitro* alternative tests that could replace the Draize test method, the four tests proposed by EPA were chosen for an extensive and detailed review by NICEATM: 1) the isolated rabbit eye (IRE) test, 2) the isolated chicken eye (ICE) test, 3) the bovine corneal opacity and permeability (BCOP) test, and 4) the hen's egg test – chorioallantoic (HET-CAM).

NICEATM, with the assistance of the Ocular Toxicity Working Group (OTWG), compiled Background Review Documents (BRD) for each of the alternative test methods. The BRDs contain information about the validation status of each alternative test method. The evaluation of the validation status of the four alternative test methods was performed by reviewing existing data that was either published or that was submitted to NICEATM in response to requests for data on chemicals evaluated by *in vitro* or *in vivo* ocular irritancy test methods (69 FR 13859, 70 FR 9661).

The remainder of Section II of this memo will describe each of the tests, relevant validation and performance data, recommendations and ICCVAM conclusions.

### A. Isolated Rabbit Eye (IRE) Test

#### 1. Background

The IRE test method is proposed for identifying substances that are severely irritating or corrosive to the cornea. The advantage of this test method is that it uses eyes from rabbits which were used for other purposes such as research or food, and therefore, it should closely model the *in vivo* test system. To perform this assay, rabbit eyes are carefully mounted in specially-designed eye holders. The test substance is applied to the isolated eyes and the effects of the test substance on the eye are assessed. Corneal swelling, corneal opacity[10], the area of corneal involvement and permeability[11] are scored and the

[9] The OECD is a multilateral organization that, for one, promotes and coordinates European and international test guidelines and policies.

[10] Opacity - the amount of light transmission

[11] Permeability - the amount of dye that passes through the corneal cells

value of the scores determines the eye irritancy or corrosivity of the test substance. Additional measurements such as histological assessments of morphological alterations are also recommended.

2. Validation and Performance

For this performance analysis, the data obtained from published literature and submissions to accurately assess ocular irritancy were incomplete. Thus, predictions of ocular damage were determined based on the available data. Based on the evaluation of the limited data, the accuracy of the IRE test method was 65% or 68%, depending on how irritancy was measured (e.g., corneal swelling, corneal opacity, etc.). Additionally, the false positive rate was 38% or 56% and the false negative rate was 30% or 0%. If only mixtures were considered, the accuracy was 67%, the false positive rate was 25% and the false negative rate was 38%.

An evaluation of intra-laboratory repeatability[12] or reproducibility[13] could not be conducted for the IRE test method because there was an insufficient amount of data.

The extent of agreement among laboratories (or inter-laboratory reproducibility) in assigning the same regulatory classification for a particular substance tended to depend upon the *in vivo* classification of the substance. For substances classified as severe irritants (14 substances) by *in vivo* tests, all of the laboratories (100%) agreed on the classification, based on the IRE test method. If all of the test substances that were accurately identified (i.e., severe, non-severe, and non-irritating) by the IRE test method (28) were considered, there was 100% agreement among laboratories in the classification of 71% (20/28) of the test substances. Overall, the percentage of test substances in which there was 100% agreement among laboratories in the IRE test method classification of test substances was 59% (35/59). However, some values may be exaggerated because they are based on limited data.

Another approach used to measure inter-laboratory reproducibility is to assess the variability (e.g., CV) of a test method endpoint (e.g., corneal swelling, corneal opacity, etc.) across laboratories; CVs less than 35% are considered to be satisfactory for biologically based test methods (BRD, 2006). There was considerable variability of test substances across laboratories (e.g., CV mean 53%, median 50% for corneal swelling; mean 64%, median 43% for corneal opacity). If only substances classified *in vivo* as severe irritants were considered, the mean and median CVs decreased (e.g., mean 37%, median 36% for corneal swelling; mean 40%, median 34% for corneal opacity).

3. Recommendations for Using Alternative Method for Determining Eye Irritancy

On January 11 and 12, 2005 (first Expert Panel review meeting), a peer review panel composed of expert scientists from industry, academia and other scientific professionals

[12] Repeatability is the replication of data within the same experiment.
[13] Reproducibility is the variability or reproducibility of the results within the same laboratory and/or among different laboratories.

organized by ICCVAM, in collaboration with NICEATM, convened to review and evaluate the validation status, make recommendations for revisions, and finally comment on the usefulness and limitations of the proposed alternative tests. They concluded the following with regard to the IRE test method:

- *The IRE test method appears to be capable of identifying ocular corrosives or severe irritants in a tiered-testing strategy, but the accuracy and reliability of the test method must be corroborated using a larger number of substances.*

On September 19, 2005, the review panel reconvened (second Expert Panel review meeting) to finalize their conclusions and recommendations after additional modifications, based on the first Expert Panel meeting and public comments, were made to the proposed alternative method documents. They recommended the following:

- *The recommendation and conclusion for the IRE test are the same as stated in the first Expert Panel review meeting.*

4. ICCVAM Conclusion

In January 2006, ICCVAM finalized its conclusions and recommendations. After reviewing the BRDs, the reports of both peer review panels, and public comments, ICCVAM concluded that the IRE test cannot be considered to be a replacement for the *in vivo* rabbit eye test. However, the following conclusion was made regarding the IRE test method:

- *The IRE test method may have utility in identifying ocular corrosives, but there is not sufficient data to substantiate the use of the IRE test method for identifying ocular corrosives and severe irritants in a tiered-testing strategy.*

B. Isolated Chicken Eye (ICE) Test

1. Background

The ICE test method is proposed as a screening assay to identify the ocular corrosive and severe irritation potential of chemicals or substances. The advantage of this test method is that it utilizes chicken eyes obtained from slaughterhouses. To perform the assay, eyes are carefully dissected from a chicken head and mounted in a specially designed apparatus. The test substance is applied to the mounted eye and damage to the eye is measured. Corneal swelling, corneal opacity and dye retention are scored and the value of the score determines the eye irritancy/corrosivity of the test substance.

2. Validation and Performance

Based on the evaluation of data from a few published studies, the overall accuracy of the ICE test method was 92%, if alcohols, surfactants, and solids were excluded. Additionally, the false positive and false negative rates were 6% and 29%, respectively, if alcohols, surfactants, and solids were excluded.

If only mixtures were evaluated, the accuracy was 97%, the false positive rate was 0%, and the false negative rate was 50% (this value is based on a small subset of test substances, n=2).

The variability of the ICE test method within the same laboratory (or intra-laboratory repeatability) depended upon the *in vitro* classification of the substance. The overall CV ranged from -87% to 346%; CVs less than 35% are considered to be satisfactory for biologically based test methods (BRD, 2006). Substances that were considered to be non-irritating by the ICE test method tended to have the highest CV values. High variability of the ICE test method may be due to the small number of substances tested, differences in test methods, or the small score values (which can inflate the variability) of the test results.

There was also more variability in intra-laboratory reproducibility of test substances that were considered to be non-irritating by the ICE test method compared to irritating and severely irritating test substances.

For inter-laboratory reproducibility, there was 100% agreement among laboratories in classification (based on the ICE test method) for 75% (44/59) of the test substances. If only substances that were accurately identified as severe by the ICE test method were considered, the reproducibility decreased (100% agreement for 64% (7/11) of the test substances), but if only substances that were accurately identified as non-severe by the ICE test method were considered, the reproducibility increased (100% agreement for 85% (22/26) of the test substances).

Another approach used to measure inter-laboratory reproducibility is to assess the variability (e.g., CV) of a test method endpoint (e.g., corneal swelling, corneal opacity, etc.) across laboratories. The CV for test substances ranged from 0% to 159%. The CV range did not change significantly if only severe irritants were considered. Surfactants, heterocyclic compounds, acetate/ester, and acids exhibited more inter-laboratory variability than any other chemical class.

3. Recommendations for Using Alternative Method for Determining Eye Irritancy

On January 11 and 12, 2005 (first Expert Panel review meeting), a peer review panel composed of expert scientists from industry, academia and other scientific professionals organized by ICCVAM, in collaboration with NICEATM, convened to review and evaluate the validation status, make recommendations for revisions, and finally comment on the usefulness and limitations of the proposed alternative tests. They concluded the following with regard to the ICE test:

- *The ICE test method can be used to screen for severe or corrosive eye irritants with caution for alcohol, surfactants, and solids but criteria for validation have not been met because of limited intra- and inter-laboratory data on reliability, repeatability, and reproducibility.*

On September 19, 2005, the review panel reconvened (second Expert Panel review meeting) to finalize their conclusions and recommendations after additional modifications, based on the First expert panel meeting, public comments, submission of additional data, and a re-analysis of the data, were made to the proposed alternative method documents. They recommended the following:

- *Recommendation and conclusion for the ICE test are the same* as stated in the first Expert Panel review meeting.

4. ICCVAM Conclusion

In January 2006, ICCVAM finalized its conclusions and recommendations. After reviewing the BRDs, the reports of both peer review panels, and public comments, ICCVAM concluded that the ICE test cannot be considered to be a replacement for the *in vivo* rabbit eye test. However, the following conclusion was made:

- *There are sufficient data to substantiate the use of the ICE test method for screening ocular corrosives and severe irritants, with the exception of alcohols, surfactants, and solids, in a tiered-testing strategy using a weight-of-evidence approach.*

C. Bovine Corneal Opacity And Permeability (BCOP) Test

1. Background

The BCOP test method is proposed as the initial test in a battery of tests to evaluate the ocular irritancy of substances. The advantage of this test method is that it uses bovine eyes collected from slaughterhouses. In addition, the BCOP should closely model human response because the corneal tissue of the bovine eye is similar to the corneal tissue of the human eye. In this assay, undamaged corneas are dissected from the bovine eye and mounted in a specially designed corneal holder (Ubel holder) that has chambers which allow direct contact of the test substance with the cornea. The cornea is treated with the test substance and opacity is measured. Immediately after the opacity assay, the cornea is rinsed and exposed again with the same test substance and permeability is measured. Eye irritancy is determined from opacity and permeability scores. Additional measurements such as corneal swelling or hydration and histological assessment of morphological alterations are recommended to further assess the extent of corneal injury and whether the damage is permanent.

2. Validation and Performance

Based on the evaluation of data from published studies, the overall accuracy of the BCOP test method was 92% if alcohols, ketones and solids were excluded. Additionally, the false positive and false negative rates were 12% and 0 %, respectively, if alcohols, ketones and solids were excluded.

A study that used mixtures (e.g., shampoos, hand soap, foam bath, facial cleaner, etc.), instead of the commonly used individual components of a mixture, showed that, for

mixtures, the BCOP test method had an accuracy of 87%, a false positive rate of 7%, and a false negative rate of 25%.

The extent of agreement for the BCOP test method within the same laboratory (or intra-laboratory repeatability) in assigning the same regulatory classification for a particular substance depended upon the *in vitro* classification of the substance. For substances classified as severe irritants by the BCOP test method, the coefficient of variation was low (CV ranged from 0.1% to 30.3%); CVs less than 35% are considered to be satisfactory for biologically based test methods (BRD, 2006). In contrast, considerable variability existed within the same laboratory in their classification of test substances as mild, moderate, or non-irritants (e.g., CV ranged from 11% to 312%).

The extent of agreement among laboratories (or inter-laboratory reproducibility) in assigning the same regulatory classification for a particular substance tended to also depend upon the *in vivo* classification of the substance. For substances classified as severe irritants by *in vivo* tests, there was 100% agreement among laboratories in the classification of the test substance for 76% (13/17), 67% (4/6), or 100% (4/4) (depending on the study evaluated) of the substances that were accurately identified as severe by the BCOP test method. Overall, the percentage of test substances in which there was 100% agreement among laboratories ranged from 68% to 94%, depending on the study evaluated. The substances that did not have 100% agreement in classification among laboratories included organic solvents such as alcohols, ketones, heterocyclic compounds, and surfactants.

Another approach used to measure inter-laboratory reproducibility is to assess the variability (e.g., CV) for a test method endpoint (i.e., *in vitro* prediction) across laboratories. Specifically, the CV for severe irritants ranged from 8% to 89% and for non-severe irritants or non-irritants ranged from 17% to 4511% (test substances with ocular injury scores similar to background scores resulted in unusually high CVs and may be outliers).

3. Recommendations for Using Alternative Method for Determining Eye Irritancy

On January 11 and 12, 2005 (first Expert Panel review meeting), a peer review panel composed of expert scientists from industry, academia and other scientific professionals organized by ICCVAM, in collaboration with NICEATM, convened to review and evaluate the validation status, make recommendations for revisions, and finally comment on the usefulness and limitations of the proposed alternative tests. They concluded the following regarding the BCOP test:

- *The BCOP test method has been shown to have adequate accuracy and reliability for detecting corrosive or severe eye irritants in a tiered-testing strategy for regulatory hazard classification and labeling purposes (except for testing alcohols, ketones, and solids).*

On September 19, 2005, the review panel reconvened (second Expert Panel review meeting) to finalize their conclusions and recommendations after additional modifications, based on the First expert panel meeting, public comments, submission of additional data, and a re-analysis of the data, were made to the proposed alternative method documents. They recommended the following:

- *The recommendation and conclusion for the BCOP test are the same* as stated in the first Expert Panel review meeting.

4. ICCVAM Conclusion

In January 2006, ICCVAM finalized its conclusions and recommendations. After reviewing the BRDs, the reports of both peer review panels, and public comments, ICCVAM concluded that the BCOP test cannot be considered to be a replacement for the *in vivo* rabbit eye test. However the following conclusion was made:

- *There are sufficient data to substantiate the use of the BCOP test method for identifying ocular corrosives and severe irritants, with the exception of alcohols, ketones, and solids, in a tiered-testing strategy using a weight-of-evidence approach.*

D. Hen's Egg Test – Chorioallantoic (HET-CAM)

1. Background

The HET-CAM test method is proposed for identifying substances that are severely irritating or corrosive to the conjunctiva. The advantage of this test method is that it uses chorioallantoic membranes (CAM) from chicken embryos, a proposed model of the conjunctiva. CAMs are composed of blood vessels and proteins that are believed to mimic the response of exposures of test substances in the eye. It is believed that exposure of CAMs to toxic substances will cause damage to the CAM that is related to the damage that would be induced if the same toxic substances were placed in the eye of a rabbit. The test substance is applied to the CAM of fertilized hen eggs. Following exposure, the development of hyperemia[14], hemorrhage, and coagulations is scored and the value of the score is used to determine eye irritancy.

2. Validation and Performance

Several methods are utilized in the published literature to calculate eye irritancy scores for the HET-CAM test method. In addition, methods utilized to analyze the data also depended on the study. This inconsistency in test method protocol or analysis affected the CV values of the HET-CAM test method results.

Based on the evaluation of data from published studies, the overall accuracy of the HET-CAM test method ranged from 41% to 83%, depending on the method of analysis. Additionally, the false positive rate ranged from 0% to 91% and false negative rate from 0 % to 75%.

[14] Hyperemia – an increased amount of blood in a tissue or organ.

If only mixtures were evaluated, the accuracy ranged from 50% to 83%, the false positive rate from 0% to 33%, and the false negative rate from 0% to 75%.

The CV values for intra-laboratory repeatability and reproducibility of the HET-CAM test method ranged from 7.6% to 53% for the mean and 2.2% to 34% for the median (depending on the method of analysis). CVs less than 35% are considered to be satisfactory for biologically based test methods (BRD, 2006).

The extent of agreement among laboratories (or inter-laboratory reproducibility) in assigning the same regulatory classification for a particular substance depended highly upon the study and the analysis method. For substances classified as severe irritants by *in vivo* tests, there was 100% agreement among laboratories in the classification of the test substance for 60% to 100% of the substances that were accurately identified as severe by the HET-CAM test method, depending on the study evaluated and the method of evaluation used. If all test substances that were accurately identified by the HET-CAM test method (i.e., severe, non-severe, and non-irritating) were considered, there was 100% agreement among laboratories for 50% to 100% of the test substances. Overall, the percentage of test substances in which there was 100% agreement in regulatory classification ranged from 45% to 82%.

Another approach used to measure inter-laboratory reproducibility is to assess the variability (e.g., CV) for a test method endpoint (i.e., irritancy score) across laboratories. The CV for substances classified as severe irritants by the HET-CAM test method ranged from 8% to 95%, depending on the study and the analysis method. The CV for non-severe and non-irritant substances ranged from 0% to 1196% (test substances with ocular injury scores similar to background scores resulted in unusually high CVs and may be outliers).

3. Recommendations for Using Alternative Method for Determining Eye Irritancy

On January 11 and 12, 2005 (first Expert Panel review meeting), a peer review panel composed of expert scientists from industry, academia and other scientific professionals organized by ICCVAM, in collaboration with NICEATM, convened to review and evaluate the validation status, make recommendations for revisions, and finally comment on the usefulness and limitations of the proposed alternative tests. They concluded the following with regard to the HET-CAM:

- *The HET-CAM has been shown to be useful for the identification of severe or corrosive ocular irritants in a tiered-testing strategy but positive results must be further confirmed.*

On September 19, 2005, the review panel reconvened (second Expert Panel review meeting) to finalize their conclusions and recommendations after additional modifications, based on the first expert panel meeting and public comments, were made to the proposed alternative method documents. They recommended the following:

- *Based on newly submitted data and a re-analysis of the data, the HET-CAM may have limited utility for the identification of severe ocular irritants or corrosives although it may be useful for the identification of mild to moderate ocular irritants.*

4. ICCVAM Conclusion

In January 2006, ICCVAM finalized its conclusions and recommendations. After reviewing the BRDs, the reports of both peer review panels, and public comments, ICCVAM concluded that the HET-CAM cannot be considered to be a replacement for the *in vivo* rabbit eye test. However the following conclusion was made:

- *The HET-CAM test method may have utility in identifying ocular corrosives, but additional optimization studies are needed to reduce the false positive and false negative rates of the HET-CAM analysis method for identifying ocular corrosives and severe irritants in a tiered-testing strategy.*

## III. Related Events Regarding Ocular Toxicity Testing

Two scientific symposia, sponsored by ICCVAM, NICEATM, the European Centre for the Validation of Alternative Methods (ECVAM), and the European Cosmetic, Toiletry, and Perfumery Association (COLIPA), were held from May 11 to 13, 2005 on ocular toxicity. The two symposia ("*Mechanisms of Chemically-Induced Ocular Injury and Recovery*" and "*Minimizing Pain and Distress in Ocular Toxicity Testing*") were organized to review current awareness of the mechanisms of chemically-induced ocular injury and recovery. The objective of the symposia was to identify research needed to "advance the development of test systems necessary to meet regulatory requirements that will reduce, refine, and/or replace the use of animals." Many of the issues discussed at the May 2005 symposia fed into the second Expert Panel review meeting held in September 2005.

## IV. ICCVAM Recommendations

ICCVAM recommendations were finalized in January 2006. ICCVAM recommends utilizing the BCOP test method for identifying substances as ocular corrosive and severe irritants, with the exception of alcohols, ketones, and solids using a weight-of-evidence and tiered-testing approach. ICCVAM also recommends utilizing the ICE test method for screening ocular corrosive and severe irritants, with certain limitations (excluding alcohols, surfactants, and solids). In the tiered-testing strategy, test substances that test positive by either the BCOP or the ICE test method can be classified as ocular corrosives or severe irritants without testing in animals. ICCVAM does not recommend using the IRE or the HET-CAM test methods until more optimization studies are done.

## V. Discussion by CPSC Staff

Staff agrees that each of the *in vitro* alternative methods can provide information about damage to the eye. However, some *in vitro* tests are better at gauging damage on certain parts of the eye than on other parts. For example, the IRE, ICE, and BCOP primarily evaluate corneal injury, whereas the HET-CAM primarily evaluates damage to the conjunctiva.

Staff agrees with ICCVAM that the four alternative test methods are based on sound science and are scientifically valid for their proposed uses. However, some of the alternative test methods have not met all the criteria for validation.

## VI. Options

The Commission can vote to:

1. Accept the ICCVAM recommendations and instruct staff to draft a letter to ICCVAM indicating acceptance of its recommendations.

2. Reject the ICCVAM recommendations and instruct staff to draft a letter to ICCVAM indicating rejection of its recommendations.

## VII. Recommendations by CPSC Staff

Staff recommends accepting the ICCVAM recommendations. Thus, staff recommends utilizing the BCOP test method for identifying substances as ocular corrosive and severe irritants, with the exception of alcohols, ketones, and solids using a weight-of-evidence and tiered-testing approach. Staff also recommends utilizing the ICE test method for screening ocular corrosive and severe irritants (excluding alcohols, surfactants, and solids) with similar limitations.

Labeling of a consumer product regarding the hazards associated with that product is required by the FHSA. In order to determine the appropriate cautionary labeling for acute eye irritation or corrosion, animal testing may be necessary. However, the Commission supports minimizing the number of animals used and reducing the pain or suffering associated with animal testing and encourages the development and use of alternatives to animal test models. Thus the staff recommends that the Commission accept the ICCVAM recommendations because the alternative *in vitro* test methods encourage the reduction, refinement, or replacement of animals in testing and the data indicate that the methods are scientifically valid methods. By using BCOP and ICE in a tiered-testing strategy, corrosives and severe irritants can be labeled based upon these tests and alleviate the need to test severe irritants and corrosives *in vivo*.

Staff will draft a letter to ICCVAM indicating the Commission's actions with regard to the ICCVAM recommendations. The ICCVAM website (http://iccvam.niehs.nih.gov/home.htm) will link to the Commission website where we will post our acceptance or non-acceptance of the four *in vitro* ocular toxicity test methods for determining ocular corrosives and severe irritants. In the section of the ICCVAM website, Pertinent Regulations, Guidelines and Laws (http://iccvam.niehs.nih.gov/agencies/regs.htm), there will be an announcement of the Commission's action on the acceptance or non-acceptance of the four *in vitro* ocular toxicity test methods. Once ICCVAM receives responses from all the agencies, it will publish a Federal Register notice announcing all the agencies responses.

References

49 FR 22522. Consumer Product Safety Commission. Animal Testing Policy. 1984

69 FR 13859. Department of Health and Human Services. Second Request for Data on Chemicals Evaluated by *In Vitro* or *In Vivo* Ocular Irritancy Test Methods. 2005

70 FR 9661. Department of Health and Human Services. Request for Public Comment on the Nomination for Ocular Toxicity Test Methods and Related Activities and Request for Data on Chemicals Evaluated by *In Vitro* or *In Vivo* Ocular Irritancy Test Methods. 2005

BRD. Background Review Documents. *In Vitro* Test Methods for Detecting Ocular Corrosives and Severe Irritants. 2006
http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd.htm

Draize, J.H., Woodard, G. and Calvery, H.O. Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. *J. Pharmacol. and Exp. Therapeutics.* 82, 377–390. 1944

Expert Panel Report. Evaluation of the Current Validation Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants. 2005
http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_report.htm

ICCVAM. Test Method Evaluation Report. 2006
http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm

Young J.R., How, M.J., Walker A.P., Worth W.M.H. Classification as Corrosive or Irritant to Skin of Preparations Containing Acidic or Alkaline Substances, Without Testing on Animals. *Toxic In Vitro.* 2, 19-26. 1987