



**UNITED STATES
 CONSUMER PRODUCT SAFETY COMMISSION
 4330 EAST WEST HIGHWAY
 BETHESDA, MD 20814**

BALLOT VOTE SHEET

This document has been electronically approved and signed.

Date: February 23, 2011

TO : The Commission
 Todd A. Stevenson, Secretary

FROM : Cheryl A. Falvey, General Counsel
 Kenneth R. Hinson, Executive Director

SUBJECT : Staff Response to the ICCVAM Recommendations on Four Test Method
 Evaluation Reports Regarding Ocular Toxicity Testing

BALLOT VOTE due: March 2, 2011

The attached memorandum from CPSC staff summarizes the recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) regarding 2010 test method evaluation reports on ocular toxicity testing. Staff recommends that the Commission accept the ICCVAM recommendations, and instruct staff to so inform ICCVAM by letter, and direct staff to update the CPSC's animal testing policy to reference the ICCVAM recommendations.

Please indicate your vote.

- I. Accept the ICCVAM recommendations, and instruct staff to so inform ICCVAM by letter, and direct staff to update the CPSC's animal testing policy to reference the ICCVAM recommendations.

 Signature

 Date

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

 Signature

 Date

III. Take other action (Please specify).

(Signature)

(Date)

Attachment - Staff Response to the ICCVAM Recommendations on Four Test Method Evaluation Reports, memorandum from Leslie Patton, Ph.D., Directorate for Health Sciences, to the Commission, February, 2011.



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approved and signed.

Memorandum

Date: February 23, 2011

TO: The Commission
Todd A. Stevenson, Secretary

THROUGH: Kenneth R. Hinson, Executive Director
Cheryl A. Falvey, General Counsel

FROM: Robert J. Howell, Assistant Executive Director
Office of Hazard Identification and Reduction

Leslie Patton, Ph.D., Toxicologist
Directorate for Health Sciences

SUBJECT: Staff Response to the ICCVAM Recommendations on Three Test Method
Evaluation Reports Regarding Ocular Toxicity Testing

This memorandum discusses three of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommendations for the U.S. Consumer Product Safety Commission ("Commission" or "CPSC" or "Agency") action on the 2010 Test Method Evaluation Reports: (1) the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress in ocular safety testing; (2) the current validation status of five *in vitro* test methods proposed for identifying eye injury hazard potential of chemicals and products; and (3) the discontinuation of the use of the low volume eye test for ocular safety testing. In addition, information is provided on whether these recommendations 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). The fourth report, the current validation status of a proposed *in vitro* testing strategy for the U.S. Environmental Protection Agency ocular hazard classification and labeling of antimicrobial cleaning products, will not be reviewed and commented on by CPSC staff because the report addresses a strategy for products that are not within CPSC's jurisdiction.

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 130 1). 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. The Committee comprises representatives from 15 federal regulatory and research agencies (members from the CPSC included, prior to her retirement, ICCVAM chair Marilyn Wind, and current ICCVAM vice chair Joanna Matheson); these agencies generate, use, or provide information from toxicity test methods for risk assessment purposes. 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. 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.

In September 2010, ICCVAM forwarded four reports to the Commission with recommendation for action on: (1) the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress in ocular safety testing, (2) the current validation status of five *in vitro* test methods proposed for identifying eye injury hazard potential of chemicals and products, and (3) the discontinuation of the use of the low volume eye test for ocular safety testing. As mentioned previously, the fourth report (the current validation status of a proposed *in vitro* testing strategy for U.S. Environmental Protection Agency ocular hazard classification and labeling of antimicrobial cleaning products), was not reviewed by CPSC staff because the report addresses a strategy for products that are not within the CPSC's jurisdiction. For each action item, the CPSC must review the recommendations made by ICCVAM on acute ocular toxicity testing, and determine if the recommendations would be acceptable, particularly with respect to their compatibility with the Federal Hazardous Substances Act (FHSA). 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. The Commission needs to notify ICCVAM by Monday, March 7, 2011.

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. Typically, performance is evaluated by calculating the accuracy,¹ false positive rate,² false negative rate,³ sensitivity,⁴ or specificity⁵ 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), percentage of agreement among laboratories) 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.

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 albino rabbits are tested. A test substance is placed directly into the animal’s eye, and after a specified period of time, the eyes are evaluated for injury. If the test substance produces any signs⁶ of eye injury, the animal is scored as exhibiting a positive reaction. The substance is deemed an eye irritant if four or more of the animals exhibit a positive reaction, and the substance is deemed 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 considered an eye irritant. If only one or two

¹ Accuracy—proportion of correct outcomes.

² False positive rate—proportion of all negative substances that are falsely identified as positive.

³ False negative rate—proportion of all positive substances that are falsely identified as negative.

⁴ Sensitivity—the proportion of all positive substances that are classified as positive.

⁵ Specificity—the proportion of all negative substances that are classified as negative.

⁶ 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 of the lids, or a diffuse crimson-red with individual blood vessels not easily discernible.

animals in the second test exhibit a positive reaction, the test should be repeated a third time in a new group of six rabbits. If any rabbit in the third group exhibits a positive response, the substance is considered an eye irritant.

In 1984, the Commission adopted a policy to reduce the number of animals tested and lower 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 further advised that topical anesthetics are to be applied to the eyes of test animals prior to *in vivo* testing to reduce the pain associated with testing, which eliminated the need for restraining test rabbits, allowing them to have full mobility and access to food and water.

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 whether a consumer product that 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, and/or corrosive properties of the substance (16 CFR §1500.5).

D. Current Eye Irritancy or Corrosivity 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. The extent of eye irritancy is determined by evaluating the eyes for injury.

For regulatory purposes, the Draize method allows for the categorization of substances as corrosive, mild, moderate, or severe irritants, and 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 Organization for Economic Co-operation and Development (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 rabbits may be used on a case-by-case basis to confirm inconclusive results). TG 405 was revised in 1987, and revised 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 that would reduce the number of animals tested

In 2006, NICEATM, with the assistance of the Ocular Toxicity Working Group (OTWG), compiled Background Review Documents (BRD) for four *in vitro* alternatives to the Draize eye test: the bovine corneal opacity and permeability (BCOP) test; the hen's egg test—chorioallantoic membrane (HET-CAM); the isolated rabbit eye (IRE) test; and the isolated chicken eye (ICE) test (NICEATM 2006a, 2006b, 2006c, 2006d, 2006e). The BRDs comprised information on the validation status of each alternative test method, determined by reviewing existing published or submitted data. From these documents, ICCVAM recommended that positive results in the BCOP and ICE test methods could be used as part of a weight-of-evidence approach to identify ocular corrosives and severe irritants for certain chemical classes (ICCVAM/NICEATM 2006). As a result in 2008, U.S. federal regulatory agencies, including the CPSC, as well as the OECD, accepted ICCVAM's recommendations and adopted test guidelines for the BCOP and ICE tests. The following sections of the memo describe each of the four tests separately, the ICCVAM recommendations, and CPSC staff recommendations for Commission acceptance or rejection.

II. Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing

A. Background

Current CPSC test guidelines for animal testing seek to reduce the number of animals tested and decrease the pain and suffering associated with testing. The CPSC has published an Animal Testing Policy, “which is intended to reduce the number of animals tested to determine hazards associated with household products and to reduce any pain that might be associated with such testing” (49 FR 22522). The policy states that the CPSC and manufacturers of substances covered by the Federal Hazardous Substances Act (FHSA) “should wherever possible utilize existing alternatives to conducting animal testing [including] prior human experience, literature sources which record prior animal testing or limited human tests, and expert opinion.” The FHSA gives preference to studies based on humans over animals and states that the CPSC “resorts to animal testing only when the other information sources have been exhausted.” Under this policy, for example, a Draize assay would not be performed on a substance that is a known skin irritant.

However, not all federal agencies or international entities emphasize the routine use of such endpoints. The U.S. Environmental Protection Agency (EPA), European Commission (EC), and United Nations Economic Commission for Europe (UNECE) recognize and accept certain humane endpoints during mandatory ocular hazard testing, including severe and enduring signs of pain or distress, and irreversible eye lesions that necessitate ending testing early. However, current ocular testing guidelines, most notably the Draize rabbit eye test, underemphasize the routine use of such endpoints. Therefore, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) began an evaluation of practices and activities related to reducing, refining, and replacing the use of rabbits in the current *in vivo* eye irritation test method.

The ICCVAM Ocular Toxicity Working Group (OTWG) worked with the National Toxicology Program Interagency Center for the Evaluation of Alternative Methods (NICEATM) to prepare a background review document (BRD) of data and experiences with topical anesthetics, systemic analgesics, and humane endpoints that alleviate pain in rabbits during mandatory ocular irritation testing. Methods proposed to alleviate distress and pain in rabbits during mandatory ocular irritation testing fall under one of three categories: (1) preemptive pain management comprising the use of topical anesthetics and systemic analgesics that eliminate or reduce suffering that may occur as a result of the application process, as well as from the test substance itself; (2) routine post-treatment with systemic analgesics for pain relief; and (3) humane endpoints comprising scheduled observations, monitoring, and recording of clinical signs of distress and pain, and also of the nature, severity, and progression of eye injuries. Humane endpoints also included actions like the early termination of an experiment when suffering is considered extreme; consideration of existing ocular and dermal irritation data before committing animals to testing; and performing animal studies only when absolutely necessary.

The OTWG solicited and considered public comments and stakeholder involvement throughout the BRD evaluation process; and an independent international scientific review panel (the “Panel”) evaluated the extent to which the draft BRD addressed established validation and acceptance criteria and the extent to which the draft BRD supported ICCVAM’s draft test method recommendations. Based upon this extensive review process, ICCVAM finalized its recommendations in a Test Method Evaluation Report (TMER) submitted to federal agencies in 2010. The remainder of Section II will describe ICCVAM’s TMER, including validation and performance data and recommendations for pain management and avoidance using topical anesthetics, systemic analgesics, and humane endpoints in mandatory ocular toxicity testing.

B. Validation and Performance

ICCVAM evaluated previously published scientific literature on the use of anesthetics and analgesics in animal testing. Studies showed that the efficacy of topical ocular anesthetics (*i.e.*, applied directly to the eye) can be dependent upon a variety of factors, including the anesthetic used, the dose, the application procedure, and the species tested. ICCVAM, NICEATM, and the European Centre for the Validation of Alternative Methods (ECVAM) organized a symposium in 2005, where experts (*e.g.*, human and veterinary ophthalmologists, anesthesiologists, experts in ocular toxicity testing, and research and industrial scientists) discussed the effect of topical anesthetic pretreatment of the eye on the outcome of the Draize rabbit eye test. The consensus was that the effect would be slight, if any, and would more likely err on the side of a false positive result.

Subsequently, NICEATM evaluated effects of pretreatment of rabbits with tetracaine hydrochloride, a typical ocular topical anesthetic, on the potential ocular irritancy of 97 chemical formulations. Results indicated that pretreatments have no statistically significant impact on the severity of irritation to the eye and resulting hazard classification of the formulation. Furthermore, for most of the formulations tested, pretreatment with tetracaine hydrochloride did not affect the variability in ocular irritation responses among animals treated with the same test material or the number of days required for an ocular lesion to clear. When a difference was seen, the response with anesthetic was usually more severe, but not statistically so, an artifact that can be monitored routinely because the eye not treated with test chemical in a Draize test routinely will receive the topical anesthetic.

The efficacy of a number of drugs and drug combinations was compared in the TMER, including an extensive comparison of proparacaine and tetracaine, two common topical ocular anesthetics. Proparacaine is used commonly, and its properties (*e.g.*, onset and duration of action, dosage requirements, instillation pain) are well described for both human and animal ocular applications as are its impacts on corneal wound healing and irritant hazard classification. Compared to tetracaine, the instillation of proparacaine to the eye is considerably less painful. Similarly, the systemic analgesic buprenorphine, an opioid, has an established record in managing pain in rabbits and other small animals, with a wide safety margin. Sedation is minimal and duration of analgesia is long: six to 12 hours. Meloxicam, a nonsteroidal anti-inflammatory drug (NSAID), has well-

known analgesic properties in humans and animals. Balancing an opioid and a NSAID is an established pain management strategy in human and veterinary medicine.

Humane endpoints for animal testing are already recognized and accepted by a number of domestic agencies and international entities including the U.S. EPA, the U.S. Department of Agriculture (USDA), the European Union (EU), and the Organization for Economic Co-operation and Development (OECD). For example, OECD regulatory guidelines for humane endpoints in animal testing include: designing studies to reduce pain, distress, or suffering, consistent with the scientific objective of the study; sacrificing animals at the earliest indication of severe pain, distress, or impending death; avoiding severe pain, suffering, or death as endpoints; terminating animal studies once study objectives are achieved or once it is clear objectives will not be achieved; including knowledge about the test substance in the study design; and defining in the study protocol conditions under which humane sacrifice is warranted.

As previously mentioned, a 2005 symposium on reducing pain in ocular toxicity testing comprised an expert panel of eye experts from a variety of backgrounds. This panel collaborated on the list (shown in section II.C below) of adverse responses that could serve as early humane endpoints to terminate testing.

C. Recommendations Regarding the Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing

A balanced three-part preemptive pain management strategy comprises specific recommendations regarding the Draize rabbit eye test method made by ICCVAM and the international expert review Panel (described above). Part one involves pretreatment of animals with a topical anesthetic and systemic analgesic before applying test substances. The ICCVAM-recommended protocol calls for administration of 0.01 mg/kg buprenorphine by subcutaneous injection 60 minutes before application of the test substance, plus 1 to 2 drops per eye of 0.5 percent proparacaine hydrochloride or 0.5 percent tetracaine hydrochloride five minutes before applying the test substance. The Panel stated a preference for the use of proparacaine because it is less painful to administer than tetracaine, but ICCVAM maintained its recommendation to give the option to use tetracaine. ICCVAM suggested that this topical application could be repeated in five-minute intervals, as needed, before test substance administration, while the Panel disagreed, citing the potential for proparacaine to change the hazard classification of the test substance. As a result, ICCVAM qualified its recommendation with a warning that multiple applications could increase the severity or longevity of ocular lesions.

The second part of the pain management strategy is adherence to a routine schedule of systemic analgesia after test substance administration. ICCVAM recommended a “rescue” dose of 0.03 mg/kg buprenorphine given every eight hours, and 0.5 mg/kg meloxicam every 12 hours following test substance administration for a distressed animal. If the test animal is not in distress, ICCVAM’s protocol called for 0.01 mg/kg

buprenorphine plus 0.5 mg/kg meloxicam to be delivered subcutaneously eight hours after test article administration. If ocular lesions and/or clinical signs of pain and distress persisted after this combination dose, another dose of buprenorphine could be given at 12-hour intervals and another dose of meloxicam at 24-hour intervals.

The third part of ICCVAM's recommended pain management strategy was scheduled observations, monitoring, and recording of the nature, severity, and progression of all eye injuries. ICCVAM recommended maintaining a written record of all observations to facilitate decisions on the progression or resolution of ocular lesions. Specific methods emphasized by ICCVAM to help detect and measure ocular endpoints included fluorescein staining, slit-lamp biomicroscopy, and digital photography. The expert Panel and ICCVAM also identified specific ocular lesions indicative of a severity and irreversibility of damage that should warrant termination of studies before the end of the scheduled 21-day observation period. These included the following:

- Draize corneal opacity score of 4 (indicating the most severe irritation) that persists for 48 hours;
- corneal perforation or significant corneal ulceration including staphyloma⁷;
- 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;
- destruction of more than 75 percent of the limbus;
- depth of injury to the cornea (routinely using slit-lamp and fluorescein staining) in which corneal ulceration extends beyond superficial layers of the stroma;
- vascularization of the corneal surface (*i.e.*, pannus⁸)
- no diminishment in area of fluorescein staining and/or increase in depth of injury over time; and
- lack of re-epithelialization five days after application of the test substance.

The independent peer review Panel recommended that the last three endpoints were useful in clinical decisions of early study termination only in combination. The Panel also noted that animals should be examined at least daily so that termination decisions are made as soon as required.

In terms of future studies, ICCVAM and the expert Panel recommended that detailed injury and pain data be collected including, where possible, histopathology analysis and photographic records of injuries during routine regulatory ocular testing to help improve the pain management strategy. New animal studies should be performed only when absolutely necessary to develop new pain management strategies. Studies are also needed to determine optimal timing and dosing of systemic analgesics and topical

⁷ Protrusion of the sclera or cornea, usually lined with uveal tissue, due to inflammation.

⁸ A specific type of corneal inflammation that begins within the conjunctiva, and with time, spreads to the cornea.

anesthetics to prevent the misclassification of test substances. Finally, future studies are needed to optimize the choice of analgesic and anesthetic drugs.

D. ICCVAM Conclusions

ICCVAM finalized its conclusions and recommendations in the 2010 TMER after reviewing the background document, the conclusions and recommendations of the expert review Panel, comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and public comments. ICCVAM concluded that the Draize rabbit eye test protocol, currently used for regulatory safety assessments of potential ocular hazards, should be conducted with the modifications described and outlined above. ICCVAM emphasized the importance of continuing studies on the use of anesthetics, analgesics, and humane endpoints in ocular irritancy/corrosivity testing.

E. ICCVAM Recommendations

ICCVAM recommendations were written into the Test Method Evaluation Report and finalized in September 2010 as follows:

“ICCVAM recommended that pain management procedures should always be used to avoid or minimize pain and distress when it is determined necessary to conduct the Draize rabbit eye test for regulatory safety assessments. These procedures include the routine use of topical anesthetics, systemic analgesics, and humane endpoints.”

ICCVAM recommended that ocular safety testing protocols include a pain management procedure and schedule and identified specific ocular lesions that warrant the early termination of a Draize test.

F. Discussion by CPSC Staff

CPSC staff supports ICCVAM's recommendations. The animal testing strategy outlined in the TMER does not appear to be at odds with standard ocular hazard testing under the FHSA. In fact, many of the recommended testing strategies are standard already or are recommended procedures for ocular hazard testing under the Commission's 1984 animal testing policy (49 FR 22522). The Commission's policy is intended to reduce the number of animals tested and decrease the pain and suffering associated with assessing the potential hazards of household products for the FHSA. The utilization of laboratory animals for testing is recommended in a tiered and sequential approach. In a tiered-testing strategy, the test substance is examined *in vivo* if the appropriate hazard determination cannot be made from physicochemical characteristics, expert opinion, prior human experience, or animal testing. For example, the CPSC animal testing policy states that known skin irritants need not be tested for ocular irritancy. The CPSC animal testing policy also eliminates the use of restraints for test rabbits, allowing them full access to food and water. In addition, for topical anesthetics and systemic analgesics, the CPSC's

current Animal Testing Policy recommends two preapplications of tetracaine hydrochloride, administered 10 to 15 minutes apart, for all rabbit eye testing.

Staff agrees that the TMER presented sufficient evidence on the scientific validity of these test methods and that ICCVAM's specific recommendations regarding future testing will ensure these procedures continue to be fine-tuned and optimized. In summary, CPSC staff agrees that the specific pain management and prevention procedures recommended by ICCVAM in its 2010 TMER should be adopted.

G. 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 and direct staff to update the CPSC's animal testing policy to reference the ICCVAM recommendations; or
2. Reject the ICCVAM recommendations, and instruct staff to draft a letter to ICCVAM indicating rejection of its recommendations.

H. Recommendations by CPSC Staff

CPSC staff recommends accepting the ICCVAM suggestions for pain management procedures. Staff recommends the three-tiered strategy for reducing and alleviating animal suffering during ocular hazard testing: (1) pretreatment with a topical anesthetic and systemic analgesic; (2) a routine schedule of systemic analgesia that depends on the severity of the animal's response to the test substance; and (3) scheduled observations, monitoring, and recording of the nature, severity, and progression of all eye injuries with an early termination plan.

Labeling a consumer product for the hazards associated with that product is required by the FHSA. To determine the appropriate cautionary labeling for acute eye irritation or corrosion, *in vivo* animal testing may be necessary. However, if animal testing is needed, the Commission supports reducing the number of animals used and decreasing the pain or suffering associated with animal testing models. Thus, the staff recommends that the Commission accept the ICCVAM recommendations because the pain management strategy outlined encourages the reduction, refinement, or replacement of animals in testing, and the data indicate that the methods are scientifically valid, and therefore, ultimately protective of the public health. In addition, staff recommends that the CPSC update its animal testing policy to reflect these changes.

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 nonacceptance of the use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress in ocular safety testing. On the ICCVAM website, there will be an announcement of the Commission's

action on the acceptance or nonacceptance of the use of topical anesthetics, systemic analgesics, and humane endpoints. Once ICCVAM receives responses from all the agencies, it will publish a *Federal Register* notice announcing all the agencies' responses.

III. *In vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products: Current Validation Status

In 2003, the U.S. Environmental Protection Agency (EPA) nominated for evaluation by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), four *in vitro* alternative tests to be used 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 the EPA were chosen for an extensive review by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (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 membrane (HET-CAM) test. In 2006, NICEATM, with the assistance of the ICCVAM Ocular Toxicity Working Group (OTWG), compiled Background Review Documents (BRD) for these four alternative test methods (NICEATM 2006a, 2006b, 2006c, 2006d, 2006e). Additionally, a BRD summarizing the available data on the Cytosensor® Microphysiometer (CM) test method (an *in vitro* test for chemicals which measures changes in cellular metabolism) was obtained from the European Centre for the Validation of Alternative Methods (see Curren et al. 2008). The BRDs comprised information on the validation status of each alternative test method. Test methods were determined by reviewing published or submitted data. Based upon this 2006 review, ICCVAM recommended that positive results in the BCOP and ICE test methods could be used as part of a weight-of-evidence approach to identify ocular corrosives and severe irritants for certain chemical classes (ICCVAM/NICEATM 2006). In 2008, U.S. federal regulatory agencies, including the CPSC, accepted ICCVAM's recommendations; and in 2009, the Organization for Economic Co-operation and Development (OECD) adopted test guidelines for the BCOP and ICE tests, following review by an independent, international peer-review Panel.

The 2010 Test Method Evaluation Report (TMER) contains ICCVAM's recommendations regarding the BCOP, HET-CAM, ICE, and IRE test methods for identifying nonsevere ocular irritants and substances not labeled as irritants, and the CM test method for identifying corrosives and severe irritants and substances not labeled as irritants under the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), EU, FHSA, and EPA ocular irritancy classification systems.⁹ The remainder of Section III of this memo will describe briefly the tests, the relevant validation and performance data, the Panel's recommendations, and ICCVAM's conclusions. This memo will not discuss the data presented in the 2006 ICCVAM TMER of alternative *in vitro* methods because they were reviewed and acted upon by the CPSC in January and February 2008 (see response at <http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/transmit/CPSCResponse.pdf>).

⁹ The FHSA ocular hazard classification system is a binary system that does not distinguish between ocular corrosives/severe irritants and less severe irritants. For this reason, test methods were evaluated only with respect to the FHSA for their ability to distinguish compounds that would be considered "Not Labeled" as irritants.

A. Bovine Corneal Opacity and Permeability Test Method

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 BCOP assay uses isolated bovine cornea to predict irritation, as measured by corneal opacity and permeability. The BCOP test should closely model human response because the corneal tissue of the bovine eye is similar to the corneal tissue of the human eye. Another advantage of this test method is that it uses bovine eyes collected from slaughterhouses; therefore, animals are not being slaughtered for the express purpose of ocular testing. Undamaged corneas are dissected from the bovine eye and mounted in a specially designed corneal holder (Ubel holder) that has chambers allowing for 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. The *in vitro* irritation score (IVIS) is determined from opacity and permeability scores, which categorize the hazard of the substances. The BCOP classification scheme is listed below in Table 1, along with the corresponding FHSA binary classification scheme.

Table 1: Ocular Irritancy Classification Scheme for the BCOP Test Method and Corresponding FHSA Classification (from NICEATM 2006a)

<i>In vitro</i> Score Range	BCOP Classification	FHSA Classification
0–3.0	Not Labeled	Not Labeled
3.1—25	Mild Irritant	Irritant
25.1–55	Moderate Irritant	
≥55.1	Severe Irritant	

As noted above, ICCVAM concluded in 2006 that the BCOP test cannot be considered a replacement for the *in vivo* rabbit eye test, but it can be used 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 (ICCVAM/NICEATM 2006).

2. Validation and Performance

In the current evaluation, ICCVAM assessed the validation status of the BCOP test method for the purpose of identifying nonsevere ocular irritants and substances not labeled as irritants. These included substances that induce reversible ocular damage (*i.e.*, EPA Category II and III, EU R36, GHS Category 2A and 2B) and substances not labeled as irritants (*i.e.*, EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems. ICCVAM also reassessed the use of BCOP as a screening test for the identification of severe irritants and corrosives (*i.e.*, EPA Category I, EU R41, GHS Category 1).

The current BCOP validation database contains 211 substances, representing a wide variety of chemical and product classes, including 135 commercial products or formulations. The overall correct classifications ranged from 49 percent (91/187) to 55 percent (102/187), depending on whether the EPA, EU, or GHS classification system was used when evaluating the entire database. Accuracy of the BCOP test method to distinguish substances not labeled as irritants from all other ocular hazard categories ranged from 64 percent (76/118) to 83 percent (148/179, 155/187, or 161/194), depending on the classification system under comparison. The rates of false positives (*i.e.* overpredictions of irritancy) were high (53 percent (24/45 or 25/47) to 70 percent (63/90)), while the false negative rates were low (0 percent (0/54 or 0/97) to 6 percent (8/142)). The rate of false negatives for the FHSA and EPA classification systems was 5 percent (6/132) and 6 percent (8/142), respectively, and included substances producing significant lesions *in vivo*.

Although the false negative rate was 0 percent (0/97) for the GHS classification scheme, the GHS does not classify as eye hazards substances that produce some corneal and conjunctival injuries. Such substances are required to be labeled "eye hazards, according to the EPA and FHSA classification systems. Prompted by these results, NICEATM formally evaluated how the GHS classification criteria for the Draize eye test compared to the classification criteria of the FHSA and EPA. Based upon evaluation of two independent datasets, NICEATM found that approximately 30 percent of substances surveyed that required eye irritation labeling under the EPA or FHSA classification criteria did not under the GHS, implying that the GHS was not sufficiently protective. As a result, ICCVAM and NICEATM proposed additional GHS categories to be considered by the international regulatory community in the near future.

In the 2010 TMER, ICCVAM performed a qualitative analysis of interlaboratory reproducibility in the ability of the BCOP to distinguish between all ocular hazard categories of the EPA, EU, and GHS and between substances not labeled as irritants and all other eye hazard categories. The FHSA scheme was not included in this part of the reliability evaluation because the performance of the BCOP was very similar under both the FHSA classification system and the EPA classification system. Data were derived from three independent studies from a total of 19 laboratories. For the first analysis, there was approximately 100 percent agreement among the laboratories for a majority of the Draize ocular corrosives and severe irritants based on all three classification systems, whether the chemicals were identified correctly or underclassified. There was also 100 percent agreement among the laboratories for most of the overpredicted "Not Labeled" substances and for at least 50 percent of the correctly identified "Not Labeled" substances.

For the second analysis of interlaboratory reproducibility, there was 100 percent agreement in the classification of 65-88% substances tested *in vitro*, depending upon the study and the classification scheme. For substances not labeled as irritants, there was 100 percent agreement in the classification of 50 to 100 percent of these substances. All laboratories agreed on 83 to 96 percent of all other irritant class substances, depending upon the study and which classification system was used.

3. Recommendations Regarding the BCOP Method

ICCVAM and the independent peer review Panel concluded that the accuracy and reliability of the BCOP test method does *not* support its use as a screening test to distinguish substances not labeled as irritants from all other hazard categories when results are to be used specifically to classify and label substances under the EPA, EU, FHSA, or GHS classification systems. All positive results from these tests would require additional testing in a valid test system that can characterize accurately whether such substances require hazard labeling (*i.e.*, the Draize eye test).

ICCVAM deferred its final recommendation on the usefulness of using the BCOP test method as a screening test to identify substances not labeled as irritants according to the GHS classification system. The decision to defer its final recommendation was based upon the disconnect discovered between this system and that of the EPA and FHSA. ICCVAM will revisit this recommendation if the GHS eye hazard classification criteria are updated.

Because of the high rate of false negatives, ICCVAM did not recommend use of the BCOP in identifying moderate or mild ocular irritants, as defined by the EPA, EU, or GHS classification systems.

ICCVAM and the independent review Panel also agreed that BCOP can continue to be used as a screening test to identify severe irritants and ocular corrosives as a part of a tiered testing strategy, for which ICCVAM recommends the updated BCOP protocol described in Appendix B to the 2010 TMER. This strategy calls for a follow-up *in vivo* study when a negative result is obtained via BCOP. A positive result does not require further testing.

For improving BCOP as a tool to identify ocular irritants, ICCVAM recommended additional studies to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants, as well as additional studies to further assess BCOP's reliability and accuracy. Specifically, ICCVAM proposed that histopathological evaluation of the corneal tissue be included in the BCOP. Finally, ICCVAM requested that users of the BCOP test method provide all data that are generated in order to further characterize the usefulness and limitations of the BCOP test method in identifying all ocular hazard categories.

4. ICCVAM Conclusions

In 2010, ICCVAM finalized its conclusions and recommendations in the TMER after reviewing the BRD, the conclusions and recommendations of the expert review Panel, comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and public comments. ICCVAM concluded that the BCOP test method should *not* be used as a screening test to distinguish substances not labeled as irritants (*i.e.*, EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not

Classified) from all other hazard categories (*i.e.*, EPA Category I, II, and III; EU R41 and R36; FHSA Irritant; GHS Category 1, 2A, and 2B) when results are to be used specifically to classify and label substances under the EPA, EU, FHSA, or GHS classification systems. Likewise, the BCOP test method is not recommended for identifying reversible eye irritants (*i.e.*, EPA Category II and III; EU R36; GHS Category 2A and 2B), as defined by the EPA, EU, and GHS classification systems.

B. Cytosensor® Microphysiometer Test Method

1. Background

The Cytosensor Microphysiometer (CM) test method indirectly measures the metabolic activity of mouse L929¹⁰ cells exposed to an increasing series of test substance concentrations (Curren et al. 2008). Decision criteria for ocular hazard classification for the CM assay are based on the MRD₅₀,¹¹ where a low value indicates a severe irritant, and a high value indicates a mild or nonirritant because it takes *less* of a highly hazardous chemical to produce an adverse effect. An advantage of the CM test method is that its endpoint is a reversible cell change, which may be more appropriate for assessing ocular irritation potential than cell death. Also, good correlations have been found between results of the CM and *in vivo* eye irritation data.

In the current report, the CM test method was evaluated by ICCVAM as an *in vitro* alternative to the Draize rabbit eye test for identifying ocular corrosives and irritants (*i.e.*, EPA Category I, EU R41, GHS Category 1) and substances not labeled as irritants (*i.e.*, EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified). Note that because the FHSA system only distinguishes between irritants and nonirritants, evaluating the CM test method as a screen for severe irritants/corrosives is not possible using the FHSA classification system.

2. Validation and Performance

Performance of the CM test method depends on the type of chemical under evaluation and the hazard classification. For 53 water-soluble surfactants¹²/surfactant-containing products, the CM ocular classification matched that of the Draize eye test for 66 percent to 93 percent of the substances not labeled as irritants, depending on which classification scheme was used. The FHSA-20 percent¹³ and FHSA-67 percent systems had overall

¹⁰ A cell line originally derived from mice.

¹¹ The concentration of test material that reduces cellular metabolic rate to 50 percent of the control rate.

¹² A compound that lowers the surface tension of a liquid. Examples include: detergents, wetting agents, emulsifiers, foaming agents, and dispersants.

¹³ Because the FHSA classification system is based on a sequential testing strategy that uses up to 18 animals, only a small percentage of the substances in the database would be classifiable if the FHSA criteria were strictly applied. To maximize the number of substances included in these analyses, “proportionality” criteria were applied for the purpose of assigning an FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy. Under the criterion FHSA-20 percent for example, 20 percent of the animals must show a positive response for the substance to be labeled an irritant.

accuracies of 92 percent and 93 percent, respectively, for identifying surfactants not labeled as irritants. There was one false negative under both the EPA and FHSA-20 percent classification systems; the false positive rate ranged from 50 percent to 69 percent. For distinguishing ocular corrosives and severe irritants from all other ocular hazard categories, the overall accuracy for surfactants ranged from 85 percent to 94 percent, with false positive rates of 3 percent to 10 percent and false negative rates of 9 percent to 22 percent.

For 29 water-soluble nonsurfactants, the CM results were accurate for distinguishing 63 percent to 76 percent of the substances not labeled as irritants. The FHSA-20 percent and FHSA-67 percent systems had overall accuracies of 64 percent and 63 percent, respectively, for identifying nonsurfactants not labeled as irritants. False negative rates ranged from 24 percent to 40 percent, and false positive rates were 25 percent to 40 percent. For distinguishing ocular corrosives and severe irritants from all other ocular hazard categories, the overall accuracy for nonsurfactants ranged from 79 percent to 92 percent, with a false positive rate of 0 percent for all classification systems and false negative rates of 29 percent to 50 percent.

The coefficients of variation (CVs) for MRD₅₀ values for two different studies performed in the same laboratory were 10 percent to 24 percent. This intralaboratory reproducibility was slightly higher for surfactants than nonsurfactants.

Mean CVs calculated to assess interlaboratory reproducibility ranged from 16 percent to 37 percent for surfactant substances and up to 51 percent for nonsurfactant substances. For surfactant materials, all four laboratories had 100 percent agreement for 55 percent (6/11) of the test substances; 75 percent of the laboratories had identical results for 27 percent (3/11) of the test substances; and 50 percent of the laboratories had agreement for 18 percent (2/11) of the test substances. For nonsurfactant substances, agreement among the laboratories was 100 percent for 48 percent (11/23) of the test substances, 75 percent for 22 percent (5/23) of the test substances, 67 percent for 4 percent (1/23) of the test substances, and 50 percent for 13 percent (3/23) of the test substances.

3. Recommendations for Using the Cytosensor Microphysiometer Test Method

ICCVAM recommended that the CM test method be used only as a screening test to distinguish substances that are not labeled as irritants from all other hazard categories under the EPA, FHSA, and EU classification systems, limited to water-soluble surfactants and surfactant-containing cosmetics and personal care products (but not pesticides). The CM test method was not recommended for this purpose for water-soluble *nonsurfactant* substances. Also, until the issues associated with the GHS classification system are overcome (see discussion under BCOP section), ICCVAM deferred final recommendations on the benefits and limitations of using the CM test method as a screening test to identify substances not labeled as irritants according to the GHS classification system.

In addition, ICCVAM recommended the CM as a screening test to identify water soluble substances (surfactants, nonsurfactants, and surfactant-containing formulations) as ocular corrosives and severe irritants in a tiered-testing strategy as part of a weight-of-evidence approach. Under this approach, a substance testing negative in the CM method would still need to be tested in the Draize rabbit eye test to ensure that the result is not a false negative and to distinguish mild from moderate ocular irritants.

The CM test method INVITTOX Protocol 102 was the proposed method for both of the above-mentioned screening tests. For the specific substance types not compatible with the CM test method (*i.e.*, water-soluble substances that are not identified as ocular corrosives and severe irritants or water-soluble surfactants and the aforementioned types of surfactant-containing formulations that are not identified as nonirritants) the Draize test must be performed. In addition, because of the high rate of false positives for identifying substances not labeled as irritants, users wishing to make this identification *first* may not want to use the CM test method.

Finally, ICCVAM recommended that additional studies be considered and undertaken to expand the applicability domain of the CM test method for the identification of the types of substances discussed here. Additional research should include the development of a list of reference substances for assessing utility of the CM test method as a screening test and optimization studies directed toward increasing the performance of the CM test method for identifying all levels of irritation.

The independent peer review Panel agreed that the CM test method could be used as a screening test to identify water-soluble surfactant substances as ocular corrosives and severe irritants and substances not labeled as irritants in a tiered-testing strategy as part of a weight-of-evidence approach. However, the Panel expressed concern that the CM test method will not be used widely because of the limited availability of the instrument.

4. ICCVAM Conclusions

In 2010, ICCVAM finalized its conclusions and recommendations in the TMER after reviewing the BRD, the conclusions and recommendations of the expert review Panel, comments from ICCVAM's SACATM, and public comments. ICCVAM concluded that the CM test method can be used as a screening test to distinguish water-soluble surfactant chemicals and certain types of surfactant-containing formulations that are not labeled as irritants (*i.e.*, EPA Category IV; EU Not Labeled; and FHSA Not Labeled) from all other hazard categories (*i.e.*, EPA Category I, II, III; EU R41, R36; FHSA Irritant) when results are to be used specifically for hazard classification and labeling purposes under the EPA, EU, and FHSA classification systems. A substance that tests positive in a CM test identifying nonirritants would require additional testing in a valid test system that can characterize accurately whether it requires hazard labeling (*i.e.*, the Draize test). ICCVAM further concluded that the accuracy and reliability of the CM test method support its use as a screening test to identify water-soluble substances (water-soluble surfactants, surfactant-containing formulations, and nonsurfactants) as ocular corrosives

and severe irritants (EPA Category I, EU R41, GHS Category 1) in a tiered-testing strategy as part of a weight-of-evidence approach.

C. The Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) Test Method

1. Background

The HET-CAM test method is proposed for identifying substances that are severely irritating or corrosive to the conjunctiva. This test method 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. In the HET-CAM test, a substance is applied to the CAM of fertilized hen eggs, after which the development of hyperemia, hemorrhage, and coagulations is scored, and the value of the score is used to determine eye irritancy. 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.

Previously, ICCVAM evaluated the validation status of the HET-CAM test method as an *in vitro* alternative to the Draize rabbit eye test to identify ocular corrosives and severe irritants (*i.e.*, EPA Category I, EU R41, GHS Category 1) and determined that the reproducibility and accuracy were not sufficient to support use of the HET-CAM for this purpose (ICCVAM 2006).

2. Validation and Performance

In the present TMER, ICCVAM evaluated HET-CAM for its ability to identify nonsevere/reversible ocular irritants (*i.e.*, EPA Category II and III, EU R36, GHS Category 2A and 2B) and substances not labeled as irritants (*i.e.*, EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems. ICCVAM reviewed HET-CAM classifications as compared to the Draize rabbit eye test for each classification system (EPA, EU, and GHS) using each of the six HET-CAM protocols (IS[A], IS[B], Q-Score, S-Score, IS, and ITC), and determined that IS(A) was the most valid.

No new data were available for validation of the HET-CAM test method since the 2006 ICCVAM TMER. The HET-CAM database consists of 260 substances representing a wide range of chemicals. The overall accuracy of classification for all categories of ocular irritation ranged from 38 percent (23/60) to 41 percent (24/59) depending on the classification system used. The overall accuracy for the identification of substances not labeled as irritants from all other categories ranged from 62 percent (36/58) to 80 percent (44/55), with false positive and false negative rates ranging from 60 percent (9/15) to 69 percent (22/32) and 0 percent (0/26 or 0/36) to 9 percent (4/45 or 4/47), respectively. Under the FHSA system, HET-CAM had an overall accuracy of 78 percent to 80 percent for identifying substances not labeled as irritants. An assessment of the accuracy of

HET-CAM in identifying mild or moderate irritants in the EPA, EU, and GHS systems was hindered by the small database of chemicals in those categories.

Because the database has not changed, the analysis of HET-CAM test method reliability is the same as in the 2006 report. In some additional qualitative analyses of HET-CAM's interlaboratory reproducibility for identifying all ocular hazard categories according to the EPA, EU, and GHS systems, there was very high to complete agreement among laboratories when assigning ratings of severe irritation/corrosivity, moderate, mild, or nonirritation under all three classification systems. For identifying substances not labeled irritants, there was also high interlaboratory agreement (≥ 76 percent) for the EPA, EU, and GHS classification systems.

3. Recommendations Regarding the HET-CAM Test Method

ICCVAM and the independent peer review Panel did not recommend the HET-CAM test method for the identification of substances not labeled irritants when results are to be used for EPA, FHSA, EU, or GHS hazard classifications. Nor did they recommend the HET-CAM assay for the identification of moderate and mild ocular irritants, or for the screening and identification of severe irritants and corrosive ocular irritants in a tiered-testing strategy as part of a weight-of-evidence approach.

ICCVAM's recommended HET-CAM test method protocol is the IS(A) analysis, a more detailed version of the old HET-CAM protocol. Evaluation of several HET-CAM protocols indicated that the IS(A) analysis protocol performed best on substances not labeled as irritants.

ICCVAM recommended that additional studies be conducted to further optimize the HET-CAM test method decision criteria that would be used to identify substances in each of the ocular hazard categories. The Panel did not support additional studies for using the HET-CAM test method to identify all categories of ocular irritants. ICCVAM also encouraged experimentation with a wider range of chemical substances in the HET-CAM assay. Finally, to further optimize the test protocol, ICCVAM encouraged test users to generate and share all data from HET-CAM test runs for the identification of all ocular hazard categories.

4. ICCVAM Conclusions

ICCVAM finalized its conclusions and recommendations in the 2010 TMER after reviewing the BRD, the conclusions and recommendations of the expert review Panel, comments from SACATM, and public comments. ICCVAM concluded that the scientific validity of the HET-CAM test method has been evaluated adequately, and HET-CAM is *not* recommended as a screening test to distinguish substances not labeled irritants (*i.e.*, EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other hazard categories (*i.e.*, EPA Category I, II, III; EU R41, R36; GHS Category 1, 2A, 2B) or to identify moderate and mild ocular irritants (*i.e.*, EPA Category II, III; EU R36; GHS Category 2A, 2B) when results are to be used specifically

for hazard classification and labeling purposes under any of the hazard classification systems.

D. The Isolated Chicken Eye (ICE) Test Method

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. Eyes are dissected from a chicken head, mounted in a specially designed apparatus, and exposed to the test substance. Corneal swelling, opacity, and dye retention are scored, and the value of the score determines the eye irritancy/corrosivity of the test substance.

In 2006, ICCVAM evaluated the validation status of the ICE test method as an *in vitro* alternative to the Draize rabbit eye test to identify ocular corrosives and severe irritants and determined that the reproducibility and accuracy were sufficient to support its use for this purpose for some types of substances. Regulatory agencies in the United States and international organizations have adopted this test method for this purpose.

2. Validation and Performance

In the present evaluation report, ICCVAM assessed the validation status of ICE for its ability to identify nonsevere ocular irritants (*i.e.*, those that induce reversible eye damage, such as EPA Category II and III, EU R36, and GHS Category 2A and 2B), as well as substances not labeled eye irritants (*i.e.*, EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified). No new ICE data were obtained for the validation database since the previous ICCVAM report was issued in 2006.

The overall correct classifications ranged from 59 percent (83/141) to 77 percent (118/153), depending upon the classification system used. Substances not labeled irritants were identified correctly for 78 percent (110/141) to 85 percent (130/153), with a high false negative rate of 6 percent to 22 percent and a false positive rate of 11 percent to 34 percent. The false negative rate was 9 percent (7/76) for the FHSA-67 percent system and 12 percent (10/84) for the FHSA-20 percent system.

Quantitative and qualitative evaluations of ICE test method reliability were conducted in the previous TMER. Additional qualitative analysis of interlaboratory reproducibility was conducted on data from four laboratories based on: (1) the use of the ICE test method for identifying all ocular hazard categories according to the EPA, EU, or GHS systems; and (2) the use of the ICE test method to distinguish substances not labeled as irritants. Using the first approach, there was 100 percent agreement among the four laboratories for a majority of the Draize ocular corrosives and severe irritants for all three classification systems. The interlaboratory reproducibility was lower for the lower irritation classes. There was 100 percent agreement for at least 50 percent of moderate irritants and for 0 percent to 13 percent of correctly identified mild irritants. The majority

of substances not classified as irritants were overclassified, but consistently so across the laboratories, with at least 75 percent agreement for 76 percent of the EU Not Labeled substances. Using the second approach, there was 100 percent agreement for 61 percent to 75 percent of substances in the Balls et al. (1995) study. Laboratories agreed 100 percent on 81 percent of substances correctly identified as irritants (Categories I, II, and III) under the EPA system. None of the EPA Category IV substances was identified correctly by the ICE test method, but there was 75 percent agreement by the laboratories in this overclassification. A similar pattern of reproducibility was observed for the other two classification systems.

3. Recommendations for Using the ICE Test Method for Determining Eye Irritancy

ICCVAM and the independent peer review Panel did *not* recommend the ICE test as a screening test to distinguish substances not labeled as irritants from other hazard categories when results are to be used for EPA, FHSA, EU, or GHS hazard classifications and labeling. Nor did they recommend the ICE assay for the identification of moderate and mild ocular irritants. As reported in the 2006 ICCVAM TMER, the ICE test is acceptable for the screening and identification of severe irritants and corrosive ocular irritants in a tiered-testing strategy as part of a weight-of-evidence approach, where a negative result would require a follow-up Draize eye test.

An updated ICE test method protocol appended to the TMER is recommended by ICCVAM and the independent peer review Panel.

ICCVAM and the Panel recommended additional studies to further optimize the ICE test method decision criteria for identifying substances in the moderate, mild, and Not Labeled ocular hazard categories. ICCVAM and the Panel also recommended that histopathological evaluations of corneal tissue accompany the ICE test method for more accurate classifications. Finally, to further optimize test protocol, ICCVAM encouraged users to generate and provide all data from ICE test runs for the identification of all ocular hazard categories.

4. ICCVAM Conclusions

ICCVAM finalized its conclusions and recommendations in the 2010 TMER after reviewing the BRD, the conclusions and recommendations of the expert review Panel, comments from SACATM, and public comments. ICCVAM concluded that the scientific validity of the ICE test method has been evaluated adequately and it did *not* recommend ICE as a screening test to distinguish substances not labeled as irritants (*i.e.*, EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other hazard categories (*i.e.*, EPA Category I, II, III; EU R41, R36; GHS Category 1, 2A, 2B), nor to identify moderate and mild ocular irritants (*i.e.*, EPA Category II, III; EU R36; GHS Category 2A, 2B) when results are to be used specifically for hazard classification and labeling purposes under any of the hazard classification systems. ICCVAM maintained its previous conclusion that the ICE test method can be used as an *in vitro* alternative to the Draize rabbit eye test to identify ocular corrosives/severe

irritants (*i.e.*, EPA Category I, EU R41, and GHS Category 1) for some types of substances.

E. The Isolated Rabbit Eye (IRE) Test Method

1. Background

The IRE test method is proposed for identifying substances that are severely irritating or corrosive to the cornea. To perform this assay, the test substance is applied to rabbit eyes mounted in specially designed holders, and the effects of the test substance on the eye are assessed. Four endpoints are scored for the evaluation of ocular irritancy and corrosivity: corneal swelling, corneal opacity, the area of corneal involvement, and permeability. Additional measurements, such as histological assessments of morphological alterations, are also recommended. The advantage of this test method is that it uses eyes from rabbits that were sacrificed for other purposes, such as research or food.

In 2006, ICCVAM evaluated the validation status of the IRE test method as an *in vitro* alternative to the Draize rabbit eye test to identify ocular corrosives and severe irritants. ICCVAM determined that the test's reproducibility and accuracy were not sufficient to support its use for this purpose.

2. Validation and Performance

In the present TMER, ICCVAM attempted to evaluate the validation status of IRE for its ability to identify nonsevere ocular irritants (*i.e.*, those that induce reversible eye damage, such as EPA Category II and III, EU R36, and GHS Category 2A and 2B), as well as substances not labeled as eye irritants (*i.e.*, EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified). The available validation database, which has not changed since the 2006 ICCVAM evaluation of 149 substances, was insufficient in making this determination. Data from single studies using all four recommended IRE endpoints did not specify criteria decisions for any classification beyond severe irritant/corrosive.

3. Recommendations for Using the IRE

Because the available validation database has not changed since the original ICCVAM evaluation of the IRE test method to identify and classify irritants, the original recommendations, as presented to the federal regulatory agencies in 2006, did not change. Briefly, these included the rejection of the IRE test method for screening and identifying ocular corrosives and severe irritants and all ocular hazard categories.

The IRE test protocol also did not change from the previous TMER. ICCVAM maintained its recommendation that additional studies should be undertaken and shared to increase the current IRE database and further validate this test method. ICCVAM also recommended that a histopathological evaluation of the corneal tissue be included in the IRE test method.

The independent peer review Panel drew similar conclusions and recommended development of specific standard procedures for eye handling and test article administration.

4. ICCVAM Conclusions

ICCVAM finalized its conclusions and recommendations in the 2010 TMER after reviewing the BRD, the conclusions and recommendations of the expert review Panel, comments from SACATM, and public comments. ICCVAM concluded that the IRE test cannot be considered a replacement for the *in vivo* rabbit eye test and should not be used to classify substances by ocular irritancy.

F. ICCVAM Recommendations for the *In vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products

ICCVAM recommendations for *in vitro* test methods for identifying eye injury hazard potential were written into the TMER and finalized in September 2010, as follows:

“ICCVAM recommended that the Cytosensor Microphysiometer (CM) test method can be used as a screening test to identify some types of substances that may cause permanent or severe eye injuries. ICCVAM also recommended that the CM can be used to determine if a limited range of substances will not cause sufficient injury to require hazard labeling for eye irritation. ICCVAM evaluated four other in vitro test methods for their usefulness and limitations for identifying substances with the potential to cause reversible and nonsevere ocular injuries and substances that do not require ocular hazard labeling. ICCVAM concluded that the performance of these methods must be improved before they can be used in regulatory safety testing to classify such substances. The report includes ICCVAM recommendations for future studies that could potentially improve these test methods.”

ICCVAM did not recommend the BCOP, HET-CAM, IRE, or ICE test methods—in their present manifestations—for use in regulatory safety testing to classify substances as having the potential to cause reversible, nonsevere eye injuries, or as not requiring hazard labeling for eye irritation. The BCOP and ICE tests, as per ICCVAM’s 2006 TMER, are recommended for identifying certain substances as ocular corrosive and severe irritants, using a weight-of-evidence and tiered-testing approach.

ICCVAM deferred final recommendations on the usefulness of certain test methods as screening tests to identify substances not labeled as irritants according to the GHS classification system based on the disconnect between this system and that of the EPA and FHSA. ICCVAM will revisit this recommendation if the GHS eye hazard classification criteria are updated.

G. Discussion by the CPSC Staff

Staff agrees that each of the *in vitro* alternative methods can provide information about damage to the eye but that some tests are more accurate and reliable at this task, depending on the level of ocular hazard, the type of test substance being evaluated, and the classification scheme in question. The FHSA classification system is binary, dividing all substances into *FHSA Irritant* or *FHSA Not Labeled*. Therefore, ICCVAM's recommendation to use the CM test method as a screening test to distinguish certain substances as not labeled as irritants from all other hazard categories is of particular interest to the CPSC. CPSC staff agrees that the reliability of the CM test method is sufficient to support its use as a screening test to distinguish water-soluble surfactant chemicals and certain types of surfactant-containing formulations not labeled as irritants from other hazard categories for hazard classification and labeling. The false negative rate for this application of the CM was low for all classification systems. However, the false positive rate was high (50 percent for the FHSA classification system), and came from a small dataset (n=6 for FHSA). Therefore, staff believes that using the CM test method to distinguish between the two FHSA classifications may not be particularly useful until more data are available for this validation.

Indeed, the CM test method is proposed "*as a screening test to identify... substances not labeled as irritants.... However testing in another test method would be necessary for ... water-soluble surfactant chemicals and specific types of surfactant-containing formulations that are not identified as substances not labeled as irritants.*" In other words, testing positive for irritancy using the CM test has a high (50 percent according to the existing dataset) chance of being a false positive under the FHSA classification system. In this event, the substance would have to be retested using the Draize rabbit eye test.

If a substance tests negative for irritation using the CM test, no further testing would be necessary, and the substance could be classified *FHSA Not Labeled* or the corresponding category under one of the other three classification schemes.

The other ICCVAM recommendations, which deal with differentiating levels of irritancy, are important to the mission of ICCVAM but not germane to the FHSA classification scheme, which only has two categories: irritant or not irritant. Nevertheless, staff agrees with ICCVAM's conclusions on these points.

H. 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.

I. Recommendations of CPSC Staff

Staff recommends accepting the ICCVAM recommendations for the four *in vitro* alternatives to the Draize eye test because the alternative *in vitro* test methods encourage the reduction, refinement, or replacement of animals in testing, and the data indicate that the methods recommended by ICCVAM are scientifically valid. By using the CM test method to identify certain types of substances that are not irritants, fewer animals will be needed for testing. However, a positive result with the CM test would require some further *in vivo* testing, as discussed above. Staff also maintains its recommendations contained in the previous response to ICCVAM's review of *in vitro* alternative test methods (<http://www.cpsc.gov/library/foia/foia08/brief/ocular.pdf>), which recommended using the BCOP and ICE test methods as screening tests in a tiered-testing strategy to identify corrosives and severe irritants. This will also decrease the number of test animals used and will alleviate the need to test severe irritants and corrosives *in vivo*.

Staff will draft a letter to ICCVAM indicating the Commission's actions regarding 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 nonacceptance of using alternative *in vitro* tests for ocular safety testing. On the ICCVAM website, there will be an announcement of the Commission's action on the acceptance or nonacceptance of the use of *in vitro* test methods proposed for identifying eye injury hazard potential of chemicals and products. Once ICCVAM receives responses from all the agencies, it will publish a *Federal Register* notice announcing all the agencies' responses.

IV. Discontinuation of the Low-Volume Eye Test for Ocular Safety Testing

A. Background

The low volume eye test (LVET) is an *in vivo* test method that assesses the potential ocular irritancy of a test substance. It was developed as an alternative to the Draize rabbit eye test, which had been criticized for overpredicting actual hazard; in some instances, Draize results indicate that innocuous substances are severe ocular irritants or corrosives. The LVET was proposed as a more accurate representation of accidental ocular exposure in humans in terms of exposure volume and duration. In the Draize test, 100- μ L of test substance is administered to the conjunctival sac, and eyelids are held closed for one second. In the LVET, a 10- μ L volume is exposed directly to the rabbit's cornea, and the eyes are not forced closed. Quantification of corneal, iridal, and conjunctival lesions is the same in the two assays. However, the LVET is not considered to be an adequately valid *in vivo* reference test method; to date, no regulatory agency has formally adopted it.

ICCVAM reviewed the validity of the LVET because this protocol was being used to validate an *in vitro* ocular irritancy test method intended for use with antimicrobial cleaning products (AMCPs). As previously summarized, in 2008, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and ICCVAM's Ocular Toxicity Working Group (OTWG) received a background review document (BRD) from the U.S. Environmental Protection Agency (U.S. EPA) Office of Pesticide Programs (OPP) of an *in vitro* testing strategy for the evaluation, categorization, and labeling of ocular hazards of AMCPs. In response, ICCVAM and NICEATM produced a summary review document of the LVET, detailing its current validation status, as well as their recommendations. This document was reviewed by an independent expert review Panel and the public, and ICCVAM produced the Test Method Evaluation Report (TMER) under consideration here. The remainder of section IV presents results of the TMER.

B. Validation and Performance

LVET's classification of ocular irritants underpredicts severe irritants and corrosives compared to the Draize eye test. For instance, one comparison found that the LVET underpredicted 60 percent of U.S. EPA Category I (severe irritant/corrosive) substances as Category III (mild irritants). A similar comparison and finding were made with respect to GHS hazard classification. However, the performance of corrosives and moderate to severe irritants in the LVET is not well described; only a few personal and household cleaning products have been tested for ocular irritation using this protocol, and those were all chemically similar substances. Comparative human data from clinical studies and accidental exposures are available for mild or nonirritating substances only, and often these do not have accurate descriptions of amount and duration of exposure. Therefore, the usefulness of these data is limited too. Thus, while the LVET is thought to better model exposure conditions when a substance enters the human eye accidentally, there are limited data to indicate whether it can identify accurately the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries.

C. Recommendations for the Low Volume Eye Test Method

Because ICCVAM could not recommend the LVET as a complete replacement for the Draize rabbit eye test, it recommended discontinuing its use in ocular safety testing altogether. When animals are required in ocular testing, ICCVAM instead recommends the Draize rabbit eye test modified to include anesthetics, analgesics, and humane endpoints, as described under section II of this memo. ICCVAM also recommended that LVET data *not* be used in the validation of alternative *in vitro* ocular toxicity methods, as noted under section IV of the memo with respect to *in vitro* testing of antimicrobial cleaning products.

ICCVAM encouraged companies with LVET data to share them for use in a weight-of-evidence approach to ocular safety testing.

The independent peer review Panel did not make a recommendation or draw a conclusion with respect to the validation status of the LVET citing insufficient data.

D. ICCVAM Conclusions

In 2010, ICCVAM finalized its conclusions and recommendations in the TMER after reviewing the response of the expert review Panel, comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and public comments. ICCVAM concluded that the LVET is not a suitable replacement for the Draize rabbit eye test and therefore should not be used in ocular testing beyond a weight-of-evidence approach. ICCVAM concluded instead that, in the cases where *in vivo* testing is required, analgesics, anesthetics, and humane endpoints should be used in an otherwise standard Draize rabbit eye test.

E. ICCVAM Recommendation to Discontinue Use of the Low-Volume Eye Test for Ocular Safety Testing

The ICCVAM recommendation to discontinue use of the low volume eye test for ocular safety testing was written into the Test Method Evaluation Report and finalized in September 2010, as follows:

“ICCVAM recommended that a proposed low volume rabbit eye test should not be used for regulatory testing due to performance issues when compared to the current standard rabbit eye test.”

F. Discussion by the CPSC Staff

Staff agrees that, because the LVET is not a complete replacement of the Draize eye test, it should be discontinued for use in regulatory ocular safety testing. Based on a dual goal of reducing animal suffering and maintaining the highest standard for public safety, the

better choice when *in vivo* testing is required is the Draize rabbit eye test, modified to include pain management/reduction strategies, such as the use of topical anesthetics, systemic analgesics, and humane endpoints.

G. 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.

H. Recommendations of CPSC Staff

Staff recommends accepting the ICCVAM recommendation to discontinue use of the low volume eye test based on the conclusions stated above.

Staff will draft a letter to ICCVAM indicating the Commission's actions regarding 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 nonacceptance of the recommendation to discontinue the low-volume eye test for ocular safety testing. On the ICCVAM website, there will be an announcement of the Commission's action on the acceptance or nonacceptance of the recommendation to discontinue use of the LVET for ocular safety testing. Once ICCVAM receives responses from all the agencies, it will publish a *Federal Register* notice announcing all the agencies' responses.

V. Overall Recommendations by CPSC Staff

CPSC staff recommends accepting the ICCVAM recommendations regarding pain management procedures. Staff recommends the three-tiered strategy for reducing and alleviating animal suffering during ocular hazard testing, including: pretreatment with a topical anesthetic and systemic analgesic; a routine schedule of systemic analgesia, which depends on the severity of the animal's response to the test substance; and scheduled observations, monitoring, and recording of the nature, severity, and progression of all eye injuries, with an early termination plan. In addition, staff recommends that the CPSC update its animal testing policy to reflect these changes.

Staff further recommends accepting the ICCVAM recommendations for the four *in vitro* alternatives to the Draize eye test because the alternative *in vitro* test methods encourage the reduction, refinement, or replacement of animals in testing, and the data indicate that the methods recommended by ICCVAM are scientifically valid. Specific recommendations include: using the Cytosensor Microphysiometer test method to identify certain types of substances that are not irritants, as well as continuing use of the BCOP and ICE test methods as screening tests in a tiered-testing strategy to identify corrosives and severe irritants.

Finally, CPSC staff recommends accepting the ICCVAM recommendation to discontinue use of the low-volume eye test.

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