United States
Consumer Product Safety Commission

OS 1

Staff's original briefing memo was updated on 7/17/23 in accordance with the Commission vote on 7/14/23.

This document has been electronically approved and signed.

Ballot Vote Sheet

TO: The Commission
Alberta E. Mills, Secretary

DATE: July 5, 2023

THROUGH: Austin C. Schlick, General Counsel
Jason K. Levine, Executive Director

FROM: Daniel R. Vice, Assistant General Counsel, Regulatory Affairs

SUBJECT: OECD Test Guideline No. 496: In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

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BALLOT VOTE DUE: Tuesday, July 11, 2023

The Federal Hazardous Substances Act, 15 U.S.C. §§ 1261-1275, requires that hazardous substances bear cautionary statements on their labels. Manufacturers may perform toxicological tests to determine whether products require cautionary labeling. In 2022, the Commission adopted guidance outlining CPSC's procedures for evaluating alternative test methods in place of animal testing.[1] Any such alternative test method, if accepted by the Commission, will be considered a reliable test method for evaluating compliance with certain FHSA labeling requirements. The Commission's guidance states that acceptance of a test method is not irrevocable; subsequent data and experience with the test method may lead to a loss or affirmation of its acceptability status.

Atul Jhalani, President of InVitro International, contacted CPSC staff requesting a review of the Organisation for Economic Co-operation and Development Test Guideline No. 496: "In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals not Requiring Classification for Eye Irritation or Serious Eye Damage." This test guideline is an animal-free methodology to identify substances that can induce serious eye damage and eye irritation.

Staff has reviewed the request and recommends that the Commission accept the use of OECD Test Guideline 496 in toxicological testing of consumer products for eye irritation and

[1] https://www.cpsc.gov/FAQ/CPSCs-Policy-on-Animal-Testing

U.S. Consumer Product Safety Commission
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Bethesda, MD 20814

National Product Testing and Evaluation Center
5 Research Place
Rockville, MD 20850

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Ballot Vote Sheet

damage, as part of a tiered testing and risk assessment strategy, as described in the accompanying staff memorandum.

Please indicate your vote on the following options:

I. Approve the use of OECD Test Guideline No. 496 as an acceptable method for toxicological testing of consumer products for eye irritation and damage as part of a tiered testing and risk assessment strategy.

_____ _____
(Signature) (Date)

II. Do not approve the use of OECD Test Guideline No. 496 as an acceptable method for toxicological testing of consumer products for eye irritation and damage.

_____ _____
(Signature) (Date)

III. Take other action specified below.

_____

_____

_____

_____

_____ _____
(Signature) (Date)

Attachment: Staff recommendation on the acceptance or rejection of the OECD Test Guideline No. 496: “In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals not Requiring Classification for Eye Irritation or Serious Eye Damage”

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National Product Testing and Evaluation Center
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This briefing memo was revised on July 17, 2023 in accordance with the Commission vote on 7/14/2023.

United States
Consumer Product Safety Commission

Memorandum

TO: The Commission
Alberta E. Mills, Secretary

DATE: July 17, 2023

THROUGH: Austin C. Schlick, General Counsel
James K. Levine, Executive Director
DeWane Ray, Deputy Executive Director for Operations

FROM: Duane Boniface, Assistant Executive Director
Office of Hazard Identification and Reduction
John D. Gordon, Ph.D., Project Manager
Division of Toxicology and Risk Assessment, Directorate for Health Sciences

SUBJECT: Staff recommendation on the acceptance or rejection of the OECD Test Guideline No. 496: "In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals not Requiring Classification for Eye Irritation or Serious Eye Damage" submitted to CPSC for review.

Atul Jhalani, President of InVitro International, contacted CPSC staff requesting a review of the Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 496: "In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals not Requiring Classification for Eye Irritation or Serious Eye Damage" (OECD 2019).[1] OECD TG 496 is a New Approach Method (NAM) that is intended to be used to identify substances that can induce serious eye damage and/or eye irritation. In this memorandum, CPSC staff responds to the request for review, using the new CPSC Guidance (CPSC 2022), "Guidance for Industry and Test Method Developers: CPSC Staff Evaluation of Alternative Test Methods and Integrated Testing Approaches and Data Generated from Such Methods to Support FHSA Labeling Requirements."

This memorandum will cover applicability of the method for use in conforming CPSC requirements; the reliability and relevancy of the method; and limitations of use, in the context of support for labeling under the Federal Hazardous Substances Act (FHSA) (15 U.S.C. §§ 1261-1278).

[1] https://www.oecd-ilibrary.org/environment/test-no-496-in-vitro-macromolecular-test-method-for-identifying-chemicals-inducing-serious-eye-damage-and-chemicals-not-requiring-classification-for-eye-irritation-or-serious-eye-damage_970e5cd9-en

In the "Staff Recommendations" at the end of this memo, staff recommends that CPSC follow the recommendations set forth by the OECD and Interagency Coordinating Committee on the Validation of Alternative Methods reviews. Staff recommends that OECD 496 may be used as part of a tiered testing and risk assessment strategy.

Advantages of this method include a simple, animal-free methodology, using standard equipment that most laboratories will have, and applicability domain areas (e.g., solids) not covered by existing eye testing methods listed in the CPSC Animal Testing Policy webpage.

Staff further recommends that the CPSC Animal Testing Policy web page be updated to reflect the staff recommendations.

Introduction

In 2012, CPSC issued an updated policy related to toxicity testing in animals, which strongly encourages non-animal or alternative testing methods to support labeling requirements in the FHSA (codified 16 C.F.R. § 1500.232).[2] The policy encourages using scientifically validated alternatives to animal testing and using a weight-of-evidence[3] analysis evaluating existing information, including prior human experience, prior animal testing results, and expert judgment to determine whether a product constitutes a hazard under the FHSA. Accordingly, since CPSC's animal testing policy has been in place, toxicologists in CPSC's Directorate for Health Sciences are tasked with reviewing alternative test methods and resulting data provided by manufacturers to assess whether the test methods and data are scientifically valid and defensible to support a product's labeling under the FHSA.[4] CPSC's animal testing policy website[5] lists alternative test methods that are currently accepted by CPSC for specific conditions of use, including two eye toxicity tests: Bovine Corneal Opacity and Permeability (BCOP) (OECD TG 437) (OECD 2017a), and the Isolated Chicken eye (ICE) (OECD TG 438) (OECD 2017b).

Under the FHSA, manufacturers must evaluate household products to determine whether such products present a hazard to consumers during reasonably foreseeable handling and use; and, if so, manufacturers must provide with the products, precautionary labeling to address the hazard (FHAS 2011, CPSC 2012).[6]

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[2] https://www.cpsc.gov/FAQ/CPSC-Policy-on-Animal-Testing

[3] Weight-of-evidence approach means expert consideration of all available data and information, with evaluation of strengths, limitations, and relevance of each study and information source, to determine relative support for hypotheses or answers to questions.

[4] For example, under the FHSA, manufacturers must evaluate household products to determine whether they require precautionary labeling to address the hazards associated with their handling or use. When manufacturers present data from non-animal or alternative methods to CPSC in support of a FHSA labeling determination, such data is first sent to the Office of Compliance. If Compliance requires a technical evaluation, Compliance sends the information to Health Sciences for their input.

[5] https://www.cpsc.gov/FAQ/CPSC-Policy-on-Animal-Testing

[6] https://www.cpsc.gov/Business--Manufacturing/Business-Education/Business-Guidance/FHSA-Requirements

"Corrosive" and "irritant" are two of the six hazards defined under the FHSA in sections 2(i) and 2(j) (15 U.S.C. § 1261(i) and (j), respectively; and restated in 16 C.F.R. § 1500.3(b)(7) and (b)(8)):

> (i) *The term ''corrosive'' means any substance which in contact with living tissue will cause destruction of tissue by chemical action; but shall not refer to action on inanimate surfaces.*
>
> (*j) The term ''irritant'' means any substance not corrosive within the meaning of subparagraph (i) which on immediate, prolonged, or repeated contact with normal living tissue will induce a local inflammatory reaction.*

The following supplemental definitions of corrosive and irritant, located in the FHSA at C.F.R. § 1500.3(c)(3) and (c)(4), respectively, interpret and supplement the statutory definitions. These supplemental definitions provide information on the extent of injury (*e.g.,* irreversible changes or reversible changes) to differentiate between when a substance is corrosive or an irritant. They also provide information on data types to consider when assessing whether a substance is corrosive or irritant.

> *Corrosive means a substance that causes visible destruction or irreversible alterations in the tissue at the site of contact. A test for a corrosive substance is whether, by human experience, such tissue destruction occurs at the site of application. A substance would be considered corrosive to the skin if a weight-of-evidence analysis suggests that it is corrosive, or validated in vitro test method suggests that it is corrosive, or if, when tested by the in vivo technique described in § 1500.41, the structure of the tissue at the site of contact is destroyed or changed irreversibly in 24 hours or less. Other appropriate tests should be applied when contact of the substance with other than skin tissue is being considered. A substance could also be labeled corrosive based on the outcome of any of the approved test methods described in the CPSC's animal testing policy set forth in 16 CFR 1500.232, including data from in vitro or in silico test methods that the Commission has approved; or a validated weight-of-evidence analysis comprising all of the following that are available: Existing human and animal data, structure activity relationships, physicochemical properties, and chemical reactivity data.* 16 C.F.R. § 1500.3(c)(3)
>
> *Irritant includes primary irritant to the skin, as well as substances irritant to the eye or to mucous membranes. Primary irritant means a substance that is not corrosive and that human experience data indicate is a primary irritant; and/or means a substance that results in an empirical score of five or more when tested by the method described in 1500.41; and/or a substance that can be considered a primary irritant based on the outcome of any of the approved test methods described in the CPSC's animal testing policy set forth in 16 CFR 1500.232, including data from in vitro or in silico test methods that the Commission has approved; or a validated weight- of-evidence analysis comprising all of the following that are available: existing human and animal data, structure activity relationships, physicochemical properties, and chemical reactivity data. Eye irritant means a substance that human experience data indicate is an irritant to the eye; and/or means a substance for which a positive test is obtained when tested by the method described in 1500.42; and/or means a substance that can be considered an eye irritant based on the outcome of any of the approved test methods described in the CPSC's animal testing*

> *policy set forth in 16 CFR 1500.232, including data from in vitro or in silico test methods that the Commission has approved; or a validated weight-of-evidence analysis comprising all of the following that are available: existing human and animal data, structure activity relationships, physicochemical properties, and chemical reactivity data.* 16 C.F.R. § 1500.3(c)(4)

Other groups also define corrosion similarly. For example, the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) defines skin corrosion as the production of irreversible damage to the skin, manifested as visible necrosis through the epidermis and into the dermis, following the application of a test chemical (United Nations 2023).

Depending on the purpose and context, testing for the corrosivity or irritancy of chemicals or products may be conducted using laboratory animals, New Approach Methods (NAMs), or a combination of approaches, to identify substances that potentially pose this hazard to humans. Test results are then used to classify and label substances with regard to this potential hazard to consumers and ensure appropriate precautionary labeling. Traditionally, chemical safety testing for the eye is performed using the Draize method (Draize 1944), as modified by Kay and Calandra (Kay & Calandra 1962). This procedure involves applying the substance under evaluation under the lower eyelid of an albino rabbit and is the method described in 16 C.F.R. § 1500.42. Observations and signs of toxicity are recorded for the cornea, iris, and conjunctiva at regular time intervals. If the test substance produces any signs of eye injury, the animal is scored as exhibiting a positive reaction. The substance is regarded as an eye irritant if four or more out of six of the animals exhibit a positive reaction and is considered negative if no more than one rabbit exhibits a positive reaction. Testing is repeated up to a third round, if fewer than four animals are positive.

Under the CPSC animal testing policy, eye irritancy testing is not performed if a product is already known to be corrosive or irritant to the skin. Furthermore, the policy recommends a weight-of-evidence analysis to evaluate existing information before any irritation testing using animals is considered. This analysis should incorporate any existing data on humans and animals, validated *in vitro* or *in silico* test data, data on the substance's dermal corrosivity/irritation, evidence of eye irritation of one or more structurally related substances or mixtures of such substances, data demonstrating high acidity or alkalinity of the substance, and any other relevant physicochemical properties that indicate the substance might be a dermal corrosive/irritant or eye irritant. If the weight of evidence is insufficient to determine a substance's potential eye irritation, a Commission-approved *in vitro* or *in silico* assay for eye irritancy should be run to assess irritation potential and determine labeling, if available and appropriate for the substance. If no valid *in vitro* test exists, the test strategy for determining dermal corrosion/irritation outlined in the CPSC animal testing policy can be followed to determine eye irritation. If the dermal test strategy leads to a conclusion of not corrosive, a tiered *in vivo* eye irritation test should be performed, in which a single rabbit is exposed to the substance initially.

The GHS and the Environmental Protection Agency (EPA) both define eye hazard classification systems differently though both employ the Draize method (Draize, 1944; Kay, 1962). The GHS includes Category 1 (serious eye damage or corrosion with irreversible effects on the eye) and Category 2 (eye irritation/reversible effects on the eye). For authorities who use the GHS classification system and who want more than one designation for reversible eye irritation,

where data are sufficient, substances can be classified in Category 2A or 2B based on observation of effects reversing within 21 days or 7 days, respectively.

The EPA classification includes hazard categories I (corrosive), II (moderate irritant), III (mild irritant, includes lesions that persist for 24 hours), and IV (no significant damage 24 hours after exposure, but may include adverse eye effects that occur prior to 24 hours but then clear below an acceptable limitation by 24 hours). EPA specifies that the classification is determined by the single most severe animal response and in accordance with the guidelines in the Label Review Manual (US EPA 2003), and the test methods described in the Acute Eye Irritation Health Effects Test Guideline (US EPA 1998).

Staff notes that both the GHS and EPA eye hazard classification systems include alternative test methods, with the *in vivo* Draize test conducted as a last resort. The GHS suggests a tiered approach consisting of existing human and animal data, followed by Defined Approaches[7] and *in vitro* or *ex vivo*[8] data for eye effects, existing data on skin corrosion from human, laboratory animal, *in vitro*, or *ex vivo* studies, other existing animal skin or eye data, extreme pH and acid/alkaline reserve, and finally non-test methods.

Variability in results of the Draize eye test is well known and can result in misclassification errors (Barroso et al. 2017). Multiple studies have demonstrated that the largest variability in Draize test results is with mild and moderate irritants (Earl 1997, Gettings 1998, York 1998, Balls 1999, CPSC 2013). One study showed at least 11 percent of GHS Category 1 substances were also identified as Category 2; similarly, about 12 percent of Category 2 substances were not classified as irritants (Adriaens et al. 2014). The variability in results is believed to be due to the technical performance of the test and the subjective nature of the visual evaluations of effects. Furthermore, most of the studies were performed before the introduction of Good Laboratory Practices (GLP). In a European Union analysis, even if alternative methods were perfectly reproducible, the variability in the Draize test to which they are being compared results in moderate correlation between Draize test results and results of alternative methods (e.g., correlation coefficients ranging from 0.65 to 0.80) for weaker to moderate irritants (Balls 1999, Earl 1997). These authors recommended before initiating a validation study of a proposed NAM to assess whether the NAM was mechanistically relevant and to decide how much variability is acceptable.

Validation of NAMs

Reliability and relevance testing, sometimes known as validation testing, of alternative methods is recommended before evaluation for regulatory acceptance and use 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

[7] Defined approaches consist of a rule-based combination of data obtained from a predefined set of different information sources (e.g., *in chemico* methods, *in vitro* methods, physico-chemical properties, non-test methods).

[8] ex vivo refers to experimentation or measurements done in or on tissue from an organism in an external environment with minimal alteration of natural conditions.

measuring the biological effect of interest, such as sensitization, irritation, or corrosion) (OECD 2005, CPSC 2022).

The reliability and relevancy of an alternative test method can be assessed from the statistical analysis of data produced by the method. 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,[9] false positive rate,[10] false negative rate,[11] sensitivity,[12] and specificity[13] (as well as any other relevant endpoints) of the alternative test method. The reliability of the alternative test method can be determined from the reproducibility of test method results within and among laboratories. For further information on these concepts, see the "Guidance for Industry and Test Method Developers: CPSC Staff Evaluation of Alternative Test Methods and Integrated Testing Approaches and Data Generated from Such Methods to Support FHSA Labeling Requirements" (CPSC 2022).

OECD Test Guideline 496

A. Introduction

OECD Test Guideline 496[14] (OECD 496) is an *in vitro* method that can be used to identify potential eye irritants. This method is based on macromolecular damage following test chemical exposure. The OECD 496 protocol includes a pre-screen assessment to identify test chemicals that are outside the applicability domain of the test method, and therefore not suitable for evaluation using this method. Other preliminary tests are used to determine the optimal test procedure specific to the chemicals of interest. The test system contains a macromolecular reagent composed of a mixture of proteins, glycoproteins, carbohydrates, lipids, and low molecular weight components, that when rehydrated forms a complex macromolecular matrix which mimics the highly ordered structure of the transparent cornea of the eye. Test substances presenting an eye hazard will produce turbidity (cloudiness) of the matrix by causing disruptions in the proteins, as well as disruption and disaggregation of the matrix components. The test method determines if a substance is an eye irritant by measuring and comparing chemical models for three variables:

1. Damage to the corneal stroma (water-soluble molecules)
2. Damage to phospholipid bilayers (water-insoluble molecules), and
3. The potential to induce pH extremes in a system (pH buffering system of the eye).

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[9] Accuracy - proportion of correct outcomes among all test results.
[10] False positive rate - proportion of all negative substances that are falsely identified as positive.
[11] False negative rate - proportion of all positive substances that are falsely identified as negative.
[12] Sensitivity –in the context of evaluating a method by comparing test results to known information about chemicals, sensitivity is defined as the probability of a positive test result given a true positive. In the context of assessing the technical capabilities of a method, sensitivity refers to the ability of laboratory instrument or other analytical method to detect low signals or concentrations of a substance being measured.
[13] Specificity - the probability of a negative test result given a true negative.
[14] https://www.oecd-ilibrary.org/environment/test-no-496-in-vitro-macromolecular-test-method-for-identifying-chemicals-inducing-serious-eye-damage-and-chemicals-not-requiring-classification-for-eye-irritation-or-serious-eye-damage_970e5cd9-en

The OECD 496 method addresses a broad range of potential mechanisms of chemical injury. The test can be performed by an entry-level technician familiar with using a pH meter, pipette, and spectrophotometer. The method is available as a test kit that includes all necessary reagents and most consumables. Kits have at least a 1-year shelf life.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) published a validation report on the OptiSafe method in 2020 (Choksi et al., 2020). The ICCVAM Validation Management Team (VMT) monitored all phases of the validation study and selected the substances tested. The National Toxicology Program provided coded (blinded) vials of the selected test substances to the lead lab and two naïve laboratories. Three laboratories (Lebrun Labs (lead lab), NICEATM, and ILS) participated in the inter-laboratory validation study. After training, each lab was instructed to test the five Phase I (demonstration of competency) coded vials in triplicate. After the demonstration of competency was completed, the results were presented to the VMT, which then recommended proceeding with Phases II (accuracy and transferability) and III (application domain). For the accuracy and transferability study, 30 coded substances and kits were sent to each lab to complete the study; the kits were from three distinct lots so that lot-to-lot variability could be determined. For the application domain study, the testing of 60 coded substances was performed in triplicate by the lead lab only. During the testing phases, each lab sent their data and results directly to the VMT as weekly updates. Their results included both a summary spreadsheet with EPA and GHS classification predictions and raw data. Prior to the receipt of coded vials, the lead lab opted to select and test 16 additional un-coded substances (these data are provided as “retrospective data”).

B. Accuracy

For the ICCVAM validation study, Phase I tested five substances in an effort to demonstrate that the method could be transferred to naive laboratories. Thirty coded substances were evaluated in Phase II of the validation study to demonstrate both intra- and inter-laboratory reproducibility. Phase III evaluation of an additional 60 substances by the lead laboratory provided a comprehensive assessment of test method accuracy and defined the applicability domain of the method. The following table summarizes the results of the study:

Table 1. Summation of ICCVAM Validation study looking at reproducibility, accuracy, false negative and false positive rates.

Study Phase	Measurement Calculated	Measurement Determination	
Phase I	Within Lab Reproducibility	93% - 100%	
Phase II	Intralaboratory Reproducibility	93% - 99%	
	Interlaboratory Reproducibility	91%	
		EPA	GHS
	Accuracy	82% - 88%	78% - 88%
	False Negative	0% - 7%	0% - 15%
	False Positive	25%	23%
Phase II and III	Accuracy	83%	79%
	False Negative	4%	0%
	False Positive	40%	42%

Note: Table adapted from data in Choksi 2020. All terms in this table are defined on CPSC's Animal Testing Page https://www.cpsc.gov/FAQ/CPSCs-Policy-on-Animal-Testing and in the "Guidance for Industry and Test Method Developers: CPSC Staff Evaluation of Alternative Test Methods and Integrated Testing Approaches and Data Generated from Such Methods to Support FHSA Labeling Requirements": https://www.cpsc.gov/s3fs-public/Guidance-for-Industry-and-Test-Method-Developers-CPSC-Staff-Evaluation-of-Alternative-Test-Methods-and-Integrated-Testing-Approaches.pdf?VersionId=6EJxcMXMu4PzZEQFQivF3AUZODrMRK5J

Recent studies by Lebrun et. al. (2023), intending to optimize the OptiSafe™ method have shown that modifying the procedure by adding of 0.1 mg/mL ascorbic acid to the testing solution resulted in reducing the false positive rate from 40% to 22.2%.

The EURL-ECVAM Scientific Advisory Committee (ESAC) conducted a validation study between 2009 and 2012, using substances known to induce serious eye damage (GHS Category 1) (ESAC 2016). ESAC followed their validation study with an independent peer-review in 2016. A total of 89 test substances, including 13 mixtures and 76 substances, were assessed during the validation study. The tested substances covered a broad spectrum of hazard potency categories and applicability domains, including 25 solids, 57 liquids and 7 viscous test substances. The test method is applicable to solid and liquid substances whose 10% solution/dispersion (v/v or w/v as appropriate) has a pH in the range $4 \leq pH \leq 9$. The liquids may be viscous or non-viscous. Solids may be soluble or insoluble in water, as they are tested neat unless they have surfactant properties. Gases and aerosols have not been assessed yet in a validation study and are therefore outside of the applicability domain. The accuracy of the test method was compared to the *in vivo* classification (i.e., the Draize test). In the ESAC validation study, the test method was found to have an overall accuracy of 75% (66.5/89), a specificity of 81% (55.8/69), a sensitivity of 54% (10.7/20), a false positive rate of 19% (13.1/69), and a false negative rate of 44% (8.3/19), as compared to the Draize test. Any false negative in this method (*i.e.*, GHS Category 1 classified with *in vivo* data, but identified as not being GHS Category 1 by this OECD 496 test) is not a critical consideration because all test substances that come out negative are subsequently tested with other adequately validated *in vitro* test(s), or as a last option, in rabbits, depending on regulatory requirements, using a sequential testing strategy in a weight-of-evidence approach according to OECD Guidance Document (GD) 263.

ESAC also conducted a study to identify substances that do not require classification for eye irritation. Overall accuracy of 75% (67.0/89), sensitivity of 91% (41.7/46), specificity of 59%

(25.3/43), false positive rate of 41% (17.7/43), and false negative rate of 7% (3.3/45) were calculated based on a weighted approach (ESAC 2016).

C. Initial Considerations and Limitations

Substances that fail objective internal quality checks (for example, optical density that exceeded the photometric range) are classified as OAD (outside of the application domain). Of the 30 substances in the ICCVAM Phase II study, three (benzalkonium chloride [5%], sodium lauryl sulfate [3%], and acetyl pyridinium bromide [0.1%]) did not qualify for further analysis based on the results of the pre-screen protocol. These substances were identified during the pre-screen procedure as surfactants. According to the method, some surfactants may interfere with the test system. Interference may include inhibition of the proper functioning of the macromolecular matrix reflected as specific OD405 readings for sets of controls and test chemicals. Therefore these 3 compounds were considered outside the applicability domain of the OECD 496 procedure.

Several limitations have been identified for OECD 496 for substances such as intensely colored substances, substances which caused salting-out precipitation, high concentrations of some surfactants, and highly volatile substances, which may interfere with the test system. Interference may include inhibition of the proper functioning of the macromolecular matrix reflected in inaccurate spectrophotometer readings for sets of controls.

The test method is applicable to both individual substances and mixtures. When considering testing of mixtures, difficult-to-test substances (*e.g.*, unstable, and polymerizing substances such as those containing acrylates), or test substances not clearly within the applicability domain, consideration should be given to whether the results of such testing will yield results that are scientifically meaningful for FHSA labeling purposes.

OECD 496 can be used for identifying chemicals that cause serious eye damage. This method can also be used to identify chemicals that do not require classification for eye irritation or serious eye damage. OECD 496 is not recommended for the identification of test chemicals that should be classified as irritating to the eyes (*e.g.*, UN GHS Category 2, 2A or 2B). In other words, the method should not be used for identification of mild or moderate eye irritants. This is due to the considerable number of over- and under-classifications that result from testing such substances.

The OECD does not recommend OECD 496 as a stand-alone replacement for the *in vivo* rabbit eye test. However, the OECD recommends it as an initial step of a Top-Down testing strategy approach (as described within OECD GD 263) to positively identify substances inducing serious eye damage without further testing, *i.e.*, substances to be classified as UN GHS Category 1.

In the ICCVAM study, no substances classified as corrosives (*i.e.*, GHS Category 1 or EPA Category I) were mis-identified by OECD 496. These ICCVAM results support that the method can accurately classify substances that produce severe or corrosive effects.

Good Laboratory Practice Compliance

The ICCVAM validation study was conducted in compliance with the OECD GLPs. Quality control checks were performed for each run in all laboratories. Following completion of each

testing phase, quality assurance personnel from the lead laboratory audited the data collection worksheets from all participating laboratories (OECD 1998).

Staff Recommendations

Staff recommends that CPSC follow the recommendations set forth by the OECD and ICCVAM reviews. Staff recommends that OECD 496 may be used as part of a tiered testing and risk assessment strategy. In this approach, negative responses are not sufficient for determining labeling under the FHSA. A positive response would require no further testing for FHSA labeling as corrosive to the eye, unless the testing party is concerned about a potential false positive response. The method is not sufficient for labeling as an eye irritant under the FHSA.

Advantages of this method include a simple, animal-free methodology, using standard equipment that most laboratories will have, and applicability domain areas (*e.g.*, solids) not covered by existing eye testing methods listed in the CPSC Animal Testing Policy webpage.

Staff further recommends that the CPSC Animal Testing Policy web page be updated to reflect the staff recommendations.

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