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ICCVAM Test Method Evaluation Report: Identifying Chemical Eye Hazards with Fewer Animals

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

About the Interagency Coordinating Committee on the Validation of Alternative Methods and The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

In 1997, the National Institute of Environmental Health Sciences (NIEHS), one of the National Institutes of Health, established the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to:

- Coordinate interagency technical reviews of new and revised toxicological test methods, including alternative test methods that reduce, refine, or replace the use of animals
- Coordinate cross-agency issues relating to validation, acceptance, and national and international harmonization of new, modified, and alternative toxicological test methods

On December 19, 2000, the ICCVAM Authorization Act (42 U.S.C. 285*l*-3) established ICCVAM as a permanent interagency committee of NIEHS under the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

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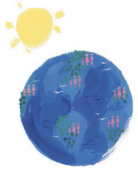

The NICEATM-ICCVAM earth-and-sun graphic symbolizes the important role of new and alternative toxicological and safety testing methods in protecting and advancing the health of people, animals and the environment.

ICCVAM Test Method Evaluation Report

Identifying Chemical Eye Hazards with Fewer Animals

Interagency Coordinating Committee on the
Validation of Alternative Methods

National Toxicology Program (NTP) Interagency Center for the
Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
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List of Abbreviations and Acronyms

CFR	U.S. Code of Federal Regulations
CPSC	U.S. Consumer Product Safety Commission
EPA	U.S. Environmental Protection Agency
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FR	*Federal Register*
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
JaCVAM	Japanese Center for the Validation of Alternative Methods
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Interagency Ocular Toxicity Working Group
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
U.S.C.	United States Code

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Preface

Each year, an estimated 2 million eye injuries occur in the United States. Of these, more than 40,000 cause permanent visual impairment (McGwin et al. 2006a). Chemicals and compounds are the third most common cause of eye injuries, with household cleaning products comprising the second leading type of product associated with consumer eye injuries (McGwin et al. 2006b). To warn consumers and workers of the potential for chemicals and products to cause eye injuries, eye safety testing is performed to determine if substances may cause temporary or permanent eye damage. Test results are then used for hazard classification of chemicals and products using appropriate national and/or international hazard classification systems.

Eye safety testing procedures vary among U.S. agencies. Current testing procedures specified in the U.S. Code of Federal Regulations (16 CFR 1500.42) require 6 animals per test and may require up to three sequential tests for each substance, thereby requiring 6, 12, or 18 animals to reach a hazard decision (CPSC 2010). The requirement for second and third sequential tests is based on the number of positive responses in the previous test.

Based on previous initiatives in the United States to reduce the number of animals used for eye safety testing, some U.S. and international test guidelines for eye irritation/corrosion testing have been modified. The maximum number of animals currently used is typically 3 (OECD 2002; EPA 1998). U.S. agencies will accept data generated in accordance with test guidelines by the Organisation for Economic Co-operation and Development (OECD) that require only 3 animals per test. However, current testing procedures (16 CFR 1500.42) do not provide criteria to classify results from 3-animal tests. Therefore, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), conducted an analysis (Haseman et al. 2011) to determine classification criteria based on results from a 3-animal test that would maintain hazard classification equivalent to that provided by current testing procedures.

ICCVAM is charged by law with reviewing and evaluating alternative methods and approaches that can reduce animal use in testing. This test method evaluation report provides ICCVAM's recommendations for using fewer animals to identify chemical eye hazards while maintaining hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42). The process for developing these recommendations began with a critical review of the analysis (Haseman et al. 2011) and existing data by the ICCVAM Interagency Ocular Toxicity Working Group (OTWG). As part of ICCVAM's ongoing international collaborations, scientists from the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) served as liaisons to the OTWG.

The analysis (Haseman et al. 2011) was provided to the Scientific Advisory Committee on the Validation of Alternative Toxicological Methods (SACATM) for comment. The public was also given the opportunity to comment. The OTWG then developed draft proposed ICCVAM recommendations regarding classification criteria based on results from a 3-animal test that would maintain hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42). The draft ICCVAM recommendations and the supporting analysis (Haseman et al. 2011) were made available on the NICEATM–ICCVAM website (http://iccvam.niehs.nih.gov) for comment by the broad stakeholder community. ICCVAM considered all public and SACATM comments before finalizing these recommendations. This ICCVAM test method evaluation report presents the recommendations and supporting analysis.

As required by the ICCVAM Authorization Act (42 U.S.C. 285*l*-3), ICCVAM will forward the recommendations to U.S. Federal agencies for consideration. Federal agencies are required to respond to ICCVAM within 180 days after receiving the ICCVAM recommendations. This report is available

to the public on the NICEATM–ICCVAM website (http://iccvam.niehs.nih.gov), and agency responses will be made available on the website as they are received.

We gratefully acknowledge the many individuals who contributed to the preparation, review, and revision of this report. We thank the OTWG for assuring a meaningful and comprehensive review. We especially thank Dr. Jill Merrill (U.S. Food and Drug Administration Center for Drug Evaluation and Research) for serving as Chair of the OTWG. Integrated Laboratory Systems, Inc., the NICEATM support contractor, provided excellent technical support, for which we thank Drs. David Allen, Elizabeth Lipscomb, Lori Rinckel, and Mr. James Truax. Finally, we thank the OTWG liaisons from our partner organizations in the International Cooperation on Alternative Test Methods for their participation in this review. Drs. João Barroso and Valerie Zuang were the liaisons from EURL ECVAM, and Dr. Hajime Kojima was the liaison from JaCVAM.

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Executive Summary

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), recently conducted an analysis to determine classification criteria that would identify chemical eye hazards with fewer animals (Haseman et al. 2011). NICEATM–ICCVAM analyzed results from 3-animal tests that would maintain eye hazard classification equivalent to that provided by current testing procedures specified in the U.S. Code of Federal Regulations (16 CFR 1500.42). Current testing procedures require 6 animals per test and may require up to three sequential tests for each substance, thereby requiring 6, 12, or 18 animals to reach a hazard decision (CPSC 2010).

In 2002, the Organisation for Economic Co-operation and Development (OECD) Test Guidelines Programme adopted U.S. proposed revisions to Test Guideline 405: Acute Eye Irritation/Corrosion (OECD 2002) to reduce the maximum number of animals required for eye hazard classification from 6 to 3. The Animal Welfare Act (7 U.S.C. 2131 et seq.) requires that only the minimum number of animals necessary to obtain scientifically valid results be used for testing. The Public Health Service Policy on Humane Care and Use of Laboratory Animals requires that a rationale for the appropriateness of the number of animals be provided to and approved by the Institutional Animal Care and Use Committee (OLAW 2002). In light of these policies and regulations, most *in vivo* eye safety testing would be expected to adhere to the 3-animal procedure described in the OECD and U.S. Environmental Protection Agency test guidelines (EPA 1998; OECD 2002). However, current testing procedures (16 CFR 1500.42) do not provide criteria to classify results obtained from a 3-animal test. Therefore, NICEATM–ICCVAM conducted an analysis to determine classification criteria based on results from a 3-animal test that would maintain hazard classification equivalent to that provided by current testing procedures. This analysis (Haseman et al. 2011) forms the basis for the ICCVAM recommendations described herein.

ICCVAM Recommendations

ICCVAM recommends that alternative *in vitro* test methods should always be considered and used where appropriate for eye safety testing. While currently approved *in vitro* test methods can identify some eye hazards (OECD 2009a, OECD 2009b), they are not sufficiently validated and accepted to completely replace all animal testing. When eye safety testing for those regulatory authorities still requiring the use of animals is necessary, testing should be conducted using the minimum number of animals in the most humane manner possible consistent with testing objectives.

ICCVAM concludes that using a classification criterion of one or more positive animals in a 3-animal test to identify chemicals and products that are eye hazards will maintain hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42 [CPSC 2010]), while using up to 50% to 83% fewer animals. ICCVAM therefore recommends consideration of the use of this classification criterion together with eye safety testing procedures that use a maximum of 3 animals per test substance. Consistent with ICCVAM's duty to foster national and international harmonization (42 U.S.C. 285*l*-3)), this recommendation also harmonizes the number of animals used for eye safety testing across U.S. regulatory agencies and international test guidelines.

Analysis Supporting the Use of Fewer Animals for Evaluating Eye Hazards

The percentage of substances that would be classified as eye irritants was calculated for each of the three different classification criteria:

- Strategy 1: current testing procedures (16 CFR 1500.42)
- Strategy 2: at least one positive animal in a 3-animal test (≥1/3)
- Strategy 3: at least two positive animals in a 3-animal test (≥2/3)

In order to compare the frequency with which each strategy would identify substances as eye irritants, NICEATM–ICCVAM examined a number of different underlying population positive response rates. (The population positive response rate is the overall likelihood that an animal will show a positive response for a given substance.) In a separate approach, a NICEATM database of 481 rabbit eye test studies was analyzed using a mixture of three binomial distributions to estimate rates of over- and underprediction for each criterion.

In each instance, a classification criterion of at least one positive animal in a 3-animal test (≥1/3) more closely matched the expected outcome based on current testing procedures (16 CFR 1500.42) than did a criterion of at least two positive animals in a 3-animal test (≥2/3), which identified far fewer irritants. These results showed that using a classification criterion of at least one positive animal in a 3-animal test (≥1/3) to identify eye hazards will provide eye hazard classification the same as or greater than current testing procedures, while using up to 50% to 83% fewer animals.

ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process incorporates a high level of transparency. This process is designed to provide numerous opportunities for stakeholder involvement, including written comments and oral comments at the public meetings of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). In finalizing this test method evaluation report and the supporting analysis (Haseman et al. 2011), ICCVAM considered comments provided by SACATM and the public.

Four different opportunities for public comments were provided during the ICCVAM evaluation process. Three public comments, which supported using fewer animals to identify chemical eye hazards, were received (**Section 4.0**). SACATM members and two *ad hoc* experts agreed that the proposed 33% positive response rate provides appropriate criteria for eye safety testing compared to current testing procedures (16 CFR 1500.42).

1.0 Introduction

In 2002, the Organisation for Economic Co-operation and Development (OECD) Test Guidelines Programme adopted U.S. proposed revisions to Test Guideline 405: Acute Eye Irritation/Corrosion (OECD 2002) to reduce the maximum number of animals required for eye hazard classification from 6 to 3. The Animal Welfare Act (7 U.S.C. 2131 et seq.) requires that only the minimum number of animals necessary to obtain scientifically valid results be used for testing. The Public Health Service Policy on Humane Care and Use of Laboratory Animals requires that a rationale for the appropriateness of the number of animals be provided to and approved by the Institutional Animal Care and Use Committee (OLAW 2002). In light of these policies and regulations, most *in vivo* eye safety testing would be expected to adhere to the 3-animal procedure described in the OECD and U.S. Environmental Protection Agency test guidelines (EPA 1998; OECD 2002). However, current testing procedures specified in the U.S. Code of Federal Regulations (16 CFR 1500.42) do not provide criteria to classify results obtained from a 3-animal test (CPSC 2010). Therefore, the National Toxicology Program Interagency Committee for the Evaluation of Alternative Toxicological Methods (NICEATM), in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), conducted an analysis to determine classification criteria based on results from a 3-animal test that would maintain hazard classification equivalent to that provided by current testing procedures. This analysis (Haseman et al. 2011) forms the basis for the ICCVAM recommendations described herein.

In accordance with the ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*-3), ICCVAM coordinates the technical evaluation of new, revised, and alternative test methods with regulatory applicability. The ICCVAM Interagency Ocular Toxicity Working Group (OTWG) worked with NICEATM in conducting the analysis. The European Union Reference Laboratory for Alternatives to Animal Testing and the Japanese Center for the Validation of Alternative Methods designated liaison members to the OTWG.

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the analysis to determine classification criteria based on results from a 3-animal test that would maintain hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42) for discussion at their meeting on June 17–18, 2010. Public stakeholders were given the opportunity to comment at the meeting. A second opportunity for SACATM and public comments was provided at the SACATM meeting on June 16–17, 2011. On August 12, 2011, ICCVAM announced the availability of the draft ICCVAM recommendations. The draft ICCVAM recommendations and the supporting analysis (Haseman et al. 2011) were posted on the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov/). A detailed timeline of the ICCVAM evaluation for identifying chemical eye hazards with fewer animals is included with this report (**Appendix A**).

ICCVAM considered all public and SACATM comments (**Appendix B**) before finalizing its recommendations. The recommendations and the supporting analysis (Haseman et al. 2011) are presented in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act (42 U.S.C. 285*l*-3), ICCVAM will forward the recommendations to U.S. Federal agencies for consideration. Federal agencies are required to respond to ICCVAM within 180 days after receiving the ICCVAM recommendations. This report is available to the public on the NICEATM–ICCVAM website, and agency responses will be made available on the website as they are received.

2.0 ICCVAM Recommendations: Identifying Chemical Eye Hazards with Fewer Animals

2.1 Introduction and Applicable Regulatory Requirements

Relevant U.S. and international ocular toxicity regulations and test guidelines are summarized in **Appendix C**. Eye safety testing procedures vary among U.S. agencies. Current testing procedures specified in 16 CFR 1500.42 provide criteria and procedures for identifying eye hazards based on rabbit eye test results (CPSC 2010). However, current testing procedures do not provide criteria to classify results from a 3-animal test. Therefore, NICEATM–ICCVAM conducted an analysis to determine classification criteria based on results from a 3-animal test that would maintain hazard classification equivalent to that provided by current testing procedures (Haseman et al. 2011).

In the analysis (Haseman et al. 2011), the frequency with which current testing procedures (16 CFR 1500.42) identify substances as eye irritants was compared with the frequency with which a classification criterion of either at least one or two positive animals in a 3-animal test would identify these substances. A number of different underlying population positive response rates for identifying substances as eye irritants were examined. A NICEATM database of 481 rabbit eye test studies using 6 animals per test was also used to estimate over- and underprediction rates for each criterion using a mixture of three binomial distributions. In each instance, a classification criterion of at least one positive animal in a 3-animal test more closely matched the expected outcome based on current testing procedures, while a criterion of at least two positive animals in a 3-animal test identified far fewer irritants. These results showed that using a classification criterion of at least one positive animal in a 3-animal test to identify eye hazards will provide the same as or greater than level of eye hazard classification as current testing procedures, while using up to 50% to 83% fewer animals. ICCVAM developed the following recommendations based on the results of this analysis (Haseman et al. 2011).

2.2 ICCVAM Recommendations

ICCVAM recommends that alternative *in vitro* test methods should always be considered and used where appropriate for eye safety testing. While currently approved *in vitro* test methods can identify some eye hazards (OECD 2009a, 2009b), they are not sufficiently validated and accepted to completely replace all animal testing. When eye safety testing for those regulatory authorities still requiring the use of animals is necessary, testing should be conducted using the minimum number of animals in the most humane manner possible consistent with testing objectives.

ICCVAM concludes that using a classification criterion of one or more positive animals in a 3-animal test to identify chemicals and products that are eye hazards will maintain hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42 [CPSC 2010]), while using up to 50% to 83% fewer animals. ICCVAM therefore recommends consideration of the use of this classification criterion together with eye safety testing procedures that use a maximum of 3 animals per test substance. Consistent with ICCVAM's duty to foster national and international harmonization (42 U.S.C. 285*l*-3), this recommendation also harmonizes the number of animals used for eye safety testing across U.S. regulatory agencies and international test guidelines.

3.0 Analysis Supporting the Use of Fewer Animals for Evaluating Eye Hazards

3.1 Current Testing Procedures (16 CFR 1500.42)

Current testing procedures specified in 16 CFR 1500.42 provide information on conducting the rabbit eye test, a description of positive responses for individual animals, and a testing strategy for determining the overall results of the test (CPSC 2010) (**Table 3-1**). Testing is conducted using an initial group of 6 albino rabbits, and 0.1 mL or 0.1 gram of the test substance is placed in the conjunctival sac of one eye with the contralateral eye serving as a negative or solvent control. The eyes are examined 24, 48, and 72 hours after test substance administration. Severity scores are recorded for the following eye injuries: corneal ulceration/opacity, iritis, conjunctival swelling, and conjunctival redness (**Table 3-2**). Positive responses for individual animals are based on meeting or exceeding the minimum severity criteria for any of the types of eye injuries at any of the three time points (**Table 3-2**). Significant corneal ulceration can be used as a humane endpoint to terminate a study (OECD 2002). The number of animals exhibiting a positive response in each test group determines whether the hazard test result is positive, negative, or if a second or third test is required (**Table 3-1**).

Table 3-1 Current Testing Procedures (16 CFR 1500.42)

Positive Responses for Individual Animals[a]	Corneal ulceration[b] or corneal opacity[c] ≥1 Iritis[d] ≥1 Conjunctival swelling[e] ≥2 Conjunctival redness[e] ≥2
Testing Strategy – **Positive, Negative, or Repeat Test**	<u>First Test: Test 6 animals</u> • If ≥4/6 animals are positive, the test is positive. • If ≤1 animal is positive, the test is negative. • If 2/6 or 3/6 animals are positive, a second test is conducted using a different group of 6 animals. <u>Second Test: Test 6 animals</u> • If ≥3/6 animals are positive, the test is positive. • If 0/6 are positive, the test is negative. • If 1/6 or 2/6 is positive, a third test is conducted using a different group of 6 animals. <u>Third Test: Test 6 animals</u> • If ≥1/6 animals are positive, the test is positive. • If 0/6 are positive, the test is negative.

[a] Based on meeting or exceeding the minimum severity criteria for any of the types of eye injuries at 24, 48, and 72 hours, as outlined in the *Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances*, referenced in 16 CFR 1500.42 (see **Table 3-2**).

[b] Ulceration of the cornea (other than a fine stippling)

[c] Opacity of the cornea (other than a slight dulling of the normal luster)

[d] Inflammation of the iris (other than a slight deepening of the folds [or rugae] or a slight circumcorneal injection of the blood vessels)

[e] Obvious conjunctival swelling with partial eversion of the lids or conjunctival redness with diffuse crimson red; individual vessels not easily discernible

Table 3-2 Scores for Grading Severity of Eye Lesions

Lesion[a]	Score[b]
Cornea	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	**1**
Easily discernible translucent areas, details of iris slightly obscured	**2**
Opalescent areas, no details of iris visible, size of pupil barely discernible	**3**
Complete corneal opacity, iris not discernible	**4**
Iris	
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any one of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	**1**
No reaction to light, hemorrhage, gross destruction (any one or all of these)	**2**
Conjunctiva	
A. Redness (refers to palpebral and bulbar conjunctiva only)	
Normal	0
Some vessels definitely injected above normal	1
Diffuse, crimson red, individual vessels not easily discernible	**2**
Diffuse beefy red	**3**
B. Chemosis	
Normal	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of the lids	**2**
Swelling with lids about half closed	**3**
Swelling with lids about half closed to completely closed	**4**

Table is adapted from the *Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances*, referenced in 16 CFR 1500.42.

[a] Positive responses for individual animals are based on meeting or exceeding the minimum severity criteria for any of the types of eye injuries at any of the three time points.

[b] Scores in **bold** indicate positive responses.

The United States proposed revisions to OECD Test Guideline 405: Acute Eye Irritation/Corrosion (OECD 1987) to reduce the maximum number of required animals by 50% from 6 to 3 (de Silva et al. 1997; OECD 1999; Springer et al. 1993). The revised Test Guideline 405 was adopted in 2002

(OECD 2002). In accordance with the OECD Mutual Acceptance of Data Treaty (OECD 1981), U.S. agencies accept for review test data generated in accordance with OECD test guidelines.

The Animal Welfare Act (7 U.S.C. 2131 et seq.) requires that only the minimum number of animals necessary to obtain scientifically valid results be used for testing. The Public Health Service Policy on Humane Care and Use of Laboratory Animals requires that a rationale for the appropriateness of the number of animals be provided to and approved by the Institutional Animal Care and Use Committee (OLAW 2002). In light of these policies and regulations, most *in vivo* eye safety testing would be expected to adhere to the 3-animal procedure described in the OECD and U.S. Environmental Protection Agency test guidelines (EPA 1998; OECD 2002). However, current testing procedures (16 CFR 1500.42) do not provide criteria to classify results from a 3-animal test. Therefore, NICEATM–ICCVAM conducted an analysis (Haseman et al. 2011) to determine classification criteria based on results from a 3-animal test that would maintain hazard classification equivalent to that provided by current testing procedures.

3.2 Optimization of the Number of Positive Animals Required to Identify a Substance as an Irritant

To determine the optimal number of positive animals required to identify a substance as an irritant, the minimum number of positive animals necessary to classify an irritant by current testing procedures (16 CFR 1500.42) was evaluated for each of the possible test outcomes. As indicated in **Table 3-3**, the weakest possible response that is considered positive by the current sequential testing strategy is 22% (2/6 + 1/6 + 1/6, or 4/18), while a response of 17% (1/6 or 3/18) is considered negative. Therefore, it could be argued that the threshold positive response rate for considering a substance as an irritant for current testing procedures should logically lie between 17% and 22%, perhaps 20%. However, this conclusion is complicated by the fact that an observed response rate of 28% (3/6 + 2/6 + 0/6, or 5/18) may occur and result in a chemical to not be classified as an irritant (**Table 3-3**). Ideally, a testing strategy should not produce inconsistent results, in which the percentage of positive animal responses that can result in an irritant classification overlaps with the percentage that do not result in an irritant classification.

Table 3-3 Number of Positive Animals and Sequential Tests Required for Assignment of an Irritant Classification According to Current Testing Procedures (16 CFR 1500.42)

Positive Test Criteria for Irritant Classification	**Positive Animals**					
First Test Results	≥4/6	2/6 or 3/6	3/6	3/6	2/6	2/6
Results from Second Test (when required)	Second test not required	≥3/6	2/6	1/6	2/6	1/6
Results from Third Test (when required)	Third test not required	Third test not required	≥1/6	≥1/6	≥1/6	≥1/6
Minimum Number of Positive Animals for Irritant Classification	**4/6 (67%)**	**5/12 (42%)**	**6/18 (33%)**	**5/18 (28%)**	**5/18 (28%)**	**4/18 (22%)**
Maximum Number of Positive Animals for Not Classified as an Irritant	**1/6 (17%)**	**3/12 (25%)**	**5/18 (28%)**	**4/18 (22%)**	**4/18 (22%)**	**3/18 (17%)**

3.3 Comparison of Three Strategies for Reducing Animal Use

The percentage of substances that would be classified as eye hazards was calculated for each of three different decision strategies. The first strategy (Strategy 1) used current testing procedures (16 CFR 1500.42) to identify eye hazards. The second strategy (Strategy 2) used a minimum threshold of ≥1/3 (33%) positive animals. The third strategy (Strategy 3) used a minimum threshold of ≥2/3 (67%) positive animals.

The frequency with which each strategy would identify substances as eye irritants was calculated for a number of different underlying population positive response rates. This population positive response rate, denoted by *p*, is the overall likelihood that an animal will show a positive response for a given substance. Importantly, it is a ''population'' response rate, not the response rate observed in a given sample of 3 to 6 animals. However, for a specified value of *p*, it is possible to compute the likelihood of observing various responses in a given sample using binomial probabilities. This is illustrated in **Table 3-4** for a general *p*, and for *p* = 20% and *p* = 60% to provide specific examples. For example, for a substance with an underlying positive response rate of *p* = 60%, the likelihood is 0.311 (31.1%) that there will be exactly 4 positive animals in a sample of 6 animals.

Table 3-4 Probability of Observing 0 to 6 Positive Animals in a Sample of n = 3 or n = 6 for Various Population Positive Response Rates (*p*) Assuming a Binomial Model

No. Positive Animals in a Sample	Probability of Response in Sample		Probability of Response in Sample			
	n = 3	n = 6	n = 3	n = 3	n = 6	n = 6
			p = 20%	*p* = 60%	*p* = 20%	*p* = 60%
0	$(1-p)^3$	$(1-p)^6$	0.512	0.064	0.262	0.004
1	$3p(1-p)^2$	$6p(1-p)^5$	0.384	0.288	0.393	0.037
2	$3p^2(1-p)$	$15p^2(1-p)^4$	0.096	0.432	0.246	0.138
3	p^3	$20p^3(1-p)^3$	0.008	0.216	0.082	0.276
4	-	$15p^4(1-p)^2$	-	-	0.015	0.311
5	-	$6p^5(1-p)$	-	-	0.002	0.187
6	-	p^6	-	-	<0.001	0.047

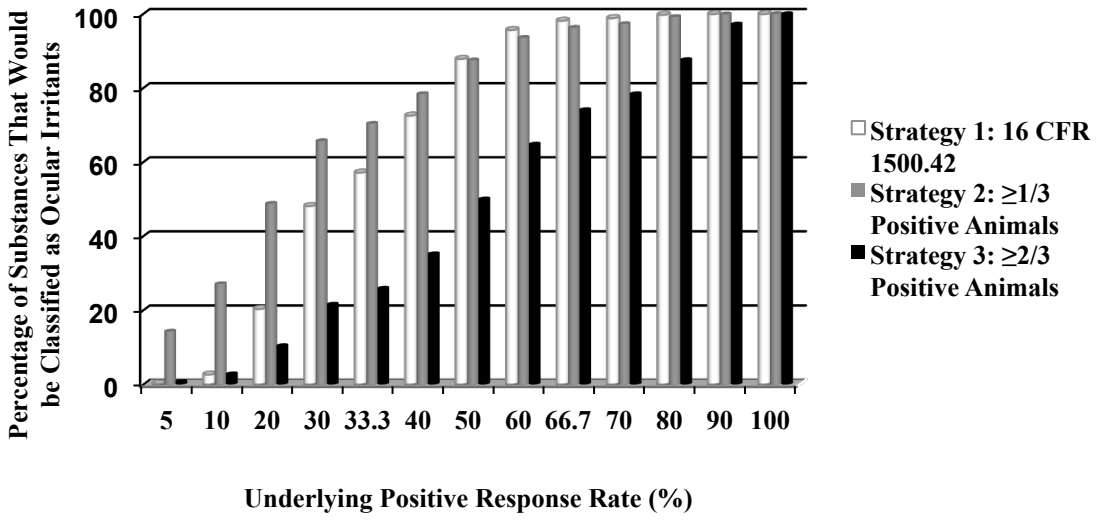
Table 3-5 presents the likelihood of classifying a substance as an eye irritant for various underlying values of *p*. However, it does not show whether or not this classification is ''correct'' because this would require knowledge of the underlying positive response rate that differentiates irritants from nonirritants. However, because the underlying positive response rates in a population that are characteristic of an irritant or a nonirritant are not definitively known (see **Table 3-3**), a range of different underlying positive response rates were compared (**Table 3-5**) and presented graphically in **Figure 3-1**.

Table 3-5 Percentage of Substances Classified as Eye Irritants Based on Various Population Positive Response Rates (*p*) for the Three Strategies

Population Positive Response Rate (*p*)	Percentage of Substances That Would be Classified as Eye Irritants		
	Strategy 1 16 CFR 1500.42	Strategy 2 ≥1/3 Positive Animals	Strategy 3 ≥2/3 Positive Animals
1.7%[a]	0.0%	5.0%	0.1%
5%	0.2%	14.3%	0.8%
10%	2.7%	27.1%	2.8%
20%	20.4%	48.8%	10.4%
30%	48.2%	65.7%	21.6%
33.3%	57.2%	70.4%	25.9%
40%	72.6%	78.4%	35.2%
50%[a]	87.9%	87.5%	50.0%
60%	95.7%	93.6%	64.8%
66.7%	98.2%	96.3%	74.1%
70%	98.9%	97.3%	78.4%
80%	99.8%	99.2%	87.6%
90%	100%	99.9%	97.2%
97.8%[a]	100%	100%	99.9%
100%	100%	100%	100%

[a] Estimated underlying positive response rates for the NICEATM database (see **Table 3-7**)

Figure 3-1 Strategy 2 Provides Eye Hazard Classification the Same as or Greater Than Current Testing Procedures (16 CFR 1500.42)



For purposes of illustration, consider p = 20%. **Table 3-6** summarizes all the possible ways in which Strategy 1 could lead to a negative classification for a substance with a 20% population positive

response rate. The probabilities in **Table 3-6** are derived from **Table 3-4**. Thus, by subtraction from 1.0, the likelihood of a positive classification for Strategy 1 for p = 20% is 1 - 0.796, or 0.204 or 20.4% (see **Table 3-5**).

Table 3-6 Probability That Strategy 1 Will Result in a Negative Classification for p = 20%

Strategy 1 Test Result			Probability
Test 1	Test 2	Test 3	
0/6	-	-	0.262
1/6	-	-	0.393
2/6	0/6	-	(0.246)(0.262) = .00645
3/6	0/6	-	(0.082)(0.262) = 0.0215
2/6	1/6	0/6	(0.246)(0.393)(0.262) = 0.0253
3/6	1/6	0/6	(0.082)(0.393)(0.262) = 0.0084
2/6	2/6	0/6	(0.246)(0.246)(0.262) = 0.0159
3/6	2/6	0/6	(0.082)(0.246)(0.262) = 0.0053
Total	-	-	0.796

These calculations are much simpler for Strategies 2 and 3. The likelihood of a positive classification using Strategy 2, assuming p = 20%, is just the likelihood of observing 1/3, 2/3, or 3/3 positive responses. Using the probabilities in **Table 3-4**, the likelihood is 0.384 + 0.096 + 0.008 = 0.488, or 48.8% (see **Table 3-5**). For Strategy 3 and p = 20%, the likelihood of a positive classification is the sum of the likelihood of observing 2/3 or 3/3 positive responses, which is 0.096 + 0.008 = 0.104, or 10.4% (see **Table 3-5**).

Even though it uses fewer animals, Strategy 2 is more powerful than current testing procedures (16 CFR 1500.42) for detecting positive response rates of up to 40% and has approximately the same power for response rates of 50% and greater (see **Figure 3-1**). Strategy 3 identifies far fewer irritants than Strategy 2 for underlying positive response rates of 80% and less. Strategy 3 considers a single positive response (1/3) to not indicate an irritant response. Strategy 3 also has lower power than current testing procedures for underlying positive response rates of 20% to 80%.

These calculations were based on a variety of underlying positive response rates without consideration of whether or not they reflect the positive response rates seen in practice. Rather than assuming that each irritant and nonirritant has its own unique (and unknown) underlying positive response rate, a potentially useful approach is to derive a mathematical model that accurately describes the observed distribution of positive responses seen for a large database of test substances. If a definitive structure can be imposed upon the data (and if the model fits the data), then the model parameters can be used to estimate over- and underprediction rates. With this in mind, NICEATM-ICCVAM analyzed a database of 481 rabbit eye test studies that each used 6 animals per test. This database includes a wide range of chemical and product categories (Haseman et al. 2011).

To calculate the estimated over- and underprediction rates for the three strategies using the NICEATM database, the first step was to find a model that fit the observed outcomes (**Table 3-7**), some of which are irritants and some of which are nonirritants. NICEATM–ICCVAM used a model that assumed a mixture of three binomial distributions because it is unlikely that every irritant has exactly the same likelihood of producing a positive response in an animal. Irritants were categorized into two groups. Irritants with a high underlying positive response rate in an animal were designated

as Type I irritants. Irritants with a smaller underlying positive response rate in an animal were designated as Type II irritants.

From the observed distribution of positive animals in a 6-animal test, five key parameters were estimated: the underlying positive response rates for nonirritants and Type I and Type II irritants, and the percentage of Type I and Type II irritants in the database (the percentage of nonirritants in the database can then be calculated by subtraction from 100%). The following parameter estimates provided the best fit to the NICEATM database (**Tables 3-7** and **3-8**):

- Type I irritants: underlying positive response rate = 97.8%
- Type II irritants: underlying positive response rate = 50.0%
- Nonirritants: underlying positive response rate = 1.7%
- Percentage of Type I irritants in the sample: 54% or 260 substances
- Percentage of Type II irritants in the sample: 12.9% or 62 substances
- Percentage of nonirritants in the sample: 33.1% or 159 substances

Given this excellent fit to the data as indicated in **Table 3-7**, NICEATM–ICCVAM calculated the percentage of substances that would be classified as eye irritants using each of the three strategies (**Table 3-8**). The likelihood that a Type I irritant would be classified as an eye irritant is close to 100% for all three strategies. The likelihood that a Type II irritant would be classified as an eye irritant is approximately 88% for Strategies 1 and 2 but 50% for Strategy 3. The likelihood of classifying a nonirritant as an eye irritant is 0% for Strategy 1, 5.0% for Strategy 2, and 0.1% for Strategy 3 (**Table 3-8**).

Table 3-7 Goodness of Fit for a Database of 481 Test Results Using a Mixture of Three Binomial Distributions

Number of Positive Animals in a 6-Animal Test	Predicted Type I Irritants	Predicted Type II Irritants	Predicted Nonirritants	Total Predicted by NICEATM Model	Total Observed in NICEATM Database
0	0	1.0	143.4	144.4	142
1	0	5.8	15.0	20.8	21
2	0	14.5	0.6	15.1	19
3	0.1	19.4	0	19.5	15
4	1.7	14.5	0	16.2	20
5	30.7	5.8	0	36.5	35
6	227.5	1.0	0	228.5	229
Total	260 (54.0%)	62 (12.9%)	159 (33.1%)	481	481

Table 3-8 Percentage of Substances Classified as Eye Irritants Based on Estimated Underlying Positive Response Rates for Three Strategies: Three Binomial Distributions

Binomial Distribution	Estimated Underlying Positive Response Rate	Percentage of Substances That Would be Classified as Eye Irritants		
		Strategy 1 16 CFR 1500.42	Strategy 2 ≥1/3 Positive Animals	Strategy 3 ≥2/3 Positive Animals
Nonirritants	1.7%	0%	5.0%	0.1%
Type II Irritants	50%	87.9%	87.5%	50.0%
Type I Irritants	97.8%	100%	100%	99.9%

Based on these outcomes, the underlying over- and underprediction rates associated with this model were then calculated. All three strategies have a very low underprediction rate for Type I irritants. However, for Type II irritants, Strategies 1 and 2 have underprediction rates of approximately 12%, while Strategy 3 has a 50% underprediction rate. For nonirritants, Strategies 1 and 3 have very low overprediction rates, while the overprediction rate for Strategy 2 is 5% (**Table 3-9**).

It is important to note that this approach is similar to the approach used by Springer et al. (1993) except for the fact that NICEATM–ICCVAM assumed two different underlying positive response rates for irritants, whereas Springer et al. used only one (i.e., they assumed that every irritant has exactly the same likelihood of producing a positive response in an animal). Based on the distribution of positive animals in a 6-animal test in the NICEATM database, the use of two different underlying positive response rates for irritants provided a much better fit to the data.

Table 3-9 Percentage of Substances That Would be Over- and Underpredicted for the Three Strategies

Three Binomial Distribution	Strategy 1 16 CFR 1500.42	Strategy 2 ≥1/3 Positive Animals	Strategy 3 ≥2/3 Positive Animals
Percentage of Substances That Would be Overpredicted			
Nonirritant	0%	5.0%	0.1%
Percentage of Substances That Would be Underpredicted			
Type II Irritants	12.1%	12.5%	50.0%
Type I Irritants	0%	0%	0.1%

3.4 Previous Proposals to Reduce the Number of Animals Used for Eye Safety Testing

Results from DeSousa et al. (1984) and Talsma et al. (1988) showed that using 3 rabbits per test provided accuracy of up to 94% in predicting a 6-animal test (using subsets of 3 animals). Springer et al. (1993) also conducted analyses to determine if the standard group size of 6 rabbits for eye safety testing could be reduced in order to use fewer animals and concluded that a 3-animal test and a decision rule requiring at least 2 positive animals to classify a substance as an irritant yielded accuracy of 98%. As indicated above, the model used by Springer et al. assumed two mutually exclusive populations, irritants and nonirritants, each population having a single underlying positive response rate estimated from the data. Springer et al. fit a mixture of two binomial models to each of four different databases, but the only database with a distribution of outcomes that closely matched

the NICEATM database of 481 rabbit eye test studies was an EPA database of 48 substances. Springer et al. reported the following parameter estimates for the EPA database:

- Irritants: underlying positive response rate = 95.0%
- Nonirritants: underlying positive response rate = 8.6%
- Percentage of nonirritants in the sample: 35%
- Percentage of irritants in the sample: 65%

Note that the estimated percentage of nonirritants in the EPA database (35%) is very similar to NICEATM–ICCVAM's estimate (33.1%) for the much larger NICEATM database, but the Springer et al. model does not differentiate between Type I and Type II irritants. As a result, their parameter estimates provided a poor fit to the NICEATM database of 481 studies (**Table 3-10**). In fact, NICEATM–ICCVAM found that the Springer et al. model did not provide a good fit to the EPA data upon which their parameter estimates were based (e.g., predicting only 0.2 outcomes when three outcomes were actually observed for 3/6 positive responses, a 15-fold underprediction). This lack of model fit was more apparent using the NICEATM database of 481 substances, which was approximately 10-fold larger than the Springer et al. (1993) EPA database.

The largest database used by Springer et al. (1993) was the 139-substance Marzulli and Ruggles database, but the pattern of response seen in this database was quite different from that seen in the NICEATM database of 481 studies. Even so, the best-fitting Springer et al. model showed the same lack-of-fit problem. For example, ten 3/6 positive responses were observed compared with only 3.1 predicted by the best-fitting Springer et al. model.

It is important to understand the factors that led to different conclusions in the NICEATM–ICCVAM evaluation, which favored Strategy 2, and that of Springer et al. (1993), which favored Strategy 3. For example, Table 1 in Springer et al. suggests that Strategy 2 may have an unacceptably high overprediction rate.

Table 3-10 Lack of Fit Using the Springer et al. (1993) Model on the NICEATM Database

Number of Positive Animals in a 6-Animal Test	Springer Model Predicted Irritants	Springer Model Predicted Nonirritants	Total Predicted by Springer Model	Total Observed in NICEATM Database
0	0	98.2	98.2	142
1	0	55.5	55.5	21
2	0	13.0	13.0	19
3	0.7	1.6	2.3	15
4	9.5	0.1	9.6	20
5	72.6	0	72.6	35
6	229.8	0	229.8	229
Total	312.6 (65%)	168.4 (35%)	481	481

The primary reason for the different conclusions is that the EPA 48-substance database was of insufficient size to detect the Type II irritants that were producing positive response rates of approximately 50%. By not taking these irritants into account, the Springer et al. (1993) model underestimated the underprediction rate for Strategy 3 because this strategy does not perform well for detecting positive response rates of approximately 50% (see **Table 3-5**).

Another consequence of Springer et al. (1993) ignoring the Type II irritants was a 5-fold overestimation of the positive response rate of nonirritants. This difference is important because the overprediction rate of Strategy 2 increases substantially as the assumed positive response rate for nonirritants increases (see **Table 3-5**). It is the Springer et al. overestimation of the positive response rate for nonirritants that produced the artificially high overprediction rate for Strategy 2 shown in their Table 1.

3.5 Animal Welfare Considerations

This analysis (Haseman et al. 2011) reduces animal use because it should facilitate regulatory decisions on classification criteria that will support the adoption of test methods using fewer animals. It also harmonizes the number of animals used for eye safety testing with current EPA (1998) and OECD (2002) testing guidelines, thereby reducing the number of tests that should need to be performed.

4.0 ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process provides numerous opportunities for public stakeholder involvement, including submission of written comments and oral comments at the public SACATM meetings. **Table 4-1** lists the four opportunities for public comments that were provided during the ICCVAM evaluation process (**Appendix A**). The number of public comments received in response to each of the opportunities is indicated. Three public comments were received. Comments received in response to or related to the *Federal Register* notices are included in **Appendix B** and are accessible on the NICEATM–ICCVAM website (http://iccvam.niehs.nih.gov/). The following sections briefly discuss the public comments received.

Table 4-1 Opportunities for Public Comment

Opportunity for Public Comment	Date	Number of Public Comments Received
75 FR 26757: Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)	May 12, 2010	0
SACATM Meeting, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina	June 17–18, 2010	1
SACATM Meeting, Hilton Arlington, Arlington, Virginia	June 16–17, 2011	0
76 FR 50220: Availability of Draft ICCVAM Recommendations; Request for Comments	August 12, 2011	2

4.1 Public Comments in Response to 75 FR 26757 (May 12, 2010)

Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

The SACATM meeting was announced, and written and public oral comments on the agenda topics were requested.

No written public comments were received in response to this *Federal Register* notice.

4.2 Public and SACATM Comments: SACATM Meeting on June 17–18, 2010

The SACATM meeting included a discussion of the current issues in the validation of alternative methods for assessing chemically induced eye injuries, which included an overview of the analysis (Haseman et al. 2011) conducted to determine classification criteria based on results from a 3-animal test that would maintain eye hazard classification equivalent to that provided by current testing procedures specified in 16 CFR 1500.42 (CPSC 2010).

SACATM Comment

SACATM members and two *ad hoc* experts praised the statistical analysis (Haseman et al. 2011) and agreed that the proposed 33% positive response rate provides appropriate criteria for eye safety testing compared to current testing procedures (16 CFR 1500.42).

Public Comment

One oral comment relevant to this discussion was provided.

An individual shared her story of having been in a serious automobile accident many years ago in which airbags deployed, and she suffered chemical burns to her eyes from the chemical powder in the airbag. The chemical eye injuries caused permanent damage, with complete loss of vision in one eye and severe visual impairment in the other eye. She urged the committee to bring more attention to the

danger of serious eye injuries associated with the chemicals in airbags in older cars and the importance of warnings for consumers about the presence of chemicals that can cause severe or permanent eye injuries.

ICCVAM Response
ICCVAM appreciates her unique perspective on the importance of eye hazard labeling and her willingness to share her story, which emphasizes the need for accurate testing and appropriate hazard classification and labeling.

4.3 Public and SACATM Comments: SACATM Meeting on June 16–17, 2011

The NICEATM–ICCVAM update presented at the SACATM meeting included a brief summary of the analysis (Haseman et al. 2011) conducted to determine classification criteria based on results from a 3-animal test that would maintain eye hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42).

SACATM Comment
No SACATM member comments specific to this agenda topic were provided.

Public Comment
No public comments specific to this agenda topic were provided.

4.4 Public Comments in Response to 76 FR 50220 (August 12, 2011)

Availability of Draft ICCVAM Recommendations on Using Fewer Animals to Identify Chemical Eye Hazards: Revised Criteria Necessary to Maintain Equivalent Hazard Classification; Request for Comments
NICEATM requested public comments on the draft ICCVAM recommendations that were based on the analysis (Haseman et al. 2011) conducted to determine classification criteria based on results from a 3-animal test that would maintain eye hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42).

NICEATM received two written comments in response to this *Federal Register* notice.

A comment from an individual supported using fewer animals but further encouraged the use of non-animal methods.

ICCVAM Response
Current U.S. animal welfare laws, regulations, and policies require that the fewest animals necessary for statistically significant results should be used. Investigators proposing the use of animals for eye testing must provide written documentation of their consideration of alternative methods that can reduce or avoid the use of animals and lessen or avoid unrelieved pain and distress. Alternative methods should be used when determined to be appropriate. Adequate consideration and appropriate use of available reduction, refinement, and replacement alternatives must be documented and approved by Institutional Animal Care and Use Committees before tests are conducted in animals (OLAW 2002). However, while currently approved *in vitro* test methods can identify some eye hazards (OECD 2009a, 2009b), they are not currently sufficiently validated to completely replace all animal testing. Until there are valid *in vitro* alternatives that can completely replace the use of animals for eye safety testing, reduction and refinement strategies will be critical to promoting animal welfare (ICCVAM 2010).

A second comment provided by the Association for Research in Vision and Ophthalmology supported the draft ICCVAM recommendation that eye safety testing should adhere to the 3-animal procedure, as described in Test Guideline 405 (OECD 2002) and by the EPA (1998). The association stated that the proposed regulatory change is in the spirit of “the 3Rs” and would be good for

harmonizing guidelines. The association also found the statistical approach used in the analysis (Haseman et al. 2011) to be reasonable.

ICCVAM Response

ICCVAM appreciates the time and effort that was dedicated to review of the analysis (Haseman et al. 2011) and thanks the association for their support of the draft ICCVAM recommendations and the statistical approach. ICCVAM agrees that harmonizing the number of animals used for eye safety testing with current testing guidelines for the EPA (1998) and the OECD (2002) is in the spirit of "the 3Rs" and will reduce the number of tests that should need to be performed.

5.0 References

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Appendix A

ICCVAM Evaluation Timeline
Identifying Chemical Eye Hazards with Fewer Animals

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ICCVAM Evaluation Timeline

2009–2011	NICEATM–ICCVAM conduct an analysis to determine classification criteria based on results from a 3-animal test that would provide eye hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42).
June 17-18, 2010	SACATM public meeting – SACATM and public comments on the analysis to determine classification criteria based on results from a 3-animal test that would provide eye hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42).
April 27, 2011	ICCVAM approves release of draft recommendations on using fewer animals to identify chemical eye hazards for public comment.
June 16–17, 2011	SACATM public meeting – Second opportunity for SACATM and public comments on the analysis to determine classification criteria based on results from a 3-animal test that would provide eye hazard classification equivalent to that provided by current testing procedures (16 CFR 1500.42).
August 12, 2011	*Federal Register* notice (76 FR 50220) – Availability of Draft ICCVAM Recommendations on Using Fewer Animals to Identify Chemical Eye Hazards: Revised Criteria Necessary to Maintain Equivalent Hazard Classification; Request for Comments
November 29, 2011	NICEATM requests ICCVAM-OTWG consideration of public and SACATM comments on draft TMER for Identifying Chemical Eye Hazards with Fewer Animals by December 13, 2011.
December 13, 2011	At the request of ICCVAM-OTWG, NICEATM extends the commenting period for draft TMER for Identifying Chemical Eye Hazards with Fewer Animals to January 13, 2012.
January 20, 2012	NICEATM receives comments from ICCVAM-OTWG on draft TMER for Identifying Chemical Eye Hazards with Fewer Animals.
March 27, 2012	NICEATM requests ICCVAM-OTWG concurrence on draft final TMER for Identifying Chemical Eye Hazards with Fewer Animals by April 10, 2012.
April 10, 2012	ICCVAM-OTWG provides concurrence on draft final TMER for Identifying Chemical Eye Hazards with Fewer Animals to ICCVAM.
June 27, 2012	ICCVAM approves forwarding of TMER for Identifying Chemical Eye Hazards with Fewer Animals to Federal agencies (180 days to respond).
2012 (published within two weeks after transmittal)	*Federal Register* notice – Availability of ICCVAM TMER for Identifying Chemical Eye Hazards with Fewer Animals.

2012-2013 (within 180 days of transmittal receipt)	Agency responses submitted to ICCVAM. Responses provided to the public on the NICEATM–ICCVAM website. *Federal Register* notice published announcing availability of agency responses.

Abbreviations: CFR = U.S. Code of Federal Regulations; FR = *Federal Register*; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; OTWG = Interagency Ocular Toxicity Working Group; SACATM = Scientific Advisory Committee on Alternative Toxicological Methods; TMER = Test Method Evaluation Report.

Appendix B

Federal Register Notices and Public Comments

B1 75 FR 26757 - Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) B-3

B2 76 FR 50220 - Availability of Draft ICCVAM Recommendations on Using Fewer Animals to Identify Chemical Eye Hazards: Revised Criteria Necessary to Maintain Equivalent Hazard Classification B-9

B3 Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments: SACATM Meeting on June 17 - 18, 2010 ..... B-17

B4 Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments: SACATM Meeting on June 16 - 17, 2011 ..... B-19

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Appendix B1

75 FR 26757

Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

(No public comments received)

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Federal Register / Vol. 75, No. 91 / Wednesday, May 12, 2010 / Notices **26757**

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP); Office of Liaison, Policy and Review; Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

AGENCY: National Institute of Environmental Health Sciences

26758 **Federal Register** / Vol. 75, No. 91 / Wednesday, May 12, 2010 / Notices

(NIEHS), National Institutes of Health (NIH).

ACTION: Meeting announcement and request for comments.

SUMMARY: Pursuant to section 10(a) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of a meeting of SACATM on June 17–18, 2010, at the U.S. Environmental Protection Agency, 109 T.W. Alexander Drive, Research Triangle Park, NC 27711. The meeting is open to the public with attendance limited only by the space available. The meeting will be videocast through a link at (*http://www.niehs.nih.gov/news/video/live*). SACATM advises the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the Director of the NIEHS and NTP regarding statutorily mandated duties of ICCVAM and activities of NICEATM.

DATES: The SACATM meeting will be held on June 17 and 18, 2010. The meeting is scheduled from 8:30 a.m. Eastern Daylight Time to 5 p.m. on June 17 and 8:30 a.m. until adjournment on June 18, 2010. All individuals who plan to attend are encouraged to register online at the NTP Web site (*http://ntp.niehs.nih.gov/go/32822*) by June 10, 2010. In order to facilitate planning, persons wishing to make an oral presentation are asked to notify Dr. Lori White, NTP Designated Federal Officer, via online registration, phone, or e-mail by June 10, 2010 (see **ADDRESSES** below). Written comments should also be received by June 10, 2010, to enable review by SACATM and NIEHS/NTP staff before the meeting.

ADDRESSES: The SACATM meeting will be held at the U.S. Environmental Protection Agency, 109 T.W. Alexander Drive, Research Triangle Park, NC 27711. Public comments and other correspondence should be directed to Dr. Lori White (NTP Office of Liaison, Policy and Review, NIEHS, P.O. Box 12233, MD K2–03, Research Triangle Park, NC 27709; telephone: 919–541–9834 or e-mail: *whiteld@niehs.nih.gov*). Courier address: NIEHS, 530 Davis Drive, Room 2136, Morrisville, NC 27560. Persons needing interpreting services in order to attend should contact 301–402–8180 (voice) or 301–435–1908 (TTY). Requests should be made at least 7 days in advance of the meeting.

SUPPLEMENTARY INFORMATION:

Preliminary Agenda Topics and Availability of Meeting Materials

Preliminary agenda topics include:

- NICEATM–ICCVAM Update.
- Regulatory Acceptance of ICCVAM-Recommended Alternative Test Methods.
- Assessment of Acute and Chronic Pain in Animals.
- Federal Agency Research, Development, Translation, and Validation Activities Relevant to the NICEATM–ICCVAM Five-Year Plan.
- Current Issues in the Validation of Alternative Methods for Assessing Chemically Induced Eye Injuries.
- Alternative Methods for Vaccine Potency Testing.
- Update from the European Centre for the Validation of Alternative Methods.
- Update from Health Canada.
- Update from the Korean Center for the Validation of Alternative Methods.

A copy of the preliminary agenda, committee roster, and additional information, when available, will be posted on the NTP Web site (*http://ntp.niehs.nih.gov/go/32822*) or available upon request (*see* **ADDRESSES** above). Following the SACATM meeting, summary minutes will be prepared and available on the NTP Web site or upon request.

Request for Comments

Both written and oral public input on the agenda topics is invited. Written comments received in response to this notice will be posted on the NTP Web site. Persons submitting written comments should include their name, affiliation (if applicable), and sponsoring organization (if any) with the document. Time is allotted during the meeting for presentation of oral comments and each organization is allowed one time slot per public comment period. At least 7 minutes will be allotted for each speaker, and if time permits, may be extended up to 10 minutes at the discretion of the chair. Registration for oral comments will also be available on-site, although time allowed for presentation by on-site registrants may be less than for pre-registered speakers and will be determined by the number of persons who register at the meeting. In addition to in-person oral comments at the meeting, public comments can be presented by teleconference line. There will be 50 lines for this call; availability will be on a first-come, first-served basis. The available lines will be open from 8 a.m. until 5 p.m. on June 17 and 8:30 a.m. to adjournment on June 18, although public comments will be received only during the formal public comment periods, which will be indicated on the preliminary agenda. The access number for the teleconference line will be provided to registrants by e-mail prior to the meeting.

Persons registering to make oral comments are asked to do so through the online registration form (*http://ntp.niehs.nih.gov/go/32822*) and to send a copy of their statement to Dr. White (see **ADDRESSES** above) by June 10, 2010, to enable review by SACATM, NICEATM–ICCVAM, and NIEHS/NTP staff prior to the meeting. Written statements can supplement and may expand the oral presentation. If registering on-site and reading from written text, please bring 40 copies of the statement for distribution and to supplement the record.

Background Information on ICCVAM, NICEATM, and SACATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the development, scientific validation, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 [42 U.S.C. 285*l*–3] established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of U.S. Federal agencies. Additional information about ICCVAM and NICEATM, guidelines for nomination of test methods for validation studies, and guidelines for submission of test methods for ICCVAM evaluation are available at: *http://iccvam.niehs.nih.gov.*

SACATM was established in response to the ICCVAM Authorization Act [Section 285*l*–3(d)] and is composed of scientists from the public and private sectors. SACATM advises ICCVAM, NICEATM, and the Director of the NIEHS and NTP regarding statutorily mandated duties of ICCVAM and activities of NICEATM. SACATM

provides advice on priorities and activities related to the development, validation, scientific review, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods. Additional information about SACATM, including the charter, roster, and records of past meetings, can be found at *http://ntp.niehs.nih.gov/go/167.*

Dated: May 4, 2010.

John R. Bucher,

Associate Director, National Toxicology Program.

[FR Doc. 2010–11318 Filed 5–11–10; 8:45 am]

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Appendix B2

76 FR 50220

Availability of Draft ICCVAM Recommendations on Using Fewer Animals to Identify Chemical Eye Hazards: Revised Criteria Necessary to Maintain Equivalent Hazard Classification

Public Comments Received in Response to 76 FR 50220

- Dr. Bobbie Ann Austin (Association for Research in Vision and Ophthalmology).....B-13
- J. Public.....B-15

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50220 **Federal Register** / Vol. 76, No. 156 / Friday, August 12, 2011 / Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Availability of Draft ICCVAM Recommendations on Using Fewer Animals to Identify Chemical Eye Hazards: Revised Criteria Necessary to Maintain Equivalent Hazard Classification; Request for Comments

AGENCY: Division of the National Toxicology Program (DNTP), National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, HHS.

ACTION: Availability of Recommendations; Request for Comments.

SUMMARY: The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), conducted an analysis to determine classification criteria using results from 3-animal tests that would provide eye hazard classification equivalent to testing conducted in accordance with current U.S. Federal Hazardous Substances Act (FHSA) regulations, which require the use of 6 to 18 animals. The results showed that using a classification criterion of at least 1 positive animal in a 3-animal test to identify eye hazards will provide the same or greater level of eye hazard classification as current FHSA requirements, while using 50% to 83% fewer animals. ICCVAM developed draft recommendations based on the results of this analysis. NICEATM invites public comments on these draft ICCVAM recommendations.

DATES: Written comments on the draft recommendations should be received by September 26, 2011.

ADDRESSES: NICEATM prefers that comments be submitted electronically via the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm*) or via e-mail to *niceatm@niehs.nih.gov.* Written comments may also be sent by mail or fax to Dr. William S. Stokes, Director, NICEATM, NIEHS, P.O. Box 12233, Mail Stop: K2–16, Research Triangle Park, NC 27709; (fax) 919–541–0947. Courier address: NICEATM, NIEHS, Room 2034, 530 Davis Drive, Morrisville, NC 27560.

FOR FURTHER INFORMATION CONTACT: Dr. William S. Stokes: (telephone) 919–541–2384, (fax) 919–541–0947, or (e-mail) *niceatm@niehs.nih.gov.*

SUPPLEMENTARY INFORMATION:

Background

Testing requirements necessary to determine the eye hazard potential for substances regulated under the FHSA (FHSA, 2008) are provided in 16 CFR 1500.42 (U.S. Consumer Product Safety Commission [CPSC], 2010). Current FHSA regulations provide procedures to determine the eye hazard classification and labeling requirements for chemicals and products to which consumers may be exposed. The current procedure requires a minimum of 6 animals per test and may require up to 3 sequential tests for each substance, thus requiring 6, 12, or 18 animals to reach a hazard classification decision. The requirement for second and third sequential tests is based on the number of positive responses in the previous test.

In 2002, the Organisation for Economic Co-operation and Development (OECD) Test Guidelines Program adopted U.S. proposed revisions to Test Guideline 405: Acute Eye Irritation/Corrosion (OECD, 2002) that reduce the maximum number of required animals per test from 6 to 3. The Animal Welfare Act (7 U.S.C. 2131 *et seq*) and the Public Health Service (PHS) Policy (PHS, 2002) similarly require that only the minimum number of animals necessary to obtain scientifically valid results should be used and that a rationale for the appropriateness of the number of animals used be provided to and approved by the Institutional Animal Care and Use Committee. In light of this policy and regulations, most *in vivo* ocular safety testing is expected to adhere to the 3-animal procedure described in OECD Test Guideline 405 (OECD, 2002) and in a test guideline issued by the U.S. Environmental Protection Agency (EPA, 1998). However, current FHSA regulations do not provide criteria to classify results from a 3-animal test. Therefore, an analysis was conducted to determine classification criteria based on results from a 3-animal test that would provide eye hazard classification equivalent to procedures in current FHSA regulations (Haseman *et al.,* 2011). The results showed that using a classification criterion of at least 1 positive in a 3-animal test to identify eye hazards will provide the same or greater level of eye hazard classification as current FHSA requirements, while using 50% to 83% fewer animals. Based on these results, ICCVAM developed draft recommendations to use this classification criterion for ocular safety testing procedures that use only a maximum of 3 animals per test substance.

Availability of the Documents

The draft ICCVAM recommendations and the supporting publication describing the results of the analysis are available on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/methods/ocutox/reducenum.htm*), and may also be obtained by contacting NICEATM (see **FOR FURTHER INFORMATION CONTACT**).

Request for Public Comments

NICEATM invites the submission of written comments on the draft ICCVAM recommendations and the extent to which the NICEATM analysis supports the recommendations by September 26, 2011. When submitting written comments, please refer to this **Federal Register** notice and include appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, if applicable). NICEATM will post all comments on the NICEATM–ICCVAM Web site (*http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm*) identified by the individual's name and affiliation or sponsoring organization (if applicable). ICCVAM will consider all public comments and comments made by the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) at the June 17–18, 2010 meeting (75 FR 26757) when finalizing its recommendations. Final ICCVAM recommendations will be forwarded to relevant Federal agencies for their consideration. These recommendations will also be available to the public on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/methods/ocutox/reducenum.htm*).

Background Information on ICCVAM, NICEATM, and SACATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information. ICCVAM conducts technical evaluations of new, revised, and alternative safety testing methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological and safety testing methods that more accurately assess the safety and hazards of chemicals and products and that reduce, refine (decrease or eliminate pain and distress), or replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*–3) established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM, provides scientific and operational support for ICCVAM-related activities, and conducts independent validation studies to assess the usefulness and limitations of new, revised, and alternative test methods and strategies. NICEATM and ICCVAM welcome the public nomination of new, revised, and alternative test methods and strategies for validation studies and technical evaluations. Additional information about NICEATM and ICCVAM can be found on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov*).

SACATM was established in response to the ICCVAM Authorization Act [Section 285*l*–3(d)] and is composed of scientists from the public and private sectors. SACATM advises ICCVAM, NICEATM, and the Director of the NIEHS and NTP regarding statutorily mandated duties of ICCVAM and activities of NICEATM. SACATM provides advice on priorities and activities related to the development, validation, scientific review, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods. Additional information about SACATM, including the charter, roster, and records of past meetings, can be found at *http://ntp.niehs.nih.gov/go/167.*

References

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CPSC. 2010. Hazardous Substances and Articles; Administration and Enforcement Regulations. 16 CFR part 1500. Available: *http://www.gpo.gov/fdsys/pkg/CFR-2010-title16-vol2/xml/CFR-2010-title16-vol2-part1500.xml.*

EPA. 1998. Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. Washington, DC: U.S. Environmental Protection Agency. Available: *http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/EPA/EPA_870_2400.pdf.*

FHSA. 2008. 15 U.S.C. 1261–1278. Public Law 86–613. Available: *http://www.cpsc.gov/businfo/fhsa.pdf.*

Haseman JK, Allen DG, Lipscomb EA, Truax JF, Stokes WS. 2011. Using fewer animals to identify chemical eye hazards: Revised criteria necessary to maintain equivalent hazard classification [published online ahead of print]. Regul Toxicol Pharmacol. Available: *http://dx.doi.org/10.1016/j.yrtph.2011.06.006.*

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PHS. 2002. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Bethesda, MD: Office of Laboratory Animal Welfare, National Institutes of Health. Available: *http://grants.nih.gov/grants/olaw/references/phspol.htm.*

Dated: August 3, 2011.

John R. Bucher,

Associate Director, National Toxicology Program.

[FR Doc. 2011–20537 Filed 8–11–11; 8:45 am]

BILLING CODE 4140–01–P

Subject: FR Notice Comments - 76 FR 50220 - Recommendations on Using Fewer Animals to Identify Chemical Eye Hazards
Date: Monday, September 26, 2011 1:14 PM

Below is the result of your feedback form. It was submitted by () on Monday, September 26, 2011 at 13:14:12

Comment_date: September 26, 2011

Prefix: Dr.

FirstName: Bobbie Ann

LastName: Austin

Degree: PhD

onBehalfOf: yes

Title: Assistant Director

Department: Executive

Company: Association for Research in Vision & Ophthalmology (ARVO)

Country: United States

Phone:

EMail:

Comments: Submitted on behalf of Brian Gilger, DVM, MS, ARVO's Animals-in-Research committee chair

Dear Dr. Stokes:

Thank you for welcoming comments from ARVO about the proposed reduction in the number of animals to identify chemical eye hazards and also for answering our questions via e-mail about the manuscript referenced in the federal register.

ARVO's Animals-in-Research committee reviewed the Federal Register notice (Vol . 76, No. 156, August 12, 2011) about the request for feedback on draft ICCVAM recommendations to

use fewer animals to identify chemical eye hazards. They also reviewed the referenced publish ahead of print article [Regulatory Toxicology and Pharmacology 61 (2011) 98–104], which discusses the Scientific Advisory Committee on Alternative Toxicological Methods meeting, held on June 17-18, 2010.

ARVO's Animals-in-Research committee supports the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommendation that in vivo ocular safety testing adhere to the 3-animal procedure, as described in the test guidelines issued by the Organisation for Economic Co-operation and Development (OECD Test Guideline 405) and by the U.S. Environmental Protection Agency (OPPTS 870.2400). The committee thinks the proposed regulatory change is good for harmonizing guidelines across government agencies and is in the spirit of the 3R's concept for humane research animal use (reduction, refinement and replacement). They also thought the statistical argument made in the referenced manuscript, from the Scientific Advisory Committee on Alternative Toxicological Methods meeting, seemed reasonable.

ARVO is the largest and most respected vision research organization in the world. Members include more than 12,763 researchers from over 80 countries. The membership is multidisciplinary and consists of both clinical and basic scientists who contribute to the quality of life by increasing knowledge about sight. The Association encourages and assists members in research, training, publication and dissemination of knowledge in vision and ophthalmology, and in the ethical and humane use of animals in vision research. Continued progress in many areas of vision inquiry requires research with animals to investigate complex systems and functions. For more information, visit: www.arvo.org/animals.

Thank you for your time.

Sincerely,

Bobbie Ann Austin

Subject: public comment on federal register
Date: Thursday, August 25, 2011 4:28 PM
From: usacitizen1 usacitizen1
To: NIEHS NICEATM

i suppost using 3 animals instead of the hordes of animals you used to use.
but still feel hurting and abusing 3 animals for these tests is much too much.
i want to see you use other methods. jean public address if required

(complete text of FR notice originally appended)

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Appendix B3

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments

SACATM Meeting on June 17 - 18, 2010

The meeting minutes are available online at
http://ntp.niehs.nih.gov/go/8202

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Appendix B4

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments

SACATM Meeting on June 16 - 17, 2011

The meeting minutes are available online at
http://ntp.niehs.nih.gov/go/8202

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Appendix C

Ocular Toxicity Regulations and Testing Guidelines

C1 Table of Eye Irritation/Corrosion Testing: Regulations and Guidelines C-3

C2 Federal Hazardous Substances Act – Public Law 86-613; 15 U.S.C. 1261-1278 ..... C-7

C3 Organisation for Economic Co-operation and Development Test Guideline 405 ..... C-9

C4 Test for Eye Irritants (16 CFR 1500.42) C-25

C5 U.S. Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances Guidance Document 870.2400 ..... C-29

C6 U.S. Environmental Protection Agency Label Review Manual C-39

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Appendix C1

Table of Eye Irritation/Corrosion Testing: Regulations and Guidelines

Note to the Reader:
Regulations may be updated in the future. It is recommended that users review the most current version of all regulations identified.

Electronic versions of United States Code (U.S.C.) can be obtained at:
http://www.gpoaccess.gov/uscode/index.html

Electronic versions of the Code of Federal Regulations (CFR) can be obtained at:
http://www.gpoaccess.gov/cfr/index.html

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Eye Irritation/Corrosion Testing: Relevant U.S. Federal Laws, Regulations, Guidelines, and Recommendations				
Agency, Center, or Office	**Regulated Products**	**Statutory Safety Requirements**	**Regulations**	**Guidelines and Recommendations**
CPSC	Consumer Products	Federal Hazardous Substances Act (U.S.C. Title 15, Chapter 47)	16 CFR 1500.3 (Definitions) 16 CFR 1500.42 (Current test procedure) 16 CFR 1500.121 (Labeling)	Animal Testing Policy (1984)
EPA/OPPTS	Chemicals as defined by the Toxic Substances Control Act Pesticides	Toxic Substances Control Act (U.S.C. Title 15, Chapter 53) Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6)	40 CFR 716 (Safety Data) 40 CFR 717 (Adverse Reactions) 40 CFR 720 (Premanufacture Notification) 40 CFR 156 (Labeling) 40 CFR 158 (Pesticide Data)	OPPTS 870.2400 (1998) Label Review Manual (2007)
FDA/CFSAN FDA/CDER	Cosmetics[1] Pharmaceuticals	Federal Food, Drug, and Cosmetic Act (U.S.C. Title 21, Chapter 9) Public Health Service Act (U.S.C. Title 42, Chapter 6A)	21 CFR 70 (Color additives in food, medical devices, and cosmetics) 21 CFR 312 (IND Application) 21 CFR 314 (IND Approval) 21 CFR 701 (Cosmetic Labeling) 21 CFR 740 (Cosmetic Warning Statement)	No Specific Guidelines or Recommendations on Eye Irritation/Corrosion Testing are Provided

[1] FDA does not have authority for pre-market approval of cosmetics or cosmetic ingredients with the exception of color additives. However, the FDA may enforce action against products or ingredients that are in violation of Federal labeling laws, including provision of adequate safety information.

Eye Irritation/Corrosion Testing: Relevant U.S. Federal Laws, Regulations, Guidelines, and Recommendations				
Agency, Center, or Office	**Regulated Products**	**Statutory Safety Requirements**	**Regulations**	**Guidelines and Recommendations**
OSHA	Chemicals	Occupational Health and Safety Act of 1970 (U.S.C. Title 29, Chapter 15)	29 CFR 1910.1200 (Hazard Communication Standard) 16 CFR 1500.42 (Test for Eye Irritants)	No Specific Guidelines or Recommendations on Eye Irritation/Corrosion Testing are Provided

Abbreviations: CDER = Center for Drug Evaluation and Research; CFR = Code of Federal Regulations; CPSC = U.S. Consumer Product Safety Commission; EPA = U.S. Environmental Protection Agency; FDA = U.S. Food and Drug Administration; OPPTS = Office of Prevention, Pesticides and Toxic Substances; OSHA = U.S. Occupational Safety and Health Administration; US = United States; U.S.C. = United States Code

Appendix C2

Federal Hazardous Substances Act – Public Law 86-613; 15 U.S.C. 1261-1278

Available at:
http://www.cpsc.gov/businfo/fhsa.html

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Appendix C3

Organisation for Economic Co-operation and Development Test Guideline 405

Available at:
http://www.oecd-ilibrary.org/environment/test-no-405-acute-eye-irritation-corrosion_9789264070646-en

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OECD/OCDE **405**

Adopted:
24th April 2002

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Acute Eye Irritation/Corrosion

INTRODUCTION

1. OECD Guidelines for Testing of Chemicals are periodically reviewed to ensure that they reflect the best available science. In the review of this Guideline, special attention was given to possible improvements through the evaluation of all existing information on the test substance in order to avoid unnecessary testing in laboratory animals and thereby address animal welfare concerns. This updated version of Guideline 405 (adopted in 1981 and first revised in 1987) includes the recommendation that prior to undertaking the described *in vivo* test for acute eye irritation/corrosion, a weight-of-the-evidence analysis be performed (1) on the existing relevant data. Where insufficient data are available, it is recommended that they be developed through application of sequential testing (2)(3). The testing strategy includes the performance of validated and accepted *in vitro* tests and is provided as a Supplement to the Guideline. In addition, the use of an *in vivo* dermal irritation/corrosion test to predict eye corrosion prior to consideration of an *in vivo* eye test is recommended in this Guideline.

2. Definitions of acute eye irritation and corrosion are set out in the Annex to the Guideline.

INITIAL CONSIDERATIONS

3. In the interest of both sound science and animal welfare, *in vivo* testing should not be considered until all available data relevant to the potential eye corrosivity/irritation of the substance has been evaluated in a weight-of-the-evidence analysis. Such data will include evidence from existing studies in humans and/or laboratory animals, evidence of corrosivity/irritation of one or more structurally related substances or mixtures of such substances, data demonstrating high acidity or alkalinity of the substance (4)(5), and results from validated and accepted *in vitro* or *ex vivo* tests for skin corrosion and irritation (6)(7). The studies may have been conducted prior to, or as a result of, a weight-of-the-evidence analysis.

4. For certain substances, such an analysis may indicate the need for *in vivo* studies of the ocular corrosion/irritation potential of the substance. In all such cases, before considering the use of the *in vivo* eye test, preferably a study of the *in vivo* dermal effects of the substance should be conducted first and evaluated in accordance with Testing Guideline 404 (8). The application of a weight-of-the-evidence analysis and the sequential testing strategy should decrease the need for *in vivo* testing for eye corrosivity/irritation of substances for which sufficient evidence already exists from other studies. If a determination of eye corrosion or irritation potential cannot be made using the sequential testing strategy, even after the performance of an *in vivo* study of dermal corrosion and irritation, an *in vivo* eye corrosion/irritation test may be performed.

5. A preferred sequential testing strategy, which includes the performance of validated *in vitro* or *ex vivo* tests for corrosion/irritation, is included as a Supplement to this guideline. The strategy was developed at, and unanimously recommended by the participants of, an OECD workshop (9), and has been adopted as the recommended testing strategy in the Globally Harmonised System for the Classification of Chemical Substances (GHS) (10). It is recommended that this testing strategy be followed prior to undertaking *in vivo* testing. For new substances it is the recommended stepwise testing approach for

developing scientifically sound data on the corrosivity/irritation of the substance. For existing substances with insufficient data on skin and eye corrosion/irritation, the strategy should be used to fill missing data gaps. The use of a different testing strategy or procedure, or the decision not to use a stepwise testing approach, should be justified.

PRINCIPLE OF THE *IN VIVO* TEST

6. The substance to be tested is applied in a single dose to one of the eyes of the experimental animal; the untreated eye serves as the control. The degree of eye irritation/corrosion is evaluated by scoring lesions of conjunctiva, cornea, and iris, at specific intervals. Other effects in the eye and adverse systemic effects are also described to provide a complete evaluation of the effects. The duration of the study should be sufficient to evaluate the reversibility or irreversibility of the effects.

7. Animals showing continuing signs of severe distress and/or pain at any stage of the test should be humanely killed, and the substance assessed accordingly. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document (11).

PREPARATIONS FOR THE *IN VIVO* TEST

Selection of species

8. The albino rabbit is the preferable laboratory animal, and healthy young adult animals are used. A rationale for using other strains or species should be provided.

Preparation of animals

9. Both eyes of each experimental animal provisionally selected for testing should be examined within 24 hours before testing starts. Animals showing eye irritation, ocular defects, or pre-existing corneal injury should not be used.

Housing and feeding conditions

10. Animals should be individually housed. The temperature of the experimental animal room should be 20°C (± 3°C) for rabbits. Although the relative humidity should be at least 30% and preferably not exceed 70%, other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unrestricted supply of drinking water.

TEST PROCEDURE

Application of the test substance

11. The test substance should be placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control.

Irrigation

12. The eyes of the test animals should not be washed for at least 24 hours following instillation of the test substance, except for solids (see paragraph 16), and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate.

13. Use of a satellite group of animals to investigate the influence of washing is not recommended unless it is scientifically justified. If a satellite group is needed, two rabbits should be used. Conditions of washing should be carefully documented, e.g., time of washing; composition and temperature of wash solution; duration, volume, and velocity of application.

Dose level

(1) Testing of liquids

14. For testing liquids, a dose of 0.1 mL is used. Pump sprays should not be used for instilling the substance directly into the eye. The liquid spray should be expelled and collected in a container prior to instilling 0.1 mL into the eye.

(2) Testing of solids

15. When testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 mL or a weight of not more than 100 mg. The test material should be ground to a fine dust. The volume of solid material should be measured after gently compacting it, e.g., by tapping the measuring container. If the solid test substance has not been removed from the eye of the test animal by physiological mechanisms at the first observation time point of 1 hour after treatment, the eye may be rinsed with saline or distilled water.

(3) Testing of aerosols

16. It is recommended that all pump sprays and aerosols be collected prior to installation into the eye. The one exception is for substances in pressurised aerosol containers, which cannot be collected due to vaporisation. In such cases, the eye should be held open, and the test substance administered to the eye in a simple burst of about one second, from a distance of 10 cm directly in front of the eye. This distance may vary depending on the pressure of the spray and its contents. Care should be taken not to damage the eye from the pressure of the spray. In appropriate cases, there may be a need to evaluate the potential for "mechanical" damage to the eye from the force of the spray.

17. An estimate of the dose from an aerosol can be made by simulating the test as follows: the substance is sprayed on to weighing paper through an opening the size of a rabbit eye placed directly before the paper. The weight increase of the paper is used to approximate the amount sprayed into the eye. For volatile substances, the dose may be estimated by weighing a receiving container before and after removal of the test material.

Initial test (*in vivo* eye irritation/corrosion test using one animal)

18. As articulated in the sequential testing strategy (Supplement to Guideline), it is strongly recommended that the *in vivo* test be performed initially using one animal.

19. If the results of this test indicate the substance to be corrosive or a severe irritant to the eye using the procedure described, further testing for ocular irritancy should not be performed.

Local anaesthetics

20. Local anaesthetics may be used on a case-by-case basis. If the weight-of-the-evidence analysis indicates that the substance has the potential to cause pain, or initial testing shows that a painful reaction will occur, a local anaesthetic may be used prior to instillation of the test substance. The type, concentration, and dose of the local anaesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use. The control eye should be similarly anaesthetised.

Confirmatory test (*in vivo* eye irritation test with additional animals)

21. If a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals. If a severe irritant effect is observed in the initial test indicating a possible strong (irreversible) effect in the confirmatory testing, it is recommended that the confirmatory test be conducted in a sequential manner in one animal at a time, rather than exposing the two additional animals simultaneously. If the second animal reveals corrosive or severe irritant effects, the test is not continued. Additional animals may be needed to confirm weak or moderate irritant responses.

Observation period

22. The duration of the observation period should be sufficient to evaluate fully the magnitude and reversibility of the effects observed. However, the experiment should be terminated at any time that the animal shows continuing signs of severe pain or distress (9). To determine reversibility of effects, the animals should be observed normally for 21 days post administration of the test substance. If reversibility is seen before 21 days, the experiment should be terminated at that time.

Clinical observations and grading of eye reactions

23. The eyes should be examined at 1, 24, 48, and 72 hours after test substance application. Animals should be kept on test no longer than necessary once definitive information has been obtained. Animals showing continuing severe pain or distress should be humanely killed without delay, and the substance assessed accordingly. Animals with the following eye lesions post-instillation should be humanely killed: corneal perforation or significant corneal ulceration including staphyloma; blood in the anterior chamber of the eye; grade 4 corneal opacity which persists for 48 hours; absence of a light reflex (iridial response grade 2) which persists for 72 hours; ulceration of the conjunctival membrane; necrosis of the conjuctivae or nictitating membrane; or sloughing. This is because such lesions generally are not reversible.

24. Animals that do not develop ocular lesions may be terminated not earlier than 3 days post instillation. Animals with mild to moderate lesions should be observed until the lesions clear, or for 21 days, at which time the study is terminated. Observations should be performed at 7, 14, and 21 days in order to determine the status of the lesions, and their reversibility or irreversibility.

25. The grades of ocular reaction (conjunctivae, cornea and iris) should be recorded at each examination (Table I). Any other lesions in the eye (e.g. pannus, staining) or adverse systemic effects should also be reported.

26. Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device. After recording the observations at 24 hours, the eyes may be further examined with the aid of fluorescein.

27. The grading of ocular responses is necessarily subjective. To promote harmonisation of grading of ocular response and to assist testing laboratories and those involved in making and interpreting the observations, the personnel performing the observations need to be adequately trained in the scoring system used.

DATA AND REPORTING

Evaluation of results

28. The ocular irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. The individual scores do not represent an absolute standard for the irritant properties of a material, as other effects of the test material are also evaluated. Instead, individual scores should be viewed as reference values and are only meaningful when supported by a full description and evaluation of all observations.

Test report

29. The test report must include the following information:

Rationale for *in vivo* testing: weight-of-the-evidence analysis of pre-existing test data, including results from sequential testing strategy:

- description of relevant data available from prior testing;
- data derived in each step of testing strategy;
- description of *in vitro* tests performed, including details of procedures, results obtained with test/reference substances;
- description of *in vivo* dermal irritation / corrosion study performed, including results obtained;
- weight-of-the-evidence analysis for performing *in vivo* study

Test substance:

- identification data (e.g. CAS number, source, purity, known impurities, lot number);
- physical nature and physicochemical properties (e.g. pH, volatility, solubility, stability, reactivity with water);
- in case of a mixture, composition and relative percentages of components;
- if local anaesthetic is used, identification, purity, type, dose, and potential interaction with test substance.

Vehicle:

- identification, concentration (where appropriate), volume used;
- justification for choice of vehicle.

Test animals:

- species/strain used, rationale for using animals other than albino rabbit;
- age of each animal at start of study;
- number of animals of each sex in test and control groups (if required);

- individual animal weights at start and conclusion of test;
- source, housing conditions, diet, etc.

Results:

- description of method used to score irritation at each observation time (e.g., hand slitlamp, biomicroscope, fluorescein);
- tabulation of irritant/corrosive response data for each animal at each observation time up to removal of each animal from the test;
- narrative description of the degree and nature of irritation or corrosion observed;
- description of any other lesions observed in the eye (e.g., vascularization, pannus formation, adhesions, staining);
- description of non-ocular local and systemic adverse effects, and histopatho-ogical findings, if any.

Discussion of results.

Interpretation of the results

30. Extrapolation of the results of eye irritation studies in laboratory animals to humans is valid only to a limited degree. In many cases the albino rabbit is more sensitive than humans to ocular irritants or corrosives.

31. Care should be taken in the interpretation of data to exclude irritation resulting from secondary infection.

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TABLE: GRADING OF OCULAR LESIONS

Cornea

Opacity: degree of density (readings should be taken from most dense area)*

No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured	2
Nacrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4

Maximum possible: 4

* The area of corneal opacity should be noted

Iris

Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect	1
Hemorrhage, gross destruction, or no reaction to light	2

Maximum possible: 2

Conjunctivae

Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)

Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson colour; individual vessels not easily discernible	2
Diffuse beefy red	3

Maximum possible: 3

Chemosis

Swelling (refers to lids and/or nictating membranes)

Normal	0
Some swelling above norma	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4

Maximum possible: 4

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ANNEX

DEFINITIONS

1. Eye irritation is the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.

2. Eye corrosion is the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.

SUPPLEMENT TO TEST GUIDELINE 405

A Sequential Testing Strategy for Eye Irritation and Corrosion

GENERAL CONSIDERATIONS

1. In the interests of sound science and animal welfare, it is important to avoid the unnecessary use of animals, and to minimise testing that is likely to produce severe responses in animals. All information on a substance relevant to its potential ocular irritation/corrosivity should be evaluated prior to considering *in vivo* testing. Sufficient evidence may already exist to classify a test substance as to its eye irritation or corrosion potential without the need to conduct testing in laboratory animals. Therefore, utilizing a weight-of-the-evidence analysis and sequential testing strategy will minimise the need for *in vivo* testing, especially if the substance is likely to produce severe reactions.

2. It is recommended that a weight-of-the-evidence analysis be used to evaluate existing information pertaining to eye irritation and corrosion of substances and to determine whether additional studies, other than *in vivo* eye studies, should be performed to help characterise such potential. Where further studies are needed, it is recommended that the sequential testing strategy be utilised to develop the relevant experimental data. For substances which have no testing history, the sequential testing strategy should be utilised to develop the data are needed to evaluate its eye corrosion/irritation. The testing strategy described in this Supplement was developed at an OECD workshop (1). It was subsequently affirmed and expanded in the Harmonised Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, in November 1998 (2).

3. Although this testing strategy is not an integrated part of Test Guideline 405, it expresses the recommended approach for the determination of eye irritation/corrosion properties. This approach represents both best practice and an ethical benchmark for *in vivo* testing for eye irritation/corrosion. The Guideline provides guidance for the conduct of the *in vivo* test and summarises the factors that should be addressed before considering such a test. The sequential testing strategy provides a weight-of-the-evidence approach for the evaluation of existing data on the eye irritation/corrosion properties of substances and a tiered approach for the generation of relevant data on substances for which additional studies are needed or for which no studies have been performed. The strategy includes the performance first of validated and accepted *in vitro* or *ex vivo* tests and then of Guideline 404 skin irritation/corrosion studies under specific circumstances (3)(4).

DESCRIPTION OF THE STEPWISE TESTING STRATEGY

4. Prior to undertaking tests as part of the sequential testing strategy (Figure), all available information should be evaluated to determine the need for *in vivo* eye testing. Although significant information might be gained from the evaluation of single parameters (e.g., extreme pH), the totality of existing information should be assessed. All relevant data on the effects of the substance in question, and its structural analogues, should be evaluated in making a weight-of-the-evidence decision, and a rationale for the decision should be presented. Primary emphasis should be placed upon existing human and animal data on the substance, followed by the outcome of *in vitro* or *ex vivo* testing. *In vivo* studies of corrosive substances should be avoided whenever possible. The factors considered in the testing strategy include:

5. Evaluation of existing human and animal data (Step 1). Existing human data, e.g. clinical and occupational studies, and case reports, and/or animal test data from ocular studies should be considered first, because they provide information directly related to effects on the eyes. Thereafter, available data from human and/or animal studies investigating dermal corrosion/irritation should be evaluated. Substances with known corrosivity or severe irritancy to the eye should not be instilled into the eyes of animals, nor should substances showing corrosive or severe irritant effects to the skin; such substances should be considered to be corrosive and/or irritating to the eyes as well. Substances with sufficient evidence of non-corrosivity and non-irritancy from previously performed ocular studies should also not be tested in *in vivo* eye studies.

6. Analysis of structure activity relationships (SAR) (Step 2). The results of testing of structurally related chemicals should be considered, if available. When sufficient human and/or animal data are available on structurally related substances or mixtures of such substances to indicate their eye corrrosion/irritancy potential, it can be presumed that the test substance will produce the same responses. In those cases, the substance may not need to be tested. Negative data from studies of structurally related substances or mixtures of such substances do not constitute sufficient evidence of non-corrosivity/non-irritancy of a substance under the sequential testing strategy. Validated and accepted SAR approaches should be used to identify the corrosion and irritation potential for both dermal and ocular effects.

7. Physicochemical properties and chemical reactivity (Step 3). Substances exhibiting pH extremes such as ≤2.0 or ≥11.5 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid/alkaline reserve (buffering capacity) may also be taken into consideration (5)(6). If the buffering capacity suggests that a substance may not be corrosive to the eye, then further testing should be undertaken to confirm this, preferably by the use of a validated and accepted *in vitro* or *ex vivo* test (see paragraph 9).

8. Consideration of other existing information (Step 4). All available information on systemic toxicity via the dermal route should be evaluated at this stage. The acute dermal toxicity of the test substance should also be considered. If the test substance has been shown to be highly toxic by the dermal route, it may not need to be tested in the eye. Although there is not necessarily a relationship between acute dermal toxicity and eye irritation/corrosion, it can be assumed that if an agent is highly toxic via the dermal route, it will also exhibit high toxicity when instilled into the eye. Such data may also be considered between Steps 2 and 3.

9. Results from *in vitro* or *ex vivo* tests (Steps 5 and 6). Substances that have demonstrated corrosive or severe irritant properties in an *in vitro* or *ex vivo* test (7)(8) that has been validated and accepted for the assessment specifically of eye or skin corrosivity/irritation, need not be tested in animals. It can be presumed that such substances will produce similar severe effects *in vivo*. If validated and accepted *in vitro/ex vivo* tests are not available, one should bypass Steps 5 and 6 and proceed directly to Step 7.

10. Assessment of *in vivo* dermal irritancy or corrosivity of the substance (Step 7). When insufficient evidence exists with which to perform a conclusive weight-of-the-evidence analysis of the potential eye irritation/corrosivity of a substance based upon data from the studies listed above, the *in vivo* skin irritation/corrosion potential should be evaluated first, using Guideline 404 (4) and the accompanying Supplement (9). If the substance is shown to produce corrosion or severe skin irritation, it should be considered to be a corrosive eye irritant unless other information supports an alternative conclusion. Thus, an *in vivo* eye test would not need to be performed. If the substance is not corrosive or severely irritating to the skin, an *in vivo* eye test should be performed.

11. *In vivo* test in rabbits (Steps 8 and 9): *In vivo* ocular testing should begin with an initial test using one animal. If the results of this test indicate the substance to be a severe irritant or corrosive to the eyes, further testing should not be performed. If that test does not reveal any corrosive or severe irritant effects, a confirmatory test is conducted with two additional animals. Depending upon the results of the confirmatory test, further tests may be needed. [see Test Guideline 405 (10)]

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FIGURE

TESTING AND EVALUATION STRATEGY FOR EYE IRRITATION/CORROSION

	Activity	Finding	Conclusion
1	Existing human and/or animal data showing effects on eyes	Severe damage to eyes	Apical endpoint; consider corrosive to eyes. No testing is needed.
		Eye irritant	Apical endpoint; consider irritating to eyes. No testing is needed.
		Not corrosive/not irritating to eyes	Apical endpoint; considered non-corrosive and non-irritating to eyes. No testing required.
	Existing human and/or animal data showing corrosive effects on skin	Skin corrosive	Assume corrosivity to eyes. No testing is needed.
	Existing human and/or animal data showing severe irritant effects on skin	Severe skin irritant	Assume irritating to eyes. No testing is needed
	↓ *no information available, or available information is not conclusive* ↓		
2	Perform SAR for eye corrosion/irritation	Predict severe damage to eyes	Assume corrosivity to eyes. No testing is needed.
		Predict irritation to eyes	Assume irritating to eyes. No testing is needed.
	Perform SAR for skin corrosion	Predict skin corrosivity	Assume corrosivity to eyes. No testing is needed.
	↓ *No predictions can be made, or predictions are not conclusive or negative* ↓		
3	Measure pH (buffering capacity, if relevant)	pH ≤ 2 or ≥ 11.5 (with high buffering capacity, if relevant)	Assume corrosivity to eyes. No testing is needed.
	↓ *2<pH < 11.5, or pH≤2.0 or ≥11.5 with low/no buffering capacity, if relevant* ↓		

4	Evaluate systemic toxicity via the dermal route	Highly toxic at concentrations that would be tested in the eye.	Substance would be too toxic for testing. No testing is needed.
	↓ *Such information is not available, or substance is not highly toxic* ↓		
5	Perform validated and accepted *in vitro* or *ex vivo* test for eye corrosion	Corrosive response	Assume corrosivity to eyes. No further testing is needed.
	↓ *Substance is not corrosive, or internationally validated in vitro or ex vivo testing methods for eye corrosion are not yet available* ↓		
6	Perform validated and accepted *in vitro* or *ex vivo* test for eye irritation	Irritant response	Assume irritancy to eyes. No further testing is needed.
	↓ *Substance is not an irritant, or internationally validated in vitro or ex vivo testing methods for eye irritation are not yet available* ↓		
7	Experimentally assess *in vivo* skin irritation/corrosion potential (see OECD Guideline 404)	Corrosive or severe irritant response	Assume corrosivity to eyes. No further testing is needed.
	↓ *Substance is not corrosive or severely irritating to skin* ↓		
8	Perform initial *in vivo* rabbit eye test using one animal	Severe damage to eyes	Consider corrosive to eyes. No further testing is needed.
	↓ *No severe damage, or no response* ↓		
9	Perform confirmatory test using one or two additional animals	Corrosive or irritating	Consider corrosive or irritating to eyes. No further testing is needed
		Not corrosive or irritating	Consider non-irritating and non-corrosive to eyes. No further testing is needed.

Appendix C4

Test for Eye Irritants (16 CFR 1500.42)

Available at:
http://www.gpo.gov/fdsys/pkg/CFR-2012-title16-vol2/pdf/CFR-2012-title16-vol2-sec1500-42.pdf

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§ 1500.42 Test for eye irritants.

(a)(1) Six albino rabbits are used for each test substance. Animal facilities for such procedures shall be so designed and maintained as to exclude sawdust, wood chips, or other extraneous materials that might produce eye irritation. Both eyes of each animal in the test group shall be examined before testing, and only those animals without eye defects or irritation shall be used. The animal is held firmly but gently until quiet. The test material is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance is dropped. The lids are then gently held together for one second and the animal is released. The other eye, remaining untreated, serves as a control. For testing liquids, 0.1 milliliter is used. For solids or pastes, 100 milligrams of the test substance is used, except that for substances in flake, granule, powder, or other particulate form the amount that has a volume of 0.1 milliliter (after compacting as much as possible without crushing or altering the individual particles, such as by tapping the measuring container) shall be used whenever this volume weighs less than 100 milligrams. In such a case, the weight of the 0.1 milliliter test dose should be recorded. The eyes are not washed following instillation of test material except as noted below.

(2) The eyes are examined and the grade of ocular reaction is recorded at 24, 48, and 72 hours. Reading of reactions is facilitated by use of a binocular loupe, hand slit-lamp, or other expert means. After the recording of observations at 24 hours, any or all eyes may be further examined after applying fluorescein. For this optional test, one drop of fluorescein sodium ophthalmic solution U.S.P. or equivalent is dropped directly on the cornea. After flushing out the excess fluorescein with sodium chloride solution U.S.P. or equivalent, injured areas of the cornea appear yellow; this is best visualized in a darkened room under ultraviolet illumination. Any or all eyes may be washed with sodium chloride solution U.S.P. or equivalent after the 24-hour reading.

(b)(1) An animal shall be considered as exhibiting a positive reaction if the test substance produces at any of the readings ulceration of the cornea (other than a fine stippling), or opacity of the cornea (other than a slight

dulling of the normal luster), or inflammation of the iris (other than a slight deepening of the folds (or rugae) or a slight circumcorneal injection of the blood vessels), or if such substance produces in the conjunctivae (excluding the cornea and iris) an obvious swelling with partial eversion of the lids or a diffuse crimson-red with individual vessels not easily discernible.

(2) The test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction. If only one animal exhibits a positive reaction, the test shall be regarded as negative. If two or three animals a positive reaction, the test is repeated using a different group of six animals. The second test shall be considered positive if three or more of the animals exhibit a positive reaction. If only one or two animals in the second test exhibit a positive reaction, the test shall be repeated with a different group of six animals. Should a third test be needed, the substance will be regarded as an irritant if any animal exhibits a positive response.

(c) To assist testing laboratories and other interested persons in interpreting the results obtained when a substance is tested in accordance with the method described in paragraph (a) of this section, an ''Illustrated Guide for Grading Eye Irritation by Hazardous Substances'' will be sold by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.[1] The guide will contain color plates depicting responses of varying intensity to specific test solutions. The grade of response and the substance used to produce the response will be indicated.

[38 FR 27012, Sept. 27, 1973; 38 FR 30105, Nov. 1, 1973; 62 FR 46667, Sept. 4, 1997]

[1] The Illustrated Guide is out of print and, as of January 1, 1981, no longer available. However, information about the test method, and black and white photocopies may be obtained by writing to the Directorate for Epidemiology and Health Sciences, CPSC, Washington, D.C. 20207, (301) 504–0957.

Appendix C5

U.S. Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances Guidance Document 870.2400

Available at:
http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0156-0006

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United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–98–195
August 1998

Health Effects Test Guidelines OPPTS 870.2400 Acute Eye Irritation

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.2400 Acute eye irritation.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPTS 798.4500 Primary Eye Irritation; OPP 81–4 Acute Eye Irritation—Rabbit (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD 405 Acute Eye Irritation/Corrosion.

(b) **Purpose.** (1) In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant and/or corrosive effects on eyes of mammals is an important initial step. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the eyes and associated mucous membranes to the test substance.

(2) Data on primary eye irritation are required by 40 CFR 158.340 to support the registration of each manufacturing-use product and end-use product. (See § 158.50 to determine whether these data must be submitted and which purity/grade of the test substance should be tested.)

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Eye corrosion is the production of irreversible tissue damage in the eye following application of a test substance to the anterior surface of the eye.

Eye irritation is the production of reversible changes in the eye following the application of a test substance to the anterior surface of the eye.

(d) **Principle of the test method.** The substance to be tested is applied in a single dose to one of the eyes in each of several experimental animals; the untreated eye is used to provide control information. The degree of irritation/corrosion is evaluated and scored at specified intervals and is fully described to provide a complete evaluation of the effects. The duration of the study should be sufficient to permit a full evaluation of the reversibility or irreversibility of the effects observed. The period of observation should be at least 72 h, but need not exceed 21 days. Animals showing severe and enduring signs of distress and pain may need to be killed in a humane fashion.

(e) **Initial considerations.** (1) Strongly acidic or alkaline substances, for example, with a demonstrated pH of 2 or less or 11.5 or greater, need not be tested owing to their predictable corrosive properties. Buffer capacity should also be taken into account.

(2) Materials which have demonstrated definite corrosion or severe irritation in a dermal study need not be further tested for eye irritation. It may be presumed that such substances will produce similarly severe effects in the eyes.

(3) Results from well validated and accepted *in vitro* test systems may serve to identify corrosives or irritants such that the test material need not be tested *in vivo*.

(f) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** A variety of experimental animals has been used, but it is recommended that testing should be performed using healthy adult albino rabbits. Commonly used laboratory strains should be used. If another mammalian species is used, the tester should provide justification/reasoning for its selection.

(ii) **Number of animals.** A single animal should be considered if marked effects are anticipated. If the results of this test in one animal suggest the test substance to be a severe irritant (reversible effect) or corrosive (irreversible effect) to the eye using the procedure described, further tests may not need to be performed. In cases other than a single animal test, at least three animals should be used. Occasionally, further testing in additional animals may be appropriate to clarify equivocal responses.

(2) **Dose level.** For testing liquids, a dose of 0.1 mL is recommended. In testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 mL, or a weight of not more than 100 mg (the weight must always be recorded). If the test material is solid or granular, it should be ground to a fine dust. The volume of particulates should be measured after gently compacting them (e.g. by tapping the measuring container). To test a substance contained in a pressurized aerosol container, the eye should be held open and the test substance administered in a single burst of about 1 sec from a distance of 10 cm directly in front of the eye. The dose may be estimated by weighing the container before and after use. Care should be taken not to damage the eye. Pump sprays should not be used but instead the liquid should be expelled and 0.1 mL collected and instilled into the eye as described for liquids. For volatile substances, the dose may be estimated by weighing the container before and after use.

(3) **Examination of eyes prior to test.** Both eyes of each experimental animal provisionally selected for testing should be examined within 24 h before testing starts by the same procedure to be used during the

test examination. Animals showing eye irritation, ocular defects, or pre-existing corneal injury should not be used.

(4) **Application of the test substance.** (i) The test substance should be placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about 1 sec in order to limit loss of the material. The other eye, which remains untreated, serves as a control. If it is thought that the substance may cause extreme pain, local anesthetic may be used prior to instillation of the test substance. The type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use. The control eye should be similarly anesthetized.

(ii) The eyes of the test animals should not be washed out for 24 h following instillation of the test substance. At 24 h, a washout may be used if considered appropriate. This is to show whether washing with water palliates or exacerbates irritation.

(iii) For some substances shown to be irritating by this test, additional testing using animals with eyes washed soon after instillation of the substance may be indicated. Half a minute after instillation, the eyes of the animals are washed with water for 30 sec, using a volume and velocity of flow which will not cause injury.

(5) **Observation period.** The duration of the observation period is at least 72 h, and should not be fixed rigidly, but should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed. The observation period normally need not exceed 21 days after instillation.

(6) **Clinical examination and scoring.** (i) The eyes should be examined at 1, 24, 48, and 72 h. If there is no evidence of irritation at 72 h, the study may be ended. Extended observation (e.g. at 7 and 21 days) may be necessary if there is persistent corneal involvement or other ocular irritation in order to determine the progress of the lesions and their reversibility or irreversibility. In addition to the observations of the cornea, iris and conjunctivae, any other lesions which are noted should be recorded and reported. The grades of ocular reaction using the following table should be recorded at each examination.

Grades for Ocular Lesions	
Cornea	
Opacity: Degree of density (area most dense taken for reading). No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	*1
Easily discernible translucent area, details of iris slightly obscured	*2
Nacrous area, no details or iris visible, size of pupil barely discernible	*3
Opaque cornea, iris not discernible through the opacity	*4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	*1
No reaction to light, hemorrhage, gross destruction (any or all of these)	*2
Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae, excluding cornea and iris).	
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	*2
Diffuse beefy red	*3
Chemosis (refers to lids and/or nictitating membranes)	
No swelling	0
Any swelling above normal (includes nictitating membranes)	1
Obvious swelling with partial eversion of lids	*2
Swelling with lids about half closed	*3
Swelling with lids more than half-closed	*4

*Starred figures indicate positive grades.

(ii) Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device. After recording the observations at 24 h, the eyes of any or all rabbits may be further examined with the aid of fluorescein.

(iii) The grading of ocular responses is subject to various interpretations. To promote harmonization and to assist testing laboratories and those involved in making and interpreting the observations, an illustrated guide in grading eye irritation should be used.

(g) **Data and reporting—**(1) **Data summary.** Data should be summarized in tabular form, showing for each individual animal the irritation scores at observation time up until reversal (nonpositive grades) or 21 days when the test is concluded; a description of the degree and nature of irritation; the presence of serious lesions and any effects other than ocular which were observed.

(2) **Evaluation of the results.** The ocular irritation scores should be evaluated in conjunction with the nature and reversibility or otherwise of the responses observed. The individual scores do not represent an absolute standard for the irritant properties of a material. They should be viewed as reference values and are only meaningful when supported by a full description and evaluation of the observations.

(3) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i)Species, strain, sex, age, and source of test animal.

(ii) Rationale for selection of species (if species is other than the species preferred.

(iiii) Tabulation of irritant/corrosive response data for each individual animal at each observation time point (e.g. 1, 24, 48, and 72 h until reversibility of lesions or termination of the test).

(iv) Description of any lesions observed.

(v) Narrative description of the degree and nature of irritation or corrosion observed.

(vi) Description of the method used to score the irritation at 1, 24, 48, and 72 h (e.g. hand slit-lamp, biomicroscope, fluorescein stain).

(vii) Description of any nonocular effects noted.

(viii) Description of any pre-test conditioning, including diet, quarantine, and treatment of disease.

(ix) Description of caging conditions including number (and any change in number) of animals per cage, bedding material, ambient temperature and humidity, photoperiod, and identification of diet of test animal.

(x) Manufacturer, source, purity, and lot number of test substance.

(xi) Physical nature, and, where appropriate, concentration and pH value for the test substance.

(xii) Identification, composition, and characteristics of any vehicles (e.g., diluents, suspending agents, emulsifiers, and anesthetics) or other materials used in administering the test substance.

(xiii) A list of references cited in the body of the report, i.e., references to any published literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results.

(h) **References.** The following references should be consulted for additional background information on this test guideline

(1) Buehler, E.V. and Newmann, E.A. A Comparison of Eye Irritation in Monkeys and Rabbits. *Toxicology and Applied Pharmacology* 6:701–710 (1964).

(2) Draize, J.H. *Dermal Toxicity. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959) 3rd printing 1975, pp. 49–52.

(3) Draize, J.H. et al. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Journal of Pharmacology and Experimental Therapeutics.* 83:377–390 (1944).

(4) Loomis, T.A. *Essentials of Toxicology.* Lea and Febicer, Philadelphia 3rd ed. 1978 pp. 226–232.

(5) Kay, J.H. and Calandra, J.C., Interpretation of eye irritation tests. *Journal of the Society of Cosmetic Chemists* 13:281–289 (1962).

(6) National Academy of Sciences. *Principles and Procedures for Evaluating the Toxicity of Household Substances.* A report propared by the Committee for the revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(7) World Health Organization. *Part I. Environmental Health Criteria 6. Principles and Methods for Evaluating the Toxicity of Chemicals.* World Health Organization, Geneva (1978).

Appendix C6

U.S. Environmental Protection Agency Label Review Manual

Available at:
http://www.epa.gov/oppfead1/labeling/lrm/

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