

Using Fewer Animals to Identify Chemical Eye Hazards: Revised Classification Criteria Necessary to Maintain Equivalent Hazard Labeling

W. Stokes¹, J. Haseman², E. Lipscomb³, J. Truax³, N. Johnson³, B. Jones³, and D. Allen³.

¹NICEATM/NIEHS/NIH/DHHS, RTP, NC; ²Consultant, Raleigh, NC; ³ILS Inc., RTP, NC.

Abstract

U.S. Federal Hazardous Substances Act (FHSA) regulations specify eye safety testing procedures and hazard classification criteria for chemicals and products regulated by CPSC. Current regulations require up to 3 sequential tests of 6 animals per test, with decisions on the need for subsequent tests based on the number of positive responses observed. Testing conducted in accordance with the OECD test guideline for eye irritation and corrosion can also be used, but current FHSA regulations do not provide criteria to classify results from this 3-animal test. An analysis was therefore conducted to determine classification criteria for results from a 3-animal test that would provide hazard labeling equivalent to that provided by current FHSA regulations. The frequency that current FHSA classification criteria identify substances as ocular irritants was compared with the frequency that a classification criterion of either one or two positive animals out of three would identify these substances. The classifications assigned by each of the three criteria using four different underlying response rates (20%, 40%, 50%, and 75%) were compared. For these response rates, current FHSA requirements would identify 20%, 73%, 88%, and >99% of substances as irritants, respectively, while using a criterion of at least one out of three positive animals in a 3-animal test would identify 49%, 78%, 88%, and 98% of substances as irritants, respectively. In contrast, using a criterion of at least two out of three positive animals in a 3-animal test would identify far fewer irritants, with detection rates of 10, 35, 50, and 84%, respectively. We conclude that using a classification criterion of at least one out of three positive animals in a 3-animal test will provide the same or greater level of eye hazard labeling as current FHSA requirements, while using up to 83% fewer animals.

Introduction

- Each year, approximately 2 million eye injuries occur in the U.S. (McGwin et al. 2006a).
 - Of these, more than 40,000 result in permanent visual impairment.
- Household cleaning chemicals and other chemical products are the leading cause of consumer product-related eye injuries in children under age 10 (McGwin et al. 2006b).
- The Federal Hazardous Substances Act (FHSA; FHSA 2008) “requires that certain hazardous household products (“hazardous substances”) bear cautionary labeling to alert consumers to the potential hazards that those products present and to inform them of the measures they need to protect themselves from those hazards” (<http://www.cpsc.gov/businfo/fhsa.html>).
- The U.S. Consumer Product Safety Commission (CPSC) issues regulations implementing the FHSA.
- The regulations for hazardous substances under the FHSA are found in Title 16 CFR part 1500 (CPSC 2010).
- The “Test for Eye Irritants” (16 CFR 1500.42 [CPSC 2010]) provides criteria and procedures for identification of ocular hazards based on rabbit eye test results as follows (**Figure 1**):
 - Each test requires 6 animals. Up to 3 sequential tests may be required, with each substance requiring 6, 12, or 18 animals to reach a hazard decision (**Table 1**).
 - The requirement for second and third sequential tests is based on the number of positive animals in the previous test.
 - Observations and severity scores are recorded at 24, 48, and 72 hours after test substance administration for four types of ocular injuries (**Table 2**):
 - Corneal ulceration/opacity

- Iritis
- Conjunctival redness
- Conjunctival swelling
- Reading of reactions is facilitated by:
 - Use of a binocular loupe, hand slit-lamp, or other expert means
 - After 24 hours, eyes may be further examined after applying fluorescein (aids in detecting areas of corneal ulceration).
- Guidelines for eye irritation/corrosion testing were revised in 2002 to reduce the maximum number of required animals from 6 to 3 (see **Background**). 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 hazard classification equivalent to that provided by current FHSA regulations that require the use of 6 to 18 animals.

Figure 1. Schematic of FHSA Hazard Classification Criteria and Procedures

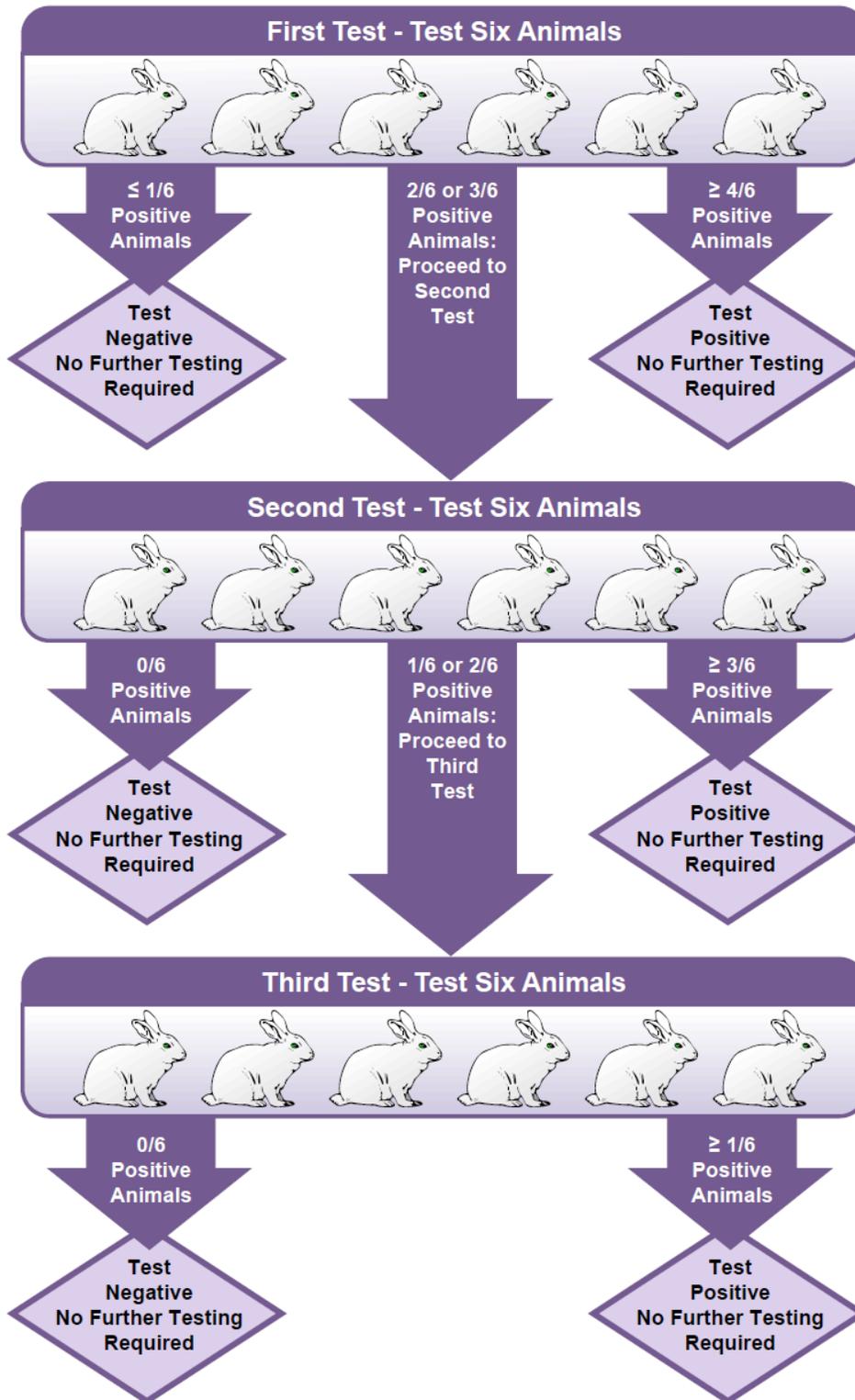


Table 1. FHSA Classification Criteria and Procedures for Identification of Ocular Hazards (16 CFR 1500.42)

<p>Criteria for a Positive Response in a Single Rabbit (Based on one or more of the following for any reading at 24, 48, and 72 hours)</p>	<p>1. Corneal ulceration or corneal opacity¹ ≥1 2. Iritis² ≥1 3. Conjunctival swelling³ ≥2 4. Conjunctival redness³ ≥2</p>
<p>Hazard Classification Criteria and Procedures</p>	<p><u>First Test: Test 6 animals</u></p> <ul style="list-style-type: none"> • 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. <p><u>Second Test: Test 6 animals</u></p> <ul style="list-style-type: none"> • 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. <p><u>Third Test: Test 6 animals</u></p> <ul style="list-style-type: none"> • If ≥1/6 animals are positive, the test is positive. • If 0/6 are positive, the test is negative.

¹ Ulceration of the cornea (other than a fine stippling), or opacity of the cornea (other than a slight dulling of the normal luster)

² Inflammation of the iris (other than a slight deepening of the folds [or rugae] or a slight circumcorneal injection of the blood vessels)

³ Obvious conjunctival swelling with partial eversion of the lids or conjunctival redness with diffuse crimson-red, individual vessels not easily discernible

Table 2. Scores for Grading Severity of Ocular Lesions¹

Lesion ²	Score ³
Cornea	
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	
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)	
Some vessels definitely injected above normal	1
Diffuse, crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
B. Chemosis	
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

¹ Table is adapted from the *CPSC Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances*, available by written request: Directorate for Epidemiology and Health Sciences, CPSC, Washington, DC 20207.

² Positive responses for individual animals are based on meeting or exceeding the minimum severity criteria for any one of the four types of eye injuries at any of the three time points.

³ Scores in **bold** indicate positive responses. Scores of 0 are assigned for each parameter if the cornea, iris, or conjunctiva is normal.

Background

- The U.S. Interagency Regulatory Animal Group (IRAG) previously conducted analyses to determine if a group size of 6 rabbits for ocular safety testing could be reduced in order to use fewer animals (Springer et al. 1993).
 - Use of 3 rabbits per test provided accuracy of up to 97% in predicting a 6-animal test.
- The U.S. subsequently proposed revisions to Organisation for Economic Co-operation and Development (OECD) Test Guideline 405: Acute Eye Irritation/Corrosion (OECD 1987) to reduce the maximum number of required animals from 6 to 3 (DeSilva et al. 1997; OECD 1999; Springer et al. 1993).
 - The revised Test Guideline 405 was adopted in 2002 (OECD 2002).
 - U.S. agencies agree to accept test data for review generated in accordance with OECD test guidelines.

Optimization of the Number of Positive Animals Required for FHSA Hazard Classification and Labeling

- The minimum number of animals that would be required under the FHSA sequential testing strategy to assign a definitive test classification as positive or negative was evaluated for each of the possible test outcomes (**Table 3**).
 - The minimum percentage of positive animal responses that can result in a positive FHSA hazard classification is 22% (4/18).
 - The maximum percentage of positive animal responses that can result in a negative FHSA hazard classification ranges from 17% (1/6) to 28% (5/18).

Table 3. Number of Positive Animals and Sequential Tests Required for Assignment of an Irritant Classification According to the Current FHSA Requirements

Positive Test Criteria for Irritant Classification:	Positive Animals					
	≥4/6	2/6 or 3/6	3/6	3/6	2/6	2/6
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 Labeled Classification	1/6 (17%)	3/12 (25%)	5/18 (28%)	4/18 (22%)	4/18 (22%)	3/18 (17%)

Reducing the Number of Animals Required for FHSA Hazard Classification: Comparison of Three Strategies

- The hazard classifications assigned by each of the three different classification strategies were compared using four different underlying positive response rates (20%, 40%, 50%, and 75%) (see **Table 4**).
 - Classification Strategy 1: The current sequential testing strategy used to assign an FHSA classification, which uses a minimum threshold of 22% (4/18) positive animals
 - Classification Strategy 2: A minimum threshold of $\geq 1/3$ (33%) positive animals
 - Classification Strategy 3: A minimum threshold of $\geq 2/3$ (67%) positive animals
- In order to compare the frequency that each strategy would identify substances as ocular irritants, a number of different hypothetical positive response rates and the resulting classification that would be assigned by each strategy were compared.
- Example of Strategy 1 calculations, assuming an underlying positive response rate of 20% in all animals tested:
 - Based on the binomial distribution, the likelihood of observing:
 - 0/6 positive responses is 0.262 or 26.2%
 - The likelihood of observing six consecutive negative responses, each of which has a probability of 0.8 (1.0 – 0.2 [underlying positive response rate]) or $0.8^6 = 0.262$.
 - 1/6 positive responses is 0.393 or 39.3%
 - 2/6 positive responses is 0.246 or 24.6%
 - 3/6 positive responses is 0.082 or 8.2%
 - >3/6 positive responses is 0.017 or 1.7%

- The probability that the first test will produce a negative classification is simply the sum of the likelihood of observing 0/6 and 1/6 positive response ($0.262 + 0.393 = 0.655$) (i.e., the only results that can result in a negative classification with the first 6-animal test).
 - Thus, 65.5% of the time, no further testing would be necessary, and the substance would not be labeled.
- A second 6-animal test would be needed if the first test resulted in 2/6 or 3/6 positive responses, which would occur with a likelihood of 32.8% ($0.246 + 0.082 = 0.328$).
 - The second test would result in a negative classification only if 0/6 positive responses were observed, making the likelihood of a negative classification by the second test 8.6% ($0.328 \times 0.262 = 0.086$).
- A third 6-animal test would be needed if the second test resulted in 1/6 or 2/6 positive responses, which would occur with a likelihood of 63.9% ($0.393 + 0.246 = 0.639$).
 - The third test would produce a negative classification if 0/6 positive responses were observed.
 - Thus, the likelihood that a negative classification will result from the third test is 5.5% ($0.328 \times 0.639 \times 0.262 = 0.055$).
- Adding these three probabilities results in the overall likelihood of a negative classification of $0.655 + 0.086 + 0.055$ or 0.796 (79.6%), and thus the likelihood of a positive classification by subtraction is $1 - 0.796$ or 0.204 (20.4%; see **Table 4**).
- Example of Strategy 2 calculations, assuming an underlying positive response rate of 20% in all animals tested:
 - The likelihood of a positive classification using Strategy 2 is just 1 minus the likelihood of observing 0/3 positives or $1 - (0.8 \times 0.8 \times 0.8)$ or 0.488 (48.8%; see **Table 4**).

- Example of Strategy 3 calculations, assuming an underlying positive response rate of 20% in all animals tested:
 - A positive response rate of 1/3 (likelihood = 0.384) would also lead to a negative classification, making the overall likelihood of a positive classification $0.488 - 0.384$ or 0.104 (10.4%; see **Table 4**).

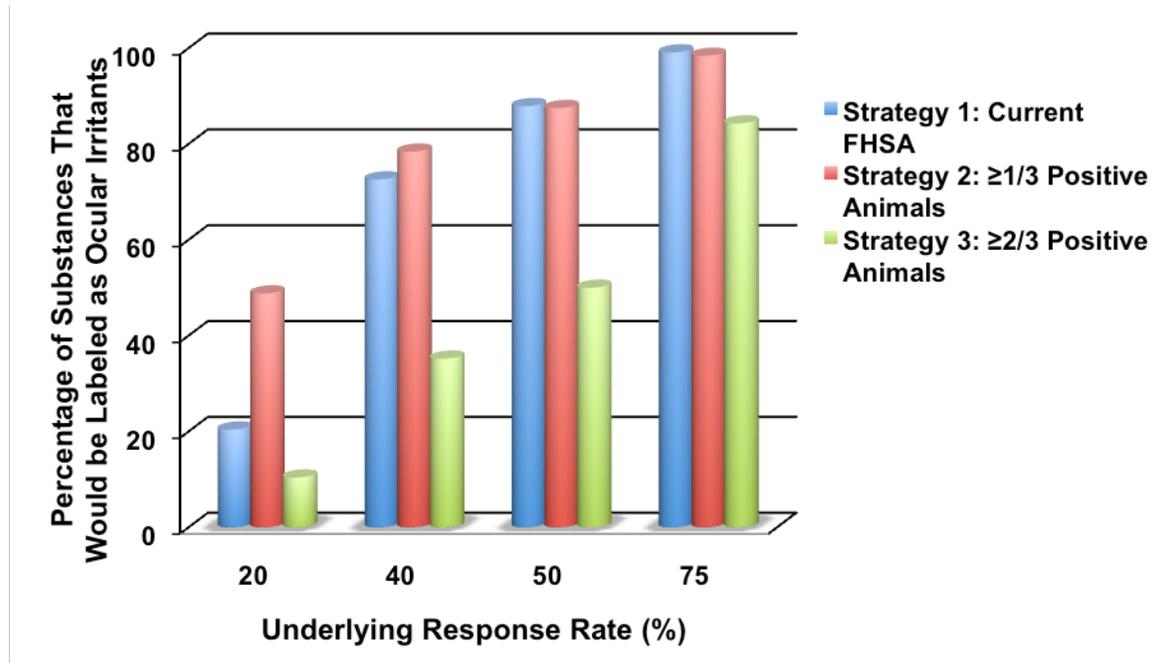
Table 4. Percentage of Substances Labeled as Ocular Irritants Based on Three Strategies

Underlying Positive Response Rate	Percentage of Substances That Would be Labeled as Ocular Irritants		
	Strategy 1 Current FHSA	Strategy 2 ≥1/3 Positive Animals	Strategy 3 ≥2/3 Positive Animals
20%	20.4%	48.8%	10.4%
40%	72.6%	78.4%	35.2%
50%	87.9%	87.5%	50.0%
75%	>99%	98.4%	84.3%

Results of Comparing Three Strategies

- Even though it uses fewer animals, Strategy 2 is more powerful than the current FHSA requirements for detecting positive response rates of 20% to 40%, and has approximately the same power as current FHSA requirements for response rates of 50% and 75% (**Figure 2**).
- Strategy 3 will identify far fewer irritants than either Strategy 1 (current FHSA requirements) or Strategy 2.
 - Strategy 3 considers a single positive response (1/3) to not be indicative of an irritant response and has lower power than the current FHSA requirements in all cases considered.

Figure 2. Strategy 2 Provides the Same or Greater Level of Eye Hazard Labeling as Current FHSA Requirements



Conclusions

- Using a classification criterion of at least one out of three positive animals in a 3-animal test for the identification of eye hazards will provide the same or greater level of eye hazard labeling as current FHSA requirements, while using up to 83% fewer animals.
- Using a criterion of at least two out of three positive animals in a 3-animal test will identify far fewer irritants.

References

- CPSC. 2010. 16 CFR 1500. Washington, DC:U.S. Code of Federal Regulations.
- DeSilva O, Cottin M, Dami N, Roguet R, Catroux P, Toufic A, et al. 1997. Food Chem Toxicol 35:159-164.
- FHSA. 2008. Federal Hazardous Substances Act. Public Law 86-613, 15 U.S.C. 1261-1278.
- McGwin G, Hall TA, Xie A, Owsley C. 2006a. Invest Ophthalmol Vis Sci 47:521-527.
- McGwin G, Hall TA, Seale J, Xie A, Owsley C. 2006b. J Safety Res 37:501-506.
- OECD. 1987. Test Guideline 405. Paris:Organisation for Economic Co-operation and Development.
- OECD. 1999. Detailed Review Document Number 14. ENV/JM/MONO(99)4. Paris:Organisation for Economic Co-operation and Development.
- OECD. 2002. Test Guideline 405. Paris:Organisation for Economic Co-operation and Development.
- Springer J, Chambers W, Green S, Gupta K, Hill R, Hurley P, et al. 1993. Food Chem Toxicol 31:105-109.

Acknowledgements

ILS, Inc. staff are supported by NIEHS contract N01-ES 35504. The views expressed above do not necessarily represent the official positions of any Federal agency. This poster reflects the views of the authors. Since the poster was written as part of the official duties of the authors, it can be freely copied.