



Summary of Current Ocular Safety Testing Guidelines and Criteria Used for Hazard Classification and Labeling in the U.S.

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Bethesda, MD
January 19, 2011

Outline

- Current guidelines for ocular safety testing
 - Statutes and regulations
 - Testing guidelines
 - The rabbit eye test
- Criteria used for ocular hazard classification and labeling in the U.S.
 - EPA
 - FHSA

Statutes and Regulations Requiring Ocular Safety Testing

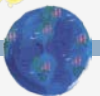
| Agency | Authority | Regulation | Test Description or Guideline |
|--------|-----------------------------|----------------|-------------------------------|
| EPA | FIFRA (1947) TSCA (1977) | 40 CFR 156 | OPPTS 870.2400 |
| CPSC | FHSA (1964) | 16 CFR 1500 | 16 CFR 1500.42 |
| FDA | FDCA (1938) | 21 CFR 501-523 | 16 CFR 1500.42 |
| OSHA | OHSA (1970) | 29 CFR 1910 | 16 CFR 1500.42 |

Abbreviations: CFR: Code of the Federal Register; CPSC: Consumer Product Safety Commission; EPA: Environmental Protection Agency; FDA: Food and Drug Administration FDCA: Food, Drug and Cosmetic Act; FHSA: Federal Hazardous Substances Act; FIFRA: Federal Insecticide, Fungicide, and Rodenticide Act; OPPTS: EPA, Office of Prevention, Pesticides, and Toxic Substances; OSHA: Occupational Safety and Health Administration; TSCA: Toxic Substances Control Act

Relevant Testing Guidelines

| Test Method Component | EPA (TG OPPTS 870.2400 [1998]) | FHSA (16 CFR 1500.42 [2005]) | OECD (TG 405 [2002]) |
|------------------------|---|---|---|
| Number of Animals | n=1 to screen for corrosive, then n≥2 | n≥6 (or n=1-3 using TG 405) | n=1 to screen for corrosive, then n≥2 |
| Quantity | 0.1 mL or 0.1 g | 0.1 mL or 0.1 g | 0.1 mL or 0.1 g |
| Observation Times | 1, 24, 48, 72 hr, and daily thereafter until lesions clear or 21 days | 24, 48, 72 hr | 1, 24, 48, 72 hr, and daily thereafter until lesions clear or 21 days |
| Post-dosing Irrigation | 24 hr* | 24 hr | Liquids: 24 hr Solids: 1 hr |
| Use of Anesthetics | Prior to dosing if pain anticipated and no interference with test | Prior to dosing if pain anticipated and no interference with test | Prior to dosing if pain anticipated and no interference with test |

*For substances shown to be irritating by this test, additional testing using animals with eyes washed 30 seconds after instillation may be indicated



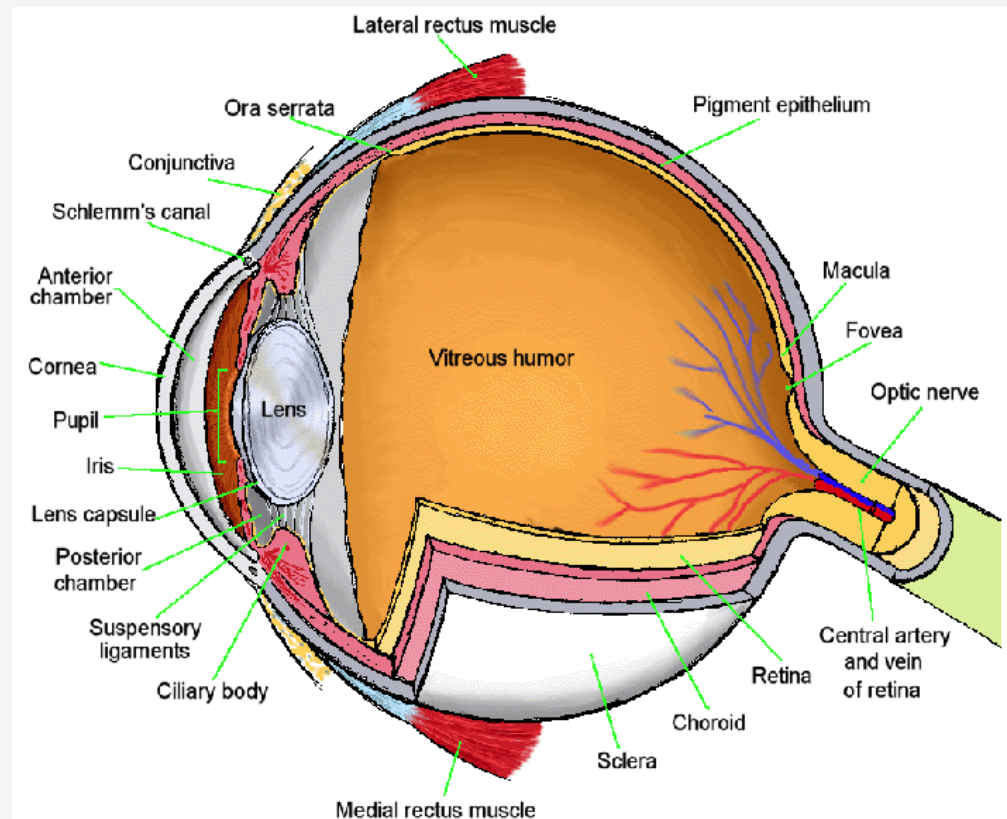
The Rabbit Eye Test

- 1-3 healthy adult albino rabbits (e.g., White New Zealand)
- 0.1mL (liquid) or 0.1g (solid) placed in the conjunctival sac of one eye
 - Untreated eye serves as control
- Observation period: 1, 24, 48, 72 hr, and daily thereafter until lesions clear or 21 days

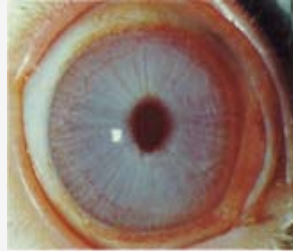
Rabbit Eye Injury Scoring

- Eyes scored using the rabbit eye test for injuries in three different tissues:

- A. Cornea
- B. Iris
- C. Conjunctiva



Eye Injury Scoring: Corneal Opacity Score¹

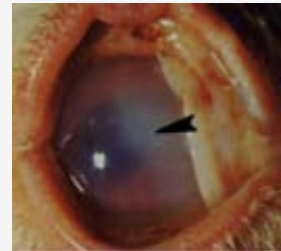


0 = Normal, no ulceration or opacity

Positive Lesions



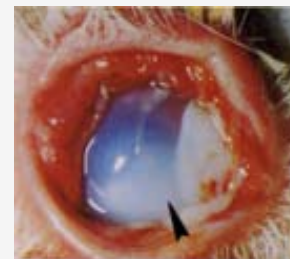
1 = Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible



3 = Nacreous area, no details of iris visible, size of pupil barely discernable



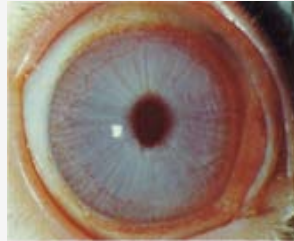
2 = Easily discernible translucent area, details of iris slightly obscured



4 = Opaque cornea, iris not discernible through the opacity

¹Photos obtained from the CPSC Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances. Grades for ocular lesions reproduced from EPA. 1998. Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195.

Eye Injury Scoring: Iritis Score¹

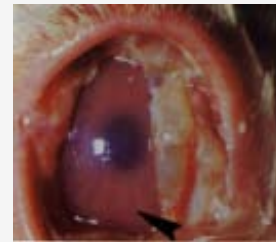


0 = Normal

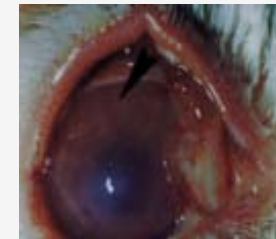
Positive Lesions



1 = Markedly deepened rugae, congestion, swelling moderate circumcorneal hyperemia, or injection, any of these or combination thereof, iris still reacting to light (sluggish reaction is positive)

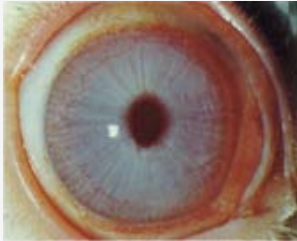


2 = No reaction to light, hemorrhage, gross destruction (any or all of these)



¹Photos obtained from the CPSC Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances. Grades for ocular lesions reproduced from EPA. 1998. Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195.

Eye Injury Scoring: Conjunctival Redness Score¹



0 = Normal

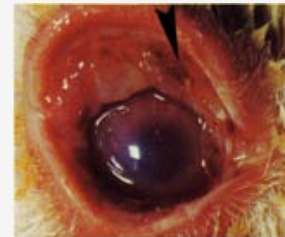


1 = Some blood vessels definitely hyperemic (injected)

Positive Lesions

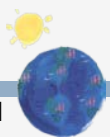


2 = Diffuse, crimson color, individual vessels not easily discernable

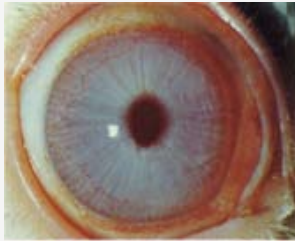


3 = Diffuse, beefy red

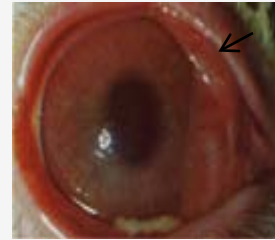
¹Photos obtained from the CPSC Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances. Grades for ocular lesions reproduced from EPA. 1998. Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195.



Eye Injury Scores: Conjunctival Chemosis (Swelling) Score¹



0 = Normal

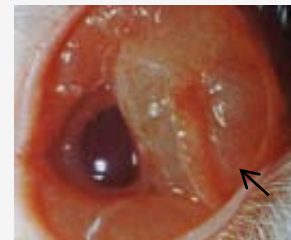


1 = Any swelling above normal (includes nictitating membrane)

Positive Lesions



2 = Obvious swelling with partial eversion of the lids



4 = Swelling with lids more than half-closed

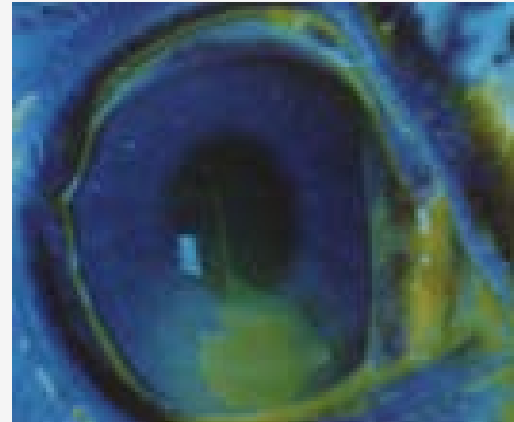
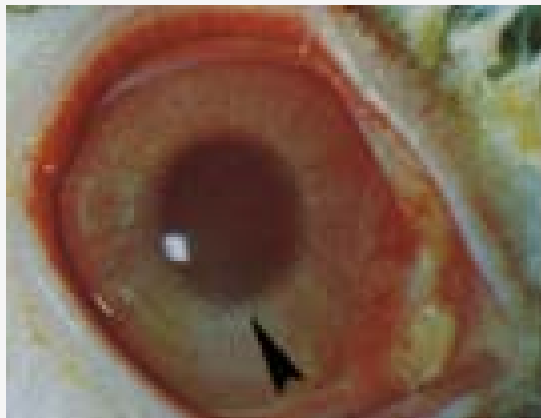


3 = Swelling with lids about half closed

¹Photos obtained from the CPSC Illustrated Guide for Grading Eye Irritation Caused by Hazardous Substances. Grades for ocular lesions reproduced from EPA. 1998. Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195.

Optional Assessments of Ocular Effects (1)

- OECD TG 405 (2002); EPA TG OPPTS 870.2400 (1998); CPSC 16CFR1500.42 (2005):
 - “Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device.”
 - “After recording observations at 24 hours, the eyes [of any or all rabbits] may be further examined with the aid of fluorescein.”



Corneal Opacity = 1

Optional Assessments of Ocular Effects (2)

- In practice, information from these optional assessments is rarely provided to support ocular hazard classification and labeling

Assessments Not Currently Required or Suggested in Current Testing Regulations and Guidelines

- Corneal thickness changes
- Depth of corneal injuries
- Aqueous humor changes
- Subtle corneal opacities
 - Detectable by slit lamp biomicroscopy
- Histopathology
- Photographic documentation

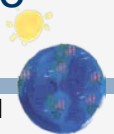
Testing Guidelines: Summary

- All three testing guidelines are based on the rabbit eye test (Draize et al. 1944)
- All three testing guidelines permit the use of anesthetics
- EPA and OECD require studies to be carried out to 21 days to evaluate reversible/irreversible effects, while FHSA only requires observations out to 3 days
- All three test guidelines allow irrigation of eyes after 24 hr
 - OECD allows for irrigation at 1 hr for solid substances



Interpretation of Ocular Lesions Varies Among Hazard Classification Systems

- The minimum number/proportion of animals with positive eye injury responses required for classifying a substance as an eye hazard differs significantly
 - EPA eye hazards labeled when **any single test animal** exhibits a positive response, regardless of the number of animals tested
 - FHSA eye hazards labeled based on as few as **22%** positive animals
 - In most cases, classification according to GHS requires more severe and/or longer duration of eye injuries
 - Ex: Need **at least 67% (2/3)** positive animals for classification as a Category 2 eye hazard
- There is a significant difference in the basis for eye injuries to be considered a positive response
 - EPA and FHSA positive animals based on the highest severity response at **any daily time point**
 - GHS positive animals based on the **average** severity across three daily time points



EPA Classification System

- At least 3 animals per test (one-animal screen permitted)
 - OR no test due to physical chemical properties and take TOX I label
- Maximum score in any animal used for classification
 - Positive: CO or IR ≥ 1 or CC or CR ≥ 2
- Most severe response used for classification of substance

| EPA Category | <i>In Vivo</i> Effect |
|--------------|--|
| I | Corrosive; (irreversible) corneal involvement or irritation persisting more than 21 days |
| II | Corneal involvement or irritation clearing in 8-21 days |
| III | Corneal involvement or irritation clearing in ≤ 7 days |
| IV | Minimal effects clearing within 24 hr. |

CC: Conjunctival Chemosis; CO: Corneal Opacity; CR: Conjunctival Redness; IR: Iritis

EPA Eye Irritation Studies - Conventional Chemicals

| Tox 1 - Liner Database (reviewed before 2000) | | |
|---|----------------|------------------|
| EPA Category | No. of Studies | Percent of Total |
| I | 451 | 25 |
| II | 273 | 15 |
| III | 793 | 45 |
| IV | 269 | 15 |
| Total | 1786 | 100 |
| IHAD* (reviewed after 2000) | | |
| I | 877 | 17 |
| II | 753 | 14 |
| III | 2194 | 42 |
| IV | 1434 | 27 |
| Total | 5258 | 100 |

•IHAD = Integrated Hazard and Assessment Database

Eye Irritation Studies - Antimicrobial Chemicals

| Antimicrobial (Dec 19, 2007 - Jan 15, 2009) | | |
|---|----------------|------------------|
| EPA Category | No. of Studies | Percent of Total |
| I | 73 | 64 |
| II | 13 | 11 |
| III | 16 | 14 |
| IV | 13 | 11 |
| Total | 115 | 100 |

Category I - supported by studies or cited as similar -----43
 - waived commonly due to pH or corrosivity -----30
 Category II - supported by studies or cited as similar -----13
 Category III - supported by studies or cited as similar -----16
 Category IV - supported by studies of cited as similar -----12
 Category IV - waived -----1

EPA Labeling Categories¹

| EPA Category | Signal Word | Statements | Protective Equipment/Actions |
|--------------|--------------------|--|---|
| I | DANGER | Corrosive. Causes irreversible eye damage. Do not get in eyes or on clothing. | Wear protective [eyewear goggles, face shield, or safety glasses]. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Remove and wash contaminated clothing prior to reuse. |
| II | WARNING | Causes substantial but temporary eye injury. Do not get in eyes or on clothing | Same as Category I above |
| III | CAUTION | Causes moderate eye irritation. Avoid contact with eyes or clothing. | Wear protective [eyewear goggles, face shield, or safety glasses]. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. |
| IV | (CAUTION optional) | None required | None required, but may choose Cat. III |

¹EPA Label Review Manual, Chapter 7, Precautionary Labeling (August 2007) available at: <http://www.epa.gov/oppfead1/labeling/lrm/2007-lrm-chap-07.pdf>

FHSA Classification System (16 CFR 1500.42)¹

- At least 6 animals per test
- Corrosive: ≥ 1 animal with destruction or irreversible alterations at the site of contact
- For irritants, maximum score in any animal on any day used for classification
 - Positive: CO or IR ≥ 1 or CC or CR ≥ 2
- Testing may be carried out in multiple tiers (6 animals/tier)
 - Tier 1:
 - ≥ 4 positive animals = Irritant
 - 2-3 positive animals = Go to Tier 2
 - 1 positive animal = negative
 - Tier 2:
 - ≥ 3 positive animals = Irritant
 - 1-2 positive animals = Go to Tier 3
 - 0 = negative
 - Tier 3:
 - 1 positive animal = Irritant

¹ FHSA (15 U.S.C. 1261; 2008) requires labeling based on *Eye Irritation Testing* 16 CFR 1500.42

Classification Systems: Summary

- All ocular toxicity classification systems are based on the proportion and severity of eye injury scores
- EPA and GHS allow for classification of corrosive based on a one-animal screen
 - If the initial animal indicates corrosivity, no additional testing is required
- Classification according to EPA and FHSA is based on the most severe lesion in any animal and on any day
- In most cases, classification according to GHS requires more severe and/or longer duration of eye injuries

Acknowledgements

- ICCVAM
- ICCVAM Interagency Ocular Toxicity Working Group
- NICEATM Staff