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

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ICCVAM Best Practices Workshop
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National Institutes of Health
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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

<table>
<thead>
<tr>
<th>Agency</th>
<th>Authority</th>
<th>Regulation</th>
<th>Test Description or Guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>FIFRA (1947)</td>
<td>40 CFR 156</td>
<td>OPPTS 870.2400</td>
</tr>
<tr>
<td></td>
<td>TSCA (1977)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPSC</td>
<td>FHSA (1964)</td>
<td>16 CFR 1500</td>
<td>16 CFR 1500.42</td>
</tr>
<tr>
<td>FDA</td>
<td>FDCA (1938)</td>
<td>21 CFR 501-523</td>
<td>16 CFR 1500.42</td>
</tr>
</tbody>
</table>

## Relevant Testing Guidelines

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Number of Animals</td>
<td>n=1 to screen for corrosive, then n≥2</td>
<td>n≥6 (or n=1-3 using TG 405)</td>
<td>n=1 to screen for corrosive, then n≥2</td>
</tr>
<tr>
<td>Quantity</td>
<td>0.1 mL or 0.1 g</td>
<td>0.1 mL or 0.1 g</td>
<td>0.1 mL or 0.1 g</td>
</tr>
<tr>
<td>Observation Times</td>
<td>1, 24, 48, 72 hr, and daily thereafter until lesions clear or 21 days</td>
<td>24, 48, 72 hr</td>
<td>1, 24, 48, 72 hr, and daily thereafter until lesions clear or 21 days</td>
</tr>
<tr>
<td>Post-dosing Irrigation</td>
<td>24 hr*</td>
<td>24 hr</td>
<td>Liquids: 24 hr</td>
</tr>
<tr>
<td>Use of Anesthetics</td>
<td>Prior to dosing if pain anticipated and no interference with test</td>
<td>Prior to dosing if pain anticipated and no interference with test</td>
<td>Prior to dosing if pain anticipated and no interference with test</td>
</tr>
</tbody>
</table>

*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 Score

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

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

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

4 = Opaque cornea, iris not discernible through the opacity

Eye Injury Scoring: Iritis Score\(^1\)

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)

Eye Injury Scoring: Conjunctival Redness Score

0 = Normal

1 = Some blood vessels definitely hyperemic (injected)

Positive Lesions

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

3 = Diffuse, beefy red

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Eye Injury Scores: Conjunctival Chemosis (Swelling) Score¹

0 = Normal

1 = Any swelling above normal (includes nictitating membrane)

2 = Obvious swelling with partial eversion of the lids

3 = Swelling with lids about half closed

4 = Swelling with lids more than half-closed

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 \( \geq 1 \) or CC or CR \( \geq 2 \)
- Most severe response used for classification of substance

<table>
<thead>
<tr>
<th>EPA Category</th>
<th>In Vivo Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corrosive; (irreversible) corneal involvement or irritation persisting more than 21 days</td>
</tr>
<tr>
<td>II</td>
<td>Corneal involvement or irritation clearing in 8-21 days</td>
</tr>
<tr>
<td>III</td>
<td>Corneal involvement or irritation clearing in ( \leq 7 ) days</td>
</tr>
<tr>
<td>IV</td>
<td>Minimal effects clearing within 24 hr.</td>
</tr>
</tbody>
</table>

CC: Conjunctival Chemosis; CO: Corneal Opacity; CR: Conjunctival Redness; IR: Iritis
EPA Eye Irritation Studies - Conventional Chemicals

<table>
<thead>
<tr>
<th>EPA Category</th>
<th>No. of Studies</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>451</td>
<td>25</td>
</tr>
<tr>
<td>II</td>
<td>273</td>
<td>15</td>
</tr>
<tr>
<td>III</td>
<td>793</td>
<td>45</td>
</tr>
<tr>
<td>IV</td>
<td>269</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>1786</td>
<td>100</td>
</tr>
</tbody>
</table>

IHAD* (reviewed after 2000)

<table>
<thead>
<tr>
<th>EPA Category</th>
<th>No. of Studies</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>877</td>
<td>17</td>
</tr>
<tr>
<td>II</td>
<td>753</td>
<td>14</td>
</tr>
<tr>
<td>III</td>
<td>2194</td>
<td>42</td>
</tr>
<tr>
<td>IV</td>
<td>1434</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>5258</td>
<td>100</td>
</tr>
</tbody>
</table>

*IHAD = Integrated Hazard and Assessment Database
### Eye Irritation Studies - Antimicrobial Chemicals

<table>
<thead>
<tr>
<th>EPA Category</th>
<th>No. of Studies</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>73</td>
<td>64</td>
</tr>
<tr>
<td>II</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>IV</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>100</td>
</tr>
</tbody>
</table>

Category I - supported by studies or cited as similar
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- waived commonly due to pH or corrosivity

- Category I - supported by studies or cited as similar
  - waived commonly due to pH or corrosivity

- Category II - supported by studies or cited as similar
  - waived commonly due to pH or corrosivity

- Category III - supported by studies or cited as similar
  - waived commonly due to pH or corrosivity

- Category IV - supported by studies or cited as similar
  - waived commonly due to pH or corrosivity

- Category IV - waived

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## EPA Labeling Categories

<table>
<thead>
<tr>
<th>EPA Category</th>
<th>Signal Word</th>
<th>Statements</th>
<th>Protective Equipment/Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DANGER</td>
<td>Corrosive. Causes irreversible eye damage. Do not get in eyes or on clothing.</td>
<td>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.</td>
</tr>
<tr>
<td>II</td>
<td>WARNING</td>
<td>Causes substantial but temporary eye injury. Do not get in eyes or on clothing</td>
<td>Same as Category I above</td>
</tr>
<tr>
<td>III</td>
<td>CAUTION</td>
<td>Causes moderate eye irritation. Avoid contact with eyes or clothing.</td>
<td>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.</td>
</tr>
<tr>
<td>IV</td>
<td>(CAUTION optional)</td>
<td>None required</td>
<td>None required, but may choose Cat. III</td>
</tr>
</tbody>
</table>

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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 Test ing 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