**Introduction**

- The bovine corneal opacity and permeability (BCOP) test method is an in vitro method that provides streamlined validation of physiological and biochemical function of the bovine cornea.
- Changes in opacity and permeability are fluoresced as indicators of potential ocular irritation and corrosivity.
- Following evaluation of the validation status of the BCOP, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended that BCOP can be used to classify ocular irritants as either irritants or corrosives (ICCVAM 2003). This recommendation was updated to include severe corrosives (ICCVAM 2007) for certain chemical classes. While not a complete replacement for the in vivo rabbit eye test, BCOP can be used in a tiered testing strategy for regulatory classification and labeling. These recommendations were made after consideration of public comments and a report from an independent international peer review panel.

**Test Method Overview (Figure 1)**

- Corneas are isolated from the eyes of freshly slaughtered cattle and are maintained in a corneal holder (Figure 2).
- Corneal opacity is measured quantitatively as the amount of light transmission through the cornea.
- Permeability is measured quantitatively as the amount of sodium fluorescein dye that passes across the full thickness of the cornea when the dye is applied to the surface of the cornea and then measured in the medium in the posterior chamber.
- Damage to the test substance is assessed by qualitative observations of changes in corneal edema and epithelial integrity, which are scored as corneal sensitivity (CS) or irritative changes (IC).
- Use of BCOP will reduce reliance on use for eye safety testing and eliminate such testing in animals of most substances likely to cause severe pain and discomfort.

**BCOP Test Method Performance and Limitations**

- A complete description of the database and the resulting accuracy and reliability analysis conducted for the BCOP test method can be obtained at http://www.ecfs.ucsf.edu/behel/studies/BCOP).
- The accuracy of the BCOP test method when compared to in vivo rabbit eye tests classifications using the United Nations (UN) Global Harmonized System of Classification and Labelling of Chemicals (GHS) classification system for classify substances is reported. A validation study was performed using the BCOP test method on 58 substances that were highly ranked as corrosive or severe irritants. Thus, the BCOP is used to assign a hazard classification category.
- BCOP can be used to classify certain types of substances as ocular corrosives and severe irritants as defined by national and international hazard classification systems (Table 1).

**Proficiency Chemicals for the BCOP Test Method**

- Prior to routine use of the BCOP test method, laboratories may wish to perform proficiency testing to confirm that the test is being conducted in a responsible and consistent manner, thereby improving the accuracy of the test method. A list of proficiency chemicals is available at the ICCVAM Background Review Document for BCOP (ICCVAM 2003).
- These substances were selected to represent the range of responses for various irritants/corrosives, based on results in vivo rabbit eye tests (OECD TG 405).

**Collection, Transport, Storage, and Preparation of Eyes Used in the Test**

- Collect the eyes while preventing contact with any potentially irritating substances such as detergents.
- Immersed completely in Balanced Salt Solution (HBSS) and transport to the laboratory on ice.
- Antibiotics added to the HBSS used to store the eyes during transport are not required (e.g., penicillin, 100 U/mL, and polymyxin at 100 U/mL).
- The saline between collection of the eyes and use of the cornea in the BCOP test is minimal (typically collected and used in the same day).
- Only corneas free of defects such as excessive opacity, neovascularization, and neovascularization are to be used.
- Acceptable corneas are dissected with a 2.5–3.0 mm incision running remaining intact placed in the corneal holders (Figure 2).
- Corneas are allowed to stabilize for at least one hour to recover normal metabolic activity, to the extent possible.
- After equilibration, any corneas that show macroscopic tissue damage (e.g., scratching, pigmentary, neovascularization) or lack opacity ≥ 5% opacity units are discarded.

**Application of the Test Substance**

- Each treatment group (test substance, concurrent negative and positive controls) consists of a minimum of three eyes.
- A closed-chamber method is initially used for substances to slightly viscous liquid substances, while an open-chamber method is typically used for semisolid and viscous liquid substances and for solid or semisolid substances.
- The test substance is applied directly to the epithelial surface of the corneas using a micropipette. After dosing, the glass window is replaced on the anterior chamber to increase a closed system.
- Two different treatment protocols are used: one for liquids and semisolid substances (solids), and one for nonirritating solids.
- Liquids are tested neat.
- Solids are tested neat or solutions.
- Sodium chloride solution, distilled water, or other solvent that has not been demonstrated to have a sublime effect on the test system.
- Exposure time for (approximate exposure times may be used with appropriate scientific rationale).
- Nonirritating substances are tested at concentrations of 30% or higher of the sodium chloride solution, distilled water, or other solvent that has been demonstrated to have a sublime effect on the test system.
- Exposure time is 1 hour.
- Solids may also be tested under direct application onto the cornea surface.
- The IVIS = mean opacity value + (15 x mean permeability OD 

**Protocol: Post Application**

- After rinsing:
  - For solids: 100% (51/51) of the test substances are ocular corrosives and severe irritants as defined by national and international hazard classification systems (Table 1).
  - Endpoints measured for the IVIS = mean opacity value + (15 x mean permeability OD

**Summary**

- The BCOP test method is well suited for use in a tiered testing strategy for regulatory classification and labeling with a significant average performance ranking.
- The negative or solvent/vehicle control responses should result in sensitivities and specifities for bovine corneas treated with the negative control vehicle.

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