Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

Commonly used acronym: *BCOP, OECD TG 437*
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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Regulatory use - Routine production
Type of method	In vitro - Ex vivo
This method makes use of	Animal derived cells / tissues / organs
Species from which cells/tissues/organs are derived	Cattle
Type of cells/tissues/organs	Isolated corneas from the eyes of cattle slaughtered for commercial purposes

DESCRIPTION

Method keywords

Cornea
opacity
Permeability
In Vitro Irritancy Score (IVIS)
Laser light-based opacitometer (LLBO)
LLBO Irritancy Score (LIS)

Scientific area keywords

Classification and Labelling of chemicals
GHS
Hazard classification
Serious Eye Damage
eye irritation
Ocular irritation
Toxicology

Method description

The Bovine Corneal Opacity and Permeability test method (BCOP) has been fully described in OECD TG 437. It is an *in vitro* test method that can be used to identify chemicals (substances or mixtures) as either 1) causing “serious eye damage” (category 1 of the Globally Harmonised System for the Classification and Labelling of chemicals (GHS)), or 2) not requiring classification for eye irritation or serious eye damage according to the GHS. The BCOP uses isolated corneas from the eyes of cattle slaughtered for commercial purposes, thus avoiding the use of laboratory

animals. Each treatment group (test chemical, negative/positive controls) consists of a minimum of three eyes where the cornea has been excised and mounted to a holder. Depending on the physical nature and chemical characteristics of the test chemical, different methods can be used for its application since the critical factor is ensuring that the test chemical adequately covers the epithelial surface. Toxic effects to the cornea are measured as opacity and permeability, which when combined gives an *In Vitro* Irritancy Score (IVIS) or laser light-based opacitometer (LLBO) Irritancy Score (LIS) for each treatment group. A chemical that induces an 'IVIS > 55' or 'LIS >30 and lux/7 <=145 and OD490 <=2.5' or 'LIS >30 and lux/7 >145' is defined as a category 1 (“causing serious eye damage” according to the GHS); a chemical that induces an IVIS <= 3 or LIS <= 30 is considered as not requiring classification for eye irritation or serious eye damage according to the GHS.

Lab equipment

- Standard equipment for cell culture work;
- Dissection equipment (scissors, scalpels, forceps);
- Corneal holders;
- Electric screwdriver;
- Opacitometer (OP-KIT, Opacitometer3.0 or Laser light-based opacitometer);
- Plastic containers for collection and transport of eyes;
- Specialized window-locking ring screwdriver;
- 96-well microtiter plate reader.

Method status

Validated by an external party (e.g. OECD, EURL ECVAM,...)

PROS, CONS & FUTURE POTENTIAL

Advantages

While it is not considered valid as a stand-alone replacement for the *in vivo* rabbit eye test, the BCOP test method is recommended as an initial step within a testing strategy such as the Top-Down approach to identify chemicals inducing serious eye damage, i.e. chemicals to be classified as UN GHS Category 1, without further testing. The BCOP test method is also recommended to identify chemicals that do not require classification for eye irritation or serious eye damage, as defined by the UN GHS (UN GHS No Category) within a testing strategy such as the Bottom-Up approach. However, a chemical that is not predicted as causing serious eye damage or as not classified for eye irritation/serious eye damage with the BCOP test method would require additional testing (*in vitro* and/or *in vivo*) to establish a definitive classification. The choice of the most appropriate test method and the use of this Test Guideline should be seen in the context of the OECD Guidance Document on an Integrated Approaches on Testing and Assessment for Serious Eye Damage and Eye irritation. For more information see OECD TG nr. 437

Challenges

- The potential shortcomings of the BCOP test method when used to identify chemicals inducing serious eye damage (UN GHS Category 1) are based on the high false positive rates for alcohols and ketones and the high false negative rate for solids. However, since not all alcohols and ketones are over-predicted by the BCOP test method and some are correctly predicted as UN GHS Category 1, these two organic functional groups are not considered to be out of the applicability domain of the test method.
- The BCOP test method is not recommended for the identification of test chemicals that should be classified as irritating to eyes (UN GHS Category 2 or Category 2A) or test chemicals that should be classified as mildly irritating to eyes (UN GHS Category 2B).
- A chemical that is not predicted as causing serious eye damage or as not classified for eye irritation/serious eye damage with the BCOP test method would require additional testing (*in vitro* and/or *in vivo*) to establish a definitive classification.
- The time interval between collection of the eyes and use of corneas in the BCOP test

method should be minimized (typically collected and used on the same day) and should be demonstrated to not compromise the assay results.
- The BCOP test method does not consider conjunctival and iridial injuries, it addresses corneal effects.
- The reversibility of corneal lesions cannot be evaluated per se in the BCOP test method.
- The BCOP test method does not allow for an assessment of the potential for systemic toxicity associated with ocular exposure.
For more information see OECD TG nr. 437

Modifications

- Eyes from cattle less than 12 months of age have not traditionally been used since the eyes are still developing and the corneal thickness and corneal diameter are considerably smaller than that reported for eyes from adult cattle. However, the use of corneas from young animals (i.e., 6 to 12 months old) is permissible since there are some advantages, such as increased availability, a narrow age range, and decreased hazards related to potential worker exposure to Bovine Spongiform Encephalopathy. As further evaluation of the effect of corneal size or thickness on responsiveness to corrosive and irritant substances would be useful, users are encouraged to report the estimated age and/or weight of the animals providing the corneas used in a study.
For more information see OECD TG nr. 437

Future & Other applications

- Further scientific knowledge is required to understand how irreversible effects not linked with initial high level injury occur.
- Histopathology may be potentially useful when a more complete characterization of corneal damage is needed. Users are encouraged to preserve corneas and prepare histopathology specimens that can be used to develop a database and decision criteria that may further improve the accuracy of this test method.
- Further evaluation of the effect of corneal size or thickness on responsiveness to

corrosive and irritant substances would be useful, users are encouraged to report the estimated age and/or weight of the animals providing the corneas used in a study. For more information see OECD TG nr. 437

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

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Improvement of the Bovine Corneal Opacity and Permeability (BCOP) assay as an *in vitro* alternative to the Draize rabbit eye irritation test 2013-06 | Toxicology *in Vitro*; DOI: 10.1016/j.tiv.2013.02.018; Part of ISSN: 0887-2333

Associated documents

OECD 437_update 2020.pdf

Links

OECD Test Guideline No. 437: Bovine Corneal Opacity And Permeability Test Metho...

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