# Commission Regulation (EU) No 1152/2010 of 8 December 2010 amending, for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (Text with EEA relevance)

## COMMISSION REGULATION (EU) No 1152/2010

## of 8 December 2010

## amending, for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

## (Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1907/2006 of 18 December 2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC[(1)], and in particular Article 13(3) thereof,

Whereas:

(1) Commission Regulation (EC) No 440/2008[(2)] contains the test methods for the purposes of the determination of the physico-chemical properties, toxicity and eco-toxicity of substances to be applied for the purposes of Regulation (EC) No 1907/2006.

(2) It is necessary to update Regulation (EC) No 440/2008 to include with priority two new in vitro test methods for ocular irritation recently adopted by the OECD, in order to obtain a reduction of the number of animals to be used for experimental purposes, in accordance with Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes[(3)]. Stakeholders have been consulted on this draft.

(3) Regulation (EC) No 440/2008 should therefore be amended accordingly.

(4) The measures provided for in this Regulation are in accordance with the opinion of the Committee established under Article 133 of Regulation (EC) No 1907/2006,

HAS ADOPTED THIS REGULATION:

**Changes to legislation:** *There are currently no known outstanding effects for the Commission Regulation (EU) No 1152/2010. (See end of Document for details)*

*Article 1*

In Part B of the Annex to Regulation (EC) No 440/2008, Chapters B.47 and B.48 are added as set out in the Annex to this Regulation.

*Article 2*

This Regulation shall enter into force on the third day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 8 December 2010.

*For the Commission*

*The President*

José Manuel BARROSO

***Changes to legislation:** There are currently no known outstanding effects for the Commission Regulation (EU) No 1152/2010. (See end of Document for details)*

ANNEX

## B. 47. **BOVINE CORNEAL OPACITY AND PERMEABILITY TEST METHOD FOR IDENTIFYING OCULAR CORROSIVES AND SEVERE IRRITANTS**

### INTRODUCTION

1. The Bovine Corneal Opacity and Permeability (BCOP) test method is an *in vitro* test method that can be used, under certain circumstances and with specific limitations, to classify substances and mixtures as "ocular corrosives and severe irritants" (1) (2) (3). For the purpose of this test method, severe irritants are defined as those that induce ocular lesions that persist in the rabbit for at least 21 days after administration. While it is not considered valid as a complete replacement for the *in vivo* rabbit eye test, the BCOP is recommended for use as part of a tiered-testing strategy for regulatory classification and labelling within a specific applicability domain (4) (5). Test substances and mixtures (6) can be classified as ocular corrosives or severe irritants without further testing in rabbits. A substance that tests negative would need to be tested in rabbits using a sequential testing strategy, as outlined in OECD Test Guideline 405 (7) (chapter B. 5 of this Annex).

2. The purpose of this test method is to describe the procedures used to evaluate the potential ocular corrosivity or severe irritancy of a test substance as measured by its ability to induce opacity and increased permeability in an isolated bovine cornea. Toxic effects to the cornea are measured by: (i) decreased light transmission (opacity), and (ii) increased passage of sodium fluorescein dye (permeability). The opacity and permeability assessments of the cornea following exposure to a test substance are combined to derive an *In Vitro* Irritancy Score (IVIS), which is used to classify the irritancy level of the test substance.

3. Ocular irritants that induce lesions that resolve in less than 21 days and non-irritants have also been tested using the BCOP test method. However, the accuracy and reliability of the BCOP test method for substances in these categories have not been formally evaluated.

4. Definitions are provided in Appendix 1.

### INITIAL CONSIDERATIONS AND LIMITATIONS

5. This test method is based on the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) BCOP test method protocol (8), which was developed following an international validation study (4)(5)(9), with contributions from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM). The protocol is based on information obtained from the Institute for In Vitro Sciences (IIVS) and INVITTOX Protocol 124 (10), which represents the protocol used for the European Community-sponsored prevalidation study of the BCOP assay conducted in 1997-1998. Both of these protocols are based on the BCOP assay methodology first reported by Gautheron *et al.* (11).

6. The identified limitations for this test method are based on the high false positive rates for alcohols and ketones and the high false negative rate for solids observed in the validation database (see paragraph 44) (5). When substances within these chemical and physical classes are excluded from the database, the accuracy of BCOP across the EU, EPA, and GHS classification systems is substantially improved (5). Based on the purpose of this assay (*i.e.*, to identify ocular corrosives/severe irritants only), false negative rates are not critical since such substances would be subsequently tested in rabbits or with other adequately validated *in vitro* tests, depending on regulatory requirements, using a sequential testing strategy in a weight of evidence approach. Furthermore, the current validation database did not allow for an adequate evaluation of some chemical or product classes (*e.g.*, mixtures). However, investigators could consider using this test method for all types of test material (including mixtures), whereby a positive result could

be accepted as indicative of an ocular corrosive or severe irritant response. However, positive results obtained with alcohols or ketones should be interpreted cautiously due to risk of over-prediction.

7. All procedures with bovine eyes and bovine corneas should follow the testing facility's applicable regulations and procedures for handling animal-derived materials, which include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions are recommended (12).

8. A limitation of the test method is that, although it takes into account some of the ocular effects evaluated in the rabbit ocular irritancy test method and to some degree their severity, it does not consider conjunctival and iridal injuries. Also, although the reversibility of corneal lesions cannot be evaluated *per se* in the BCOP assay, it has been proposed, based on rabbit eye studies, that an assessment of the initial depth of corneal injury can be used to distinguish between irreversible and reversible effects (13). Finally, the BCOP does not allow for an assessment of the potential for systemic toxicity associated with ocular exposure.

9. Efforts are ongoing to further characterize the usefulness and limitations of the BCOP assay for identifying non-severe irritants and non-irritants (see also paragraph 45). Users are also encouraged to provide specimens and/or data to validation organizations for a formal evaluation of possible future uses of the BCOP test method, including for the identification of non-severe irritants and non-irritants.

10. For any laboratory initially establishing this assay, the proficiency chemicals provided in Appendix 2 should be used. A laboratory can use these chemicals to demonstrate their technical competence in performing the BCOP test method prior to submitting BCOP assay data for regulatory hazard classification purposes.

PRINCIPLE OF THE TEST

11. The BCOP test method is an organotypic model that provides short-term maintenance of normal physiological and biochemical function of the bovine cornea *in vitro*. In this test method, damage by the test substance is assessed by quantitative measurements of changes in corneal opacity and permeability with an opacitometer and a visible light spectrophotometer, respectively. Both measurements are used to calculate an IVIS, which is used to assign an *in vitro* irritancy hazard classification category for prediction of the *in vivo* ocular irritation potential of a test substance (see Decision criteria).

12. The BCOP test method uses isolated corneas from the eyes of freshly slaughtered cattle. 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, as detected in the medium in the posterior chamber. Test substances are applied to the epithelial surface of the cornea by addition to the anterior chamber of the corneal holder. Appendix 3 provides a description and a diagram of a corneal holder used in the BCOP. Corneal holders can be obtained commercially from different sources or can be constructed.

**Source and Age of Bovine Eyes and Selection of Animal Species**

13. Cattle sent to slaughterhouses are typically killed either for human consumption or for other commercial uses. Only healthy animals considered suitable for entry into the human food chain are used as a source of corneas for use in the BCOP. Because cattle have a wide range of weights, depending on breed, age, and sex, there is no recommended weight for the animal at the time of slaughter.

14. Variations in corneal dimensions can result when using eyes from animals of different ages. Corneas with a horizontal diameter > 30,5 mm and central corneal thickness (CCT)

values ≥ 1 100 μm are generally obtained from cattle older than eight years, while those with a horizontal diameter < 28,5 mm and CCT < 900 μm are generally obtained from cattle less than five years old (14). For this reason, eyes from cattle greater than 60 months old are not typically used. 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 (15). 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.

**Collection and Transport of Eyes to the Laboratory**

15. Eyes are collected by slaughterhouse employees. To minimize mechanical and other types of damage to the eyes, the eyes should be enucleated as soon as possible after death. To prevent exposure of the eyes to potentially irritant substances, the slaughterhouse employees should not use detergent when rinsing the head of the animal.

16. Eyes should be immersed completely in Hanks' Balanced Salt Solution (HBSS) in a suitably sized container, and transported to the laboratory in such a manner as to minimize deterioration and/or bacterial contamination. Because the eyes are collected during the slaughter process, they might be exposed to blood and other biological substances, including bacteria and other microorganisms. Therefore, it is important to ensure that the risk of contamination is minimized (*e.g.*, by keeping the container containing the eyes on wet ice, by adding antibiotics to the HBSS used to store the eyes during transport [*e.g.*, penicillin at 100 IU/mL and streptomycin at 100 μg/mL]).

17. The time interval between collection of the eyes and use of corneas in the BCOP should be minimized (typically collected and used on the same day) and should be demonstrated to not compromise the assay results. These results are based on the selection criteria for the eyes, as well as the positive and negative control responses. All eyes used in the assay should be from the same group of eyes collected on a specific day.

**Selection Criteria for Eyes Used in the BCOP**

18. The eyes, once they arrive at the laboratory, are carefully examined for defects including increased opacity, scratches, and neovascularisation. Only corneas from eyes free of such defects are to be used.

19. The quality of each cornea is also evaluated at later steps in the assay. Corneas that have an opacity greater than seven opacity units (NOTE: the opacitometer should be calibrated with opacity standards that are used to establish the opacity units, see Appendix 3) after an initial one hour equilibration period are to be discarded.

20. Each treatment group (test substance, concurrent negative and positive controls) consists of a minimum of three eyes. Three corneas should be used for the negative control corneas in the BCOP assay. Since all corneas are excised from the whole globe, and mounted in the corneal chambers, there is the potential for artefacts from handling upon individual corneal opacity and permeability values (including negative control). Furthermore, the opacity and permeability values from the negative control corneas are used to correct the test article and positive control-treated corneal opacity and permeability values in the IVIS calculations.

PROCEDURE

**Preparation of the Eyes**