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CON4EI: Bovine Corneal Opacity and Permeability (BCOP) test for hazard identification and labelling of eye irritating chemicals

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ABSTRACT

Assessment of ocular irritation potential is an international regulatory requirement in the safety evaluation of industrial and consumer products. None *in vitro* ocular irritation assays are capable of fully categorizing chemicals as stand-alone. Therefore, the CEFIC-LRI-AIMT6-VITO CON4EI consortium assessed the reliability of eight *in vitro* test methods and computational models as well as established a tiered-testing strategy. One of the selected assays was Bovine Corneal Opacity and Permeability (BCOP). In this project, the same corneas were used for measurement of opacity using the OP-KIT, the Laser Light-Based Opacitometer (LLBO) and for histopathological analysis.

The results show that the accuracy of the BCOP OP-KIT in identifying Cat 1 chemicals was 73.8% while the accuracy was 86.3% for No Cat chemicals. BCOP OP-KIT false negative results were often related to an *in vivo* classification driven by conjunctival effects only. For the BCOP LLBO, the accuracy in identifying Cat 1 chemicals was 74.4% *versus* 88.8% for No Cat chemicals. The BCOP LLBO seems very promising for the identification of No Cat liquids but less so for the identification of solids. Histopathology as an additional endpoint to the BCOP test method does not reduce the false negative rate substantially for *in vivo* Cat 1 chemicals.

1. Introduction

Measurement of ocular irritation potential is a necessary step in the safety evaluation of both industrial and consumer products and is therefore part of the international regulatory requirements for the testing of chemicals.

The main objective of the CON4EI (CONsortium for *in vitro* Eye Irritation testing strategy) project (2015–2016) was to develop tiered testing strategies for eye irritation assessment for the most important drivers of classification (Adriaens et al., 2014) to finally replace the *in vivo* Draize eye test (Draize et al., 1944). The irritation potency of a set of 80 well-characterized chemicals was evaluated using 8 alternative test methods, besides the use of computational models. One of the *in vitro* assays selected was the Bovine Corneal Opacity and Permeability (BCOP) test method (Gautheron et al., 1992). The BCOP assay is an *in vitro* alternative which is routinely used in several industrial and contract testing laboratories in the context of workplace safety and product safety applications (Vanparys et al., 1993). The assay can be used under certain circumstances and with specific limitations for eye hazard classification and labelling of chemicals and is described in the Organisation for Economic Co-operation and Development Test Guideline (OECD TG) 437 (OECD, 2013a). While it is not considered valid as a stand-alone replacement for the *in vivo* Draize eye test, the BCOP test method is recommended as an initial step within a testing strategy such as the 'top-down' approach suggested by Scott et al. (2010) and the OECD (2017) to identify chemicals inducing serious eye damage, *i.e.* chemicals to be classified as United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS) Category (Cat) 1 and as implemented by the European Union Classification, Labelling and Packaging regulation (EU CLP) (EC, 2008), without further

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Table 1
Distribution of the 80 reference chemicals by important driver of classification and by physical state.

Physical state	Category 1			Category 2[a]		No category
	Irreversible effects on the eye/serious eye damage			Reversible effects on the eye/eye irritation		
	N = 38			N = 27		N = 15
	Severity (mean scores of days 1–3)[b]	Persistence on day 21	Severe CO	Severity (mean scores of days 1–3)[b]		
Driver	CO mean ≥ 3	CO pers D21 in ≥ 60% of the animals, with CO mean < 3	CO = 4 in ≥ 60% of the animals[c], in the absence of persistence and with CO mean < 3 (or if unknown)	CO mean ≥ 1	Conj mean ≥ 2	CO = 0[d]
Liquid	7	4	6	8 (3 × 2A, 5 × 2B)	5 (2 × 2A, 3 × 2B)	8
Solid	7	8	6	5 (4 × 2A, 1 × 2B)	9 (4 × 2A, 5 × 2B)	7
Total	14	12	12	13	14	15

CO: corneal opacity; IR: iritis; Conj: conjunctival redness (CR) and/or conjunctival chemosis (CC); CO pers D21: CO persistence on day 21.

[a] Sub-categorized in two categories: Category 2A (irritant to eyes) when any of the eye effects in any animal is not fully reversible within 7 days of observation (*i.e.* CO, IR, CR and/or CC > 0 at 7 ≤ day < 21) and 2B (mildly irritant to eyes) when all observed eye effects are fully reversible within 7 days of observation (*i.e.* CO, IR, CR and CC = 0 on day 7 and beyond).

[b] Mean scores are calculated from gradings at 24, 48, and 72 h after instillation of the test chemical.

[c] For two liquids and two solids this was in < 60% of the animals.

[d] CO = 0 in all observation times in all animals.

testing (UN, 2015). 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 (No Cat) (UN, 2015) within a testing strategy such as the 'bottom-up' approach (Scott et al., 2010). However, the test method is not intended to differentiate between UN GHS/EU CLP Cat 1 (serious eye damage) and UN GHS/EU CLP Cat 2 (eye irritation), so 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 BCOP test method is an organotypic model that uses isolated bovine corneas from freshly harvested eyes of slaughtered cattle. The test method assesses damage caused by the test chemical by quantitative measurements of changes in corneal opacity and permeability. In the standard BCOP assay, opacity measurements are performed with an OP-KIT opacitometer and permeability is measured with a spectrophotometer as the amount of sodium fluorescein dye that passes across the cornea (Gautheron et al., 1994). In the CON4EI project, opacity was also measured with a Laser Light-Based Opacitometer (LLBO) (Verstraelen et al., 2013). Besides these two devices, the commercial Opacitometer 3.0 (Duratec GmbH) is available (Schrage et al., 2011), but was not included in the CON4EI project. The latter device is an improvement of the OP-KIT using light from a halogen lamp, but now measured as illuminance. For that reason, a slightly adapted prediction model is used for opacity measurement.

The OP-KIT opacitometer provides a center-weighted reading of light transmission by measuring changes in voltage when the transmission of white light through the cornea alters (Van Goethem et al., 2010). As a consequence, this may underestimate opacity that develops as spots or heterogeneous opaque areas on the periphery of an isolated cornea. In contrast, the LLBO allows the analysis of the entire corneal surface and is therefore able to detect more efficiently opaque spots located around the periphery of the excised corneas. Furthermore, the IVIS scaling is broader compared to OP-KIT indicating it's use for more sensitive measurements in the mild/moderate range (Verstraelen et al., 2013; Van Goethem et al., 2010). The LLBO showed promising features which potentially can improve the usefulness and applicability domain of the BCOP test. The LLBO is currently under validation and more information can be found in Van Goethem et al. (2010) and Verstraelen et al. (2013).

A set of 80 reference chemicals (Adriaens et al., 2017a) was evaluated with the BCOP test method. The primary aim of this study was an evaluation of the performance of the BCOP OP-KIT and BCOP LLBO test methods to classify the same set of 80 well-characterized chemicals. In addition, the predictive capacity in terms of *in vivo* driver of classification was investigated in more detail. Furthermore, it was investigated if histopathological processing and microscopic evaluation using methods similar to those developed for chicken eyes (Cazelle et al., 2014) can be used as an additional endpoint in the BCOP assay to reduce the number of Cat 1 false negatives.

2. Materials and methods.

2.1. Test compounds

The eye irritation potential of 80 chemicals (38 liquids and 42 solids) was assessed under blinded conditions. The set of test chemicals was composed of 15 chemicals not requiring classification (No Cat) and 65 chemicals requiring classification (27 Cat 2 and 38 Cat 1). The distribution of the chemicals according to the UN GHS category and according to the drivers of classification is presented in Table 1. The physical state of the chemicals was balanced within the categories. The corresponding No. that is used in the Tables is the chemical No. as displayed in Table 2. More information on the chemical selection can be found in Adriaens et al. (2017a). The 80 chemicals were tested at least twice by VITO (Mol, Belgium) to obtain two independent valid runs. The corneas from successful runs were subsequently fixed in neutral-buffered formalin and submitted to Envigo (Cambridgeshire, United Kingdom) for histopathological processing and evaluation.

2.2. BCOP assay

2.2.1. Study design

The BCOP assay was performed according to OECD TG 437 (OECD, 2013a) using corneas of cattle slaughtered at age 6 to 8 months. A brief overview can be found in the paper by Van Goethem et al. (2010). Opacity readings were performed with the OP-KIT opacitometer (BCOP OP-KIT; MC2, Clermont Ferrand Cedex, France) and with the LLBO (BCOP LLBO; Peira Scientific Instruments, Turnhout, Belgium). In this project, the same corneas were used for measurement of opacity using