The Bovine Corneal Opacity and Permeability Test in Routine Ocular Irritation Testing and Its Improvement Within the Limits of OECD Test Guideline 437

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Summary — Data on eye irritation are generally needed for the hazard identification of chemicals. As the Bovine Corneal Opacity and Permeability (BCOP) test has been accepted by many regulatory agencies for the identification of corrosive and severe ocular irritants since September 2009 (OECD Test Guideline 437, TG 437), we evaluated this alternative method for routine testing at BASF. We demonstrated our technical proficiency by testing the reference standards recommended in TG 437, and 21 additional materials with published BCOP and in vivo data. Our results matched the published in vitro data very well, but with some intentionally selected false negatives (FNs) and false positives (FPs), the concordance was 77% (24/31), with FN and FP rates of 20% (2/10) and 24% (5/21), respectively. In addition, we tested 21 in-house materials, demonstrating the utility of the BCOP assay for our own test material panel. Histopathological assessment of the corneas by light microscopy was also conducted, as this was suggested as a means of improving the identification of FNs. The histopathology corrected the classification of some FNs, but also increased the number of FPs. Parallel to the test method evaluation, we compared three new opacimeter models with the current standard device. We recommend the use of an opacimeter developed in our BASF laboratory, which has certified components and electronic data storage, resulting in what we consider to be excellent sensitivity, stability and reproducibility.

Key words: alternative method, BCOP, eye irritation, opacimeter, opacity, permeability.

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Introduction

New chemicals are usually tested for their potential to cause eye irritation as an important part of the toxicology programme, e.g. for occupational safety. Eye irritation has traditionally been examined with the rabbit eye test (1) and modifications of it. After assessment according to Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 405 (2), the results are used for classification according to the Globally Harmonised System (GHS; 3).

For ethical and economic reasons, and also because of new animal protection laws, alternative methods need to be developed, to reduce, refine and replace animal experiments. In addition, the rabbit eye test has been criticised for its limitations, including the individual-dependent subjective scoring of eye changes and the differences between species, such as the presence/absence of a nictitating membrane, thickness of the cornea, and occurrence of/lack of significant tearing (4).

Because adequate human data for the evaluation are rare, new alternative methods have to be compared to the so-called ‘gold standard’ — the data from rabbits — despite these insuperable difficulties with the rabbit test.

Four in vitro methods, the Hen’s Egg Test–Chorioallantoic Membrane (HET-CAM), and the Isolated Rabbit Eye (IRE), Isolated Chicken Eye (ICE) and Bovine Corneal Opacity and Permeability (BCOP) tests, have gained regulatory acceptance in some European countries for the classification of severe irritants (5, 6), and were further evaluated in the USA by ICCVAM in 2006 (7). The latter two tests are now accepted by the OECD for use in a tiered testing strategy (8, 9).

We selected the BCOP test instead of the ICE test method for establishment and evaluation in our laboratory, as its endpoints — opacity and permeability — are objectively determined with an opacimeter and a photometer, respectively, and are therefore independent of subjective scoring. Although it has been reported that the overall con-