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Proofing the relevance of the Ex Vivo Eye Irritation Test (EVEIT) as a self-contained in vitro substitute for the Draize Eye Irritation Test

Abstract

Current developments to replace the Draize eye test by in vitro methods are based on tiered testing strategies combining different alternative methods. Typically an isolated organ is used to predict the depth of injury which is an important measure to identify the severity of an eye irritant [Maurer02, Jester06]. A second test determines cytotoxicity using cell culture assays or other test methods which can determine the potential of the test substance to disrupt cellular membranes. By these testing strategies the identification of severe irritants and non-irritants is reliable in most cases. Otherwise, shortcomings in correct identification of moderate irritants and in distinct segregation of non-irritants from mild irritants are known. Additionally, the GHS categorisation of mild and moderate irritants based on recovery time is not covered by such testing strategies.

The Ex Vivo Eye Irritation Test (EVEIT) has the potential to overcome the aforementioned limitations and to allow for a self-contained in vitro substitute for the Draize eye test. In order to achieve this goal, the EVEIT pursues two objectives in striving for higher significance when compared to other testing strategies: First, corneal cultures are used to improve biologic relevance. Second, process monitoring is enhanced from static endpoint analysis to in-time dynamic observation using optical coherence tomography (OCT).

The EVEIT is based on long term culture of rabbit corneas obtained from animals slaughtered for food production [Frentz08]. It allows for in vitro observation of the metabolic stability of the living organ including the evaluation of recovery. Here, OCT enables in situ monitoring of area and depth of structural damage and the progress of healing over time [Spöler07, Spöler08]. The dependency of these parameters on concentration, exposure time, and location are also accessible. Additionally, OCT allows for quantification of endpoints covered by established organotypic in vitro assays, i.e. corneal opacity and corneal swelling. OCT is therefore an ideal analytical tool for the intended test platform.

A research project financed by the Federal Ministry of Education and Research (BMBF), scientifically advised by the ECVAM, the Federal Institute of Risk Assessment (BfR), and several EPAA members, currently develops an appropriate operating procedure and prediction model to use the EVEIT in eye irritation testing. This includes determination of adequate substance application procedures, rationalisation of processes and evaluation of ocular irritation of a wide range of chemical substances. These ongoing experiments already demonstrated that a detailed analysis of structural damage and the progress of healing during several days is a sensitive tool to identify ocular irritants that cannot be classified by other in vitro methods.

The aim of the project applied here is an inter-laboratory study of the EVEIT involving two laboratories of EPAA members and our laboratory. This study will strive for a reliable dataset to assess the performance of the EVEIT within eye irritation testing and to subsequently enter the ECVAM test submission process. Moreover, it is intended to demonstrate the applicability of the test method to be economically used by industry for screening in the risk assessment process for product development.