Sub-categorization of dermal corrosives \textit{in vitro} using the reconstructed human skin model epiCS®

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Introduction

The epiCS skin corrosion test method classifying chemicals either as corrosive (Category 1) or non corrosive (no Category) was validated (ESAC 2009) according to OECD TG 431. Recently, the EU Classification, Labeling and Packaging Regulation (EU CLP) system required the sub-categorization of corrosive chemicals into the UN GHS optional subcategories 1A and 1B/C. Protection measures for human health are not affected by sub-categorization, i.e. independent of the sub-category the protection measures for human health are the same. The purpose of the present study was to assess whether epiCS skin models can reproducibly discriminate the sub-categories of corrosive chemicals with the classifications 1A (strong corrosive) and 1B/C (weak corrosive).

Methods

MTT Test

Duplicate epiCS tissue models were topically exposed to the test or control substances (25 mg of solids or 50 µl of liquids) for 3 minutes at room temperature and 60 minutes at 37 °C. At the end of the exposure period tissues were subjected to a washing procedure using PBS (pH 7.0). The rinsed tissues were treated with 1 mg/ml MTT (300 µl for 3 hours). The relative viability of the MTT treated tissues was assessed by extraction of formazan with isopropanol and subsequent measurement of optical density at 550 nm wavelength.

Prediction Model

Fig. 2: Prediction model for the sub-categorization of corrosives.

Eighty chemicals including solids, semi-solids and liquids of different chemical classes (e.g., electrophiles, organic bases and acids, neutral organics, surfactants, inorganic salts and acids, phenols), selected by the OECD expert group on skin corrosion, were tested in two independent runs. Freeze-inactivated tissues were used to correct for direct MTT reduction and interference by colouring agents.

The classification is based on the following prediction model (PM, Fig. 2): A chemical is classified 1A corrosive, if the viability is ≤ 50% after 3 min exposure. A chemical is classified as 1B/C corrosive, if the viability is > 50% after 3 min and ≤ 15% after 1 hr exposure. Non corrosive (NC) classification is achieved if the viability is ≥ 50% after 3 min and ≥ 15% after 1 hr exposure.

Results

The results with the epiCS test method showed correctly classified corrosive and non corrosive chemicals with a high sensitivity (91.67 %) and specificity (71.62 %). The overall accuracy regarding sub-categorization into UN GHS subcategories 1A and 1B/C was 65.41 %. According to the current prediction model, predictions for Category 1B/C chemicals have quite high over-prediction rates, i.e. over-predicted as Category 1A, while predictions for Category 1A are mostly correct.

Conclusion

The results demonstrate that the epiCS skin corrosion test method is able to correctly identify corrosive and non corrosive chemicals and can distinguish between 1A and 1B/C chemical categories with the current PM. Results will be listed in the revised version of the OECD TG 431 (2014). We recommend to examine whether modifications of the current PM can lead to higher accuracy of the test method and improved prediction of 1B/C chemicals.