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Predictive performance and inter-laboratory reproducibility in assessing eye irritation potential of water- and oil-soluble mixtures using the Short Time Exposure test method

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ABSTRACT

The Short Time Exposure (STE) test method is an alternative method for assessing eye irritation potential using Statens Seruminstitut Rabbit Cornea cells and has been adopted as test guideline 491 by the Organisation for Economic Co-operation and Development. Its good predictive performance in identifying the Globally Harmonized System (GHS) No Category (NC) or Irritant Category has been demonstrated in evaluations of water-soluble substances, oil-soluble substances, and water-soluble mixtures. However, the predictive performance for oil-soluble mixtures was not evaluated. Twenty-four oil-soluble mixtures were evaluated using the STE test method. The GHS NC or Irritant Category of 22 oil-soluble mixtures were consistent with that of a Reconstructed human Cornea-like Epithelium (RhCE) test method. Inter-laboratory reproducibility was then confirmed using 20 water- and oil-soluble mixtures blind-coded. The concordance in GHS NC or Irritant Category among four laboratories was 90%–100%. In conclusion, the concordance in comparison with the results of RhCE test method using 24 oil-soluble mixtures and inter-laboratory reproducibility using 20 water- and oil-soluble mixtures blind-coded were good, indicating that the STE test method is a suitable alternative for predicting the eye irritation potential of both substances and mixtures.

1. Introduction

It is well known that eye irritation is occurs upon substances coming in contact with the corneal surface; the eye responds with, for instance, membrane lysis of the corneal cells (Maurer et al., 2002). Eye irritation potential of mixtures such as personal care products, which comprise several substances, is affected by viscosity, pH, permeability, and interaction with the substances. Therefore, it is important to assess the eye irritation potential not only of the substances but also of mixtures.

Historically, the Draize eye irritation test using rabbits was performed for assessing the eye irritation potential. However, several *in vitro* eye irritation tests have been developed as alternatives to the Draize test for addressing the marketing ban by European Union (EU) cosmetic directives and animal welfare concerns (Eskes et al., 2005). Alternative methods for the Draize test have mainly focused on cytotoxicity/cell function, reconstructed corneal tissue models, organotypic methods such as chorioallantoic membrane (CAM), and isolated organs using the cornea or eye of slaughtered animals (Eskes et al., 2005;

Abbreviations: BCOP, Bovine Corneal Opacity and Permeability; CAM, chorioallantoic membrane; CT, calcium thioglycolate; DMSO, dimethyl sulfoxide; DPBS, Dulbecco's phosphate buffered saline; EU, European Union; FL, Fluorescein Leakage; GHS, Globally Harmonized System of Classification and Labelling of Chemicals; HCE, human corneal epithelium; HCl, hydrochloric acid; ICE, Isolated Chicken Eye; MTT, methylthiazolydiphenyl-tetrazolium bromide; NC, No Category; NICEATM, National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; OECD, Organisation for Economic Co-operation and Development; RhCE, Reconstructed human Cornea-like Epithelium; SIRC, Statens Seruminstitut Rabbit Cornea; SLS, sodium lauryl sulfate; STE, Short Time Exposure; TG, test guideline; TW80, Tween 80; UN, United Nations

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McNamee et al., 2009; Scott et al., 2010). For example, the Bovine Corneal Opacity and Permeability (BCOP) test method, which uses bovine cornea, and the Isolated Chicken Eye (ICE) test method, which uses chicken eye, have already been adopted by the Organisation for Economic Co-operation and Development (OECD) as test guidelines (TGs) 437 and 438, respectively. The BCOP and ICE test methods can identify chemicals inducing serious eye damage, defined as United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Category 1 (Cat. 1), and chemicals that do not require the classification of eye irritation or serious eye damage, defined as GHS No Category (NC) (United Nations, 2015; OECD, 2013a, 2013b). Additionally, the Fluorescein Leakage (FL) test method has been adopted as TG 460 by the OECD for identifying GHS Cat. 1 (OECD, 2012). The Reconstructed human Cornea-like Epithelium (RhCE) test method has also been adopted by the OECD for identifying GHS NC as TG 492 (OECD, 2015a). In addition to BCOP and ICE, the Short Time Exposure (STE) test method has been adopted as TG491 by the OECD for identifying GHS NC and Cat. 1 (OECD, 2015b).

Mikkelson et al. (1973) reported that > 80% of a solution exposed to the eyes would be removed by lacrimation within 1–2 min in humans and 3–4 min in rabbits. This suggests that when substances are exposed to the eye, eye irritation occurs within a short time on the surface of the corneal layer. On the basis of this mechanism, the STE test method was developed having the distinctive feature of short time exposure of the test materials to the Statens Seruminstitut Rabbit Cornea (SIRC) cell line (Takahashi et al., 2008). The STE test method is applicable to mineral oil as a test solvent because of the short exposure time of SIRC cells to a substance. This is the advantage of the STE test method compared with the other cytotoxicity tests constructed using monolayer cells. Therefore, the STE test method can be applied to both water- and oil-soluble materials.

Predictive performance was evaluated in comparison with the GHS classification using substances in a previous validation study. Data analysis resulted in a concordance of 87% (95/109) for the two classifications of GHS NC and Irritant Category (two-category prediction model) in 109 substances (Takahashi et al., 2011). Moreover, the concordance was 73% (80/109) for the three classifications of GHS Cat. 1, Cat. 2, and NC (three-category prediction model) in 109 substances (Takahashi et al., 2011). In addition, these validation studies were monitored by the Japanese Society for Alternatives to Animal Experiments and the Japanese Center for the Validation of Alternative Methods (Sakaguchi et al., 2011; Kojima et al., 2013). These validation studies demonstrated the transferability of the test system using three substances and inter-laboratory reproducibility using 65 substances. Furthermore, the applicability domain was analyzed in a peer review which was conducted by the Interagency Coordinating Committee on the Validation of Alternative Methods (Hayashi et al., 2013; NICEATM, 2013). The applicability domain was accepted by the OECD, and the STE test method was adopted as TG 491 (OECD, 2015b).

The STE test method is applicable to water- and oil-soluble substances to determine the GHS classification (Sakaguchi et al., 2011; Kojima et al., 2013). Water-soluble mixtures were evaluated for the predictive performance of the GHS classification of NC or Irritant Category (Saito et al., 2015); however, the applicability of the STE test method to oil-soluble mixtures (*e.g.*, hair styling products and sunscreen products) was not assessed yet. The purpose of this study was to evaluate the predictive performance of the STE test method to determine the GHS classification of oil-soluble mixtures. To confirm the reliability of the GHS classification determined by the STE test method, the RhCE test method, which has been adopted as TG492 by OECD, was selected. Furthermore, inter-laboratory reproducibility was evaluated at three naïve laboratories and a developing laboratory using water- and oil-soluble mixtures.

2. Materials & methods

2.1. Materials

To evaluate predictive performance using oil-soluble mixtures by the STE test method, 24 oil-soluble mixtures were selected in personal care products (hair grooming, hair dressing, rouge, deodorant, body lotion, body cream, and moisturizing lotion) that are commercially available. Mineral oil was selected to dilute the mixtures in the STE test method.

To confirm the transferability of the STE test method, three water-soluble substances (sodium lauryl sulfate, SLS; calcium thioglycolate, CT; and Tween 80, TW80) and two oil-soluble substances (1-octanol; dodecane) were evaluated. SLS, TW80, 1-octanol, and dodecane were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). CT was purchased from Sigma-Aldrich Co. or Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

To assess inter-laboratory reproducibility, 18 water-soluble mixtures and two oil-soluble mixtures, totaling to 20 mixtures of personal care products (shampoo, conditioner, hair styler, hair colorant, face and skin cleanser, deodorant, and lotion/moisturizer), were selected. The selected mixtures are the product categories for which *in vitro* tests are routinely performed to confirm irritancy in personal care products. Therefore, detergents containing surfactants were mainly selected as an expected irritant. These mixtures are different from the 24 oil-soluble mixtures evaluated. These mixtures were also commercially available and evaluated as 20 blind-coded mixtures in three naïve laboratories and Kao Corporation, which developed the STE test method.

2.2. The STE test method

2.2.1. Cell culture

SIRC cells (CCL-60) were obtained from American Type Culture Collection (Manassas, VA, USA). SIRC cells were cultured in Eagle's minimum essential medium (Sigma-Aldrich Co.) containing 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin (Invitrogen Co., Carlsbad, CA, USA). After the cells proliferated in the culture flask to confluence, the cells were dispersed using trypsin-ethylenediaminetetraacetic acid solution (Sigma-Aldrich Co.). The dispersed cells were spread into 96-well flat-bottomed plates (Corning Coster Co., Cambridge, MA, USA) at 3.0×10^3 cells/well. After incubation (37 °C, 5% CO_2) for 5 days (or 6.0×10^3 cells/well for 4 days), the cells reached confluence.

2.2.2. The protocol

The STE test method was performed according to OECD TG 491 (OECD, 2015b). The test solvent was selected before the test. Physiological saline was selected as a first solvent for each test material. If the material was insoluble in physiological saline, 5% (w/w) dimethyl sulfoxide (DMSO, Sigma-Aldrich Co.) in saline was selected as a second solvent. In case it was insoluble in both physiological saline and 5% DMSO in saline, mineral oil (Sigma-Aldrich Co.) was selected as the third solvent. In the STE test method, dissolution means being dissolved or uniform suspension in 5 min at 5% (w/w) and 0.05% (v/v) in the selected solvent. The cells cultured in 96-well plates were exposed to 200 μl of 5% and 0.05% test material diluents for 5 min. After exposure, the cells were washed twice with Dulbecco's phosphate buffered saline (–) (DPBS (–); Sigma-Aldrich Co.) and 200 μl of 0.5 mg/ml methylthiazolyldiphenyl-tetrazolium bromide (MTT, Sigma-Aldrich Co.)/medium solution were added. After a 2 h reaction time, the MTT solution was discarded and MTT formazan was extracted with 0.04 N HCl-isopropanol (Sigma-Aldrich Co.) for 1 h, and the absorbance of the extract was measured at 570 nm by a plate reader.