Toxicology in Vitro 29 (2015) 617–620

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Predictive performance of the Short Time Exposure test for identifying eye irritation potential of chemical mixtures

Kazutoshi Saito *, Masaaki Miyazawa, Yuko Nukada, Kyo Ei, Takayuki Abo, Hitoshi Sakaguchi

Safety Science Research Laboratories, Kao Corporation, Tochigi, Japan

ARTICLE INFO

Article history:
Received 26 August 2014
Accepted 17 January 2015
Available online 11 February 2015

Keywords:
STE
Eye irritation
Alternative method
Chemical mixture
SIRC cell line

ABSTRACT

The Short Time Exposure (STE) test is an *in vitro* eye irritation test based on the cytotoxicity in SIRC cells (rabbit corneal cell line) following a 5 min treatment of chemicals. This study evaluated the predictive performance of the STE test to identify the globally harmonized system (GHS) Not Classified category and other irritant categories (i.e., GHS Category 1 or 2) when used to test 40 chemical mixtures that included irritants. The STE test correctly identified 30 tested mixtures classified as GHS irritant categories and 5 out of 10 tested mixtures classified as GHS Not Classified. The sensitivity, specificity, positive predictivity, negative predictivity, and overall accuracy of the STE test were 100% (30/30), 50% (5/10), 86% (25/30), 100% (5/5), and 88% (35/40), respectively. These predictive performances were comparative to or greater than those in other *in vitro* eye irritation tests that have been accepted as test guideline by the Organisation for Economic Co-operation and Development. This suggests that the STE test has sufficient predictivity for identifying the eye irritation potential of chemical mixtures. Since no false negatives in this study were found, this indicates that the STE test is applicable as a part of the bottom-up approach.
© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

The rabbit Draize test has been used for many years to evaluate the eye irritation potential of chemicals (Draize et al., 1944). However, animal welfare concerns and EU regulatory policies prohibiting the testing of cosmetic ingredients in animals for a number of toxicological endpoints (Directive 2003/15/EC, 2003) have led to the development of a greater number of alternative eye irritation methods that use various cell lines and tissues (Balls et al., 1999; Ohno et al., 1999; Eskes et al., 2005). Among them, the Bovine Corneal Opacity and Permeability (BCOP) assay, Isolated Chicken Eye (ICE) test, and a fluorescein leakage (FL) test method have recently been adopted as the Organisation for Economic Co-operation and Development (OECD) test guidelines (TGs) for predicting eye irritation (OECD, 2009a,b, 2012).

The Short Time Exposure (STE) test was submitted to the OECD in 2011 as a new alternative method and the draft TGs are currently under review. The STE test is a cytotoxicity-based alternative test that uses SIRC cells (rabbit cornea cell line) to identify chemicals that induce eye irritation (Irritant, "I") and chemicals that do not inducing eye irritation (Not irritant, "NI") as well as to classify minimal, moderate, or severe eye irritation potency (Takahashi et al., 2008). Since the STE test uses cell viability as an endpoint after 5 min of chemical exposure, the procedure is simple and quick, and the evaluation cost is low. The STE test also has the advantage of being able to evaluate the eye irritation potential of water insoluble chemicals by using mineral oil as the test vehicle. Moreover, when the STE test was used to test 44 chemicals in order to predict the globally harmonized system (GHS) irritation categories, the results demonstrated that this test has a high accuracy (>90%) between the STE irritation categories ("NI" and "I") and the two-rank GSH classification ("Not Classified" and "Category 1 or 2") (Takahashi et al., 2009).

To date, the STE test has been shown to have a good predictive performance when evaluating single substances. However, it is also important to assess the eye irritation potential of chemical mixtures (e.g., surfactant based mixtures) when conducting safety assessments, as new combinations of ingredients could potentiate the toxicity of one or more of the ingredients in a mixture (Bruner et al., 1998). If there is a significantly increased toxicity of one or more ingredients when delivered in a particular mixture, this raises the possibility that the new mixture may be poorly tolerated, or in the worst case, cause injury (Baker and Bruner, 1997). In the current study, we evaluated the utility of the STE test within the context of the GHS classification and assessed its ability to predict

* Corresponding author at: Safety Science Research Laboratories, Kao Corporation, 2606 Akabane, Ichikai-machi, Haga-gun, Tochigi 321-3497, Japan. Tel.: +81 285 68 7474; fax: +81 285 68 7452.
E-mail address: saitou.kazutoshi@kao.co.jp (K. Saito).

http://dx.doi.org/10.1016/j.tiv.2015.01.008
0887-2333/© 2015 Elsevier Ltd. All rights reserved.

the eye irritation potential of 40 chemical mixtures, which included alcohol-based or surfactant-based mixtures.

2. Materials and methods

2.1. Materials

The study analyzed 40 chemical mixtures, which included alcohol-based or surfactant-based mixtures. The GHS classifications of the 40 chemical mixtures were defined as "NI" (Not Classified, or not an irritant) or "I" (Irritant classified as Category 1 or 2) based on the historical databases.

2.2. STE test

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., St. Louis, MO, USA) 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). Once the cells proliferated in the culture flask to confluence, they were dispersed with trypsin-ethylenediaminetetraacetic acid (EDTA) 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. STE test protocol

The STE test was carried out using the procedure of Takahashi et al. (2008). Physiological saline (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was used as the vehicle for all tested mixtures and as a control substance. The cells cultured in the 96-well plates were exposed to 200 μl of 5% solutions of test mixtures for 5 min. With the STE protocol, exposure to 5% and 0.05% solutions of the test mixtures is used to predict the three-rank GHS classifications (Category 1, 2, or Not Classified) of the mixtures, whereas the 5% exposure is sufficient for the "I" or "NI" predictions (Takahashi et al., 2008). Since the purpose of this study was to assess the performance of the STE test for predicting the "I" or "NI" mixtures, the test mixtures were only tested at 5%. After exposure, the cells were washed twice with Dulbecco's phosphate buffered saline (without magnesium and calcium) [DPBS (-); Takara Bio Inc., Shiga, Japan], with 200 μl of methylthiazolydiphenyltetrazolium bromide (MTT, Sigma–Aldrich Co.) solution (0.5 mg MTT/ml of medium) then added. After a 2 h reaction time, MTT formazan was extracted with 0.04 N HCl–isopropanol (Kanto Chemical Co., Inc., Tokyo, Japan) over a 30 min period and the absorbance of the extract was measured at 570 nm with a plate reader (Corona Electric Co., Ltd., Ibaraki, Japan or BMG LABTECH GmbH, Offenburg, Germany). The ratio of absorbance (%) for cells treated with test mixtures to that of the control was represented as relative viability (triplicate determinations). The mean of three wells was calculated for one independent test. Three independent tests were conducted for each test mixture and the overall calculated mean of the three independent tests was used for estimation of the eye irritation.

2.2.3. STE test category classification of eye irritation

The STE test ("I" or "NI") determined the category classification of the eye irritation based on the relative cell viability. A tested mixture that had a relative viability of 70% or less at a 5% concentration was categorized as "I" while a tested mixture that had a relative viability greater than 70% at the same concentration was categorized as "NI" (Takahashi et al., 2008).

3. Results

Table 1 shows the summary of the results for the 40 chemical mixtures. The STE test showed that 35 mixtures exhibited less than 70% cell viability while five (2 alcohol based mixtures and 3 cationic surfactant based mixtures) exhibited more than 70% cell viability. Five out of 10 tested mixtures that were classified as GHS Not Classified showed more than 70% cell viability and were classified as "NI" by the STE test. The STE test determined that the five other mixtures (3 cationic surfactant based mixtures (B, C, and E) and 2 surfactant based mixtures (O and P)) exhibited less than 70% viability (with the viability ranging from 2.2% in surfactant based mixture O to 53.9% in cationic surfactant based mixture E). On the other hand, the STE test found that 30 of the tested mixtures that were classified as GHS irritant categories (i.e., Category 1 or 2) exhibited less than 70% cell viability (with the viability ranging from 0% to 5.1%).

Table 2 summarizes the predictive performance of the STE test for the chemical mixtures. The accuracy of the STE test was judged based on whether the tested mixtures that were classified as GHS

Table 1
Summary of the STE test results for 40 chemical mixtures.

Tested mixture		GHS	STE	
Category	Code		Viability (%)	Category
Agricultural chemical	A	I	5.1	I
Alcohol based mixture	A	NI	76.8	NI
	B	NI	75.6	NI
Anionic surfactant based mixture	A	I	0.5	I
	B	I	0	I
	C	I	0	I
	D	I	4.2	I
	E	I	0.6	I
	F	I	1.7	I
	G	I	0	I
	H	I	0	I
	I	I	2.2	I
	J	I	0.8	I
	K	I	0.6	I
	L	I	0.3	I
Cationic surfactant based mixture	A	NI	88.7	NI
	B	NI	46.5	I
	C	NI	35.7	I
	D	NI	79.1	NI
	E	NI	53.9	I
	F	NI	95.6	NI
Sodium percarbonate based mixture	A	I	1.3	I
	B	I	0.9	I
	C	I	2.7	I
Surfactant based mixture	A	I	1.9	I
	B	I	3.8	I
	C	I	1.6	I
	D	I	2.1	I
	E	I	0	I
	F	I	3.6	I
	G	I	1.4	I
	H	I	1.6	I
	I	I	1.8	I
	J	I	2.6	I
	K	I	1.7	I
	L	I	2.4	I
	M	I	0.5	I
	N	I	1.5	I
	O	NI	2.2	I
	P	NI	5.6	I

Code of mixtures was randomly allocated.
"I" in GHS; Irritation categories (category 1 or 2), "NI" in GHS; Not-classified category.
"I" in STE; Irritants, "NI" in STE; Not irritants (see Section 2.2.3).

Download English Version:

https://daneshyari.com/en/article/2602502

Download Persian Version:

https://daneshyari.com/article/2602502

Daneshyari.com