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Second-phase validation study of short time exposure test for assessment of eye irritation potency of chemicals

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ABSTRACT

A Short Time Exposure (STE) test is a cytotoxicity test that uses SIRC cells (rabbit corneal cell line) to assess eye irritation potency following a 5-min chemical exposure. This second-phase validation study assessed the predictive capacity of the STE test using 40 coded test substances at three laboratories. A Validation Management Team (VMT) then evaluated the predictivity of the STE test for United Nation (UN) Globally Harmonized System (GHS) categories using 63 test substances including the results of the first-phase validation study.

The STE test can assess not only the severe or corrosive ocular irritants (corresponding to the UN GHS Category 1) but also non-irritant (corresponding to UN GHS Non Category) from other toxicity classes, especially for limited types of test substances. The predictivity by STE test, however, was insufficient for identification of UN GHS categories (Category 1, Category 2, or Non Category).

These results suggest that the STE test can be recommended as an initial step in a top-down approach to identification of severe irritants and test substances that require classification for eye irritation (UN GHS Category 1) as well as an initial step in a bottom-up approach to identification of test substances that do not require classification for eye irritation (UN GHS Non Category) from other toxicity classes, especially for limited types of test substances. On the other hand, the STE test is not considered adequate for the identification of mild or moderate irritants (i.e., UN GHS Categories 2A and 2B) and severe irritants (UN GHS Category 1).

1. Introduction

Eye irritation potency of chemical test substances has traditionally been evaluated using Draize rabbit-eye test. Recently, consideration of the protection and welfare of animals has resulted in adoption of new regulations in the EU (Directive 2003/15/EC, 2003) that prohibit the use of animal eye irritation tests for testing cosmetics and highlight the need for alternative methods that reduce or replace animal experiments. In September 2009, the bovine corneal opacity and permeability (BCOP) assay and isolated chicken eye (ICE) assay were adopted by the Organisation for Economic Co-operation and Development (OECD) for identifying ocular corrosives and severe irritants, i.e., OECD Test Guideline 437 or 438, respectively (OECD, 2009a,b). Furthermore, a fluorescein leakage (FL) test method was also adopted by OECD Test Guideline 460 in October 2012 (OECD, 2012).

As part of this new line-up of high-throughput assays, a Japanese proposal outlining a Short Time Exposure (STE) assay was submitted to OECD in 2011. This STE test is a short-time exposure cytotoxicity test that uses SIRC cells (rabbit corneal cell line) to evaluate minimal, moderate, or severe eye irritation potency (Takahashi et al., 2008). This test uses cell viability as an endpoint after 5 min of chemical exposure and solves other problems associated with conventional cytotoxicity tests. For example, the STE test is suitable for assessing eye irritation potency using mineral

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oil as solvent for chemical that are insoluble in water. The test itself is also very simple to use and provides immediate results. In collaborative research at three laboratories evaluating the STE test, similar results were obtained for both positive and negative controls, indicating excellent transferability (Takahashi et al., 2009). In addition, an evaluation of 51 chemicals showed high reproducibility and good predictivity at each of the three laboratories, with an inter-laboratory accuracy of 98.0% or higher (Takahashi et al., 2009). None of the three attempts, however, was coded to the identity of the test substances. During the earlier first-phase validation study conducted by the Japanese Society for Alternatives to Animal Experiments (JSAAE), five laboratories confirmed the transferability of the STE test using three standard substances. The usefulness of the STE test as an alternative to animal testing in the assessment of eye-irritation potency was further demonstrated by establishing the intra- and inter-laboratory reproducibility of four assay controls as well as inter-laboratory reproducibility and predictivity (i.e., agreement with United Nation (UN) Globally Harmonized System (GHS) irritation category and STE rank) for 25 coded test substances (Sakaguchi et al., 2011). In this second-phase validation study, a new Validation Management Team (VMT) evaluated the predictivity of the STE test at three laboratories using 63 test substances including the result of the first-phase validation study.

2. Materials and methods

2.1. Organization for STE test validation study

The STE Test VMT was responsible for determining the study plan and test protocol, confirming the data analysis, and publishing the study results. The VMT was organized by the Japanese Center for the Validation of Alternative Methods (JaCVAM) and comprised a trial coordinator, specialists in eye irritation studies, a biostatistician, representatives from the lead laboratory (Kao Corporation), and liaisons from each International validation center as shown as Fig. 1. The VMT was also subdivided into a chemical management group, a quality assurance group, and a data analysis group. The three participating laboratories were:

Laboratory 1: Kanebo Cosmetics Inc., Quality Management Department.
Laboratory 2: POLA Chemical Industries, INC., Quality Research Department.
Laboratory 3: LION Corporation, Human & Environmental Safety Evaluation Center.

The participating laboratories conducted the experiments, created printouts of absorbance measurements taken during the experiment, and input values in data sheets. The printouts as well as electronic files were then submitted to the biostatistician, who confirmed the consistency of the data on the printouts with that of the electronic file. Where values differed, inquiries were made to the laboratories involved, and the revisions made to reflect the correct value.

Prior to the first-phase validation study, a technical workshop was held at Kanebo Cosmetics Inc. in Odawara, Kanagawa, Japan, on Thursday, May 29, 2008, to correlate technical issues and experimental procedures. Therefore, since the three participating laboratories for the second-phase validation study had also taken part in the first-phase validation study, the VMT omitted a new technical workshop.

Duration of second phase validation study was 6-month from June 2010 to December 2010 on schedule. The meetings of VMT were held at Kyoto University on Friday, June 11 and Wednesday, 24 November, 2010.

2.2. Methodology

2.2.1. Test substances (coded test substances) and classification

In this phase validation study, the 40 test substances selected by the chemical management group including two test substances (C07: 2-ethylhexyl p-dimethyl-amino benzoate, and C37: 1-octanol) used in the first phase validation study (see Table 1). Each of these test substances had previously been evaluated using the Draize test and classified per UN GHS Categories (ECETOC, 1998). Ten test substances were sent to all three laboratories and 20 of the remaining 30 test substances were sent to two laboratories each. Therefore, each laboratory received 30 of 40 test substances.

All test substances were coded, rotated, and distributed by JaCVAM by mid-August, 2010, using a coding list created by the quality assurance group. The test substances were coded by the data analysis group and transported by safety officers from each participating laboratory.

In order to ensure the safety of the individuals in charge of the experiment, JaCVAM forwarded the information regarding appropriate environmental conditions and the Material Safety Data Sheets (MSDS) for coded test substances to the safety officers at the laboratories. The VMT gave instructions to handle the all test substances as if they were toxic and deleterious, to store them under appropriate environmental conditions, to refer to the MSDS only in case of accidents.

UN GHS is a system of globally standardized rules for classifying chemicals according to type and degree of hazard so that the information can be understood easily on a label or in an MSDS (United Nations, 2003). There are several classifications for eye irritation, which are mainly based on Draize rabbit-eye test results: UN GHS Category 1 and 2A for irreversible eye effects, UN GHS Categories 2B for reversible eye effects, and UN GHS Non Category for a

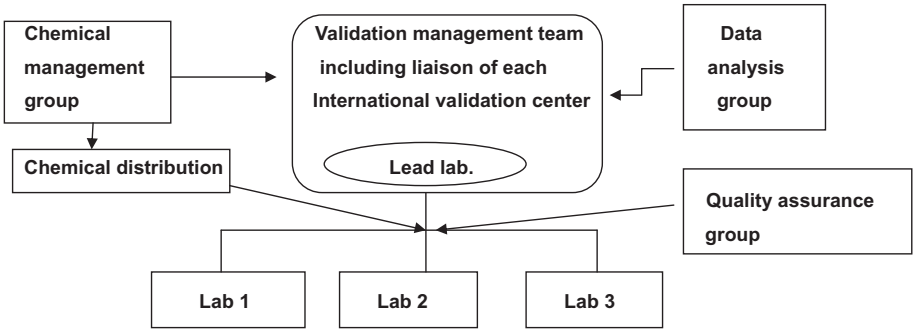

Fig. 1. Organization of STE test validation management Team Lead laboratory: Kao Corporation Lab 1: Kanebo Cosmetics Inc. (Kanebo) Lab 2: POLA Chemical Industries, Inc. (POLA) Lab 3: LION Corporation (LION).