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Brief Report

Risk Assessment of Cosmetics: KoCVAM's New Alternative Test Methods for Phototoxicity and Established Reference Chemical Database System for Skin Irritation

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ABSTRACT

Background: The Korean Center for the Validation of Alternative Methods (KoCVAM), the leading validation center of alternative test methods in Korea, has been actively promoting the Three R's principles and the utilization of alternative test methods within Ministry of Food and Drug Safety (MFDS) since 2009. Additionally, the MFDS was the first government body in Korea to begin researching alternative methods to animal testing.

Method: Both skin irritation and phototoxicity test methods using KeraSkinTM evaluate each toxicity by measuring cell viability as an endpoint of tissue death caused by test chemicals. Cell viability is determined using the MTT (3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay by calculating the OD value of the tissues exposed to a test chemical compared to those exposed to the corresponding vehicle/negative control, which is set to 100%.

Results: We have established RCDS-skin irritation and we evaluated 59 test chemicals across three RhE models and incorporated 95 test results into the RCDS-skin irritation. The KeraSkin™ phototoxicity assay demonstrated predictive performance with 100% accuracy, 100% sensitivity, and 100% specificity.

Conclusion: KoCVAM has developed RCDS-skin irritation, which includes data on 149 chemicals. The 'KeraSkinTM Phototoxicity Assay' fulfills the requirements to be included in OECD TG498.

Keywords: Phototoxicity; Photosensitivity; KeraSkin™

INTRODUCTION

The Ministry of Food and Drug Safety (MFDS) in the Republic of Korea established the Korean Center for the Validation of Alternative Methods (KoCVAM) in 2009 to 'creating a healthy country where people and animals coexist'. KoCVAM has strived to promote the advancement of alternative test methods in Korea for over 15 years [1]. In Korea, animal testing is prohibited for cosmetics and raw materials to keep up with the global trend. In detail, the Article 15-2 of the Cosmetics Act stipulates that "no responsible cosmetic distributor shall distribute or sell any cosmetics for which animal testing was conducted, or cosmetics manufactured (including manufacturing by consignment) or imported using raw materials for which animal testing was conducted". Therefore, alternative test methods are essential for toxicity assessment of chemicals including cosmetic materials before releasing products such as cosmetics into the market. In order to develop and conduct validation study of alternative test methods, a lot of data such as *in vivo* and *in vitro* data for test chemicals are required. However, there are few Databases (DB)

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suitable for development and verification of alternative test methods in the Republic of Korea. We intended to create a scientific and systematic 'Reference Chemical Database System for Skin Irritation Alternative Test (RCDS-Skin Irritation)'. Additionally, we improved the quality of the data in the RCDS-Skin Irritation by performing experiments using EpiDerm™, SkinEthic™ RHE and KeraSkin™ models.

Recently, KoCVAM completed a scientific validation study on the 'KeraSkinTM Phototoxicity Assay' using reconstructed human epidermis model (KeraSkinTM) and it was adopted in the OECD workplan for inclusion in OECD Test Guideline (TG) as a "me-too" test method of OECD TG498 (April, 2024). In this article, we would like to explain the development of the KeraSkin™ phototoxicity assay. Phototoxic reactions (phototoxicity or photoirritation), which are not mediated by immune system, can occur in anyone who is exposed to adequate levels of phototoxic chemicals [2]. This study, conducted in accordance with OECD Performance Standard No. 3563, involved one lead laboratory and four participating laboratories. The objective was to validate the relevance and reliability of the KeraSkin™ phototoxicity assay for regulatory application in the safety assessment of cosmetics and pharmaceuticals in Korea. Consequently, 'KeraSkin™ phototoxicity assay' fulfills the requirements to be included as a 'me-too' test method in OECD TG498.

METHODOLOGY

To develop the RCDS-Skin Irritation assessment, we utilized resources such as European Chemicals Agency (ECHA), Toxic Substances Control Act (TSCA), and Integrated Chemical Environment (ICE). We also gathered *in vivo* and *in vitro* data from 71 research articles and database sources pertaining to skin irritation. In total, data for 149 chemicals were compiled, and the RCDS-Skin Irritation system was constructed using this information, which encompasses fundamental details, physicochemical properties, human data, and both *in vivo* and *in vitro* data in accordance with OECD test guidelines to form the database [3]. The RCDS-Skin Irritation system is accessible *via* the KoCVAM website (http://www.nifds.go.kr/kocvamen).

We performed skin irritation tests using three RhE models: EpiDerm™ (MatTek, Massachusetts, USA), SkinEthic™ RHE (EPISKIN, rue Alexander Fleming, France), and KeraSkinTM (Biosolution Co., Ltd., Seoul, Republic of Korea). Detailed protocol parameters for each test method, including pre-incubation, test chemical application, post-incubation, and acceptability criteria, are provided in Table 1. We tested 24 chemicals using EpiDerm™ SIT, 25 chemicals using SkinEthic™ RHE, and 46 chemicals using KeraSkin™ SIT, and added the test results to the RCDS-Skin Irritation database in order to improve the quality of the database [4].

Variables	EpiDerm™ SIT	SkinEthic™ RHE	KeraSkin™ SIT
Incubation time	18-24 hours	≥ 2 hours	20-24 hours
Medium volume	0.9 mL	0.3 or 1 mL	0.9 mL
For liquids	30 µL	16 µL	40 µL
For solids	25 mg+DPBS (25 µL)	16 mg+DW (10 µL)	40 mg+DPBS (40 µL)
Use of nylon mesh	If necessary	Applied	Not used
Total application time	60 minutes	42 minutes	30 minutes
Application temperature	a) at RT for 25 minutes b) at 37°C for35 minutes	RT	37°C
Medium volume	0.9 mL × 2	2 mL	0.9 mL
MTT solution	300 µL 1 mg/mL	300 µL 1 mg/mL	300 µL 0.4 mg/mL
Mean OD of the tissue replicates treated with the NC (water or DPBS)	≥ 0.8 and ≤ 2.8	≥ 0.8 and ≤ 3	≥ 0.7 and ≤ 1.6
Mean viability of the tissue replicates treated with the positive control (SDS 5%), expressed as % of the NC	<20%	<40%	<40%
Standard deviation between tissue replicates	SD ≤ 18%	SD ≤ 18%	SD ≤ 18%

Table 1: Protocol parameters specific to each of the four test methods.

RESULTS AND DISCUSSION

The KeraSkin™ phototoxicity assay is designed to assess the phototoxic potential of test chemicals applied topically to KeraSkin™ tissues under UVA irradiation at 6 J/cm² (solar simulator, ATLAS SUNTEST® CPS+, 1500W Xenon lamp, Atlas Material Technology, Chicago, IL, USA) as well as in the absence of irradiation. We refined the assay and established its acceptance criteria through statistical analysis involving 25 test chemicals, which included 6 proficiency chemicals from OECD TG498, 6 reference chemicals from OECD Performance Standard No. 356, and 13 additional chemicals. Based on the results from these chemicals, we calculated the overall predictive performance of the KeraSkin™ phototoxicity assay, which demonstrated perfect accuracy, sensitivity, and specificity at 100% [5].

Validation of test methods necessitates comprehensive information on reference chemicals, including reliable *in vivo* and *in vitro* data. To achieve this, we gathered information on reference chemicals and explored chemical databases from Korea and other countries to develop the RCDS-Skin Irritation database. Each data element was carefully evaluated by experts, leading to the establishment of a standardized RCDS-Skin Irritation system. Furthermore, we enhanced the data quality by conducting experiments using three Reconstructed Human Epidermis (RhE) models. However, some chemicals yielded test results that were inconsistent with *in vivo* data, highlighting the need for ongoing refinement in testing methodologies. In particular, the chemicals that generated false predictions in the RhE models in OECD TG439 were mainly acids, alcohols, ketones, surfactants, electrophiles, brominated compounds, and phenolic derivatives. Therefore, caution should be exercised when testing these classes of chemicals in RhE models, as their applicability might be limited. Given these limitations, OECD Guidance Document No. 203 emphasizes the importance of the Integrated Approach on Testing and Assessment (IATA) for comprehensive evaluations of topical skin irritation. KoCVAM has successfully developed and optimized the KeraSkin™ Phototoxicity Assay, which accurately classified 25 test chemicals in line with *in vivo* results, demonstrating a remarkable predictive capacity with 100% sensitivity, specificity, and accuracy. When tested against 12 reference chemicals from OECD PS No. 356, the assay maintained these perfect metrics. Consequently, KeraSkin™ is recognized as a 'me-too' method of OECD TG498. A validation study is currently underway to further establish the reliability and relevance of the KeraSkin™ phototoxicity assay, with preparations being made for peer review. These advancements indicate a significant step toward refining testing methodologies while reducing reliance on animal testing in chemical safety assessments.

CONCLUSION

KoCVAM has established the RCDS-Skin Irritation database, which includes information on 149 chemicals compiled from literature and chemical databases in Korea. Additionally, 59 chemicals were tested using three Reconstructed human Epidermis (RhE) models, with 95 test results integrated into the database. KoCVAM has also developed an alternative phototoxicity test method based on the KeraSkin™ RhE model, qualifying it as a "me-too" test method under OECD TG498. Operating under the Ministry of Food and Drug Safety (MFDS), KoCVAM is at the forefront of developing and promoting alternative testing methods in Korea, committed to advancing these methods in alignment with the three R's principles: Replacement, Reduction, and Refinement.

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