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Catch-up validation study of an in vitro skin irritation test method based on an open source reconstructed epidermis (phase II)

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ABSTRACT

To replace the Draize skin irritation assay (OECD guideline 404) several test methods based on reconstructed human epidermis (RHE) have been developed and were adopted in the OECD test guideline 439. However, all validated test methods in the guideline are linked to RHE provided by only three companies. Thus, the availability of these test models is dependent on the commercial interest of the producer. To overcome this limitation and thus to increase the accessibility of in vitro skin irritation testing, an open source reconstructed epidermis (OS-REp) was introduced. To demonstrate the capacity of the OS-REp in regulatory risk assessment, a catch-up validation study was performed. The participating laboratories used in-house generated OS-REp to assess the set of 20 reference substances according to the performance standards amending the OECD test guideline 439. Testing was performed under blinded conditions. The within-laboratory reproducibility of 87% and the inter-laboratory reproducibility of 85% prove a high reliability of irritancy testing using the OS-REp protocol. In addition, the prediction capacity was with an accuracy of 80% comparable to previous published RHE based test protocols. Taken together the results indicate that the OS-REp test method can be used as a standalone alternative skin irritation test replacing the OECD test guideline 404.

1. Introduction

The epidermis forms the outermost barrier between the human body and the environment and is thus exposed to various potentially harmful substances. To test the potential of substances to cause skin irritation, the Draize assay was developed and implemented as test guideline 404 of the Organization for Economic Co-operation and Development (OECD) (Draize et al., 1944). However, due to the limited relevance of results from this animal assay for humans and animal welfare concerns, in vitro models have been developed to assess the skin irritation potential of chemicals (Fentem et al., 1998; Stobbe et al., 2003).The legislation worldwide endorses the introduction of alternative test methods that comply with the concept of reduction, refinement and replacement of animal experimentation introduced by Russell and Burch (Russell et al., 1959). In the European Union, clear priority is given for animal free tests due to the provisions of the legislation for the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH; EU, 2006) and the European Union Cosmetics Regulation (EU, 2009). The latter regulation put a stepwise ban of animal tests for cosmetic products and ingredients into force. The final step was enforced in

Abbreviations: EURL-ECVAM, European Union Reference Laboratory for Alternatives to Animal Testing; FHG, Fraunhofer Gesellschaft; FUB, Institute of Pharmacy at the Freie Universität Berlin; UN GHS, United Nations Globally Harmonized System of Classification and Labelling of Chemicals; hEK, Human epidermal keratinocytes; HEN, Henkel AG &Co. KGaA; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid; OECD, Organization for Economic Co-operation and Development; OS-REp, Open source reconstructed epidermis; PBS, Phosphate buffered saline; RHE, Reconstructed human epidermis; SDS, Sodium dodecyl sulfate; SOP, Standard operating procedure.

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March 2013 and bans the marketing of cosmetic products containing ingredients tested on animals after this deadline.

The prediction of skin irritation using an alternative approach is feasible by employing in vitro test methods based on reconstructed human epidermis (RHE), which is based on human epidermal keratinocytes (hEK). The models emulate the morphology, structure and metabolism of the human epidermis accurately (Rosdy and Clauss, 1990). Currently several RHE models are commercially available from various producers and academic institutes (De Wever et al., 2013; Groeber et al., 2011) and have been used for different research questions such as, skin corrosion (Kandárová et al., 2006), skin irritation (Cotovio et al., 2005), skin barrier formation (Thakoersing et al., 2013) and skin absorption (Schäfer-Korting et al., 2008). Currently the epiCS™ model by CellSystems (Pratt et al., 2014), the SkinEthic RHE (Alépée et al., 2014) and EpiSkin ™ (Alépée et al., 2016) model by L'Oréal, the EpiDerm™ model by Mattek (Chapman et al., 2014) and the ATERA-RHE by ATERA can be purchased worldwide. Additionally, the LabCyte EPI model by J-Tech (Katoh et al., 2009) and the KeraSkin-VM™ by MCTT Inc. (Jung et al., 2014) are available in Japan and Korea. Of the commercial RHE, two models, namely the EpiDerm™ and the EpiSkin™, were part of the first skin irritation validation study sponsored by the European Union Reference Laboratory for Alternatives to Animal testing (EURL-ECVAM). After optimization, the methods were found to be scientifically valid as a standalone method to identify skin irritants and were implemented by the OECD in the test guideline 439 (OECD, 2015a). Moreover, the results are the basis for the performance standards for in vitro skin irritation test methods based on reconstructed human epidermis (OECD, 2015b; Spielmann et al., 2007). Following the initial validation study, the SkinEthic™ RHE (Alépée et al., 2010) and the LabCyte EPI-MODEL™ (Kojima et al., 2013) were validated in catch-up validation studies and integrated in the test guideline 439.

However, the validated test protocols are linked to a limited number of models produced and marketed by a few companies only. Hence, all validated tests are dependent on the availability and the commercial strategies of the suppliers. An unrestricted approach has become possible by an open source reconstructed epidermis (OS-REp) established by the Henkel AG &Co. KGaA (De Wever et al., 2013, 2015).

Comparable to the open source concept in information technology, in which the source code of software is openly accessible, the OS-REp model will include the publication of the production protocol without legal restrictions (Bagozzi and Dholakia, 2006; Hertel et al., 2003; Lakhani and Von Hippel, 2003). Thus, any laboratory can use OS-REp for irritation testing and other research purposes without being dependent on commercially available models. Thereby the open source concept could foster the dissemination and implementation of skin models especially in countries with customs regulations that restrict the import of tissue models or budget restricted research laboratories. Moreover, open source allows end users to amend the source code, to improve the program or adapt it to a specific purpose. In the framework of alternative test methods it is envisioned that this philosophy will encourage a constant refinement of the model. However, it should be noted that once a model is implemented in a test procedure the model cannot be changed without again demonstrating the validity of the test methods.

The OS-REp is based on an initial protocol (Lemper et al., 2013; Poumay et al., 2004), refined by Henkel (HEN) and employed in an initial skin irritation test. In order to become accepted by regulatory authorities, we performed and describe a catch-up validation study with the OS-REp according to the EURL-ECVAM performance standards for in vitro skin irritation testing, previously an integral part of the OECD TG 439 (OECD, 2015b). In Phase I of the two-tiered approach a blinded study was conducted, in which all OS-REp models were exclusively produced at HEN and shipped to all participating laboratories. This study resulted in an overall accuracy of 75%. In addition, the skin irritation testing process was refined regarding the handling of volatile irritating test substances while leaving the procedural details of the testing protocol unchanged.

Here we present the results of the Phase II of the catch-up validation study to achieve regulatory acceptance for skin irritation testing. Due to structural, mechanistic and procedural similarity of the OS-REp model and the OS-REp skin irritation testing protocol with accepted test methods, the performance standards for in vitro skin Irritation testing are applicable to reduce the acceptance process (OECD, 2015b). In the present study HEN distributed only the standard operating procedures (SOP) for hEK isolation, OS-REp production and skin irritation testing to two additional independent laboratories. After a transfer phase all laboratories established their own hEK batches from diverse donors, generated their own OS-REp models and then only used the in-house produced models for the validation runs.

2. Material and methods

2.1. Study design

The catch up validation study was conducted according to the performance standards (OECD, 2015b) at the lead laboratory HEN, the Fraunhofer Institute for Interfacial Engineering and Biotechnology as part of the Fraunhofer Gesellschaft (FHG) and the Institute of Pharmacy at the Freie Universität Berlin (FUB). Each laboratory established own hEK batches and produced its own OS-REp in the respective facilities (Fig. 1). Subsequently, the models were employed to test the same set of 20 reference substances in each laboratory in three to five independent runs at different occasions. Test substances were coded and distributed by the Biotesys GmbH (Esslingen, Germany), safeguarding that the testing in the laboratories was conducted under blind conditions. The reference substances comprised ten irritants, i.e. category 2 substances according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS), and ten non-irritants, i.e. no category substances (Table 1) (United Nations, 2015).

In addition to the blinded test substances, two controls were included in each test run. As a negative control that controls for insufficient tissue viability, phosphate buffered saline (PBS, Life Technologies) was applied to the models. A 5% aqueous solution of sodium dodecyl sulfate (SDS, Sigma-Aldrich; Steinheim, Germany) was used as the positive control.

Per test run, each substance and each control was tested on three tissue replicates. Only test data, which met all acceptance criteria specified in the SOPs were considered valid for data analysis. In case the standard deviation between run replicates exceeded 18%, the respective substance was re-tested up to two times. However, if the acceptance criteria for the negative control and the positive control were not met,

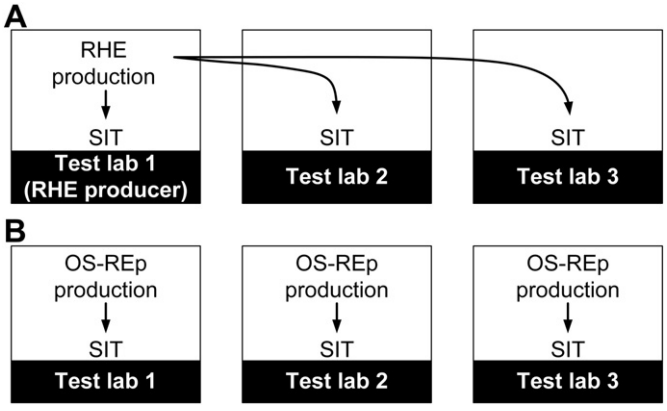

Fig. 1. Procedure for the conduction of a validation study for skin irritation testing (SIT). In the classical approach (A) the employed reconstructed human epidermis (RHE) is provided by the test developer to the additional two laboratories, where the models are used in the SIT validation test runs. In the present study (B) all participating laboratories established their own OS-REp production and then used the in-house produced models in the SIT.