Toxicology in Vitro 29 (2015) 1779–1792

Contents lists available at ScienceDirect

Toxicology in Vitro

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

An integrated testing strategy for *in vitro* skin corrosion and irritation assessment using SkinEthic™ Reconstructed Human Epidermis

Nathalie Alépée [a,*], Marie-Hélène Grandidier [a], Carine Tornier [b], José Cotovio [a]

[a] L'Oréal Research & Innovation, Aulnay sous-Bois, France
[b] Episkin SA, Lyon, France

ARTICLE INFO

Article history:
Received 3 June 2015
Revised 7 July 2015
Accepted 13 July 2015
Available online 14 July 2015

Keywords:
SkinEthic™ Reconstructed Human Epidermis
Skin corrosion and irritation
OECD Test Guideline 431 and 439
Integrated Approach on Testing and Assessment
EU Labelling and Packaging Regulation Classification
United Nations Globally Harmonized System

ABSTRACT

The SkinEthic™ Reconstructed Human Epidermis (RHE) method has been formally adopted for the regulatory assessment of skin irritation (OECD TG 439) and corrosion (OECD TG 431). Recently, the OECD adopted an Integrated Approach on Testing and Assessment (IATA) for skin corrosion and skin irritation (OECD GD 203), which provides guidance on the integration of existing and new information in a modular approach for classification and labelling. The present study aimed to evaluate the use of the SkinEthic™ RHE model within the proposed OECD IATA. Data on 86 substances were integrated in a bottom-up and top-down testing strategy to assess their capacity for EU CLP and UN GHS classifications. For EU CLP, strategies showed an accuracy of 84.8% to discriminate non-classified from classified substances, 94.4% to discriminate corrosive from non-corrosive substances, and 68.5% to discriminate the four (sub)-categories. For UN GHS, strategies showed an accuracy of 89.5% to discriminate non-classified from classified substances, 93.4% to discriminate corrosive from non-corrosive substances, and 74.2% to discriminate four GHS (sub)-categories (excluding Category 3). In conclusion, the integration of SkinEthic™ RHE irritation and corrosion data in a bottom-up and top-down testing strategy allows the classification of substances according to EU CLP and UN GHS.

1. Introduction

The assessment of the potential for a substance to cause skin corrosion and irritation is mandatory according to current regulatory requirements (OECD Test Guideline (TG) 404, 2002). The classification and labelling of substances has been harmonized worldwide by the adoption of the United Nations Globally Harmonized System (UN GHS) of Classification and Labelling of Chemicals (UN, 2011). Within the European Union (EU), the UN GHS was implemented via the Classification, Labelling and Packaging (EU CLP) regulation (EU, 2008). For skin corrosion and irritation assessment, the EU CLP regulation differentiates between non-irritant or mild irritant substances (Non-classified), irritant substances (Category 2) and corrosive substances (Category 1). For the skin corrosive Category 1 substances, three (sub)-categories (Category 1A, 1B and 1C) have been defined based on skin corrosion potential. The EU CLP regulation does not consider the optional UN GHS Category 3 for the classification of mild irritants, but classifies these substances as non-classified.

Traditionally the *in vivo* Draize skin test has been used for skin corrosion and irritation hazard assessment (Draize et al., 1944). However to improve animal welfare considerable efforts have been undertaken to develop and validate alternative *in vitro* test methods for the replacement of *in vivo* testing. The SkinEthic™ Reconstructed Human Epidermis (RHE) test methods have been formally validated for the assessment of skin irritation and corrosion of substances (ECVAM Scientific Advisory Committee 2006, 2008, 2009). Since the initial validation studies, the protocol for skin corrosion was refined to reduce the tissue surface, decrease applied test substance volume and correct for MTT reducing substances in compliance with OECD and European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM)

Abbreviations: ECVAM, European Center for the Validation of Alternative Methods; ESAC, ECVAM Scientific Advisory Committee; EU, European Union; EU CLP, European Union System of Classification of Chemicals implemented in the EU Classification, Labelling and Packaging Regulation; GD, Guidance Document; I, irritant; IATA, Integrated Approach on Testing and Assessment; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; NC, non-corrosive; NI, non-irritant; OD, optical density; OECD, Organization for Economic Co-operation and Development; RHE, Reconstructed Human Epidermis; SD, standard deviation; SOP, standard operating procedure; TG, Test Guidelines; WoE, weight-of-evidence; UN GHS, United Nations Globally Harmonized System.

* Corresponding author at: L'Oréal Research & Innovation, 1 Avenue Eugène Schueller, 93600 Aulnay-sous-bois, France.
E-mail address: nalepee@rd.loreal.com (N. Alépée).

http://dx.doi.org/10.1016/j.tiv.2015.07.012
0887-2333/

requirements (Tornier et al., 2010a,b). Because the validated test methods were shown to meet the performance and reproducibility standards defined in OECD TG 431 and TG 439, they can be used for regulatory skin corrosion and irritation assessment (Kandárová et al., 2006; Alépée et al., 2010; OECD TG 431, 2013a; OECD TG 439, 2013b).

The OECD recently adopted a guidance document on Integrated Approaches to Testing and Assessment (IATA) for skin irritation and corrosion (OECD GD 203, 2014), replacing the testing and evaluation strategy provided in the supplement to OECD TG 404 (OECD, 2002). The document provides guidance on how to integrate existing and new information on the hazard potential of substances in a modular approach for classification and labelling purposes. The proposed modular approach groups the different individual information sources of the IATA in "modules" according to the type of information. Moreover for each module the strengths and limitations as well as the potential role and contribution in the IATA are described with the purpose of minimizing the use of animals to the extent possible, while ensuring human safety.

The IATA for skin corrosion and irritation is composed of three parts, which each have a number of corresponding modules. Part 1 addresses the collection and evaluation of existing data covering human, *in vivo*, and *in vitro* data, physico-chemical properties and non-testing methods (*i.e.* read-across, QSAR and grouping). Under part 2, a weight-of-evidence (WoE) analysis is performed considering all the existing data together for decision making on classification and labelling. If the WoE analysis is inconclusive, new testing needs to be conducted under part 3, starting with *in vitro* test methods. Animal testing in compliance with regulatory requirements must only be considered as a last resort or is prohibited for cosmetics purposes in compliance with the Cosmetics Directive (76/768/EEC). The prospective testing under part 3 should be guided by the hypothesis of the WoE analysis on the skin corrosion and irritation potential of the substance. The bottom-up approach (skin irritation followed by skin corrosion testing in case the substance is irritant in the first test) should be conducted when the substance is hypothesized to be a non-irritant, whereas the top-down approach (skin corrosion followed skin irritation testing in case the substance is non-corrosive in the first test), should be used when the substance is hypothesized to be corrosive or irritant to skin (Fig. 1) as a precautionary approach (OECD GD 203).

The present study was undertaken to evaluate the use of the validated and adopted SkinEthic™ RHE model as one of the modular information sources within the modular approach of the proposed OECD IATA. SkinEthic™ RHE skin irritation and corrosion data on 86 commercially available substances was integrated into either a sequential bottom-up or top-down testing strategy to investigate if there were differences between the two approaches. Statistical data analyses were performed to evaluate the predictive capacity of both testing strategies to classify the 86 test substances according to the EU CLP and UN GHS classification systems (EU, 2008; UN, 2011).

2. Materials and methods

2.1. SkinEthic™ Reconstructed Human Epidermis (RHE) model

The SkinEthic™ Reconstructed Human Epidermis (RHE) model consists in fully differentiated three-dimensional epidermal tissue grown from normal human keratinocytes in a chemically defined medium at the air liquid interface (Rosdy and Clauss, 1990; Rosdy et al., 1993). The model is histologically similar to human epidermis, and features a functional permeability barrier, which is one of the main functions of viable skin. The SkinEthic™ tissues are manufactured according to the ISO9001 quality system (version 2008 certified) and each batch is controlled according to standard quality control criteria (Episkin SA, www.episkin.com).

2.2. Quality control criteria for the SkinEthic™ RHE model

The quality control criteria for the SkinEthic™ RHE model include cell viability for negative controls, barrier function and morphological evaluations for all batches before being released. The quality acceptance criteria for cell viability, quantified using the MTT reduction assay, is determined as OD $\geqslant$0.8. The barrier function is determined according to the property of the stratum corneum to resist the rapid penetration of cytotoxic marker chemicals, which corresponds to the exposure time required to reduce cell viability by 50% (ET-50) upon application of 1% Triton X-100. The quality acceptance criterion for the ET-50 assay is determined as: 4.0 h $\leqslant$ ET-$50_{\text{Triton-X100}}$ $\leqslant$ 10.0 h. The morphological quality control criteria include at least four viable cell layers (basal, suprabasal, spinous, granular), a stratum corneum and absence of significant histological alterations). A batch is qualified only if all quality control criteria are met.

2.3. Test substances

The set of 86 commercially available substances originally identified by the OECD Expert Panel on Skin Corrosion and Irritation to evaluate the usefulness of Reconstituted human Epidermis (RhE) models (OECD TG 431) to identify skin corrosive (sub)-categories

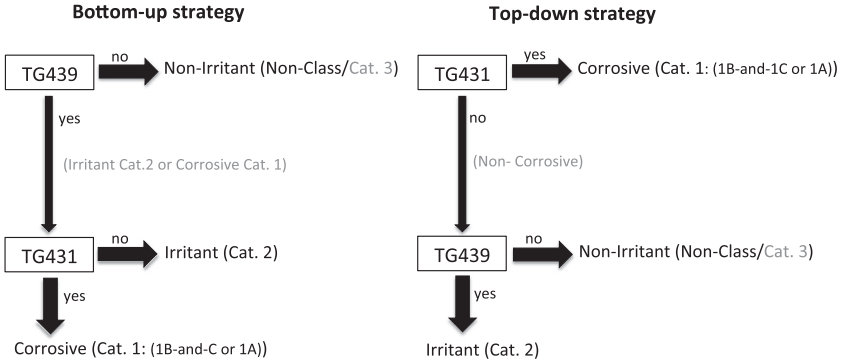

Fig. 1. Overview of the sequential bottom-up and top-down testing strategies based on the OECD IATA Guidance Document 203 for the classification of substances according to their skin corrosion and irritancy potential. The SkinEthic™ RHE skin corrosion method (OECD TG 431) was designed to classify EU CLP and UN GHS (Sub)-Categories: non-classified (Non-Class), skin corrosive Category 1B-and-1C (1B-and-1C) and Category 1A (1A). The SkinEthic™ RHE skin irritation method (OECD TG 439) was designed to classify EU CLP and UN GHS (Sub)-Categories: non-classified (Non-Class), skin irritant Category 2 (Cat. 2) or skin corrosive category 1. The methods were not designed to classify UN GHS optional Category 3 (Cat. 3) for mild-irritants, which are classified as non-classified in the EU CLP system.