Validation Study of the In Vitro Skin Irritation Test with the LabCyte EPI-MODEL24

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Summary — A validation study on an in vitro skin irritation assay was performed with the reconstructed human epidermis (RhE) LabCyte EPI-MODEL24, developed by Japan Tissue Engineering Co. Ltd (Gamagori, Japan). The protocol that was followed in the current study was an optimised version of the EpiSkin protocol (LabCyte assay). According to the United Nations Globally Harmonised System (UN GHS) of classiﬁcation for assessing the skin irritation potential of a chemical, 12 irritants and 13 non-irritants were validated by a minimum of six laboratories from the Japanese Society for Alternatives to Animal Experiments (JSAAE) skin irritation assay validation study management team (VMT). The 25 chemicals were listed in the European Centre for the Validation of Alternative Methods (ECVAM) performance standards. The reconstructed tissues were exposed to the chemicals for 15 minutes and incubated for 42 hours in fresh culture medium. Subsequently, the level of interleukin-1 alpha (IL-1α) present in the conditioned medium was measured, and tissue viability was assessed by using the MTT assay. The results of the MTT assay obtained with the LabCyte EPI-MODEL24 (LabCyte MTT assay) demonstrated high within-laboratory and between-laboratory reproducibility, as well as high accuracy for use as a stand-alone assay to distinguish skin irritants from non-irritants. In addition, the IL-1α release measurements in the LabCyte assay were clearly unnecessary for the success of this model in the classiﬁcation of chemicals for skin irritation potential.

Key words: in vitro, interleukin-1 alpha (IL-1α), MTT, reconstructed human epidermis, skin irritation, validation.

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Introduction

Since 1946, the Draize rabbit test for skin irritation has been widely used to evaluate the skin irritation potential of xenobiotics (1, 2). However, the relevance to humans of the data provided by the test is limited by species differences, so a signiﬁcant number of alternative testing methods have been developed to date, including the use of in vitro tissue constructs based on human keratinocytes (3, 4). These constructs closely resemble human epidermis with respect to biochemical proﬁle (e.g. lipid composition), tissue architecture (e.g. cell layering and formation of a stratum corneum), and the presence of a functional skin barrier.

Three commercially available test methods based on reconstructed human epidermis (RhE) have been validated by the European Centre for the Validation of Alternative Methods (ECVAM; 5–7) as being suitable for determining the potential hazardous (i.e. skin irritant) properties of xenobiotics. These methods are also in compliance with the new United Nations Globally Harmonised System (UN GHS) rules for the classiﬁcation and labelling of substances, implemented in the EU through regulations on the Classiﬁcation, Labelling and Packaging of Substances and Mixtures. In December 2008, the EU adopted a new classiﬁcation system based on the UN GHS system for Classiﬁcation and Labelling (8), but which continues to use two categories to distinguish non-irritant (No Category) chemicals from irritant (Category 2) chemicals. According to the new UN GHS rules for the classiﬁcation and labelling of skin irritants, the cut-off in vivo score to distinguish between No Category and Category