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Systematic evaluation of non-animal test methods for skin sensitisation safety assessment

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ABSTRACT

The need for non-animal data to assess skin sensitisation properties of substances, especially cosmetics ingredients, has spawned the development of many in vitro methods. As it is widely believed that no single method can provide a solution, the Cosmetics Europe Skin Tolerance Task Force has defined a three-phase framework for the development of a non-animal testing strategy for skin sensitisation

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potency prediction. The results of the first phase – systematic evaluation of 16 test methods – are presented here. This evaluation involved generation of data on a common set of ten substances in all methods and systematic collation of information including the level of standardisation, existing test data, potential for throughput, transferability and accessibility in cooperation with the test method developers. A workshop was held with the test method developers to review the outcome of this evaluation and to discuss the results. The evaluation informed the prioritisation of test methods for the next phase of the non-animal testing strategy development framework. Ultimately, the testing strategy – combined with bioavailability and skin metabolism data and exposure consideration – is envisaged to allow establishment of a data integration approach for skin sensitisation safety assessment of cosmetic ingredients.

1. Introduction

The mechanism behind skin sensitisation and the elicitation of Allergic Contact Dermatitis (ACD) has been investigated for many years and is documented by the OECD as an Adverse Outcome Pathway (AOP) (OECD, 2012). The skin sensitisation AOP captures the impact of skin exposure to sensitising chemicals as a series of biological and chemical key events, which have been reviewed extensively, e.g. by Ainscough et al. (2013), Kimber et al. (2012), Martin et al. (2011), and Toebak et al. (2009). In brief, as a prerequisite, the chemical sensitizer needs to penetrate the stratum corneum as the uppermost layer of the skin in order to become available to the viable cells of the epidermis. It binds covalently to skin proteins of the viable cells (key event 1) to form hapten-protein conjugates, which can be immunogenic. In parallel, keratinocytes become activated and release danger signals e.g. pro-inflammatory cytokines as a response to trauma (key event 2). Next, the phenotype of dendritic cells (DC) changes by the concerted recognition of hapten-protein conjugates by MHC (major histocompatibility complex) molecules and of danger signals (key event 3). The activated DCs mobilise and migrate, after maturational changes, from the skin to the draining lymph node to present the allergen to T cells. After binding to a hapten-peptide specific T cell this clone will expand (key event 4) to elicit the eventual adverse outcome in case of a second exposure with the chemical sensitiser. This level of mechanistic understanding has enabled the development of a multitude of non-animal test methods that each aim to measure the impact of substances on one or more of the AOP key events and therefore to distinguish sensitisers from non-sensitisers or to generate potency information (reviewed previously in Adler et al. (2011)). The complexity of the underlying biology has resulted in the hypothesis that no single measurement will be sufficient to predict sensitiser potency alone (Jowsey et al., 2006). Consequently efforts to apply data from these non-animal test methods for hazard characterisation or risk assessment have focussed upon integration of multiple data types (for example, MacKay et al., 2013; Jaworska et al., 2011; Bauch et al., 2012; Nukada et al., 2012; Natsch et al., 2013). Whilst these approaches continue to show promise, the majority have focused upon integrating non-animal data to predict sensitiser potential. Consequently, one major objective of the Cosmetics Europe Skin Tolerance Task Force has been to identify and evaluate test methods that could allow sensitiser potency prediction without the need for new animal test data, which is of vital importance for the cosmetics industry (Maxwell et al., 2011). This evaluation will inform the development of a non-animal testing strategy for skin sensitisation potency predictions. The resulting strategy will ultimately become an essential part – along with consideration of exposure and other information such as bioavailability or metabolism – of a data integration approach for the skin sensitisation safety assessment of cosmetic ingredients.

Here we document the first of three phases to develop such a non-animal testing strategy. Sixteen test methods were identified for systematic evaluation, following a review of the available scientific literature. The aim of this evaluation was to gain comparable detailed understanding of the test methods that would allow promising methods to be prioritised for further in-depth evaluation. Therefore, a common set of criteria was assessed involving test method characterisation and standardisation. Such criteria included AOP mapping, ease of transferability, availability and throughput, performance (in terms of reproducibility and predictivity) as well as legal aspects and information. The information was assembled for each test method in collaboration with the developers. In addition, we have compiled data on a set of ten substances for each of the methods to verify publically available data in terms of both sensitiser potential and potency prediction. The resulting analysis forms a comprehensive review of the results obtained, which informed the selection of test methods for the next evaluation phases. Finally, we present our future framework set-up for the development of a non-animal testing strategy for skin sensitisation potency predictions – a data and knowledge gap identified by a previous review of non-animal risk assessment approaches for skin sensitisation (Goebel et al., 2012).

2. Material and methods

2.1. Description of test methods

The following section provides an overview of the 16 test methods, which were analysed during the first phase of the Cosmetics Europe method evaluation process. They are presented according to their alignment to the skin sensitisation AOP (Fig. 1). The description, which covers the status at the beginning of 2013, comprises the test system, read-out parameter, prediction model, and whether the method provides only hazard identification or also includes potency prediction. Finally, the experimental conditions are summarised (including the applied dose range) as this may indicate whether the data obtained have the potential to add information to hazard characterisation beyond the currently used prediction model. As detailed information about each of the test methods is already available in the scientific literature, this is not covered here. The laboratories in which the methods have been developed are indicated and key references are included for further reading.

2.1.1. Protein reactivity test methods

Skin sensitisers show a high diversity in terms of chemical and physiochemical properties. However the AOP considers, chemicals – or in case of pre-/pro-haptens, their respective metabolites – which act as sensitisers due to their ability to react with skin proteins (haptenation). This common characteristic is used in a number of non-animal test methods to differentiate between sensitisers and non-sensitisers. Two *in chemico* assays focus on peptide reactivity using two model peptides as surrogates for cellular proteins. In addition, three cell line assays use the kelch-like ECH-associated protein 1 (Keap1) as an intracellular sensor to