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Guiding principles for the implementation of non-animal safety assessment approaches for cosmetics: Skin sensitisation

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ABSTRACT

Characterisation of skin sensitisation potential is a key endpoint for the safety assessment of cosmetic ingredients especially when significant dermal exposure to an ingredient is expected. At present the mouse local lymph node assay (LLNA) remains the 'gold standard' test method for this purpose however non-animal test methods are under development that aim to replace the need for new animal test data. COLIPA (the European Cosmetics Association) funds an extensive programme of skin sensitisation research, method development and method evaluation and helped coordinate the early evaluation of the three test methods currently undergoing pre-validation. In May 2010, a COLIPA scientific meeting was held to analyse to what extent skin sensitisation safety assessments for cosmetic ingredients can be made in the absence of animal data. In order to propose guiding principles for the application and further development of non-animal safety assessment strategies it was evaluated how and when non-animal test methods, predictions based on physico-chemical properties (including *in silico* tools), threshold concepts and weight-of-evidence based hazard characterisation could be used to enable safety decisions. Generation and assessment of potency information from alternative

Abbreviations: ACD, allergic contact dermatitis; AEL, acceptable exposure level; APC, antigen presenting cells; CEL, consumer exposure level; CIR, cosmetic ingredient review; COLIPA, the European Cosmetics Association; DC, dendritic cells; DPRA, direct peptide reactivity assay; DST, dermal sensitisation threshold; EC3, effective concentration of the test substance required to produce a three-fold increase in the stimulation index compared to vehicle-treated controls; h-CLAT, human cell line activation test; HERA, human and environmental risk assessment; HMT, human maximisation test; HPV, high production volume; HRIPT, human repeated insult patch test; KC, keratinocytes; LC, Langerhans cells; LLNA, local lymph node assay; LogPow, octanol–water partition coefficient; MUSST, myeloid U937 skin sensitisation test; NESIL, no expected sensitisation induction level; NS, non-sensitisers; OECD, organisation for economic co-operation and development; QRA, quantitative risk assessment; (Q)SAR, (quantitative) structure activity relationship; S, sensitisers; SAF, sensitisation assessment factor; SCCS, scientific committee for consumer safety; SMARTS, SMiles ARbitrary Target Specificaton; TG, test guideline; TSC, threshold of sensitisation concern; TTC, threshold of toxicological concern; WoE, weight-of-evidence.

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tools which at present is predominantly derived from the LLNA is considered the future key research area.

1. Introduction

A standard requirement within the safety assessment of cosmetic ingredients is to characterise their potential to induce skin sensitisation under product use conditions that may lead to allergic contact dermatitis (ACD) in humans. Despite extensive efforts to develop alternative methods, the sensitising potential of an ingredient currently needs to be identified on the basis of animal studies in many cases, i.e., the murine local lymph node assay (LLNA, OECD (organisation for economic co-operation and development) TG 429) (Basketter et al., 2007; OECD, 2010) and Guinea pig assays (OECD TG 406) (OECD, 1992), namely the Maximisation Test (Magnusson and Kligman, 1969) and the Buehler test (Buehler, 1965).

The LLNA is based on quantification of cell proliferation in the draining auricular lymph nodes after repeated topical applications of the chemical. By testing multiple concentrations, the assay not only identifies potential skin sensitisers, but also evaluates their sensitising potency. Guinea pig tests are based on a visual scoring of skin reactions after topical application (Buehler test) or intradermal and topical application (Maximisation test) of the chemical at a dose which induces modest irritation. Approximately three weeks later the potential of a chemical to elicit an immune response is analysed by virtue of a challenge exposure.

Immunologically, skin sensitisation can be described as a delayed-type hypersensitivity reaction induced by low molecular weight reactive chemicals (haptens). It comprises two phases, induction and elicitation (Karlberg et al., 2008). Fig. 1 schematically depicts the corresponding steps (1–7) as described in the following: as a first step in the induction phase the (possibly oxidised) chemical must penetrate the skin (steps 1 and 2) to chemically react with endogenous proteins (step 5). Some chemicals require activation through enzymatic (pro-haptens, step 4) or oxidative (pre-haptens, step 1) processes in order to become haptens capable of binding to skin proteins (step 5). The first cells to be exposed to sensitisers are epidermal keratinocytes, which respond to chemical stress with a cocktail of proinflammatory cytokines (step 3) (Corsini et al., 2009). Activated by these mediators as well as in some cases by direct hapten contact, epidermal Langerhans cells (LC) and immature dendritic cells (DC) take up and process haptenated proteins. In parallel they mature into highly effective antigen presenting cells (APC) (Toebak et al., 2009). This maturation includes the secretion of mediators like IL-8, as well as the expression of surface markers such as CD86, CD54, or chemokine receptors (step 6) (Kroeze et al., 2009). The latter facilitate the migration of LC out of the epidermis and guide them to the nearest (local) lymph node where they present haptenated protein fragments (antigens) to T cells (step 7) (Ortmann et al., 1992). This step is the link between the innate and the adaptive immune systems, i.e., recognition of the antigen by specific T cell receptors and subsequent specific T cell activation. The activated (effector) T cells home to the skin where upon repeated contact with the same allergen (elicitation phase) they orchestrate an inflammatory response that can lead to dermal injury. Hence, they are representing the immunological "memory" responsible for the specificity of the ACD (Martin and Weltzien, 1994).

COLIPA's skin sensitisation research programme aims to further refine our fundamental understanding of how each of these key pathways contribute to the induction of skin sensitisation and develop *in vitro* test methods that can predict the effect of a novel chemical on each of these key pathways (Aeby et al., 2010) the hypothesis being that integration of data from a 'toolbox' of non-animal test methods, each developed to model a different key pathway *in vitro*, will allow the precise characterisation of a chemical regarding its skin sensitising potency. Which (set) of these tools will turn out to provide an appropriate prediction is not yet determined and is under investigation. On May 26–27, 2010 COLIPA organised an expert workshop to propose how *in vitro* test methods and combinations thereof may be applied to risk assessment decision-making, in combination with other non-animal risk assessment elements.

2. Review of existing tools for evaluation of skin sensitisation risk assessment without animals

Risk assessment of cosmetic ingredients is not a standardised procedure, but a case-by-case consideration using best science. Usually a stepwise approach is employed, utilising the entire scope of information available to reach science-based decisions in a weight-of-evidence (WoE) assessment. WoE is considered as the basic principle to avoid unnecessary animal testing, since all relevant existing information is thoroughly evaluated before any new testing is undertaken, and it is iteratively applied in each step of the assessment. An expert assessment of the relevance, i.e., scientific validity or suitability of the purpose of a method or approach needs to be performed to decide how to weight individual pieces of information. New testing may be required when the existing information is not adequate to support the safety of an ingredient or when safety issues arise. In general, information can be qualitative (used for hazard identification) and/or quantitative (used for hazard characterisation and risk assessment).

WoE is broadly accepted by legislation and safety assessors as a basic principle in risk assessment and is explicitly mentioned in European chemicals and cosmetics legislation, e.g., in the recast of the European Cosmetics Directive (EU, 2009), REACH (EU, 2006), and the regulation on classification, labelling and packaging ("CLP") of substances and mixtures (EU, 2008).

Elements that are available for skin sensitisation risk assessment to gain information that can be used within WoE-based safety assessment include:

(1) ***Prediction based on physico-chemical properties*** (without experimental testing; expert judgment/*in silico*): presence or absence of ***structural alerts*** ((quantitative) structure activity relationships = (Q)SAR), indications for chemical reactivity with nucleophiles, mechanistic assignment to reactivity domains including computer-based searches for structural and functional similarities in data bases such as DEREK, TIMES, MULTICASE, OECD Toolbox, ToxTree etc.
(2) ***Read-across based on similar chemicals with available experimental data***: this is usually done by making use of WoE expert judgement, potentially assisted by *in silico* tools such as OECD toolbox, Toxtree etc.
(3) ***In vitro methods***: includes binding capacity towards proteins; responses of human cell types, i.e., primary keratinocytes (KC), dendritic cells (DC), and T cells or relevant immortalised cell lines in terms of bio-markers, cytokine secretion or gene expression (gene signature).
(4) ***Historical data***: (i) **animal studies** reliably reporting on skin sensitisation effects. (ii) **human experience** with exposure to substances and preparations regarding cutaneous