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Predicting skin sensitisation using a decision tree integrated testing strategy with an *in silico* model and *in chemico/in vitro* assays

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ABSTRACT

There is a pressing need for non-animal methods to predict skin sensitisation potential and a number of *in chemico* and *in vitro* assays have been designed with this in mind. However, some compounds can fall outside the applicability domain of these *in chemico/in vitro* assays and may not be predicted accurately.

Rule-based *in silico* models such as Derek Nexus are expert-derived from animal and/or human data and the mechanism-based alert domain can take a number of factors into account (e.g. abiotic/biotic activation). Therefore, Derek Nexus may be able to predict for compounds outside the applicability domain of *in chemico/in vitro* assays.

To this end, an integrated testing strategy (ITS) decision tree using Derek Nexus and a maximum of two assays (from DPRA, KeratinoSens, LuSens, h-CLAT and U-SENS) was developed. Generally, the decision tree improved upon other ITS evaluated in this study with positive and negative predictivity calculated as 86% and 81%, respectively. Our results demonstrate that an ITS using an *in silico* model such as Derek Nexus with a maximum of two *in chemico/in vitro* assays can predict the sensitising potential of a number of chemicals, including those outside the applicability domain of existing non-animal assays.

1. Introduction

Skin sensitisation is an important toxicological endpoint which leads to allergic contact dermatitis (ACD). ACD develops in two stages, beginning with the induction stage where a chemical (known as a hapten) forms a conjugate with nucleophilic skin proteins. This initiates a cascade, resulting in proliferation of allergen specific T-cells. The second stage, elicitation, arises when the subject is re-challenged with the same allergen (hapten). The hapten-protein complex is formed again, triggering the allergen specific T-cells to induce the release of inflammatory cytokines which leads to ACD (Kimber et al., 2002).

The prediction of skin sensitisation potential is an important requirement for a number of chemical safety assessments. This is outlined in the EURL ECVAM (European Union Reference Laboratory for alternatives to animal testing) Strategy for Replacement of Animal Testing for Skin Sensitisation Hazard Identification and Classification (European Commission, 2013a) and includes the European Union regulation Registration, Evaluation, Authorisation and restriction of Chemicals (REACH), Classification Labelling and Packaging of substances and mixtures (CLP) Regulation, and risk assessments associated with occupational exposure. Furthermore, there is considerable economic and social pressure to replace, reduce and refine the use of animal tests for the safety assessments of chemicals across a wide range of industries (Russell and Burch, 1959).

The cosmetics industry faces a particular challenge, driven by the implementation of EU Regulation 1223/2009 (replacing EU Directive 76/768/EEC) which came into effect in March 2009 (European Union, 2013). This regulation ensures that any cosmetic

Abbreviations: ACD, allergic contact dermatitis; AOP, adverse outcome pathway; CLP, classification labelling and packaging; DC, dendritic cells; DPRA, direct peptide reactivity assay; DX, Derek Nexus; EURL ECVAM, European Union Reference Laboratory for alternatives to animal testing; GPMT, guinea pig maximisation test; h-CLAT, human cell line activation test; IATA, integrated approach to testing and assessment; ITS, integrated testing strategy/strategies; KS, KeratinoSens; LC-MS, liquid chromatography–mass spectrometry; Lhasa DT, Lhasa decision tree; LLNA, murine local lymph node assay; MIE, molecular initiating event; U-SENS, myeloid U937 skin sensitisation test; OECD, Organisation for Economic Co-operation and Development.

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product or ingredient on the EU market is demonstrably safe for use but stipulates that any animal experiments must be replaced by alternative methods by March 2013. However, the current 'gold standard' regulatory accepted test methods to predict skin sensitisation are *in vivo* assays, namely the local lymph node assay (LLNA) and guinea pig maximisation test (GPMT).

An adverse outcome pathway (AOP) is the sequence of events leading from the molecular initiating event to an *in vivo* adverse outcome. The AOP for skin sensitisation caused by covalent binding of a test chemical to skin proteins has been published by the Organisation for Economic Co-operation and Development (OECD) and has been summarised as eleven steps of which four are considered key (OECD, 2012). There has been a concerted effort in recent years to develop new non-animal based assays which address these key events in the AOP such as the DPRA (Direct Peptide Reactivity Test), KeratinoSens, LuSens, h-CLAT (human Cell Line Activation Test) and U-SENS (myeloid U937 skin sensitisation test) (Fig. 1).

1.1. Integrated Testing Strategies (ITS)

It is generally accepted that no single *in chemico/in vitro* assay will be an appropriate replacement for an animal-based assay such as LLNA or GPMT. However, by using combinations of assays, it is thought that they may act in a complementary fashion and improve predictive performance. Assays can be combined using integrated testing strategies (ITS) which combine results from individual assays and/or use molecular descriptors to derive an overall assessment of hazard or risk. These can be used within integrated approaches to testing and assessment (IATA) as described by EURL ECVAM (European Commission, 2013a).

A diverse range of ITS have been investigated, for example, artificial neural networks and Bayesian networks (Hirota et al., 2015; Jaworska et al., 2013; Rorije et al., 2013; Tsujita-Inoue et al., 2015, 2014), weight of evidence (Ellison et al., 2010; Gubbels-van Hal et al., 2005; Natsch et al., 2013; Urbisch et al., 2015) or score-based test batteries (Jowsey et al., 2006; Natsch et al., 2008; Nukada et al., 2013; Takenouchi et al., 2015), decision trees (Bauch et al., 2012; van der Veen et al., 2014) and miscellaneous ITS such as gated logic algorithms, global regression analysis and machine learning (Luechtefeld et al., 2015; McKim et al., 2010; Natsch et al., 2014). Nevertheless there is still substantial interest in this area of research, and this paper will investigate ITS which utilise *in chemico/in vitro* assays alongside *in silico* models.

1.2. Limitations of in chemico/in vitro ITS

There are a number of limitations associated with *in chemico/in vitro* assays and ITS:

1.2.1. Adverse Outcome Pathway (AOP)

The skin sensitisation AOP published by the OECD is based on an electrophilic compound undergoing a covalent interaction with a skin protein, initiating a cascade of other biological events, leading to the adverse outcome of skin sensitisation (Fig. 1). The *in chemico/in vitro* assays available are designed to assess key events in this AOP only – test chemicals which undergo alternative AOPs and mechanisms of action to cause skin sensitisation may not be predicted well by these assays (e.g. metal salts). Furthermore, the AOP states that reactions with thiol and amino nucleophiles are well-characterised but less is known about reactions with other nucleophiles (OECD, 2012).

1.2.2. Applicability domain and limitations of in chemico/in vitro assays

The applicability domain of each assay is still being defined but a number of limitations are known.

(1) DPRA – This assay simulates the molecular initiating event (MIE) of the skin sensitisation AOP by measuring the reactivity of the test substance towards synthetic model peptides containing either lysine or cysteine. The sensitisation potential of a test chemical is evaluated by measuring the mean cysteine and lysine peptide depletion and assigning a class based on percentage of this depletion. One described drawback when using model peptides is that the true skin conditions are not reproduced and test chemicals with affinities for several other amino acid residues may not be adequately evaluated by the DPRA (Divkovic et al., 2005). Furthermore, a number of false positives have been reported due to test chemicals which induce cysteine oxidation (European Commission, 2013b; OECD, 2015a) resulting in a misleading quantity of peptide depletion. Similarly, LC-MS (liquid chromatography–mass spectrometry) analysis indicates that aldehydes do not form

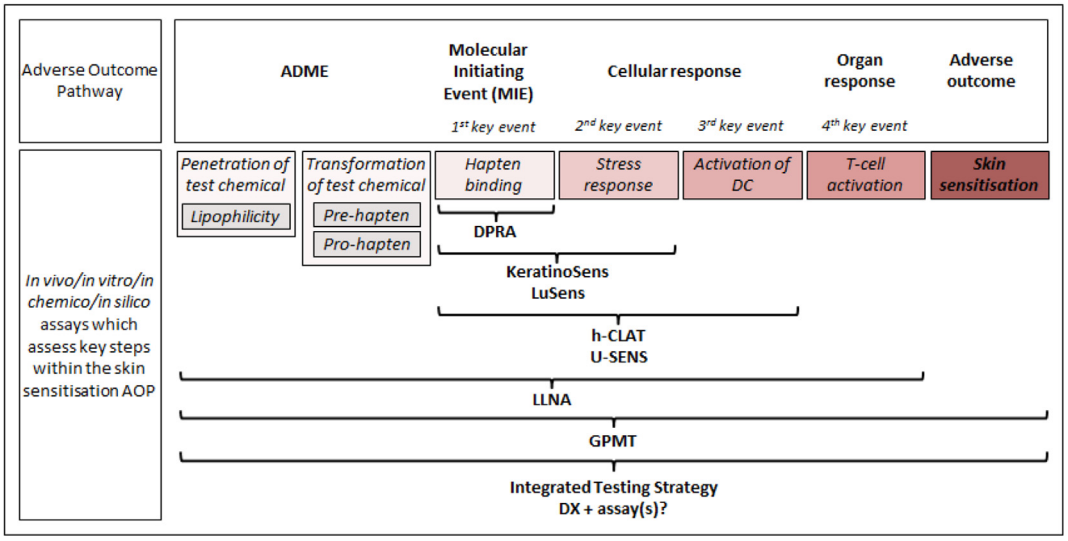

Fig. 1. Adverse outcome pathway for skin sensitisation and step/key events measured by the *in vivo*/*in chemico*/*in vitro*/*in silico* assay(s) shown. Adapted from OECD 2012.