

# Assuring Consumer Safety Without Animal Testing: A Feasibility Case Study for Skin Sensitisation

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**Summary** — Allergic Contact Dermatitis (ACD; chemical-induced skin sensitisation) represents a key consumer safety endpoint for the cosmetics industry. At present, animal tests (predominantly the mouse Local Lymph Node Assay) are used to generate skin sensitisation hazard data for use in consumer safety risk assessments. An animal testing ban on chemicals to be used in cosmetics will come into effect in the European Union (EU) from March 2009. This animal testing ban is also linked to an EU marketing ban on products containing any ingredients that have been subsequently tested in animals, from March 2009 or March 2013, depending on the toxicological endpoint of concern. Consequently, the testing of cosmetic ingredients in animals for their potential to induce skin sensitisation will be subject to an EU marketing ban, from March 2013 onwards. Our conceptual framework and strategy to deliver a non-animal approach to consumer safety risk assessment can be summarised as an evaluation of new technologies (e.g. 'omics', informatics), leading to the development of new non-animal (*in silico* and *in vitro*) predictive models for the generation and interpretation of new forms of hazard characterisation data, followed by the development of new risk assessment approaches to integrate these new forms of data and information in the context of human exposure. Following the principles of the conceptual framework, we have been investigating existing and developing new technologies, models and approaches, in order to explore the feasibility of delivering consumer safety risk assessment decisions in the absence of new animal data. We present here our progress in implementing this conceptual framework, with the skin sensitisation endpoint used as a case study.

**Key words:** allergic contact dermatitis, alternatives, animal testing, consumer safety, cosmetic testing, genomics, *in silico*, *in vitro*, proteomics, risk assessment, skin allergy, skin sensitisation, Three Rs.

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## Introduction

Consumer products such as soaps, shampoos, body lotions, antiperspirants and toothpaste (all classed as 'cosmetics' in the EU) are used globally by millions of consumers, every day. Decisions about the consumer safety of these products are made on the basis of a risk assessment (RA), in which data on the potential hazards of the ingredients are interpreted in the context of the likely exposure to the product (i.e. the concentrations of the ingredients in the product and how the product is then used by consumers). An animal testing ban on chemicals to be used in cosmetics will come into effect in the EU from March 2009, linked to an EU marketing ban on products containing any ingredients that have been subsequently tested in animals for acute toxicity, skin irritation, eye irritation, and mutagenicity (1). For various repeat-dose toxicity tests, including skin sensitisation, the EU marketing ban is due to come into effect in March 2013 (1). Given that much of the hazard data on chemicals used to inform consumer safety RAs have been generated by using animal models, the challenge to the cos-

metics industry is to continue to ensure consumer safety without the generation of new animal data.

Even without this impending EU legislation, there is a growing vision within the field of toxicology (2–4), that the application of new science and technology from medical and biological research could provide opportunities to move toward a more accurate prediction of potential health effects in man. Today, improved non-animal *in vitro* and *in silico* models are becoming increasingly available, and new 'omic' technologies and systems biology approaches enable us to generate and interpret new types of non-animal data. The term 'systems biology' has been used in a variety of contexts, but can broadly be defined as an investigation of whole biological systems through the application of mathematical modelling and/or bioinformatics analysis to large-scale datasets (5). Such approaches may be crucial for assessing repeat-dose endpoints, where there is a growing consensus that an array of different models will be required (2, 6, 7). Consequently, the need to identify new approaches for the interpretation and integration of these different forms of data is increasingly acknowledged.

A conceptual framework and strategy to deliver a non-animal approach to consumer safety risk assessment was proposed by Fentem *et al.* in 2004 (8). The aims of the conceptual framework and strategy to deliver a new non-animal approach to consumer safety RA, can be summarised as follows:

- 1) Develop *new RA approaches*;
- 2) Develop and apply *new models* for predicting adverse effects;
- 3) Evaluate the usefulness of data for risk assessment by applying *new technologies*;
- 4) Maximise the use of both *new and existing data*.

The novelty of this approach was the assertion that recent increases in our understanding of the cellular and molecular processes that underpin disease states (e.g. ACD), combined with advances in tissue culture/engineering, new analytical technologies (e.g. 'omic' approaches) and information technology platforms for handling these data, may now allow toxicology to move beyond the use of animal models for predicting effects in humans. Other publications have described similar approaches, most notably the recent publication of the NRC Toxicity Testing Vision (2). We have discussed elsewhere how these approaches complement and differ from our own (3, 4). Therefore, our focus for this publication is our ongoing feasibility case study, whereby the conceptual approach has been applied to the development of a new skin sensitisation risk assessment approach. It should be noted that we have not attempted to document all the historical or current *in vitro* toxicology approaches to the determination of the skin sensitisation endpoint, as this has been extensively reviewed elsewhere (9–12). However, as the scope of our case study was based on the knowledge of several ongoing research programmes in this field (e.g. the European Cosmetics, Toiletries and Perfumery Association [COLIPA] Skin Tolerance project team [13] and the Sens-it-iv project [an EU Framework VI integrated project to develop non-animal methods to predict skin and respiratory sensitisation potential]; 14), we have sought to highlight and acknowledge these activities to ensure that the feasibility of a non-animal RA approach to the prediction of skin sensitisation can be fully discussed.

## The Current Risk Assessment Approach

ACD is an eczematous skin reaction, which results from a specific, delayed-type hypersensitivity response to a small molecule (chemical) allergen (15). To ensure that ingredients in our products do

not induce skin sensitisation (and hence do not cause ACD in consumers), our current RA approach uses information on the concentration of the ingredient in the product, and on how the product is used by consumers, together with hazard and potency data, to assess the risk to humans and thereby inform the safety decision (16, 17). If we consider the hazard/potency information that can be generated to inform our current skin sensitisation RA approach, two broad categories exist: a) structure–activity evaluations (e.g. [Quantitative] Structure–Activity Relationships [(Q)SARs]), and b) the predictive animal tests (e.g. the mouse Local Lymph Node Assay [LLNA]; 18). Once generated, the dose–response and predicted potency from the LLNA for the material in question can be interpreted by using a Quantitative Risk Assessment (QRA) approach. QRA approaches for skin sensitisation have been reviewed elsewhere (16, 17), so only a brief description will be given here. In the QRA approach uncertainty factors (UF) or safety assessment factors (SAF) are applied to an actual or predicted Human Repeat Insult Patch Test (HRIPT) No Observed Adverse Effect Level (NOAEL), or No Effect Sensitisation Level (NESIL), as it is termed. Previous analyses (e.g. by Basketter *et al.*; 19) have demonstrated a correlation between the dose–response data obtained in the mouse LLNA with what is known about relative skin sensitiser potency in humans. Thus, in the absence of existing HRIPT data to use in a QRA, a NOAEL in humans can be predicted from a mouse LLNA. Three SAF categories are considered; inter-individual differences, vehicle or product formulation differences and exposure considerations. A default value is applied for each SAF (16, 17). This gives a theoretical safe level of exposure to material or Acceptable Exposure Level (AEL), which is then compared with the predicted consumer exposure to the material in the product. The outcome of the QRA can also be interpreted along with any existing information, such as history of use or clinical data relating to the material in question (or similar benchmark materials), in order to reach an overall risk-based safety decision. Before discussing our non-animal RA approach for skin sensitisation, it is perhaps worthwhile to consider in more detail, the two categories of hazard/potency information that inform our current RA approach.

## Structure–activity evaluations

A number of *in silico* methods exist, which aim to predict skin sensitisation potential from chemical structure alone. These (Q)SAR models/expert systems (e.g. DEREK for Windows®; 20) generate hazard and/or potency information through the identification of motifs or combinations of motifs within the chemical's structure and extrapolation

from historical animal data generated from structurally-similar compounds. Despite the lack of accepted (Q)SARs for human toxicological endpoints, or of detailed guidance on how to develop (Q)SARs for human risk assessment, structure–activity evaluations are used to inform the early phase of our current skin sensitisation RA, without the need to generate new animal data. Consequently, we envisage that similar information will also form part of our non-animal RA approach (see the *Chemical Reactivity* section, below). Several initiatives have recently emerged to increase the acceptance of (Q)SARs. The main principles for the validation of (Q)SARs have been identified, and are now referred to as the ‘OECD principles’ (21). A recently published paper (22) evaluates and compares, with reference to these principles, three different approaches to (Q)SAR development for skin sensitisation, and discusses the weaknesses, strengths and applicability domains of these models.

### The mouse LLNA

The mouse LLNA is a validated and accepted animal method for establishing skin sensitisation potential. The assay is based on the measurement of cellular proliferation in the draining auricular lymph nodes (LNs), five days after the first of three daily applications of the chemical to each ear of the mouse (23). The experiment is performed to determine a stimulation index (the extent of chemical-induced cell proliferation observed in the LNs, relative to a vehicle control) for a range of chemical doses, to establish a dose–response relationship. This relationship can then be used to determine the concentration of applied chemical necessary to provoke a stimulation index of three (commonly referred to as the ‘effect concentration’, or EC3). Based on this EC3 value, chemicals can then be assigned to skin sensitisation potency classes, from ‘weak’ to ‘extreme’ (24, 25).

As mentioned above, as a repeat-dose toxicity test, the LLNA will be impacted by the EU marketing ban due to come into effect in March 2013 (1). Consequently, the two key questions moving forward are: *What experimental data do we require to inform our new non-animal RA approach for the prediction of skin sensitisation?*; and, *How might our non-animal RA approach need to evolve to accommodate these new types of data?* To answer these questions, we separated the components of our new RA approach, as shown in Figure 1.

## The New RA Approach

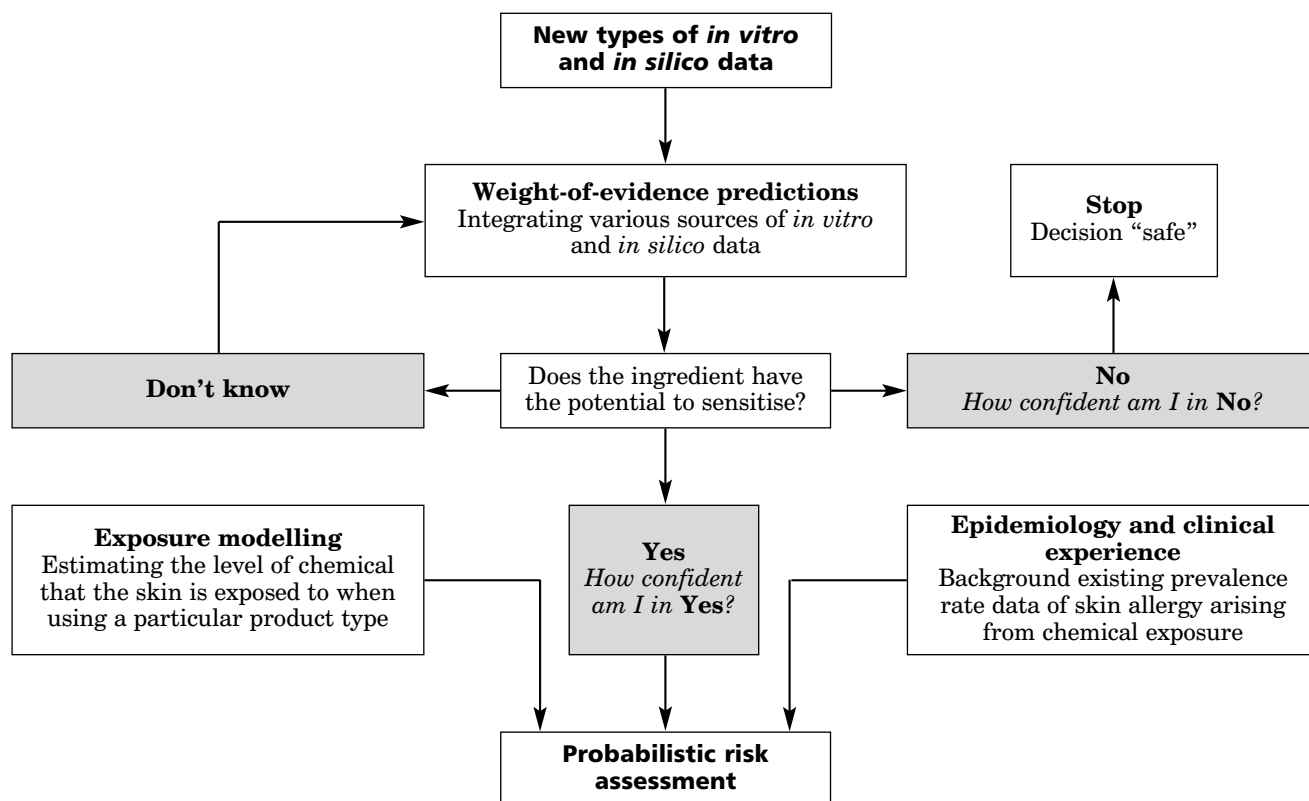
### 1) Consumer exposure information

It is recognised that not all consumers will use

products in the same ways, with respect to how much they use them, how frequently they use them, and even the mechanics of how they use them (e.g. how long a rinse-off product is left on), so this uncertainty has to be factored into the RA. The way that a product will be delivered will also vary, depending on the applicator (novel or otherwise) and the product concept. Exposure information for various product types has been generated to inform RAs, for example those which have been commissioned by industry groups such as COLIPA (26–29) and the Personal Care Products Council (PCPC; 30).

Moving forward, we aim to improve our current estimates of skin exposure to topically-applied chemicals through the application of probabilistic modelling approaches, such as the Monte Carlo techniques. These methods take into account the entire distribution of exposures in the RA process, rather than just a deterministic point estimate. This permits the refinement of our RAs, and makes the sources of uncertainty more explicit. In addition, as we make the transition from using hazard data generated in skin sensitisation animal models (where dermal exposure is intrinsic) to the adoption of *in vitro* predictive methods, it is foreseen that new types of exposure information will be required. For example, information on the local concentrations of chemical within the skin and an understanding of chemical flux through the stratum corneum, epidermis and dermis of the skin, will be valuable in establishing a relationship between the predicted exposure in man and the concentration at which an effect was observed *in vitro* (12).

A new experimental approach based on *ex vivo* human skin has recently been investigated, whereby parameters, such as skin compartmental concentrations and delivery kinetics (flux) of chemicals, can be measured (31). In addition, ongoing work funded by COLIPA aims to derive similar predictions of skin compartment-specific bioavailability through the *in silico*, mathematical modelling of chemistry parameter data sets (13). However, the true bioavailability of free chemical in the skin tissue is also influenced by skin metabolism, tissue adsorption and clearance mechanisms. A recent literature review (32) has revealed a lack of fundamental knowledge about human skin metabolism, and another COLIPA-sponsored study aims to address this knowledge gap (13, 33). It is also foreseen that quantitative data on relative skin bioavailability will be required for accurate estimation of absolute tissue doses for the purposes of RA. In addition, it must be recognised that the incorporation of metabolising systems (e.g. cytochrome P450 cocktails; 34) within any *in vitro* predictive assay, will potentially represent a significant technical challenge.

**Figure 1: A new risk assessment framework for skin allergy**

A simplified version of the RA framework defined for skin allergy. The major differences to the traditional approach are: the incorporation of new data inputs (in vitro and in silico information rather than data derived from animal studies), and the approaches being applied in modelling and integrating the data (see text).

## 2) Data interpretation and integration

Ultimately, our objective is to develop tools and approaches for integrating data of diverse types, in order to facilitate their interpretation for consumer safety RA. We are currently assessing various proposed methods to interpret and subsequently integrate non-animal hazard data, to help inform future consumer safety decisions. Jowsey *et al.* (7) initiated a discussion on a weight-of-evidence approach to the integration of several key processes known to be important mechanistically in the induction of skin sensitisation. The approach is based on scoring and integrating the outputs from a series of different non-animal hazard methods (Structural Alert, Bioavailability, Protein Binding, Dendritic Cell [DC] Maturation and T-cell Proliferation) to generate a new measure of skin sensitisation potency (7). This is a pragmatic starting point for discussion — however, it is likely that statistical approaches will be required to model and interpret assay results in a more mathematically-robust way, due to the increasing complexity of some datasets. Machine

learning and statistical approaches can be used to better identify relationships in the data, and to incorporate expert opinion and prior knowledge. Where sufficient information also exists about the underlying biological response, mechanistic modelling approaches could provide significant new opportunities for integrating and interpreting non-animal data in an overall biological context.

To test and explore the relative contributions of individual biological pathways thought to be key to the induction of skin sensitisation, we recently developed an *in silico* mathematical model (termed the Skin Sensitisation PhysioLab® [SSP] platform; 35), in collaboration with Entelos, Inc. The aim of this project was to map the biological pathways that have been consistently reported in the literature as being important in the induction of skin sensitisation to chemical sensitisers, and in so doing, to provide a systematic approach to the identification of key pathways and knowledge gaps. Our experience during the modelling exercise was that this approach provided new biological insights through combining previously-disparate data sets and drawing parallels from

other disease processes. For example, data from pathogen infection studies were used to implement a LN cell recruitment effect in the model (36, 37). Although this mechanism has not been reported as being required for the induction of skin sensitisation, the circumstantial evidence, revealed through modelling, was sufficient to justify its inclusion (35).

In addition, the sensitivity analyses performed on the SSP platform have increased our understanding of the relative contribution of individual pathways to the skin sensitisation response (7). This information has been used to focus some of our fundamental research studies and to evaluate the relative predictive power of different *in vitro* methods. We also envisage that, in the future, the SSP platform will aid in the development of a biological rationale for the categorisation and weighting of different forms of non-animal hazard information. For example, results from *in vitro* predictive methods that determine rates of flux in the skin (31) could be integrated with those measuring metabolic rates (32), in order to determine the epidermal concentration of the toxicant over time. Similar approaches have been used in Physiologically-Based Pharmacokinetic (PBPK) models (38), in order to determine systemic *absorption–distribution–metabolism–excretion* (ADME) properties. Exposure aside, modelling approaches (e.g. the SSP platform) could also be of use in making the *in vitro* to *in vivo* extrapolation, by allowing the results of *in vitro* predictive methods to be interpreted in the context of *in vivo* human biology.

## New Models and New Technologies

To determine whether a chemical has the potential to induce skin sensitisation, non-animal predictive models are being developed to encompass the events which are considered to be key to the induction of skin sensitisation. Jowsey *et al.* (7) hypothesised that no single non-animal approach could be envisaged to generate sensitiser potency information, and proposed that multiple forms of non-animal data (integrated via a weight-of-evidence approach) would be required for this purpose. Based on this hypothesis and our evaluation of the published skin sensitisation literature (by using the SSP platform), we submit that the integration of some or all of the following categories of non-animal information, in the context of human exposure, should yield a new measure of skin sensitiser potency: Chemical Reactivity; Peptide Reactivity; Epidermal Disposition/Tissue Bioavailability; Epidermal Inflammation; DC activation; T-cell proliferation. Examples of our current research, and related research being carried out by others into the development and application of non-animal predictive methods, are outlined in this section.

## Chemical reactivity

### a) *In silico* predictive models of chemical reactivity

The ability of a chemical to sensitise humans or experimental animals upon dermal exposure, is intrinsic in the properties of the chemical itself. Molecular properties in relation to chemical reactivity (in its broadest sense), e.g. oxidation potential, free radical generation, lipid and aqueous solubilities, solid/liquid state properties, volatility, and  $pK_a$ , can all be measured experimentally, or, in some cases, predicted computationally with varying degrees of confidence. Traditionally, such chemistry parameters (either measured or computationally-derived) have been used in (Q)SARs to predict toxicological endpoint effects (i.e. by using historical animal data as the basis for these predictions). It is foreseen that chemistry-based information can be more widely used in our risk assessment framework (e.g. in the context of exposure, by determining how the chemical behaves in a formulation, what chemical species the skin is exposed to prior to entry into the tissue, etc.), and in the context of defining the mechanism of action, such that chemicals can be categorised as 'similar'. This can involve applying quantitative indices of electrophilic reactivity with nucleophiles (e.g. kinetic data), together with hydrophobicity (39). Aptula *et al.* (40) illustrate the types of quantitative mechanistic models that could be built to substantiate 'mechanism of action-based similarity', by using databases of kinetic measures of reactivity and hydrophobicity, together with the exploitation of existing *in vivo* bioassay data. *In silico* models for predicting chemical reactivity parameters can also be built by using empirical measurements of chemical–nucleophile reactivity, performed within appropriate mechanistic domains (39).

### b) Peptide reactivity

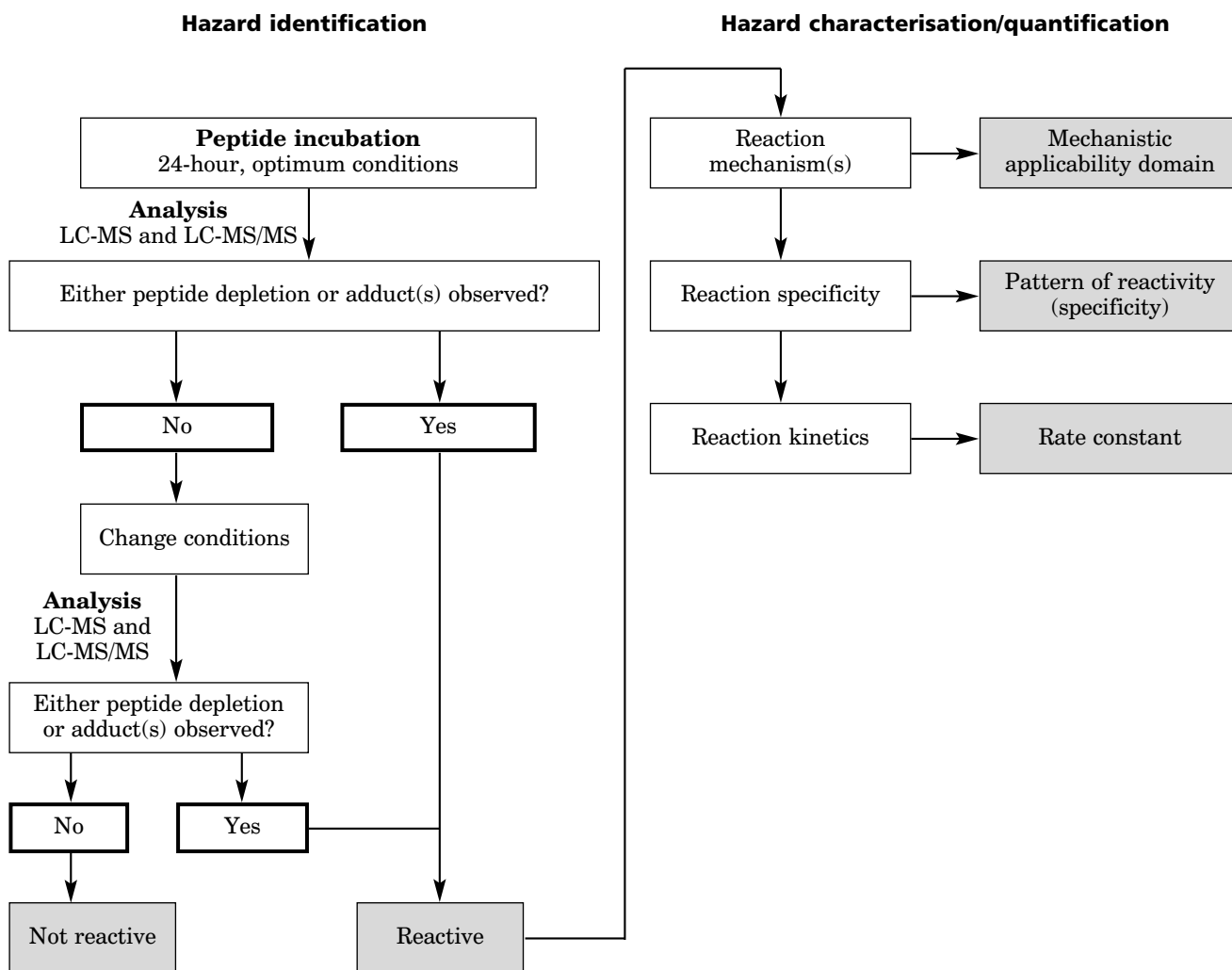
It is generally understood that any chemical (or metabolite derived from it) must form a stable (covalent) adduct with protein in the skin, in order to stimulate an immune response (41–43). Consequently, the covalent modification of a protein by a reactive chemical (haptentation) is considered to be a key step in the induction of skin sensitisation. Several *in chemico* assays for measuring the extent and nature of chemical reactions with model peptides are being developed, underpinned by this hypothesis (13, 44–46). Some of the recently developed assays are based on measuring the remaining unchanged peptide, following incubation with a test chemical (44, 47), or by observing the formation of a covalent adduct (46), or a combination of both (45).

For the purposes of deriving the maximum qualitative and quantitative information on the reactiv-

ity of a chemical with peptides for a non-animal skin allergy RA framework, we have developed an *in chemico* peptide reactivity profiling assay, which uses a panel of six single-nucleophile peptides (generic sequence AcFAAXAA, where X = Cys, Lys, Tyr, His or Arg, with H<sub>2</sub>N-FAAAAA representing the N-terminal nucleophile), with the aim of determining the reactivity profile of a chemical with a high level of confidence. In principle, the reactivity profiling in this assay is similar to that in previously-published methods (44–47), including the Direct Peptide Reactivity Assay (44) currently under evaluation within COLIPA. Each peptide is incubated with the test chemical under conditions optimal for peptide reactivity (conditions which are not necessarily physiological). The main differences are the number of nucleophiles studied, and the

addition of the adduct observation as a necessary step to distinguish a reactive chemical from non-reactive chemical. This is particularly useful in confirming whether the chemical reacted, when the peptide depletion values are less than 10%. In addition to measurements of unchanged peptide and observation of the formed adduct(s), this approach permits the determination of reaction mechanisms (40), specificity, and relative rates of reactions (Figure 2). We have tested up to 20 sensitising chemicals by using this comprehensive approach, and continue to refine the experimental procedure with regard to chemical and peptide solubility, and optimal chromatographic set-up and mechanistic adduct analysis (which are often custom-made). The tested chemicals show varied patterns of reactivity with six peptides, and exhibit specific reaction

**Figure 2: Peptide reactivity strategies**



*A strategy for the qualitative and quantitative determination of reactivity of chemicals to peptide nucleophiles, to generate data for a non-animal skin allergy RA framework.*

mechanisms which are not always theoretically foreseen (Aleksic *et al.*, manuscript in preparation).

From our current understanding, approximately a third of sensitising chemicals are not directly reactive and require activation (via interaction with the environment [e.g. air oxidation] or metabolism within the epidermis). Consequently, we are exploring the inclusion of an appropriate activating system to the peptide reactivity profiling assay. *In chemico* screening for peptide reactivity with selected nucleophiles may represent a possible route to predictive testing, particularly in combination with outputs from other relevant assays, but the immunogenic relevance of detected modifications *in vivo* is not known. The identification of the human skin protein targets of sensitising chemicals and their immunogenic potentials is the next logical step in the fundamental insight into pathophysiologically-relevant covalent protein modification, in relation to the induction of skin sensitisation.

## Immune cell activation

### *a) Epidermal inflammation*

Keratinocytes are the predominant epidermal cell type within the skin. They are known to produce large quantities of inflammatory cytokines, which serve to alert Langerhans cells (LCs; immature dendritic cells found within the epidermis) to the presence of a pathogenic infection or physical/chemical stressor (48). Consistent with this pivotal role in modulating the extent of LC activation, keratinocyte activation is currently being evaluated (as a model of epidermal inflammation), to further determine whether it can provide additional predictive information to use in the RA framework. Although no keratinocyte-based assays are currently under development specifically for the prediction of skin sensitisation potential, reconstituted skin equivalents (composed of human epidermal keratinocytes, seeded on synthetic matrices) have recently been validated by the European Centre for the Validation of Alternative Methods (ECVAM) for the prediction of skin irritation (i.e. the EpiDerm™ [MatTek, USA; 49] and Episkin™ [Episkin, France; 50] models), and several research activities to develop keratinocyte:DC co-culture models are currently under way within the Sens-it-iv Project (14).

Although not necessarily related directly to skin sensitisation due to the chemical selected, we have recently generated clinical skin inflammation datasets by using the well-characterised irritant sodium dodecyl sulphate (SDS) to evaluate the potential of the 'omic' technologies to generate datasets for use within consumer safety RAs. A moderate inflammatory response was induced on

the forearm, and skin biopsies were collected for transcriptomic (whole human genome microarrays), proteomic (Tandem Mass Spectroscopy by using ITRAQ™ peptide labelling), and immunohistochemical analyses; in addition, interstitial fluid was also collected for proteomic, metabolomic (gas chromatography-mass spectrometry), and cytokine array (Luminex multiplex arrays) analyses. These studies generated very large transcriptomic data sets that are currently being analysed by using a number of bioinformatic tools, including the Cytoscape software platform (51) in collaboration with the University of California, San Diego, CA, USA (52). Already, this network analysis approach appears to have been successful in identifying a number of novel gene sub-networks and pathways, suggesting there may be value in applying these new informatics tools and 'omic' technologies to other toxicological endpoints.

### *b) DC activation*

Independent of their interactions with keratinocytes, the phenotypic changes that LCs/DCs undergo upon direct sensitiser exposure have been extensively studied over the last 20 years, and form the basis for several DC-based predictive methods (11, 13, 53). Two DC-activation assays (based on human monocyte-like cell line models) are currently under evaluation within COLIPA (13). Both these test systems — the human Cell Line Activation Test (h-CLAT; 54–56) and the Myeloid U937 Skin Sensitisation Test (MUSST) (57) — are based upon the measurement of relative changes in cell surface receptor expression (CD54 and CD86 in the h-CLAT test, and CD86 in the MUSST). Although both of these methods appear promising, the molecular mechanisms of cell activation are currently unknown. Consequently, we have focused our activities on gaining further understanding of the mechanisms of chemical sensitiser-induced DC activation. For example, a series of studies were performed to investigate the effect of DC treatment with Toll-like receptor ligands (TLRL; pathogenic determinants that stimulate the innate immune system) prior to, or during, chemical sensitiser exposure, to explore whether these molecules could synergistically boost DC receptor or cytokine expression (58). Interestingly, the results demonstrated that chemical-induced responses (to both sensitisers and non-sensitisers) could be synergistically augmented by stimulating the Toll-like receptor pathways. However, rather than acting as an inflammatory adjuvant, the results suggested specificity, with different sensitisers requiring different TLRLs to synergistically boost DC activation. Clearly, the results of this research study and others ongoing within COLIPA (reviewed in Aeby *et al.*; 13) and the Sens-it-iv Framework VI integrated

project (14), suggest that different sensitiser may activate DCs in different ways. Consequently, in moving forward, additional endpoints (e.g. cytokine secretion; 57) may need to be added to existing DC activation methods, in order to broaden their chemical applicability domains (13).

### c) T-cell proliferation

Naïve T-cell proliferation in response to chemical treatment is a robust indicator that a substance is immunogenic, and several publications are concerned with demonstrating the experimental feasibility of inducing a naïve T-cell proliferation *in vitro* following co-culture with chemical sensitizer-treated DCs or LCs (59–61). However, the sensitivity of this approach has not been demonstrated to date, as significant proliferative responses have generally only been detectable following stimulation with sensitiser of strong potency. From our own experience, the complexity of DC:T-cell co-culture protocols make these approaches both labour intensive and difficult to standardise. Furthermore, our SSP platform analysis of draining LN cell dynamics has highlighted the importance of LN trafficking in driving the sensitizer-specific proliferative response (35), i.e. a dynamic system is needed. Such trafficking will be difficult to reproduce *in vitro*, due to the static nature of the standard DC:T-cell co-culture methods. Therefore, we are keen to investigate the potential for novel tissue engineering technologies to replace these traditional culture systems (e.g. the artificial lymph node model; 62).

## Maximising the Use of Clinical Experience and Existing Animal Data

In addition to its use within structure-based read across approaches (see the *Structure-activity evaluation* and *Chemical reactivity* sections), historical animal data have also recently been analysed to characterise and delineate the underlying biological responses embodied within these animal models. An example of this kind of data analysis was an investigation of the relationship between skin sensitizer potency and skin irritation by using historical LLNA EC3 values and the irritancy profiles of these same materials as determined in Guinea-Pig Maximisation Test (GPMT) dose range-finder studies (63). A weak but significant correlation was identified between these two parameters, thereby supporting the hypothesis that capturing the ability of a compound to induce skin irritation could be of potential value as a category within our non-animal hazard data set (see *New Models and New Technologies*).

In addition to historical animal data, risk-based safety decisions for skin sensitisation are increas-

ingly made in the context of an understanding of the epidemiology/incidence of allergy to well-characterised sensitiser which have historically been used as cosmetic ingredients. An example of such an analysis is the proactive surveillance of the antimicrobial agent, polyhexamethylene biguanide (PHMB), which is used in a number of exposure situations, including medical disinfectants and swimming pools, as well as in cosmetics and personal care products. In collaboration with the IVDK (Information Network of Departments of Dermatology), approximately 2000 patients were patch-tested over a 6-month period. It was established that the frequency of sensitisation to PHMB was low, at around 0.5%. Importantly, it was concluded that exposure via cosmetics or personal care products was unlikely to have played a role in the incidences of sensitisation that were identified, and that the major causal link was due to occupational exposure to PHMB (64, 65). In the future, such clinical data should continue to be generated (i.e. for well-characterised sensitiser which are currently in use as cosmetic ingredients), to confirm that our RA approach to the prediction of skin sensitisation is sufficiently protective for consumers.

## Discussion and Future Perspective

Assuring consumer safety without animal testing represents a formidable challenge. During the last 10 years, *in vitro* replacement methods have been adopted for several consumer safety endpoints (i.e. skin corrosion, phototoxicity, dermal absorption, and skin irritation). The development of these methods was primarily driven by the need to identify hazard, with the end result that the animal method is replaced with one non-animal method (66). However, for repeat-dose endpoints, the expectation that full replacement can be achieved through the development of a single non-animal method is now widely believed to be unrealistic (2, 6, 7). Consequently, it is now increasingly accepted that integrated testing strategies involving different types of non-animal hazard information and more-detailed exposure data, will be required, in order to derive non-animal RA approaches for these endpoints. However, the adoption of this hypothesis poses some fundamental questions. For example: *What types of information are required for the RA?*; *How should this information be integrated and/or weighted?*; *How can (indeed, can) the in vitro and in silico datasets be integrated to obtain an estimation of hazard potency?*; and, *What are we trying to predict and how can we do this?*

If we consider the first question, *What types of information are required for the RA?*, we have discussed here the different types of data that we propose could be integrated within our new skin sensitisation approach. We have outlined the fol-

lowing broad categories: consumer exposure; chemical reactivity; immune cell activation; and existing *in vivo* data. Jowsey *et al.* (7) have discussed the second question posed above (*How should this information be integrated and/or weighted?*) — however, the applicability of the weight-of-evidence scoring scheme proposed was largely restricted to integrating two of the categories we have discussed (chemical reactivity and immune cell activation). The rationale for the selection and weighting of the inputs discussed by Jowsey *et al.* (7) was largely based on the knowledge and understanding of the induction of skin sensitisation that has been derived from mouse models. The SSP platform confirmed that this selection of inputs was largely relevant. However, this approach was also based upon the available published literature, and, as such, drew predominantly on *in vivo* mouse data (35). Given that our incomplete understanding of the biological mechanisms driving skin sensitisation in humans, stems predominantly from the inherent difficulties of performing such experiments *in vivo* or *ex vivo*, we are exploring the feasibility of new models (e.g. tissue engineering approaches; 62) and technologies (e.g. ‘omic’ approaches; 52) to generate these data. However, the question of how to integrate information on consumer exposure and existing *in vivo* (human) data with hazard characterisation information from non-animal methods, still remains unanswered. Furthermore, the challenge of how to establish a measure of skin sensitisation potency from *in vitro* and *in silico* methodologies also exists and brings us to the question, *What are we trying to predict?*

We have discussed here that the key aim is to predict whether a novel chemical has the potential to induce sensitisation in consumers. However, the question beyond that aim is, *What does ‘potency’ information mean in the context of human exposure?* Historical LLNA studies (67) represent the most complete, currently-available data set for benchmarking such sensitiser potency predictions in novel assays. However, we should also consider whether a similar measure of ‘potency’ could be established, based on clinical experience. Historical HRIPT studies have been found to roughly correlate with LLNA studies for the same test compounds (20). Furthermore, studies by Kligman and colleagues in the 1960s, and later studies by Friedmann and co-workers in the 1990s (reviewed in 68), have been central to our understanding of the impact of sensitiser dose and dose/unit area on the induction of skin sensitisation in humans. However, there has been little, if any, information on the impact of the frequency and duration on the development of skin sensitisation, and unexpected results have been observed in humans when exposure parameters have been varied in practice. For example, an extended clinical study was recently performed to establish the effect of delivering

*p*-phenylenediamine (PPD, a well-characterised sensitiser used as a hair dye) via two different exposure scenarios (69). The unanticipated conclusion of this study was that infrequent, longer exposure to a higher concentration of PPD was significantly less likely to induce skin sensitisation, as compared to more-frequent, shorter duration and lower concentration exposures. This example highlights the inherent difficulty of extrapolating data generated in animal models, with fixed frequency and duration parameters, to consumer exposure scenarios. Consequently, in the future, we aim, in collaboration with dermatologists and dermatology research groups, to interrogate our understanding of how varying patterns of sensitiser exposure impact upon the induction of skin sensitisation, to ensure that the output of our non-animal RA for skin sensitisation is grounded in human experience, and that any extrapolation of data from non-animal predictive methods is appropriate.

In summary, since the publication of our conceptual non-animal RA framework (8), there has been significant progress toward assessing the feasibility of assuring consumer safety without the use of animals. With respect to our skin sensitisation feasibility case study, the building blocks of our non-animal RA approach are becoming increasingly well-characterised, and our next challenge is to begin the process of evaluating their relative abilities to inform consumer safety RA decisions.

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