Chapter 6: Local Toxicity: Sensitisation

Introduction

Skin and respiratory sensitisation are considered separately from acute dermal and ocular toxicity, since they are effects that may arise following an initial exposure to a sensitiser (by any route of exposure, and possibly via the systemic circulation), when a susceptible individual is subsequently exposed by way of the dermal or inhalation routes.

Skin sensitisation, resulting in allergic contact dermatitis is an important occupational and product-consumer health problem, leading to high costs to health-care systems. It is therefore essential that chemicals and products of various kinds are evaluated for their skin sensitising potential. Until recently, the conventional tests for the identification of skin sensitisation hazards made use of guinea-pigs. However, with increasing understanding of the complex cellular and molecular events that induce skin sensitisation and elicit allergic contact dermatitis, it is becoming possible to consider alternative approaches for hazard identification and characterisation.

Current research on *in vitro* alternatives is mainly focused on one or more of the following properties of chemicals: inherent or potential protein reactivity; penetration of the *stratum corneum* to reach the viable epidermal cell layers; induction of changes in, or in the responses of, cultured dendritic cells; and allergic stimulation of T lymphocyte activation.

The current status of these alternative approaches is reviewed below, and a stepwise testing strategy for the assessment of skin sensitisation potential is proposed.

Current Status of Alternative Methods for Skin Sensitisation

The murine local lymph node assay (LLNA)

The LLNA is an *in vivo* mouse assay for skin sensitisation, which provides a reduction and refinement alternative to the conventional guinea-pig methods — the guinea-pig maximisation test (GPMT) and the Buehler test. The LLNA also provides a more-rapid, more-quantitative and more-objective output, and uses only about half the number of animals required in the standard OECD protocols for the GPMT and the Buehler test. Furthermore, it does not require the use of Freund's complete adjuvant, intradermal injections of test substance, fur removal, occlusive dressings, the use of restraint, or the elicitation of an allergic skin reaction, all of which are features of the GPMT or the Buehler test.

The LLNA has undergone formal validation in the USA and in Europe (1), and has been endorsed as scientifically valid by the ECVAM Scientific Advisory Committee (2). It forms the basis of draft OECD Test Guideline (TG) 429 (3), which will be considered by the OECD Council in 2002. In principle, the assay evaluates the extent to which a chemical stimulates the proliferation of lymphocytes in lymph nodes draining the site of application of the chemical. A chemical is regarded as a skin sensitiser if it induces a stimulation of proliferation which is more than three times that found in concurrent vehicle-treated controls (4).

Knowledge-based computer systems

Relationships between the structure and biological properties of chemicals can be programmed into knowledge-based expert systems. One such expert system is DEREK (Deductive Estimation of Risk from Existing Knowledge; 5, 6), which is under ongoing development by LHASA Ltd (School of Chemistry, University of Leeds, UK). DEREK covers a variety of toxicological endpoints (for example, mutagenicity, carcinogenicity and skin sensitisation), and is in widespread use in the chemical industry. Other expert system approaches to the prediction of skin sensitisation include the TOPKAT (Toxicity Prediction by Computer-assisted Technology; 7) and CASE (Computer Automated Structure Evaluation; 8) systems.

DEREK embodies both a controlling programme and a chemical rulebase (see also Chapter 9). The chemical rulebase consists of descriptions of molecular substructures called "structural alerts", which correlate with specific toxicological endpoints. The user communicates with DEREK by drawing the two-dimensional structure of the chemical under investigation on the computer screen. The rulebase is then searched against that structure, and any structural alert is highlighted, together with a message indicating the nature of the toxicological hazard.

The original skin sensitisation rulebase contained around 40 rules (9), which were derived from a historical database (10) containing data from guinea-pig maximisation tests on 135 chemicals that had been classified as skin sensitisers according to EU criteria. As a result of development of the system over the last seven years (see for example, 11), the number of structural alerts for skin sensitisation currently stands at 59.

The following two-step strategy for the assessment of skin sensitisation potential is appropriate when using the DEREK rulebase.

1. The chemical is processed through the rulebase, to see if it has the potential to react with skin proteins either directly or, in some cases, after metabolism. If no structural alert is triggered, either the chemical does not possess the requisite reactivity, or its reactivity is outside the scope of the current knowledge base. In many cases, the absence of chemical reactivity can be confirmed by inspection of the chemical structure. For chemicals that do not possess the appropriate chemical reactivity, no further computational evaluation is performed. However, an evaluation of possible metabolic activation of the compound is considered by expert input.

2. For chemicals or their metabolites that do possess the appropriate chemical reactivity, the second step is to assess their skin permeability/partition parameters. This initially involves using either empirical or calculated values for computation of the logarithmic octanol–water partition coefficient (log P), and/or for the theoretical prediction of the logarithmic permeability coefficient (log K_p; 12). Molecular weight and melting point values are also used in the prediction algorithms to calculate log K_p. K_p values can also be measured by using validated *in vitro* skin-penetration models. Using *in vitro* models also allows for the visualisation, as well as the quantification, of partitioning of compounds within the skin sub-structures.

In vitro tests

At present, no *in vitro* test for skin sensitisation has been validated, although several systems are in the course of development, based on an improved understanding of the biochemical and immunological mechanisms underlying the process. Among the key steps to be considered are protein binding, metabolism, and the cellular/immunological events leading to sensitisation. Cell-based assays investigated to date have included the use of human blood-derived dendritic cell cultures, Langerhans cells, keratinocyte cultures, human skin equivalents, and dendritic cell/T cell co-cultures (for example, 13, 14). These systems have been shown to express various mediators and/or markers of activation following exposure to chemical sensitisers, although not always with a reliable outcome (15).

A human reconstructed epidermis model, containing keratinocytes, melanocytes and Langerhans cells (16), is undergoing evaluation at L'Oréal (Rainer Schmidt, personal communication). Langerhans cells integrated into the reconstructed epidermis exhibit a reactivity to allergens which is similar to that found in the *in vivo* situation. The development of this system was supported by the EU BIOTECH programme (contract BIO 4 CT 960086).

Dendritic cell culture systems (17, 18) represent a promising approach to the provision of a relevant test system. With the current increases in our knowledge of dendritic cell biology, coupled with the application of novel tools such as genomics and proteomics, it should be possible to gain new information on these systems, including a greater understanding of the interactions between a chemical and dendritic cells and other cells within the skin, for future application in *in vitro* test systems.

Possible hurdles in the development of a cell-based *in vitro* assay could be the initial haptenisation or protein binding events in sensitisation. A concerted effort to understand the mechanisms of chemical–protein interactions within the skin would provide invaluable information for the development of relevant *in vitro* test models.

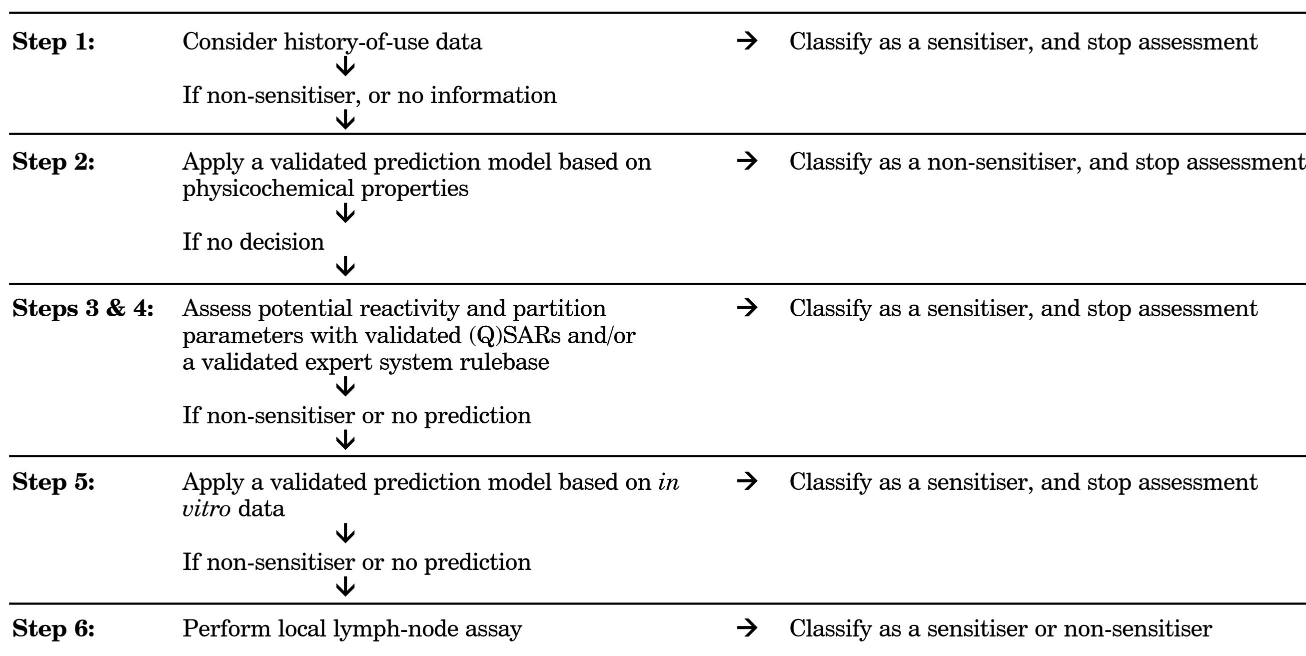
Investigations currently under way are making good progress in both of these promising areas, although much more effort will be required before standardised, robust and reliable *in vitro* tests for chemical sensitisers can be made available.

For the further development of *in vitro* tests for skin sensitisation, it is recommended that: a) basic research into the fundamental mechanisms of skin sensitisation should be continued, to identify opportunities for the development of novel, mechanistically based *in vitro* assays; and b) *in vitro* systems for skin sensitisation should be biologically relevant (i.e. based on our understanding of the key mechanisms underlying sensitisation), while focusing initially on systems that model the initiation, rather than the promotion, of the immune response.

A Tiered Testing Strategy for Skin Sensitisation

The proposal outlined below (Figure 6.1) comprises a stepwise process incorporating: knowledge of physicochemical properties; knowledge of the relationship between chemical structure/reactivity and skin sensitisation potential; use of read-across; and, finally, when necessary, the LLNA, to confirm the absence of a sensitisation potential. Before the stepwise process is conducted for a chemical, an assessment should be made of its corrosivity potential. If the chemical is classified as either R34 (causes burns) or R35 (causes severe burns), skin sensitisation testing for hazard-labelling purposes should only be conducted at non-corrosive concentrations.

Figure 6.1: A tiered testing strategy for skin sensitisation



Step	Action		Outcome
Step 1:	Consider history-of-use data ↓ If non-sensitiser, or no information ↓	→	Classify as a sensitiser, and stop assessment
Step 2:	Apply a validated prediction model based on physicochemical properties ↓ If no decision ↓	→	Classify as a non-sensitiser, and stop assessment
Steps 3 & 4:	Assess potential reactivity and partition parameters with validated (Q)SARs and/or a validated expert system rulebase ↓ If non-sensitiser or no prediction ↓	→	Classify as a sensitiser, and stop assessment
Step 5:	Apply a validated prediction model based on *in vitro* data ↓ If non-sensitiser or no prediction ↓	→	Classify as a sensitiser, and stop assessment
Step 6:	Perform local lymph-node assay	→	Classify as a sensitiser or non-sensitiser

(Q)SAR = (quantitative) structure–activity relationship

Step 1: assessment of historical data

An initial screening of the available data will determine which chemicals already have adequate data relating to sensitisation potential. History-of-use data should also be assessed at this stage.

Step 2: assessment of physicochemical properties

Some chemicals are highly unlikely to possess skin sensitisation potential, due to their physicochemical properties, for example, some inorganic salts (those elements that are sensitisers, such as nickel and chromium, are well-known). Some polymers with very high molecular masses (for example, above 5000) can also be ruled out on the basis of very low bioavailability, provided that they are free of degradation products.

Step 3: screening of structures using the DEREK skin sensitisation rulebase

This process will highlight any structural features of a chemical that indicate a potential to react with skin proteins, either directly or, in some cases, after metabolism in the skin (this is the reactivity component of skin sensitisation). However, DEREK does not possess rules for all possible cutaneous metabolic pathways, and an expert evaluation of likely metabolic activation should be conducted for molecules possessing no chemical structural alerts. Protein-binding assays can also be used to assess the potential to react with skin proteins.

Step 4: assessment of partition parameters

In order to behave as a skin sensitiser, a chemical must be able to partition through the skin and/or into an appropriate compartment for metabolism, and must possess the appropriate reactivity parameter (for covalent binding). Partition parameters can be calculated from physicochemical properties. Comparisons should be made by read-across with other chemicals possessing the same structural alerts, and for which sensitisation data are available. At this point, it may be possible to classify a chemical as a skin sensitiser on the basis of its reactivity potential and partition properties.

Step 5: in vitro *assessment of skin sensitisation*

In vitro studies should be performed for the classification of skin sensitisers, once such systems have been scientifically validated for this purpose.

Step 6: the murine local lymph node assay

In the absence of definite indications of skin sensitisation potential from steps 1 to 5, a murine LLNA should be performed according to OECD TG 429.

Skin Sensitisation: Summary, Conclusions and Recommendations

A number of methods, including QSAR models and the DEREK skin sensitisation rulebase, reconstructed epidermis models, and dendritic cells are available, and could be used for priority setting. In cases where animal testing is necessary, the LLNA should be used in preference to the conventional guinea-pig tests, except for those classes of chemicals for which the LLNA is not considered to be appropriate. In addition to its conventional use for hazard identification, the LLNA can also be used for the determination of relative potency. Further work is needed before *in vitro* systems for skin sensitisation could be used for regulatory purposes (i.e. classification and labelling, and dose–response assessment).

Short-term prospects

1. The acceptance during 2002 by the OECD Council of draft OECD TG 429, which is based on the LLNA.

2. The validation of existing QSARs and/or expert system rulebases for skin sensitisation.

Medium-term prospects

1. The development of systems, based on our current and growing understanding of mechanisms involved in skin sensitisation. Progress is being made in the area of protein binding, with efforts being focused on understanding the mechanisms of chemical–protein binding, which will ultimately yield the information required for the development of a standardised assay.

2. Human reconstructed epidermis models and human dendritic cell cultures represent promising cell-based approaches. Knowledge is growing in this area, and efforts are currently being focused on the identification of relevant cell phenotypes, and existing and novel endpoints for sensitisation.

Long-term prospects

The development and validation of new methods for skin sensitisation. The selection of a range of strong, moderate, weak and non-sensitisers should permit a determination of whether such methods could reliably provide a sufficient distinction between strong, moderate and weak sensitisers.

Recommendation

The DEREK User Group should consider making more information available on the DEREK rulebase for skin sensitisation.

Current Status of Alternative Methods for Respiratory Sensitisation

Certain chemicals are known to cause allergic sensitisation of the respiratory tract resulting in asthma and/or rhinitis. At present, no well-validated or widely accepted methods are available for the identification and characterisation of chemicals that have the potential to cause respiratory sensitisation. Originally, interest focused on the guinea-pig and the elicitation of pulmonary reactions in previously sensitised animals. More recently, alternative approaches involving mice have been described. The first of these is the mouse IgE test, in which respiratory sensitising activity is measured as a function of the ability of a chemical to cause an increase in the total serum concentration of IgE (19). The second approach is cytokine fingerprinting, in which chemicals with the ability to induce allergic sensitisation of the respiratory tract are identified on the basis of cytokine secretion patterns induced following *in vivo* exposure.

The current view is that the LLNA may be of some value in hazard identification. This is because the available evidence indicates that most, if not all, known chemical respiratory allergens elicit positive responses in the LLNA. The value of this is that chemicals which fail to induce positive LLNA responses can be regarded as lacking both contact and respiratory sensitising activity. Verification of this relationship will require further work.

When it is suspected that a chemical which is positive in a predictive test for skin sensitisation, such as the LLNA, might also be a respiratory allergen, further work should be undertaken to characterise the resulting cytokine profile, since chemical contact allergens and chemical respiratory allergens induce divergent pathways of cytokine production (20, 21).

Although there are as yet no well-characterised *in vitro* methods for predicting the potential of chemicals to cause respiratory sensitisation, the use of several systems for research purposes has been reported in the literature, including the use of human lung adenocarcinoma (A549) cells (22) and human bronchial epithelial (BEAS-2B) cells (23).

Respiratory Sensitisation: Summary, Conclusions and Recommendations

Respiratory sensitisation is an important endpoint in the context of occupational exposure to allergenic

chemicals. There is no method for respiratory sensitisation in Annex V of *Directive 67/548/EEC*, and no *in vitro* test is sufficiently well-characterised for prevalidation. Therefore, further research is required, leading to the development of alternative methods in this area, with a view to providing *in vitro* methods for respiratory sensitisation for validation, acceptance and use in the long-term.

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