Preface

This is the 59th report of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM). The main goal of ECVAM, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which have scientific relevance and which reduce, refine or replace the use of laboratory animals. One of the first priorities set by ECVAM was the implementation of procedures that would enable it to become well informed about the state-of-the-art of non-animal test development and validation, and the potential for the possible incorporation of alternative tests into regulatory procedures. It was decided that this would be best achieved by the organisation of ECVAM workshops, each addressing a specific topic, and at which selected groups of independent international experts would review the current status of various types of in vitro tests and their potential uses, and make recommendations about the best ways forward.

A workshop on Skin Sensitisation and Epidermal Disposition was held at ECVAM (Ispra, Italy) on 30–31 January 2006, under the chairmanship of David Basketter. The current status of approaches used to measure the disposition of chemicals in skin compartments was reviewed, with particular emphasis on proposing recommendations on how best to use such information to reduce, refine and/or eliminate the need for animal testing, according to the Three Rs principle. The key focus was the relevance of information on epidermal disposition, and how best to integrate such information into non-animal testing strategies for skin sensitisation.

Definitions

Definitions with respect to dermal absorption slightly diverge in various official documents and in the published literature. The definitions as proposed by the WHO (1), as given here, are used in this report.

The percutaneous/dermal absorption process is a global term which describes the passage of compounds across the skin. This process can be divided into three steps:
— penetration, which is the entry of a substance into a particular layer or structure, such as the entrance of a compound into the stratum corneum;

— permeation, which is the penetration through one layer into another, which is both functionally and structurally different from the first layer; and

— resorption, which is the uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment.

These terms have been defined in the context of the passage of compounds across the skin for the purposes of assessing systemic effects. Here, we define a new term — epidermal disposition — to represent the specific location and quantification of chemicals in the epidermal compartment, which are relevant to local toxicity endpoints, and in this case to skin sensitisation.

**Introduction**

The discussion at the workshop was underpinned by the notion that the effective evaluation of novel *in vitro* data, as derived, for example, from prospective cell-based models for sensitisation hazard prediction (2), would require an understanding of how to relate the *in vivo* dosimetry of sensitisers that penetrate into the viable epidermis via the stratum corneum of human skin (Figure 1) to the concentrations used in *in vitro* applications. This requires the need to estimate both the applied concentration of chemical onto the skin surface for given finite dose exposure conditions, expressed, for example, as µg/cm², and the permeated skin concentrations of the sensitising species (expressed in units such as total number of moles of chemical or as µg/cm²) in the different skin compartments, and to relate these parameters to the concentrations and profiles of the chemical species applied in *in vitro* assays. This will ensure that what is applied to cells *in vitro* is representative of the target dose of chemical in human skin. To estimate the epidermal dispositions of chemicals, knowledge about the kinetic processes that regulate the penetration of chemicals into and out of the different layers of the skin (stratum corneum, epidermis, dermis) is required (Figure 1).

OECD Test Guideline (TG) 428 (3), for *in vitro* skin absorption, provides general rules on how to measure systemically-available concentrations of chemicals that permeate through all layers of the skin and the overall kinetics of skin absorption. This TG, whilst providing extremely valuable approaches for estimating skin absorption for input into general systemic toxicology risk assessments, allows for no discrete evaluations of epidermal disposition (as defined above) to be derived for local skin toxicity endpoints, such as skin sensitisation. New, or substantially adapted, methods are needed in order to generate relevant data, specifically focusing on the epidermis. The early mechanisms of skin sensitisation involve the contact of a chemical entity with keratinocytes and Langerhans cells (LCs), which are located within and throughout the epidermis. It is expected that epidermal disposition data on the sensitising species that interact with keratinocytes and LCs will be a key parameter in assessing the potential for the induction of skin sensitisation, when integrated with other *in vitro* and *in silico* input data (e.g. metabolism, reactivity, and cell-based assay data) in new methods of sensitisation prediction, without using animals.

**The regulatory background**

Council Directive 86/609/EEC of 24 November 1986, on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes, foresees that each Member State should collect, and periodically make publicly available, statistical information on the use of laboratory animals (4). According to the Fourth Report by the European Commission (EC), published in 2002, 10.7 million animals were used in the then-15 European Union (EU) Member States; 51,000 of these animals were used for the assessment of skin sensitisation (5).

This number is expected to substantially increase when the new EU chemicals policy, the REACH (Registration, Evaluation and Authorisation of Chemicals) legislation comes into force. The REACH system aims to improve the protection of human health and the environment, while maintaining the competitiveness of the EU chemical industry and enhancing its innovative capability (6). Within this framework, approximately 30,000 existing chemicals, manufactured or marketed at levels of greater than 1 tonne per year, will have to be assessed for their intrinsic properties, in order to address the currently perceived lack of appropriate safety information. According to a recent estimate provided by the EC’s European Chemicals Bureau (ECB), skin sensitisation hazard assessments will need to be conducted for the highest percentage of phase-in substances (35%; 7). In addition to ethical considerations, it is acknowledged that there is an urgent need to develop effective alternative approaches, either *in vitro* or *in silico*, to allow the provisions of the REACH system to be addressed in an appropriate way. This is congruent with Article 25 (Title III) of the REACH legislation (6), which calls for the use of alternative approaches wherever possible, to avoid unnecessary animal testing: “In order to avoid animal testing, testing on vertebrate
Figure 1: A schematic representation of the structure of human skin (reproduced, with permission, from reference 30)
animals for this Regulation shall be undertaken only as a last resort.”

The development of non-animal alternative test methods is also a requirement of another recent piece of EU legislation, the 7th Amendment to the Cosmetics Directive, which seeks to gradually eliminate animal experiments for the safety toxicity testing of cosmetic ingredients (8). Under the provisions of this legislation, a ban on animal testing on cosmetic ingredients will apply from 2009 onwards, for the majority of human health endpoints. The ban on animal testing for the endpoint of skin sensitisation will apply from 2013. Currently, there are no validated non-animal alternative approaches for skin sensitisation testing.

The Immunobiology of Skin Sensitisation

Skin sensitisation resulting in allergic contact dermatitis, is induced following the topical exposure of an inherently susceptible individual to an amount of chemical allergen sufficient to induce a cutaneous immune response of the required type and vigour. Operationally, in a clinical context, skin sensitisation implies a level of immune priming, such that the subsequent exposure of a sensitised subject to the inducing chemical, at the same or at a different skin site, will result in the elicitation of a contact dermatitis reaction (9).

The successful acquisition of skin sensitisation demands varied and complex cellular–molecular interactions that are tightly regulated in time and space (9, 10). It requires that the chemical is able to gain access across the stratum corneum to the viable epidermis. A variety of important events occur here. A critical requirement is that the chemical allergen forms a stable (usually covalent) association with protein, to form an immunogenic conjugate. This complex, and possibly, in some circumstances, the free chemical, interacts in the epidermis with the resident LCs. Epidermal LCs are part of the larger family of dendritic cells derived from the bone-marrow. Collectively, they are responsible for the presentation of antigens to the immune system and the initiation of primary adaptive immune responses. In the skin, LCs have responsibility for the recognition, internalisation and processing of antigens, including chemical allergens. The activity of these cells is regulated by cutaneous cytokines, and in particular, those elaborated by epidermal cells. Many of these cytokines are induced or up-regulated following encounters with skin sensitising chemicals at skin surfaces.

Some of these cytokines (tumour necrosis factor α [TNF-α], and interleukins [IL] 1β [IL-1β] and IL-18) work in concert to mobilise LCs (including antigen-bearing LCs) at the site of exposure, and to stimulate their migration from the skin, via afferent lymphatics, to regional lymph nodes. This process is also dependent on the availability of chemokines derived from cells already resident in the draining nodes, which draw LCs into the appropriate site (the paracortex), where they come into closest apposition with T lymphocytes. Importantly, during movement from the skin, LCs are subject to a functional maturation, such that, by the time they arrive in regional nodes, they have lost the capacity for antigen processing, and have acquired instead immunostimulatory potential. This means that they are able to present antigen effectively to responsive T lymphocytes (11–14).

The central event in the acquisition of skin sensitisation is the activation of responsive T lymphocytes, which are then induced to divide and differentiate. The division of responsive T-cells results in a selective clonal expansion of allergen-reactive lymphocytes that will respond in an accelerated and more aggressive fashion following subsequent skin encounter with the inducing allergen, such that a clinically discernible cutaneous inflammatory reaction is provoked (15).

Against this background, and in the context of the purpose of this report, it is relevant to consider the events that are necessary for the successful induction of skin sensitisation. A question that is frequently posed is, what are the characteristics that confer on chemicals the ability to cause skin sensitisation? In fact, a more appropriate question is, why is it that all chemicals are not able to induce sensitisation. After all, the purpose of the adaptive immune system is to recognise and respond to non-self, and most chemicals are non-self. An appropriate metaphor is to consider chemicals as having to clear a number of discrete hurdles to reach the finishing line — which, in this case, is the successful induction of skin sensitisation. To overcome these hurdles and induce skin sensitisation, a chemical must:

— gain access to the viable epidermis across the stratum corneum, so that the necessary cellular and molecular interactions can be effected;

— form a stable association with protein such as to create an immunogenic complex; this requires that a chemical is inherently protein-reactive, or can be metabolised locally to produce a protein-reactive species;

— deliver dermal trauma sufficient to induce and up-regulate those epidermal cytokines that are necessary for the mobilisation, migration and maturation of LCs (but, in the absence of such cytokines, LC migration will fail to occur, and the chemical allergen will not reach the regional lymph node in an immunogenic form); and

— deliver a...
— be inherently immunogenic, so that a T lymphocyte response of sufficient magnitude is stimulated.

If these hurdles are not negotiated successfully, skin sensitisation will fail to develop, or at best, will be sub-optimal (16). Therefore, it is clear that the acquisition of skin sensitisation is highly dependent upon complex and coordinated chemical and biological events that culminate in the elicitation of a cutaneous immune response, characterised by the activation and proliferation of T lymphocytes in regional lymph nodes.

**Hazard Identification and Characterisation**

Currently, the method of choice for the prospective identification of skin sensitising chemicals is the mouse local lymph node assay (LLNA), in which activity is measured as a function of the vigour of lymph node cell proliferative induced responses, following the topical exposure of mice to a test chemical. In this method, skin sensitising chemicals are defined as those that provoke a three-fold or greater increase in proliferation, compared with that in vehicle-treated controls. The advantage of this approach is that the chosen endpoint represents a summation of the biological integration of the myriad cellular and molecular events that are known to be required for the initiation of a cutaneous immune response (17).

The other important point is that there is good evidence that T lymphocyte proliferative responses in the draining lymph nodes are causally and quantitatively correlated with the acquisition of skin sensitisation. That is, in the absence of T lymphocyte proliferation, skin sensitisation will fail to develop, and the extent of T lymphocyte proliferation is the main determinant of the extent to which sensitisation will be induced (that is, the degree of skin sensitisation that will be achieved). The quantitative relationship between the clonal expansion of T lymphocytes and the degree of sensitisation acquired, provides the opportunity to use the LLNA, not only for hazard identification, but also for determining relative skin sensitising potency. For this purpose, and for making direct comparisons between chemicals with regard to skin sensitisation potency, an EC3 value is derived from LLNA dose responses. This value represents the **Effective Concentration** of a skin sensitising chemical required to induce a 3-fold increase in the proliferative activity of draining lymph node cells compared with that in concurrent vehicle-treated controls. In recent years, relative potency measured as a function of derived EC3 values has proven to be invaluable in the further refinement of skin sensitisation risk assessment (18, 19).

The difficulty arises when one considers opportunities for the development of alternative approaches for skin sensitisation testing, based on one or more of the events described in the previous section, in that they do not necessarily provide an integrated view of the sensitisation process. Currently, there is interest in a number of alternative approaches that seek — ultimately — to eliminate the need for experiments on animals. The main strategies that are being developed are as follows:

**a)** Exploitation of the relationship between chemistry and skin sensitisation, since reaction chemistry underpins the mechanistic attempts to predict skin sensitisation potential from structural and physical properties, the so-called **(Quantitative) Structure–Activity Relationships (QSARs)**, some of which have become embedded in expert systems (20–22).

**b)** Assessment of the ability of reactive chemicals to modify proteins and ultimately form stable associations with proteins or peptides (23–25). First attempts have been made to measure simple peptide depletion by using biochemical assays (23, 24). Further work is needed to explore how haptenated antigens are formed via protein/peptide modification (25).

**c)** The activation of dendritic cells (DCs) or DC-like cells by exposure to chemicals in vitro; of particular interest are the stimulation of cytokine or chemokine expression and/or of elevated levels of selected plasma membrane determinants (2, 14; 26–29).

The above represent work in progress, and none of these approaches has yet reached the level of sophistication necessary for a thorough evaluation. Progress will continue to be made, however, and one interesting possibility that has recently been explored in some detail, is to consider the integration of information derived from each of the above approaches, in the hope that the collective use of these endpoints will permit a more holistic assessment of overall sensitising potential (30). A key parameter, not included in the above list, but which represents the focus of this report, is whether, and to what extent, a chemical has the ability to gain access to the viable epidermis, across the stratum corneum.

The question we have addressed here is how best we can determine whether a chemical can reach the viable epidermis, and if so, how the level of the chemical within the epidermis can be quantified, and to what extent such information could form a part of an integrated approach to hazard assessment.

In addressing this issue it is important to emphasise two points:
1) It is necessary to distinguish here between methods that are currently available for measuring skin absorption (3), in the context of assessing likely systemic exposure to chemicals, and the issue of the relevance to skin sensitisation of epidermal disposition (via permeation through the stratum corneum). That is, the important issue for skin sensitisation testing is the access of a chemical (either as the parent molecule or as a metabolite) to the viable epidermis, not merely its penetration and absorption across the skin into the systemic circulation.

2) It might be that epidermal concentrations are not sufficient for skin sensitisation to occur and/or are not quantitatively related to the extent to which sensitisation is acquired. If this is, in fact, the case, then the main value of epidermal disposition data may be to exclude from further consideration any chemicals which are unable to reach the epidermis or are unable to reach the epidermis in sufficient amounts.

Factors Affecting Epidermal Disposition

Smith and Hotchkiss (31) identified a number of factors pertaining to a chemical, which can influence the extent and kinetics of chemical absorption across the skin into the systemic circulation. These same factors are expected to influence the epidermal disposition of a chemical and its metabolites:

1) aqueous solubility of the chemical;
2) binding — non-specific adsorptive binding to (e.g. serum) proteins or lipids in the skin;
3) concentration and level of saturation in the volume of vehicle applied;
4) contact time — finite (e.g. 30 minutes then washed off) or infinite exposure;
5) dose (total amount) per unit area (µg/cm²);
6) ionisation, based on the pKₐ of the chemical and the pH of the vehicle;
7) lipophilicity (the optimal logPₒ/w for skin penetration is 2–3);
8) melting point;
9) molecular weight/molecular volume (typically, chemicals with a molecular weight above 800 do not significantly penetrate the intact stratum corneum);
10) metabolism, and rate at which chemical is transformed and cleared from the skin;
11) protein reactivity — to irreversibly bind covalently to proteins;
12) vehicle (some of which can act as penetration enhancers); and
13) volatility of the chemical.

Factors other than the permeating chemical (e.g. skin physiology) will impact epidermal bioavailability, e.g. species differences, anatomical site, age, gender, blood flow, disease/damage to the skin, hair follicle density, hydration, pH, occlusion, and skin temperature.

Skin condition (e.g. degree of hydration, irritation, abrasion, thickness) has a major effect on penetration of the stratum corneum. For example, the hydration effect on stratum corneum penetration is often estimated as a factor of 3–10. The vehicle in which the compound is applied is a second key determinate of penetration into the stratum corneum and absorption across the skin, since it affects both the thermodynamic activity of the chemical and (often) the barrier properties of the skin. This has been most effectively demonstrated for skin sensitisation by structured local lymph node studies on the impact of a range of simple vehicles on the measured potency of a range of allergens (32, 33). However, this work does not show specifically how the vehicle impacts on the local epidermal disposition of material, but rather shows its impact on the overall skin penetration of material, via topical application on the mouse ear and into the systemic circulation.

In reality, most studies in the area of skin absorption have been used to determine the systemic delivery of topically applied materials, and have examined skin penetration rather than epidermal disposition, by assessing the impact of a selection of the key factors mentioned above. Currently, there is no methodology (in vitro or in silico) for assessing the impact of these many potential influencing factors on the epidermal disposition of a chemical. However, as will be discussed later, it is not a trivial exercise to obtain basic epidermal disposition measurements on chemicals at target sites in the skin.

Measuring Epidermal Disposition

In vivo versus in vitro and choice of skin tissue

Human volunteer studies are not considered as a first option for studying either the skin absorption
More importantly, flux over epidermal membranes is not necessarily the most relevant parameter for skin sensitisers, since compounds with a (very) low flux but high residence time in the epidermis, may still cause sensitisation, depending on their site of action and how long they can act at that site. Epidermal membranes are therefore not considered to be the most suitable system for measuring the epidermal bioavailability of potential sensitisers.

In this context, focus will be mainly given to in vitro methods using ex vivo human or pig skin for making epidermal disposition measurements.

**The application of chemicals to the skin**

In aiming to apply measurements of epidermal disposition to understanding skin sensitisation potential, as derived from outcomes of both animal studies and human experience, there may be differences in exposure conditions between in vivo assays and actual human exposure. In the standard animal test for skin sensitisation (36), the preferred vehicle for the application of (industrial) chemicals to the skin is a 4:1 mixture of acetone/olive oil (AOO). The material is left on the skin for the duration of the study, and is applied at a comparatively high dose (1% w/v of neat chemical). Depending on the concentration of the chemical, such an application may approximate an infinite dose. Although this vehicle and regimen is suitable from a practical point of view in this type of study, it should be recognised that AOO, used under these conditions, may considerably alter (and increase) the dynamic properties of substances in relation to their skin permeation, in comparison with actual exposure scenarios in man (37, 38), which may be finite dose (e.g. in the case of rinse-off product types). The high-dose application of chemicals in AOO through the very thin ear skin of the mouse in the LLNA, may also effect rapid skin absorption, with rapid systemic exposure. In contrast, the low levels of chemicals in actual products may permit permeation into the epidermis of human skin in very low amounts and very slowly, thus allowing chemicals their persistence in particular skin compartments. Thus, in designing the experiments to measure epidermal disposition which is relevant to humans, appropriate dosing regimens (infinite dose, finite dose, and suitable choice of vehicle) must be employed, in order to fit the purpose for which the data are to be interpreted and used.

Vehicle and formulation can significantly affect the extent and kinetics of skin penetration and permeation. Whether the chemical is applied as an infinite dose (reaching steady-state kinetics), or a finite dose, can affect its penetration kinetics into the different skin compartments. In skin penetration studies designed for estimating values for input into systemic toxicology risk assessments, the studies

or the epidermal disposition of skin sensitisers, mainly because of the many ethical considerations and protocol complexities that are involved in the testing of compounds with unknown toxicological properties and taking skin biopsies for analysis. Nevertheless, one scenario may be to measure levels of chemicals (and their metabolites) in skin biopsies taken from already sensitised individuals exposed to eliciting doses of the relevant sensitiser, and the use of high sensitivity analytical technologies to determine the profile and concentrations of chemicals found in the different compartments of the skin. Animal studies for such measurements are technically feasible, but the data obtained require species extrapolation. Moreover, it should be taken into consideration that under the REACH system species extrapolation. Moreover, it should be taken into consideration that under the REACH system and the 7th Amendment to the Cosmetic Directive, animal studies are strongly discouraged (6).

For the above-mentioned reasons, the most attractive and ethical approach to estimating epidermal disposition should be the use of new in vitro techniques in combination with in silico modelling approaches. Ex vivo human skin would be the best choice of model for investigative in vitro measurements of the epidermal concentrations of chemicals, though human skin is not always readily available. Skin preparations of controlled thickness (dermatomed) allow for the assessment of gross compound distribution within the skin (total epidermal load). Fresh (viable) human skin is preferred, because of its biotransformation capacity, allowing for the activation of chemicals to produce reactive species (e.g. electrophiles), leading to the formation of hapten(s) and the detoxification of toxins, as would be the case in vivo (34). Since fresh human skin is generally scarce, frozen human skin can also be used for disposition measurements. Inter-individual differences, with respect to the skin barrier and metabolic capacity, should be addressed in a proper study design.

Alternatively, fresh pig skin can be used, since it shares many essential permeation characteristics with human skin, including epidermal thickness, and can be more easily obtained when needed. Skin from other experimental animals is more permeable, and has a different structure from that of human skin. Reconstructed skin models are currently being used for the testing of phototoxicity, corrosivity and irritancy, but the barrier function of these reconstructed human epidermis models is still much less developed than that of native skin (35). Therefore, such artificial models are not considered suitable tools for measuring epidermal disposition of compounds at this time.

Epidermal membranes have been used to measure kinetic flux, which can provide an initial, crude, assessment of the potential of a chemical to reach and leave the viable epidermis. However, it should be noted that tape-stripping is not possible in this system, so no distribution data can be obtained.

In this context, focus will be mainly given to in vitro methods using ex vivo human or pig skin for making epidermal disposition measurements.
are often designed by using a finite dosing regimen, which mimics the actual consumer exposure in the product type to be assessed. Hence, for the purpose of measuring epidermal disposition of sensitisers and ultimately using the data to relate to sensitisation potential as derived from the LLNA (albeit as part of an integrated strategy), it is suggested that applications should be done in both the standard AOO vehicle, as applied in the LLNA, and also in the relevant formulation(s) under relevant infinite or finite dose conditions, to assess whether there are gross differences in epidermal disposition between these two scenarios.

**Study methods for sample generation**

Currently, the existing *in vitro* methodology for assessing skin absorption could, in theory, be used and adapted to generate samples for measuring epidermal disposition in *ex vivo* skin samples. However, the difficulty of performing this task should not be underestimated. The current methodologies for the determination of systemic concentrations of skin-penetrating chemicals have been described extensively elsewhere (3; 39–45). The intralaboratory and interlaboratory reproducibility of standard skin penetration studies is an important aspect for regulatory acceptance, and has been investigated for data coming from human risk assessment after systemic exposure (43–45). However, the design of such adapted methodologies has not been attempted in relation to the specific measurement of epidermal disposition.

In standard *in vitro* skin penetration studies, the test substance (neat or formulated) is applied onto a skin sample placed in a diffusion cell (with a static or flow-through design). The test sample remains in contact with the skin on the donor side for a defined period of time. At the end of the experiment, an absorption profile in the receptor fluid (systemic approach) or in the skin tissue as a whole, is measured. Several factors which affect dermal absorption when using *in vitro* methods have been identified (40). It is not clear what impact these would have, if the experimental methods were adapted for the measurement of epidermal disposition, since these methods were not developed for this specific purpose. Additional techniques (e.g. from the area of skin pharmacology) are needed to accurately measure parameters such as μg/cm² delivered dose to the epidermis, and to relate internal skin dosimetry to *in vitro* cell-based assay dosimetry.

Mass balance data from standard dermal absorption studies using radio-labelled compounds (pesticides, industrial chemicals, cosmetic ingredients), provide crude data on the skin concentrations of the radiochemical at the end of the study (usually as percentage absorbed dose at the 24-hour time-point). It is technically possible to sub-fractionate the skin preparations further into stratum corneum, epidermis and dermis, and over more time-points, by using a more destructive sampling regime. The stratum corneum can be collected by tape stripping, while the epidermis can be peeled from the dermis after heat treatment (by placing the skin preparation on a hot-plate). However, a detailed evaluation of this technique provided the evidence that tape stripping is affected by several variables, such as applied pressure, moisture levels in the skin, and type of strips (46, 47). Although the separation of dermis and epidermis is sometimes used to evaluate the distribution of cosmetics or topical drugs within the skin, no systematic assessment of the reproducibility of this technique has been performed.

In comparison with skin absorption studies aimed at estimating the systemic availability of chemicals, the measurement of epidermal disposition will clearly involve additional labour-intensive and specifically-designed experiments. This is due to the fact that sequential destructive sampling of skin tissue is needed, in order to obtain kinetic information (e.g. over 24 hours) and information on potential tissue bioaccumulation. The determination of distribution within the skin requires the collection of many samples of each skin preparation. The skin is also a notoriously difficult tissue type to analyse and extract chemicals from, due to its heterogenous nature and high fat content. Hence, robust extraction techniques and sensitive analytical methodologies will need to be carefully designed, to ensure that the measurements of chemical epidermal disposition are truly quantitative, as well as qualitative.

**Analytical methods for identifying and quantifying chemicals in skin**

Appropriate analytical techniques will be necessary in the future, for the assessment of epidermal disposition. Some analytical methods (e.g. high performance liquid chromatography [HPLC], gas chromatography [GC], mass spectrometry [MS], etc.) require the prior extraction of the chemical from the skin, but other methods (although semi-quantitative) can be used to detect a chemical *in situ* (e.g. confocal microscopy, Raman spectroscopy). The extraction efficiencies into a range of suitable solvents should be demonstrated for the quantification, if chemicals are to be analysed from skin extracts, rather than *in situ*. When high sensitivity or high specificity are needed, radiolabelling of the test substance (with high specific activity) may be useful; alternatively, LC–MS is increasingly being used, with and without radiochemical labelling. There are several examples of skin absorption studies which employed these analytical
Methods (e.g. 43, 44, 48), including the measurements of metabolites (e.g. 34), but neither the identification nor the quantification of reactive chemical species or hapten–carrier complexes in the epidermis has ever been performed, as far as we know. Methods aimed at the visualisation of penetrating compounds, such as confocal microscopy, Raman spectroscopy or (immuno)histology, are generally semi-quantitative, and are, at present, less useful for providing a measure of epidermal disposition. However, these methods may be of use for determining the cellular distribution of chemicals. The validity and sensitivity of using different potential extraction and analytical methods for the purpose of estimating epidermal disposition of chemicals in skin tissue samples, needs significant further investigation.

Caveats on Epidermal Disposition Measurements

Despite the availability of technology that could be applied to the measurement of epidermal disposition, there are some major concerns in applying any of the above techniques in this context.

Multiple exposures

Skin sensitisation is typically a result of multiple exposures to a substance, rather than a single one-off exposure. In the LLNA, which covers the induction phase of sensitisation, the animals are initially exposed three times (once daily, on days 1, 2 and 3), then challenged with the same dose on day 5. In order to take into account the relevant kinetic processes that may occur in human skin, such as saturation of (protein) adsorptive binding (e.g. to serum proteins) and accumulation, epidermal disposition measurements of sensitisers are arguably best measured after repeated dosing. However, it should be noted that the possibilities for using multiple exposure regimens when using ex vivo tissue in an in vitro skin absorption system are limited, due to the short retention of the integrity of ex vivo skin preparations in most types of diffusion cell. Data on this issue, and improvements in the in vitro technology which is available, would be of great value, but are lacking at present.

Protein and tissue binding

Non-specific, non-covalent protein adsorptive binding can impact on both in vitro and in vivo measurements of tissue bioavailability. For example, the pharmacological effect of a drug is related to its occupancy of receptors located at the site of drug action. A well-established principle in systemic pharmacokinetics is that this occupancy is more closely-related to the free or unbound concentration of drug in the bloodstream than to the total blood concentration (49). The bound concentration serves as a reservoir, with no direct contribution to drug activity. A similar principle can presumably be applied to the disposition of sensitising chemicals in the epidermis. Sensitising chemical entities that are non-specifically bound to interstitial proteins, such as serum albumin, or that are bound to skin appendages (e.g., follicles), would presumably not be available to react covalently to form haptons with skin protein for ultimate presentation to LCs as antigens. Consequently, measurement of total chemical concentration in the epidermis may significantly overestimate the true epidermal disposition of freely-available chemical toxins that can go on to form specific haptons. This is true, whether the measurement is performed in vivo or in vitro. Hence, determination of the non-covalent protein binding of skin sensitisers would also be relevant to an integrated assessment of overall epidermal disposition and concentration of freely-available toxin.

Impaired clearance from the skin in vitro

The clearance mechanisms for diffusing permeants differ substantially in vivo and in vitro. The ex vivo skin used in in vitro systems possesses good metabolic activity and basic clearance mechanisms, such as glutathione binding. However, excised skin lacks an active microvasculature, and in vitro diffusion cells often suffer from poor stirring, leading to the less-efficient removal of permeates as compared to capillary clearance in vivo (50, 51). These features can produce artificial steady-state dermal concentrations in vitro that are higher than those in vivo, and fluxes that are lower. This problem may be avoided by using epidermal membranes combined with properly designed flow-through diffusion cells, but the methodology for measuring skin concentrations under these conditions has not been developed. It is worth noting that this issue does not impact on flux measurements, unless the permeant is highly lipophilic and rate-limited by the lower skin layers (52).

Such difficulties in measuring epidermal disposition, combined with the influence of many confounding factors on the interpretation of the measurements, lead to the consideration of an alternative or complementary approach — that of in silico model-based predictions, discussed below. A definitive solution to generating estimates of epidermal disposition will very probably require the combination of experimental measurements and model-based deconvolution of the measurements into free and bound components, with appropriate adjustment for altered clearance in vitro.
The In Silico Prediction of Epidermal Disposition

In considering the prediction of epidermal disposition by using in silico modelling approaches, it is of relevance to consider the current status of our ability to model in silico skin permeability and skin penetration per se, as there are some common models and theories.

Modelling steady-state skin absorption

Up to now, most models of the skin absorption of chemicals have aimed at predicting an infinite dose, steady-state permeability coefficient ($k_p$, for the chemical permeation through the skin as a whole and into the systemic circulation), as applied in aqueous solution. Such predictions were proposed by Flynn (53) for skin permeability coefficients. These were derived from the Flynn dataset, and the methodology was similar to that proposed originally by Potts and Guy (54), who postulated that partitioning into the skin is strongly related to lipophilicity, which can be quantified by the logarithm of the octanol–water partition coefficient (log P) and to diffusion through the skin, which is related to the size of the molecule, which in turn is easily quantified by molecular weight. Numerous workers were sufficiently encouraged by the Potts and Guy model to attempt to develop models for skin permeability coefficients, and in particular, improve statistical fit through the use of different physicochemical and structural properties (55). However, it is acknowledged here that such models do not provide relevant predictions in the context of epidermal disposition. Also, there remains the more basic need to develop simple, qualitative, rules or models for skin absorption (which provide a percentage or an amount absorbed) from finite dosing scenarios, for use in systemic toxicology risk assessments. At the moment, industry is still reliant on OECD TG 428 test methods.

However, in order to model epidermal disposition, some understanding of the common theory and mathematical expressions of skin absorption modelling is required.

Theory and mathematical expressions of skin absorption

Even though the skin is a heterogeneous membrane, skin absorption studies have shown that the steady state flux ($J_{ss}$) is well described by Fick’s first law of diffusion. In steady-state skin absorption studies, in vivo or in vitro, the dose applied is very large compared to the amount that can cross the skin barrier in the time-frame of the study. Furthermore, the concentration ($C_d$) in the donor phase is large compared with the concentration in the deeper layers of the skin/receptor phase. In this case, Fick’s first law can be written as:

$$J_{ss} = k_p C_d$$  (1)

where $k_p$ is the permeability coefficient. For the case of moderately lipophilic compounds, when the purpose is estimating skin absorption rates only, $k_p$ may be written in the simple form:

$$k_p = \frac{D_{sc} K_{sc}}{h_{sc}}$$  (2)

where $D_{sc}$ is the diffusion coefficient of the permeant in the stratum corneum, $K_{sc}$ is the stratum corneum–vehicle partition coefficient, and $h_{sc}$ is the diffusional path length through the stratum corneum. In all other cases, a more complex expression for $k_p$ must be used. For the epidermal disposition question, the simplest useful form for $k_p$ is:

$$\frac{1}{k_p} = \frac{h_{sc}}{D_{sc} K_{sc}} + \frac{h_{vt}}{D_{vt} K_{vt}}$$  (3)

In Equation 3, the subscript ‘vt’ stands for ‘viable tissue’. Equation 3 represents the solution to a two-layer diffusion problem, in which the stratum corneum and the viable tissue both play a role. It has been used extensively to describe the skin absorption of highly lipophilic permeants, and permits an estimate of epidermal concentration, as described below (56, 57). It should be noted that $k_p$ is vehicle-dependent, because it depends on $K_{sc}$. The tabulated values of $k_p$ apply to aqueous vehicles, in which case $K_{sc}$ is the stratum corneum/water partition coefficient. The most useful estimator for $K_{sc}$ has been the octanol/water partition coefficient $K_{oct}$, or its logarithm log $K_{oct}$ (often called log P). $K_{oct}$ values are readily measured or calculated, and several correlations are available between $K_{oct}$, as defined in Equation 2 or 3, and $K_{sc}$. Where the permeant can ionise, the degree of ionisation will affect the solubility and partition behaviour of the permeant, and the pH also becomes an important determinant.

Calculation and modelling of $J_{max}$ for use in predictions of epidermal disposition

In theory, a useful parameter to consider for estimating epidermal disposition is the maximum flux ($J_{max}$), which will be given by Equation 1, when $C_d$ is maximum, i.e. the vehicle solubility of the permeant $S_{veh}$. In this case, the upper layer of the stratum corneum is also saturated with the permeant, with a concentration equal to $C_{sat} = K_{sc} S_{veh}$. Thus, the value of $J_{max}$ (for the one layer model) is:

$$J_{max} = D_{sc} C_{sat}/h_{sc} = [D_{sc} K_{sc}/h_{sc}] S_{veh}$$  (4)
With the constraint that the vehicle is water, considering $S_w$ as water solubility, and substituting the more general definition of permeability coefficient $k_p$ into Equation 4, yields:

$$J_{\text{max}} = k_p S_w$$  \hspace{1cm} (5)

This readily-obtained factor is a more-useful measure of absorption potential than $k_p$ or log $K_{oc}$. The permeability coefficient alone is not a good indicator of the total amount of a permeant that will cross the skin. A major distinction between $k_p$ and $J_{\text{max}}$ is that the former is proportional (or nearly so) to the lipid/water partition coefficient, whereas the latter is proportional to lipid solubility. This can be seen by examination of Equation 4, noting that $C_{\text{sat}}$ for a lipid membrane is simply lipid solubility. Considering the skin as a two-layer membrane (c.f. Equation 3) leads directly to an expression for the steady-state concentration of the freely diffusing permeant, $C_{ss}$, at any point in the system. Taking the very simple approximation of the viable skin layers as an aqueous barrier that is cleared at a depth of 200 $\mu$m, the permeability can be expressed as (56, 57):

$$P_{vt} = 0.15 (300/MW)^{1/2} \text{ cm/h}$$  \hspace{1cm} (6)

This factor, when used in combination with Equations 3 and 5, gives the value of $C_{ss}$ associated with maximum flux conditions. Choosing the location to be the midpoint of the epidermis, where the LC density is assumed to be at its highest, this model of dermal transport and clearance yields:

$$C_{ss} = \frac{0.75 k_p}{k_p + P_{vt}} S_w$$  \hspace{1cm} (7)

Equation 7 is analogous to Mehta et al.’s $C^*$ value (58), which was defined at the basal cell layer of the epidermis. For $J_{\text{max}}$ given in $\mu g/cm^2/h$ and $k_p$ given in $cm/h$, $C_{ss}$ has units of $\mu g/cm^3$. It can easily be expressed as micromolar concentration, by multiplying by 1000 divided by the molecular weight. A consideration of the steady-state concentration profile shows that $C_{ss}$ is also equal to the average concentration of freely diffusing permeant in the epidermis under maximum flux conditions.

Magnusson et al. (59, 60) have investigated the modelling of maximal flux ($J_{\text{max}}$). They used values from an aqueous solution across human skin, which were acquired or estimated from experimental data and correlated with solute physicochemical properties. Whereas epidermal permeability coefficients, $k_p$, are optimally correlated to log P, molecular weight was found to be the dominant determinant of $J_{\text{max}}$ for these literature data. The addition of other physicochemical parameters to the molecular weight by forward stepwise regression only marginally improved the regression with a melting point (M.Pt) term ($r^2 = 0.88$), whereas addition of hydrogen bonding acceptor capability ($H_a$) was significant ($r^2 = 0.92$). Evaluation of the equation was performed with a number of other data sets, including an aqueous vehicle with full-thickness and split-thickness skin ($r^2 = 0.78$, $n = 56$).

It should be noted that the theory associated with $J_{\text{max}}$ estimation shows that molecular weight and lipid solubility $S_{lip}$, rather than molecular weight and log P, should be the governing factors. This can be seen by examining Equation 4 and noting that $C_{sat}$ for a permeant in a lipid membrane is simply $S_{lip}$. Thus, to the extent that the barrier properties of stratum are governed primarily by lipids, Equation 4 can be rewritten as (56, 61):

$$J_{\text{max}} = \frac{D_{sc}/h_{sc}}{S_{lip}}$$  \hspace{1cm} (8)

In the free volume model discussed by Kasting et al. (61), and later employed by Potts and Guy (54), permeant diffusivity in the stratum corneum decreases exponentially with molecular weight, according to the relationship:

$$D_{sc} = D_0 \exp(-\beta \cdot MW)$$  \hspace{1cm} (9)

Combining Equations 8 and 9, and taking logarithms on both sides yields:

$$\log J_{\text{max}} = \log (D_0/h_{sc}) - \beta \cdot MW + \log S_{lip}$$  \hspace{1cm} (10)

Such dependence was indeed found in earlier work on the $J_{\text{max}}$ parameter (56, 61). In fact, log $S_{lip}$ was a considerably more important factor than molecular weight for correlating log $J_{\text{max}}$ values for the data analysed in these studies. Since lipid solubility decreases exponentially with increasing melting point, the melting point dependency reported by Magnusson et al. (59, 60) may correlate with the lipid solubility factor. To demonstrate this, additional high-melting compounds should be included in the analysis, or a more appropriate functional dependence should be used.

Recent attempts at the complex modelling of skin absorption

Complex models developed from a theoretical and computational framework for skin absorption exist, e.g. the skin disposition/concentration models for transient dermal exposures to potentially volatile compounds (62–64). There are also a number of empirical models developed by Bunge and co-workers (57, 65).

The skin disposition/concentration models are more complex than the Potts and Guy approaches, but are likely to be heavily reliant on predicted properties from (Q)SAR approaches, e.g. estimates
of log P, density, water solubility and vapour pressure (66, 67). At the current time, more work is required to optimise these approaches, in order to make them into usable predictive algorithms and to deal with issues such as vehicle effects and formulations.

At present, a new computational approach to estimating epidermal disposition is being developed, and an initial overview of this work is presented below.

A Computational Skin Diffusion Model for Transient Exposures

Two important and fundamentally different parameters of epidermal disposition can be derived from skin diffusion models. One is the maximum flux, $J_{\text{max}}$, and the associated steady-state concentration, $C_{\text{ss}}$, of a diffusing permeant, achieved at some key location in the epidermis, as discussed in a previous section (c.f. Equations 1 to 10). A second measure of epidermal disposition is the concentration–time profile of a diffusing permeant at a toxicologically relevant dose. For the purposes of correlating murine LLNA data, an applied dose can be taken as equivalent to the LLNA EC3 value in $\mu$g/cm$^2$, indicating the topical exposure dose threshold for sensitisation. A reasonable general correlation has been observed between LLNA EC3 values and threshold sensitisation doses from human repeat insult patch test data, which makes such doses from the LLNA relevant for extrapolation to humans (68). In order to be able to relate the internal dose to the concentrations used in in vitro assays, determining the actual epidermal concentrations following the application of doses below, at and above a threshold sensitising dose, will contribute to our understanding of the intrinsic potency of a sensitiser, whereas $C_{\text{ss}}$ is a measure of maximum possible steady-state skin exposure. Thus, the two quantities have very different uses.

In order to represent concentration–time profiles in a concise manner, two summary parameters may be suggested. One is $C_{\text{max}}$, the peak mid-epidermal concentration achieved following the application of a sensitising dose of chemical. The second is $\text{AUC}_{120}$, the area under the epidermal concentration–time profile. This quantity is essentially the total amount of test compound that has passed into and through the epidermis. For the greatest realism, $C_{\text{max}}$ and $\text{AUC}_{120}$ should reflect the values achieved under repeat dose regimens.

In order to calculate $C_{\text{max}}$ and $\text{AUC}_{120}$ for arbitrary doses of permeant, a more sophisticated skin diffusion model than the steady-state model, leading to Equations 1–10, is required. An example of such a model, under development by Gerald Kasting (College of Pharmacy, University of Cincinnati Medical Center, Cincinnati, OH, USA) and his collaborators, is shown in Figure 2. This is a multi-layer skin diffusion model, with the capability of describing skin permeation rates under a variety of exposure conditions. The stratum corneum and dermal compartments are distributed representations of novel microtransport models, developed by their collaborator Johannes Nitsche (SUNY, Buffalo, NY, USA) and his students (50; 69–72). The stratum corneum properties vary with skin hydration (73, 74), and provision is made for the evaporation of volatile compounds from the skin surface (62–64). The in vitro clearance of diffusing permeants through a network of capillary loops in the dermis is simulated (50, 51, 71, 72). This framework provides a new tool for estimating epidermal disposition. Development is ongoing, with a combination of US and European support. A COLIPA-sponsored project is examining the relationship between epidermal disposition parameters calculated from this model and chemical reactivity and biological activity arising from emerging in vitro alternatives, including peptide reactivity and dendritic cell assays. It is hoped that internal doses of toxins in the epidermis following topical application can then be related to concentrations used in in vitro assays, to interpret any effects observed in such systems with respect to their relevance in the human-use situation. A spreadsheet version of the working bioavailability model, running under Microsoft Excel®, is available from one of the authors (kastingb@ucmail.uc.edu).

Skin layers are depicted in Figure 2. The key step in making a predictive skin and/or epidermal disposition model based on this framework, is the development of accurate underlying microtransport models for each of the layers. The microtransport model provides the basis for calculating effective transport parameters, for example $D_{\text{sc}}$ and $K_{\text{sc}}$ for the stratum corneum. Prototype models of this nature have been developed for the stratum corneum (69, 70, 75) and dermis (50, 72). As with all models, they require considerable testing and refinement, before widespread agreement as to their structure and properties can be achieved. Notably, no microtransport model for the viable epidermis is as yet available. Although much can be inferred from transport analyses in other cellular tissues (76, 77), this is a significant gap for those interested in accurate concentration estimates in this layer. Other features of the working model that require additional experimental calibration, are the effective $D_{\text{sc}}$ and $K_{\text{sc}}$ values for the partially-hydrated stratum corneum, the associated $C_{\text{sat}}$ value for the partially-hydrated stratum corneum (c.f. Equation 4), the capillary clearance factor in the dermis, $k_{\text{cl}}$, and the impact of non-covalent adsorptive protein binding on the epidermal and dermal partition coefficients, $K_{\text{ed}}$ and $K_{\text{dc}}$. Each of these factors is experimentally accessible. The measurement of these transport properties, and its interpretation in the context of a
computational model such as this, offers an attractive alternative to the difficult task of experimentally making accurate epidermal concentration measurements for a wide variety of permeants.

Gaps

Validated and accepted in vitro alternatives for the identification and assessment of potential skin sensitising chemicals do not yet exist. It is most likely that, rather than a single in vitro assay, the optimal alternative strategy will involve several tests, each of which will correspond to an essential component of the biological mechanism. Until these are in place, it may not be possible fully to define how epidermal disposition data can be integrated into an overall testing or risk assessment strategy.

Although skin sensitisation data on a substantial number of chemicals have been published (78), there is, as yet, no overview of the extent to which these cover the full diversity of organic chemicals. This topic assumes greater importance, as testing moves from a fully integrated immunobiological model (i.e. in vivo) to selected isolated elements (i.e. in vitro and in silico) which combine to make a model, and where certain assays may only be applicable for certain classes of chemical.

Only a few reports exist in the literature, which attempt to measure the total skin absorption of skin sensitisers, let alone their skin partitioning and epidermal concentrations. No empirical methods exist for measuring epidermal disposition for the purpose of defining in-tissue dosimetry, in this or any other local skin toxicity-related field. There may be some methodology and data in the pharmacology literature, which consider at-target doses of active therapeutics in the skin, and that could be drawn upon.

A further gap is that, currently, there is uncertainty about: where, at the molecular level, a chemical partitions within the epidermis; how a chemical may be transformed into other chemical species; and where the reaction occurs in the sensitising species to haptenate skin protein and result in the formation of an antigen that, in turn, can stimulate the immune system. For example, do haptenation reactions occur with extracellular proteins, intracellular proteins, or proteins on the cell surface?

Another key absence of knowledge is that of the true toxic threshold within the epidermis (in contrast to the EC3 value as an applied topical dose for defining a sensitisation threshold), which is needed to permit cut-off limits to be set in relation to in-tissue dosimetry, so that the internal dose can then be related to concentrations used in in vitro assays that lead to effects.

No widely accepted in silico models are currently available that can model systemic skin absorption for finite dose scenarios, although such models are under development, as discussed above. New mod-

Figure 2: A schematic diagram of a diffusion/evaporation model for the skin disposition of potentially volatile permeants

![Diffusion/evaporation model diagram](image-url)
els and/or additional characterisation and calibration of the newly developed models are needed, as well as new and different models for estimating epidermal disposition. The philosophy of measuring experimentally-accessible parameters for input into in silico models of epidermal disposition, rather than focusing on experimentally intractable epidermal concentration measurements, should be given serious consideration. Most likely, a combination of both types of measurements will be required, in order to obtain consensus with regard to estimations of epidermal concentrations.

Conclusions and Recommendations

The requirement for tissue dosimetry data may differ, depending on whether the question relates to simple hazard identification (e.g. in relation to the REACH system), or whether the need is for risk assessment to meet the needs of the 7th Amendment, by being able to provide robust safety evaluations of new ingredients, without the generation of new animal data.

The REACH system

In the context of the REACH system, the primary need is the identification of unambiguous “rules”, which would permit the categorisation of an untested substance as either certain to be a sensitisier, or certain not to be a sensitisier. In the view of the workshop participants, it is not possible to base such rules solely on epidermal disposition data. Instead, an integrated view of, for example, low or high epidermal concentrations of toxin, needs to be considered, in combination with other information, such as evidence of structural alerts, peptide reactivity, etc. (30). Skin bioavailability data are definitely one part of the Integrated Testing Strategy (ITS), which is highly recommended within the REACH system, for classifying chemicals according to their toxicological properties. The ITS foresees the assessment of the hazard related to the use of a chemical by integrating multiple elements, including in vitro tests, read-across or chemical grouping, in silico approaches, and exposure considerations (including in-skin absorption capacity).

Risk assessment in the context of the 7th Amendment to the Cosmetic Directive

Given that it is anticipated that any new alternative approach to risk assessment should aim toward providing a prediction of sensitisation potency relevant to humans, as well as basic hazard identification, human epidermal disposition data are considered to be essential to the achievement of this end.

It is evident that chemicals with a positive sensitisation hazard, as identified by using forced exposure conditions in assays such as the LLNA or the human repeat insult patch test (HRIPT), can, in fact, be used safely in humans, and pose minimal risk under actual exposure conditions. The permeation of the chemical through human skin, as delivered (initially as a finite low dose), must play a role here.

Novel approaches to risk assessment will need to be based on the incorporation of in vitro and in silico data, in ways which are devised to complement our existing understanding of sensitisation potential in vivo. However, firm recommendations on the use of such epidermal disposition data cannot be made until other key in vitro and in silico methods and ways of integrating the data to inform risk assessment are better developed.

Consideration is being given in some domains as to whether extrapolations of dosimetry will be easier from in vitro endpoint data obtained from tests involving reconstructed 3-D skin models, where the chemical is applied directly to the “skin” surface, and which involve some element of implicit delivery, than if the in vitro tests require exposure to occur in aqueous-based media. However, the barrier properties of 3-D skin models are poor, and we currently do not know how metabolically-active 3-D skin models are, in comparison with the activity of human skin. Thus, this is not a quick solution to the issue of human skin dosimetry versus in vitro assay dosimetry.

Recommendations for further work

1. The design of a robust integrated testing strategy will be needed, to determine whether and how estimates of epidermal disposition of chemicals can be used, and whether such data add value in determining skin sensitising potential. A reasonable starting strategy for evaluating these results is to plot LLNA potency as a function of a key skin permeation property (e.g. Jmax) and the extent of activity in a cellular or peptide-reactivity assay. The discriminating power of the descriptors for identifying allergen classes can be obtained from such a plot. Of course, in order to do this, the other assays also need to be further developed. An obvious extension is to probe the relationships among a larger number of descriptors, by using multivariate techniques. We believe the best use of epidermal disposition measurements involves such combined analyses.

2. In the same way as in vitro cell-based assays and peptide-reactivity assays are being developed to generate data for input into integrated assessments, there is a need to develop new in
vitro and in silico methodologies to a) generate input parameter data for in silico modelling and b) characterise epidermal disposition in human skin for some key benchmark sensitisers of differing potencies. To generate experimental data to complement in silico modelling, new in vitro methods will be required, based upon the significant modification of current in vitro skin absorption methods and the use of new state-of-the-art analytical techniques for analysing chemicals either in skin tissue extracts or in situ in intact skin.

3. There is little value in routinely generating skin absorption data on skin sensitisers, according to OECD TG 428, which is developed with systemic toxicity in mind. As such, it would be the recommendation not to modify the existing OECD TG 428 to accommodate the measurement of epidermal bioavailability, but to develop, in the longer term, a new guidance strategy for the purpose of generating epidermal disposition data for use in evaluating local toxicity endpoints in the context of the REACH system and the 7th Amendment.

4. A promising new approach is to develop more-complex in silico modelling approaches for predicting epidermal disposition, as described above. This should be explored and built upon further. It should be noted that the proper use of any model requires the consideration of the model's applicability domain (66).

5. Among the key elements to explore both in vitro and in silico in this area, are the assessment and rationalisation of vehicle and formulation effects, and, if possible, the incorporation of other influencing factors, as described earlier (31).

6. In terms of risk assessment, further work could be of value in the area of defining quantitative cut-off values for epidermal concentrations of chemical entities, below which sensitisation may not be of toxicological concern.

The need for greater collaboration

Given the complexity involved in determining epidermal disposition measurements, it is important that experts in both fields of skin penetration/absorption and skin sensitisation, who have traditionally worked in separate domains, should strive to work more closely together, in order to derive the correct skin exposure and epidermal disposition parameters for this local toxicity endpoint.

References


