

Using *In Vitro* Skin Absorption and Penetration Testing as an Alternative to Animal Testing

a report by

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Dr Walter Diembeck currently heads the *In Vitro* Biocompatibility/Screening Department at BDF Beiersdorf AG's Research and Development facility in Hamburg. He joined the company in 1978 and was responsible for pharmacokinetics and biochemical analytics in drug research in the pharmaceutical division until 1989. Thereafter, he continued his work in the cosmetics division, where he evaluated and developed various *in vitro* tests with his group that are now used in daily routine for safety assessment, selection of appropriate ingredients and product development purposes. Dr Diembeck is a member of The European Cosmetic Toiletry and Perfumery Association's (COLIPA's) Steering Committee on Alternatives to Animal Testing, Chairman of the COLIPA Percutaneous Absorption Task Force, a member of the COLIPA Skin Tolerance Task Force and member of the Extended Organisation for Economic Co-operation and Development Percutaneous Absorption Steering Committee. He has a Masters in chemistry from the University of Goettingen, Germany, and a PhD in biochemistry from the Max Planck Institute for Biophysical Chemistry.

The *in vitro* skin absorption and penetration testing alternative uses excised animal or human skin to replace experiments conducted *in vivo* on animals or humans for the assessment of dermal absorption and percutaneous penetration. Both aspects are essential for the risk assessment of chemicals that may make contact with, and subsequently penetrate, the skin.

There are scientific and ethical reasons for this type of testing. Animal skin is obtainable in sufficient quantities to allow replication of experiments.

Extrapolation of data obtained from appropriate animal skin is an acceptable alternative, but excised human skin obtained from surgery may be used when available. No risk or harm to living creatures is associated with this type of test.

Furthermore, Article 4a of the 7th Amendment to the EU Cosmetics Directive of March 2003 forces the industry to substitute animal testing of raw materials and products with alternative methods.

Deadlines have been set – March 2009 – for most animal tests except for those relating to repeated-dose toxicity, reproductive toxicity and toxicokinetics where the deadline has been set at March 2013.

After these deadlines, a marketing ban will be placed on products and raw materials that have been tested on animals, although accepted alternatives to those already in existence will come into effect in the EU.

This *in vitro* test is accepted by EU authorities and the Organisation for Economic Co-operation and Development (OECD).

Background

Cosmetics companies will require skin absorption and penetration data, in particular in the case of 'actives', e.g. colourants, preservatives, ultraviolet (UV) filters, substances with limitations in concentration and site of application.

This data must be provided in the safety dossier (Public Interest Report) for all ingredients (6th Amendment).

The test must be performed using relevant formulations (close to market product). The worst-case scenario should also be taken into consideration.

The 6th and 7th Amendments to the EU Cosmetics Directive include a potential ban on the use of animals in the testing of cosmetic products and their ingredients. With regard to the assessment of dermal absorption and percutaneous penetration properties of substances, *in vitro* methodologies are recommended for ethical reasons and also for reasons of feasibility.

Excised skin is readily obtainable from various animal species (pigskin is usually preferred) and, from this, suitable membranes can be prepared to enable properly controlled and reproducible experiments to be conducted. Human skin is available in limited quantities from surgical or post-mortem sources.

The value of organotypic skin models for *in vitro* absorption/penetration studies has to be demonstrated by further research. The viability of the skin is not a prerequisite for penetration testing, since the process depends on passive diffusion and not apparently on active transport.

The barrier properties of skin are usually maintained after excision from the body and appropriate storage in a freezer for up to several months. The principal diffusion barrier has been identified as the *stratum corneum*, the integrity of which has to be controlled individually prior to the experiment.

Furthermore, biotransformation is usually of minor importance for the majority of chemicals. In the exceptional case of relevance, biotransformation can be examined in separate studies utilising freshly excised skin under conditions prolonging viability. There is considerable experience within the industry to allow these *in vitro* methods to be used for the prediction of the *in vivo* situation.

Definitions

- *Stratum corneum* adsorption represents the amount of topically applied test substance that is found in the *stratum corneum* after termination of the

experiment (exposure time may be shorter). This quantity is taken as not systemically available.

- Dermal absorption represents the amount of topically applied test substance that is found in the epidermis (excluding the *stratum corneum*) and in the dermis after termination of the experiment. This quantity is taken as systemically available.
- Percutaneous penetration represents the amount of topically applied test substance that is found in the receptor fluid after termination of an *in vitro* experiment. This quantity is taken as systemically available.

Principle of the Test

The test substance, either as such or dissolved in an appropriate solvent or formulation, thereby yielding the test sample, is applied to the intact surface of the skin disc, which is positioned between the upper and lower chambers of a diffusion cell and may be either of static or flow-through design.

The test sample remains in contact with the skin on the donor side for a defined period of time (leave-on or rinse-off, depending on the intended conditions of use).

The receptor fluid may be sampled once at the end of the experiment or, preferably, at various time points in between so that a penetration profile may be constructed.

The skin and/or fluid samples are analysed by an appropriate method (e.g. scintillation counting, high-performance liquid chromatography (HPLC) and gas chromatography (GC)).

The integrity of the barrier should be checked by an appropriate method, e.g. trans-epidermal water loss (TEWL).

Description of the Method

Penetration Cell Design

The penetration cell comprises the upper donor and the lower receptor chamber, separated by a skin membrane. The *stratum corneum* faces the donor chamber and the lower skin surface faces the receptor chamber. The cells must be made from an inert and non-absorbing material (e.g. glass or polytetra-fluoroethylene).

Temperature control of the receptor fluid is crucial throughout the experiment and should be maintained at *in vivo* skin conditions. The receptor fluid must be well mixed throughout the experiment. Sampling by

appropriate cell design should be feasible without interrupting the experiment.

Receptor Fluid

The composition of the receptor fluid should not limit the extent of penetration of the test substance – in effect, the theoretical total solubility of the chemical under investigation should be guaranteed.

The receptor fluid must not affect the integrity or alter the permeability properties of the skin. A saline or buffered saline solution is recommended for hydrophilic compounds.

For lipophilic molecules, the addition of serum albumin or other appropriate solubilisers, such as non-ionic surfactants, may be appropriate but membrane integrity must be unaffected.

The appropriate total volume of the receptor fluid is determined by the solubility and analytical detectability of the test substance. It can be adapted by choosing a receptor chamber of adequate volume for static cells or by varying the setting of the pump for flow-through cells.

Skin Membranes

The skin samples used in these tests are normally prepared as discs, which are cut to fit the cell. The skin membrane may be either ‘whole’ skin, ‘split-thickness’ skin or epidermal sheets.

Whole skin may be used and this is normally prepared by cleaning any soil from the surface with a mild skin cleanser, removing any hair with clippers and removing subdermal fat and fascia. Split-thickness skin is prepared as for whole skin but the epidermis and upper dermis are removed from the bulk of the dermis with a dermatome (keratome). These membranes are usually less than 1mm thick.

Epidermal membranes may be prepared by heat, enzymic or chemical treatment of whole skin. In the case of lipophilic compounds, split-thickness skin (1mm thick layer) or epidermal preparations are preferred to limit retention in the dermis *in vitro*. Skin thickness should be measured by an appropriate method.

The test should be carried out with an appropriate number (normally a minimum of three to six) intact skin discs. ■

This article is continued, with graphics and full references, on the BBL website supporting this business briefing (www.bbriefings.com/cdps/cditem.fcm?NID=846).