



Dermal Applications



In vitro skin models for characterizing topical dosage forms during product development, drug approval procedures and safety evaluations

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Introduction

In recent years there has been increasing interest in the dermal application of drugs due to the simplicity of this type of delivery system. Despite this positive trend, costly and time-consuming clinical studies must still be performed in order to determine dermatopharmacokinetic parameters. Modern in vitro studies complement conventional clinical research and now make a significant contribution to the characterization of topically applied drug formulations. Drugs that are topically applied but that act only in deeper lying skin tissue can now be studied using novel test models and modern analytical techniques without the need for extensive and costly clinical investigations. For any new drug preparation it is now

possible to determine in vitro whether the pharmaceutically active ingredient is able to penetrate the skin "barrier".

In vitro studies also offer an interesting alternative when optimizing the composition of a formulation to achieve the required drug uptake across the skin, when investigating the interaction of chemicals with the skin, or when developing cosmetic products.

Areas of use

- Selecting drug candidates for dermal application
- Testing drug delivery strategies
- Selecting a suitable vehicle (formulation base)
- Characterization of topical dosage forms
- Preparations for clinical trials
- Product comparisons
- Quality assessment (e.g. as part of a stability testing program)
- Device development (e.g. microneedle delivery systems or other physical drug delivery techniques)
- Safety evaluation of cosmetic active ingredients, excipients and end products (approval procedures)
- Safety evaluation of industrial chemicals and pesticides

Human skin

Human skin comprises the epidermis, the dermis and the underlying subcutaneous tissue, with sebaceous and sweat glands running throughout. The outermost epidermal layer is the stratum corneum or horny layer and represents the main barrier to skin permeation by dermally applied drug formulations. The structure of the stratum corneum itself can be explained in terms of the so-called “brick and mortar” model in which horny keratinocytes (corneocytes) represent the bricks while the intercellular lipids and water-retaining natural moisturizing factors act as the mortar.

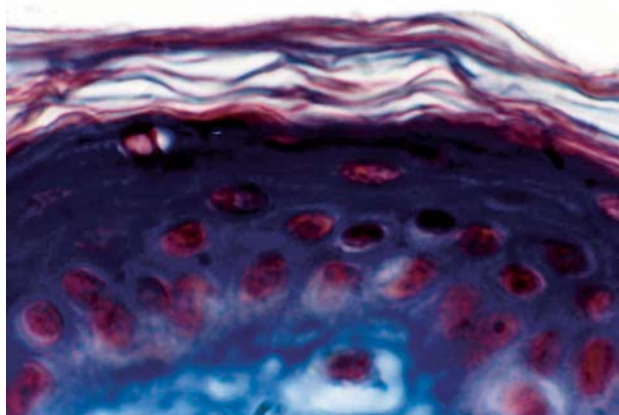


Fig.1: Cross-sectional view of human skin. The stratum corneum is clearly recognizable.



Fig. 2: Human stratum corneum (in vivo) viewed from above.

Skin models

In vitro studies on different membrane types are now a well established means of investigating questions regarding the dermal permeation and penetration of drug substances. Tests using synthetic membranes are applicable only in preclinical drug release studies or when assessing batch conformity as part of a quality assurance scheme. While animal skins can also be used as a surrogate, their ability to make reliable predictions about in vivo human data is limited. The results obtained are crucially dependent on the species of animal used, as the similarity of the skin to human skin can vary strongly. In general, animal skin has a larger number of hair follicles than human skin. This means that in addition to the transport processes already mentioned (intercellular and transcellular mechanisms), there is also the possibility of transport via transfollicular and transgranular channels. It is therefore important to take these characteristics of animal skin into account when analyzing experimental results.

As an alternative to animal skin, human skin excised during plastic surgery can also be used. Across Barriers GmbH has a skin bank that is being expanded continuously and in which samples can be stored for up to six months at a temperature of -20°C . This enables the company to use the following skin models and technologies for in vitro tests:

Human skin

- Full skin
- Dermatomic sections (300-500 μm)
- Heat-separated skin
- Isolated stratum corneum

Porcine skin

- Full skin
- Dermatomic sections (1000 μm)

Animal skins

- Mouse, rat
- Other skin models available on request

Reconstructed skin models

- e.g. EpiDerm™, Skinethic™ or Episkin™
- Other skin models available on request

Nail models

- Based on bovine hoof membranes

In vitro models and methods**Penetration - Permeation**

Across Barriers GmbH uses numerous individual and combined techniques to study the transport of active ingredients across and into the various skin layers. For the purposes of clarification, the terms used are defined below:

Permeation: *transdermal transport through the skin membrane into an acceptor compartment*

Penetration: *Transport into the various skin layers (which themselves act as individual acceptor compartments)*

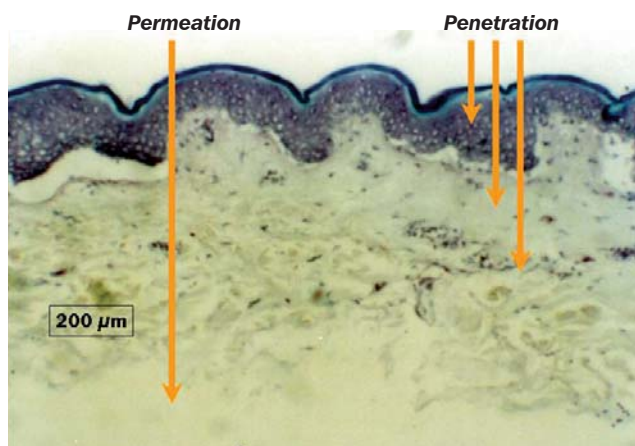


Fig. 3: Cross-section of human skin (showing the permeation and penetration processes).

In vitro penetration

Penetration studies involve determining the concentration of a topically applied substance in the various skin layers. Across Barriers uses the so-called "Saarbrücken Model" developed by Professor Helmut Loth. The model uses the tape-stripping technique to remove the various horny layers.

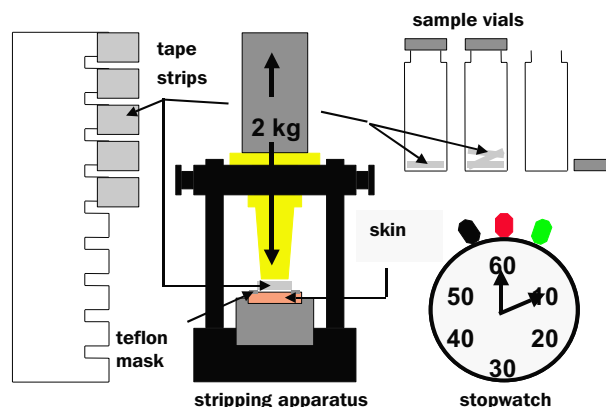


Fig. 4: Schematic diagram of the Saarbrücken model.

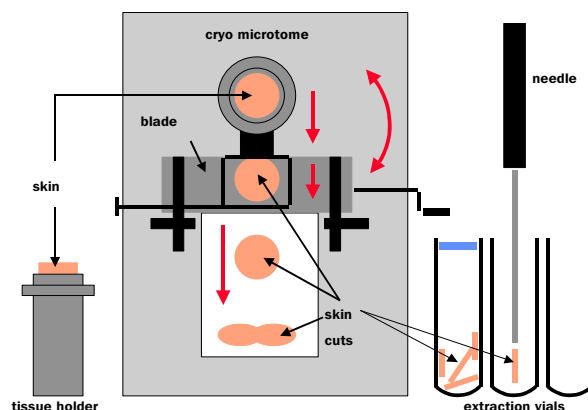


Fig. 5: Schematic diagram of the cryosectioning process.

The tape strips with the horny layer sections adhering to them are then processed and analyzed. A freeze microtome is then used to slice the remaining skin sample parallel to its surface. The substance under test is then extracted from these sections and detected analytically.

The Saarbrücken model yields a concentration profile that indicates how the concentration of the active ingredient varies with penetration depth. This allows reliable inferences to be made regarding the distribution of the active compound within the various skin layers and also provides evidence of possible layer-dependent deposits that arise from the localized enrichment of the active substance in certain skin regions.

In vivo penetration

The in vitro penetration studies mentioned above can also be used for in vivo testing.

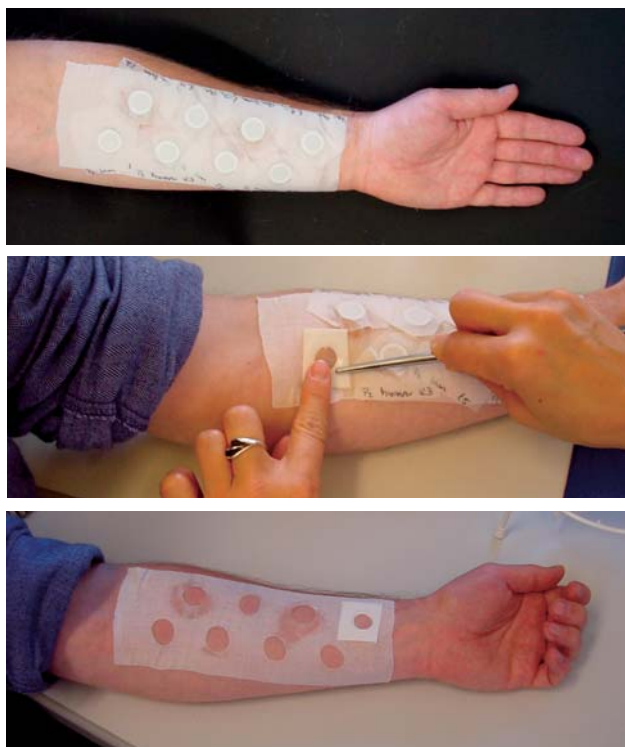


Fig. 6: In vivo penetration study using an overlay mask.

Permeation

The goal of a permeation study is to compile a kinetic profile that reflects how the concentration of an active ingredient changes in time as it diffuses through the skin.



Fig. 7: The Franz cell.

At Across Barriers we use the well-established Franz cell model for these measurements. A membrane stretched between the donor and acceptor compartments of the Franz cell acts as a diffusion barrier. The test substance is applied to the donor side from where it diffuses through the membrane into the acceptor medium. By sampling and analyzing the acceptor side at different times, a profile showing the rate of migration of the compound through the skin can be established.

Analytics

Before initiating a penetration or permeation study, an analytical method for analyzing and detecting the active ingredient is developed. This also includes a process for extracting the active ingredient from the skin. The effort expended on validating the analytical method selected will depend on the questions to be addressed in the study and the how the data is to be used. For serial testing or surveys, rapid screening methods can be used. However, if a detailed elucidation of transport phenomena is required or if the data is to be used in a product approval process, fully validated stability-indicating methods can be developed. In addition to the classical LC-UV detection method, fluorescence and electrochemical detection techniques are also available. Across Barriers also makes use of coupled techniques such as LC-UV-MS or LC-MS/MS. A scintillation counter is available for detecting radioactively labeled substances or markers. With the array of detection techniques available, Across Barriers can detect substances down to trace levels in the different skin layers and can also clarify possible metabolic pathways.

Process qualification measures (skin integrity)

The extent of percutaneous absorption (permeability) depends on numerous factors such as the concentration of the active ingredient in the formulation, its physicochemical properties, interactions with the formulation, differences in skin structure and body region as a function of gender, body weight, age and skin integrity. Application-related aspects are also relevant, including the manner and duration of application and whether finite or "infinite" dosages are applied.

These factors are taken into account by determining the following parameters in accordance with the OECD and SCCNFP guidelines:

- Stratum corneum thickness (microscopic method)
- Measurement of skin thickness
- Permeation of caffeine (the recommended OECD marker compound) or another marker (e.g. mannitol) through the skin
- Calculation of the body mass index (BMI)
- Documentation of donor data (age and gender), disinfection of the skin during the operation and the disinfectant used

Using this data, the results for unknown substances can be interpreted more easily or can be normalized.

Results

The following figures and tables, which show selected representative results from earlier studies, offer an overview of the areas in which in vitro technologies can be applied.

Penetration

This section presents the results of studies of the penetration of non-steroidal anti-inflammatories. The penetration of the active ingredient and how it is affected by the formulation was investigated by comparing two different gel preparations. The in vitro penetration data was also compared with data obtained from human in vivo studies. The following figure shows the penetration of the pure active ingredients. There is no significant difference between the two data sets.

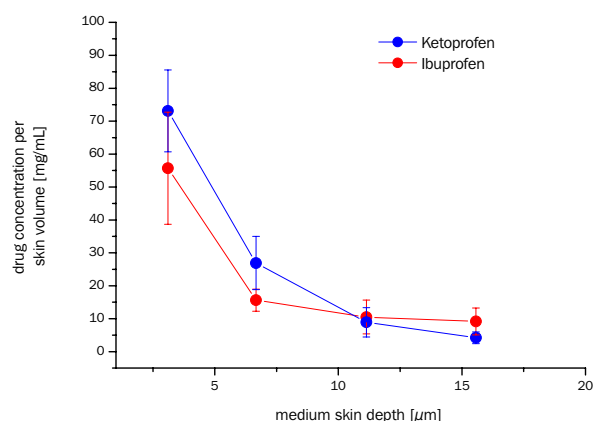


Fig. 8: Penetration of ketoprofen and ibuprofen in human full skin (in vitro). Infinite dose, 3 h at 32°C.

Figure 9 demonstrates that the different gel formulations had no effect on the penetration of the active ingredient. The two products were thus shown to be comparable.

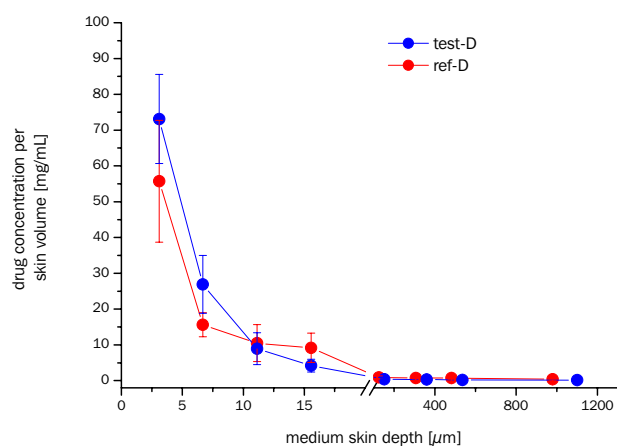


Fig. 9: Penetration of two different gel formulations of non-steroidal anti-inflammatories. Infinite dose, 3 h at 32°C.

Whenever in vitro methods are used, the question of in vitro / in vivo correlation always arises. To address this issue, penetration studies were carried out on test subjects and the results compared with the in vitro data. The following figure demonstrates the good correlation between the in vitro and in vivo results.

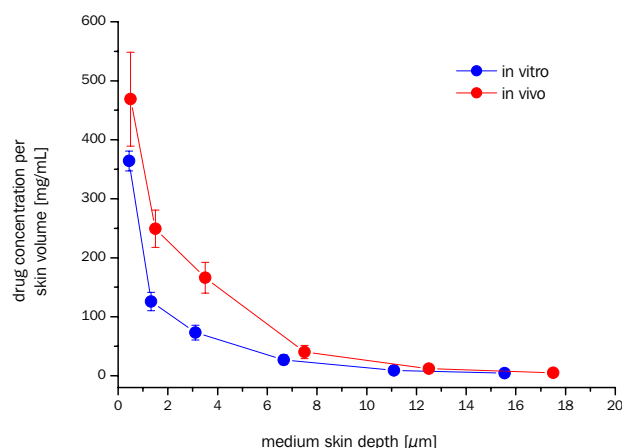


Fig. 10: Comparison of ketoprofen gel penetration data obtained from human test subjects and from an in vitro human full-skin model. Infinite dose, 3 h at 32°C.

A phenomenon frequently observed with topically applied drugs is the enrichment of the active ingredient in a specific skin layer. This indicates that the skin layer concerned is saturated and hence unable to accept more of the active ingredient. Penetration cannot therefore be increased by simply lengthening the time for which the active ingredient resides on the skin, nor by increasing its concentration

in the formulation. This type of investigation is indispensable for establishing dosage levels in a formulation or when compiling application information. The next two figures show the accumulation of caffeine in the stratum corneum (in vitro test) and the enrichment of ketoprofen in the stratum corneum (in vivo studies).

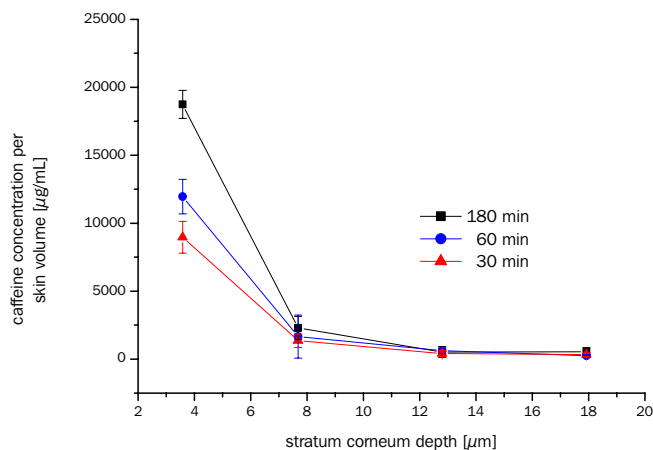


Fig. 11: Kinetic profile of caffeine penetration in human full skin. Infinite dose, 0.5, 1 and 3 h at 32°C.

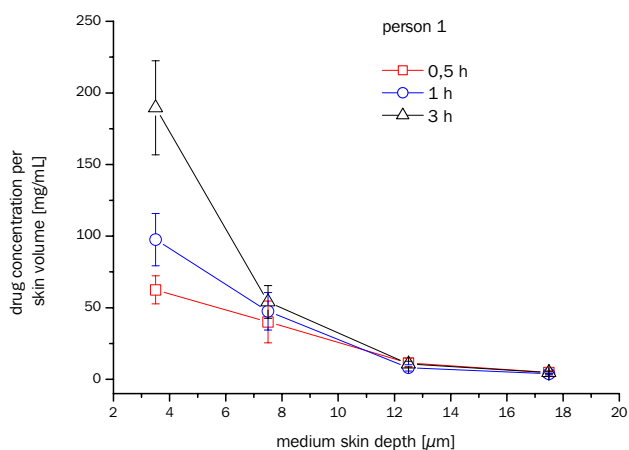


Fig. 12: Kinetic profile for in vivo penetration of ketoprofen from a gel. Infinite dose, 0.5, 1 and 3 h at 32°C.

These results demonstrate that in vitro skin studies correlate well with in vivo investigations and can therefore contribute significantly to saving time and costs when developing topically applied drug products or cosmetics.

Permeation

Permeation studies play a role whenever the systemic delivery of a topically applied drug substance is under consideration. As part of the cosmetic product development process or when evaluating substance safety, it is important to establish early on that

certain chemicals do not diffuse through skin tissue and act toxically.

In vitro permeation studies can be used either to replace costly and time-consuming animal and human trials, or for preliminary screening purposes before such in vivo studies are conducted. In vitro permeation measurements also enable the high variability between individual test subjects to be simulated. The following figure shows how the mass transport of estradiol varies across a group of different skins. Any study looking into the systemic availability of estradiol must expect a similar degree of fluctuation.

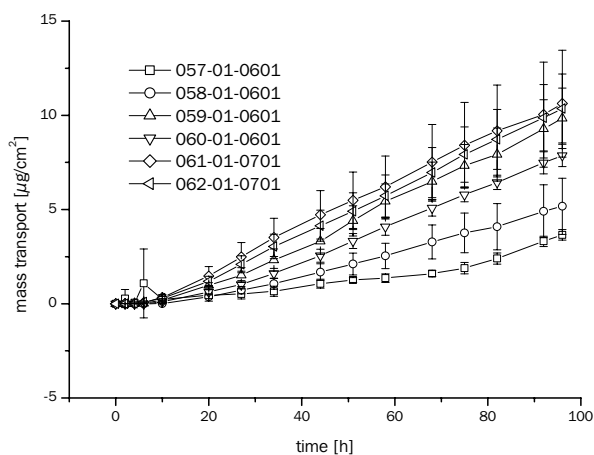


Fig. 13: Variation in the permeation of estradiol as a function of the skin sample used.

When a transdermal patch is being developed, the necessary dosage must be specified. The following figure presents a comparison of different estradiol patches. It can be seen that the significantly higher concentration of the active ingredient in TTS 1 does not lead to increased permeability.

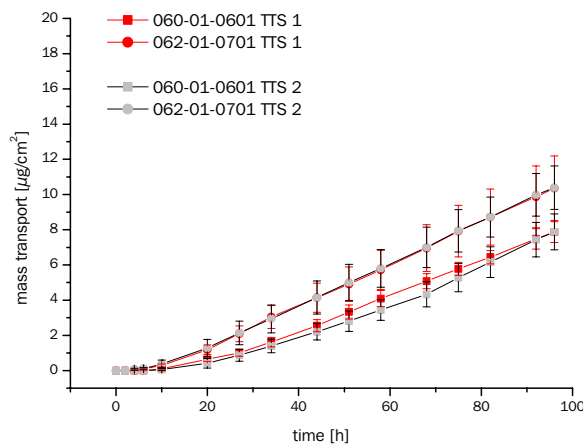


Fig. 14: Kinetic profile of the in vivo permeation of estradiol from two patches differing in the substrates used and the concentration of the active ingredient.

Using the improved patch substrate, the same permeation rate could be achieved despite the lower concentration of active ingredient.

The question of how best to store a product or of a product's stability under storage are issues that have to be addressed not only during the product development phase, but also when a product is on the market. Similarly, decisions must be made regarding the best form of packaging for a particular product. The requisite testing (for instance, to determine the decomposition products of the active substance or the matrix) is carried out primarily using physical and chemical analytical procedures. However, as the following example shows, physicochemical analytical techniques are not always sufficient. The diagram shows the permeation of estradiol from two transdermal patches.

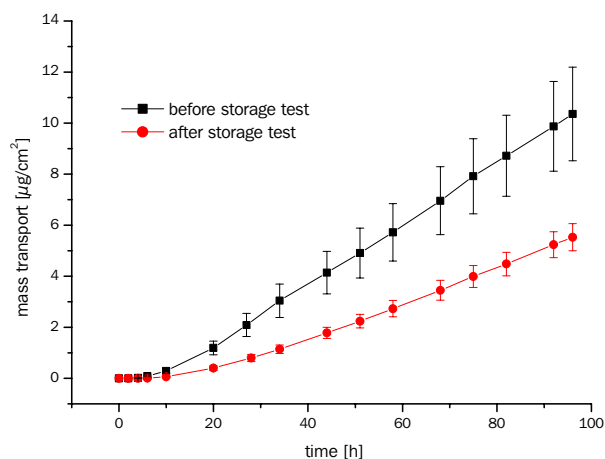


Abb. 15: Permeation of Estradiol from two patches. 96 h at 32 °C, n=3.

The measured mass transport rates are clearly different. Less active ingredient from the older patch permeates through the skin. Chemical investigations carried out previously showed no evidence of any decomposition products. The difference observed is not due to the decomposition of the active substance itself, but is attributable to a change in the matrix which causes the active ingredient to crystallize.

Research activities

Across Barriers GmbH is currently participating in the BMBF-funded joint research project "Validation study for testing skin penetration with the aid of biotechnologically prepared skin models". The project, which runs from September 1, 2002 until August 31, 2004, is a collaboration between the Free University Berlin; the Institute for Pharmaceutics (project head) at Saarland University; the Department

of Biopharmaceutics and Pharmaceutical Technology at the School of Veterinary Medicine, Hanover; the Institute for Pharmacology, Toxicology and Pharmacy of the Ludwig-Maximilian-University (LMU) in Munich; the Dermatology and Allergology Clinic and Out-Patient Unit at LMU; and the Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET) at the Federal Institute for Risk Research in Berlin

Excerpt from the Annual Report 2002 of the joint project (<http://userpage.fu-berlin.de/~msk/BMBF-Forschergruppe/Jahresbericht%202002.pdf>):

The aim of the project is to reduce the number of animal experiments used in the investigation of cutaneous penetration and permeation of xenobiotics by developing a standardized in vitro approach. The final goal is a validated alternative method based on commercially available, biotechnologically obtained human skin models that will be used for developing and testing industrial chemicals, crop protection agents and drug products. The method will significantly reduce or even replace the currently high numbers of animal experiments required up until now. A new OECD guideline on testing skin penetration and permeation by xenobiotics using artificial human skin surrogate is to be compiled.

The design of the project is based on the relevant recommendations issued by the OECD (Lodz, 1999; published online in December 2000). This means testing at least 10 test substances that differ considerably in their lipophilicity and their molecular weight. Mass balance analyses will be conducted as well as determinations of the intra- and interlaboratory variability of the data. In the first phase of the project, a generally applicable test procedure for penetration studies will be developed and prevalidated. In a second separate project phase, full validation as well as an in vitro/in vivo comparison will be performed.

Outlook

Other key research activities at Across Barriers GmbH include the dermal metabolism of drugs and innovative in vitro technologies for developing semi-solid dosage forms.

Guidance

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- OECD, Skin absorption: in vivo method. Test Guideline 427, 2002.
- COLIPA - Cosmetic Ingredients: Guidelines for Percutaneous Absorption/Penetration, 1995
- SCCNFP, Opinion concerning basic criteria for the in vitro assessment of percutaneous absorption of cosmetic ingredients- adopted by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers during the plenary session of 23 June 1999
- FDA Guidance SUPAC-SS: Nonsterile Semisolid Dosage Forms, Manufacturing Equipment Addendum, December 1998, 1722dft
- FDA Guidance, Nonsterile Semisolid Dosage Forms, Manufacturing, and Controls; in vitro Release Testing and in vivo Bioequivalence Documentation, May 1997, 1447fnl
- Test Guidelines for in vitro Assment of Dermal Absorption and Percutaneous Penetration of Cosmetic Ingredients, Diembeck et. al., Food and Chemical Toxicology 37 (1999), 191-205
- ECETOC, Percutaneous Absorption, Monograph No. 20, 1993

Selected reading

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