EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 7th plenary meeting

of 22 June 2010

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SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

As early as June 1999, before any OECD guideline on percutaneous absorption was available, important scientific articles on this methodology were published [Beck et al. 1995, Diembeck et al. 1999]. The SCCNFP discussed the scientific progress with external experts in the field and subsequently adopted its first "Basic criteria for the *in vitro* assessment of percutaneous absorption of cosmetic ingredients" [SCCNFP/0167/99]. The publication of such a set of criteria was seen as a pro-active action of the SCCNFP in order to support and speed up the introduction of *in vitro* dermal absorption studies of cosmetic ingredients in the evaluation of dossiers of cosmetic ingredients belonging to Annexes III, IV, VI or VII to Dir. 76/768/EEC. Moreover, the opinion did not only provide basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients, but also discussed some general principles and practical aspects of the methodology.

In December 2000, two draft OECD Guidelines on dermal absorption became available, namely 1) OECD Draft Guideline 427 on skin absorption: *in vivo* method [OECD 427], and 2) OECD Draft Guideline 428 on skin absorption: *in vitro* method [OECD 428]. The combination of the OECD-publication with gained practical experience, has led to the incorporation of the *in vitro* dermal absorption study in toxicological dossiers of cosmetic ingredients, plant protection products, biocides, etc [98/8/EC, 2000/6/EC, Sanco/222/2000].

Subsequently, in order to provide an up to date guidance for the application of the *in vitro* methodology for cosmetic ingredients, document SCCNFP/0167/99 was revised and updated in 2003 [SCCNFP/0750/03]. Existing documents such as the "Proposed rule for *in vitro* dermal absorption rate testing …" [US EPA 1999], the "Guidance document on dermal absorption" [Sanco/222/2000], the "Draft Guidance Document for the conduct of skin absorption studies" [OECD 2000], and the "Technical Guidance Document on Risk Assessment part 1" [ECB 2003] were taken into consideration.

The SCCP noticed later that several dossiers failed to fulfil the requirements as described in document SCCNFP/0750/03. In particular, a revision of all hair dye dossiers revealed a number of problems [Pauwels and Rogiers 2004]. An expert group was then established by SCCP with the purpose to update the "SCCP basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients" and to resolve the identified problems. On the basis of the recommendations of this expert group, the document "SCCP basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients" was updated in 2006 [SCCP/0970/06] and the chapter on dermal absorption in the SCCP Notes of Guidance, 6th revision was expanded.
Meanwhile, OECD Guideline 428 was finalized in 2004 and four years later, the protocol was copied into EC method B.45 [EU, 2008].
The application of the 2006 update of the "SCCP basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients" for the evaluation of the dermal absorption studies in dossiers submitted by the Industry, frequently resulted in disagreement on the number of skin samples and donors, concentrations to be tested, variability of the test results and the value to be used for the calculation of Margin of Safety (MoS). This led to non-acceptance of several submitted studies [Rogiers and Pauwels 2008].

Above-mentioned disagreements and the use of default values <100% for dermal absorption in the absence of appropriate experimental data, were discussed among experts from SCCP, COLIPA, as well as external experts in a "Special meeting on dermal absorption issues" in October 2008. The conclusions of this meeting are incorporated in the present updated version of the "SCCP basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients" and will be included in the next revision of the SCCS Notes of Guidance.

The dermal absorption of nanoparticles is not covered by this opinion.

2. GENERAL PRINCIPLES

Definitions

The definitions with respect to dermal absorption slightly diverge in different official documents. Hereunder, the definitions as proposed by the WHO [WHO 2005] are given:

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

The purpose of dermal absorption studies of cosmetic ingredients is to obtain qualitative and/or quantitative information on the amounts that may enter, under in-use conditions, into the systemic compartment of the human body. These quantities can then be taken into consideration to calculate the margin of safety using the NOAEL of an appropriate repeated dose toxicity study with the respective substance.

Justification for the use of *in vitro* dermal absorption studies on isolated skin is based on the fact that the epidermis, in particular the stratum corneum, forms the principal *in vivo* barrier of the skin against penetration and uptake of xenobiotics in the body.

Under *in vivo* conditions, the microcirculatory system (blood and lymph vessels) may carry compounds from the dermis into the central compartment (resorption). *In vitro*, the microcirculation is compromised, thus the potential resorption of a compound cannot be adequately studied in such a setting.

The dermal tissue may retain penetrating compounds that, *in vivo*, would have been removed into the systemic compartment. Thus, either the dermis must be removed prior to *in vitro* investigations (so-called split-thickness skin) or possible *in vitro* retention in the dermis and living epidermis (without stratum corneum) must be taken into account when interpreting the *in vitro* results.

The epidermis renews by continuous outward proliferation, differentiation and desquamation. About one layer of corneocytes is shed off per day. After topical application, xenobiotics detected *in vitro* in the skin, particularly in the stratum corneum and the pilosebaceous units, might *in vivo* have been lost from the skin via desquamation or sebum secretion, respectively. Because these processes are not present *in vitro*, the final epidermal (stratum corneum) levels *in vitro* could be elevated compared with the corresponding *in vivo* levels. Therefore, although the EU B.45 method defines the 'absorbable dose' as the *amount present on or in the skin following washing*, the amount of substance present in the stratum corneum is not considered systemically available.

According to the above principles, the following should be included in the protocol for *in vitro* dermal absorption studies:

i. Studies should be performed on appropriate standardised skin preparations. The respective choice should be justified in the protocol. The WHO recommends human skin as the gold standard.

ii. At the end of the experiment, a full mass balance should be performed.

iii. When considerable cutaneous metabolism of the test compound occurs *in vivo*, further studies may be necessary. It should be noticed that frozen skin preparations may lack the enzyme systems for biotransformation of the test compound and may not provide an accurate picture of the formation of metabolites and their dermal absorption. Therefore *in vitro* studies using frozen skin may not provide complete information on the dermal absorption of compounds that undergo biotransformation in the skin nor on their potential metabolite(s) formed. The role of cutaneous biotransformation in the absorption process is still a matter of scientific debate.

iv. Sometimes an irreversible binding of an ingredient to the epidermis may occur, followed by elimination through *in vivo* desquamation of the skin surface. When this mechanism is assumed, it must be documented by separate experiments.

3. PRINCIPLE OF THE TEST

At present only skin preparations of natural origin may be used. Although the quality of cultured or reconstituted skin has improved importantly during the last years [Bouwstra et al. 2008], these cultures do not possess a complete barrier function comparable to that of living skin.

OECD Guideline 428 / EC B.45 should be followed as close as possible, taking into account the guidance given here. Any deviation from this guideline should be documented and justified by appropriate scientific argumentation.

The test substance should be applied in an appropriate formulation on the skin sample which is placed in a diffusion cell (cf. 4.1). The skin is then positioned between the upper and lower chambers of the cell.

Diffusion cells may be of static or flow-through design. The integrity of the barrier should be checked by an appropriate method. The test sample should remain in contact with the skin on the donor side for a defined period of time, corresponding to the typical use of the cosmetic end product, such as leave-on or rinse-off conditions. The receptor fluid should be sampled at an early time point (e.g. after 30 minutes), at the end of the experiment and at appropriate time points in between in order to obtain an absorption-time profile. The skin and/or fluid samples should be analysed by appropriate and validated analytical methods, such as liquid scintillation counting, HPLC, GC or other suitable methods. Information on the sensitivity and repeatability / time-different intermediate precision of the analytical method(s) should be provided.

4. METHODOLOGY AND FACTORS AFFECTING DERMAL ABSOROPTION

Dermal absorption can be affected by several factors: e.g. physical and chemical properties of the substance, type and composition of the formulation, occlusion, concentration of the substance in the formulation, exposure pattern, skin site of the body and technical aspects of the respective *in vitro* test [Howes et al. 1996, Schaefer and Redelmeier 1996, ECETOC 1993].

In the following section, an overview is given of factors that may affect dermal absorption in *in vitro* dermal absorption studies.

4.1. Diffusion cell design

The diffusion cell consists of an upper donor and a lower receptor chamber, separated by the skin preparation under investigation. The stratum corneum faces the donor chamber. Diffusion cells should consist of inert non-adsorbing material. Temperature control of the receptor fluid is crucial throughout the experiment. The skin surface temperature in the diffusion cell should be kept at the *in vivo* skin temperature of 32 ± 1°C. Additional dermal absorption studies may be required in some specific cases, e.g. when substance exposure at a higher skin temperature may be expected. The advantage of using a flow-through system is continuous sampling; the advantage of using a static system is the increase in sensitivity for test substances only poorly penetrating the skin.

4.2. Receptor fluid

The composition of the receptor fluid is chosen so that it does not limit the extent of diffusion of the test substance, i.e. the solubility and the stability in the receptor fluid of the chemical under investigation have to be guaranteed. Saline or buffered saline solutions are commonly used for hydrophilic compounds. For lipophilic molecules, serum albumin or appropriate solubilisers / emulsifiers are added in amounts which must not interfere with membrane integrity. The fluid should not interfere with the analytical procedure.

As a general rule, the receptor fluid should have a physiological pH. Any deviation from this principle should be justified. E.g. in the case of 50/50 ethanol/water, as proposed in the OECD Guideline [OECD 2004], evidence should be included in the dossier, showing that this does not significantly affect the integrity of the skin.

In order to avoid serial non-detects at early sample points, the receptor fluid volume should be kept to a minimum.

The receptor fluid, preferably degassed in order to avoid formation of air bubbles during the experiment, should be thoroughly stirred (static cells) or continuously replaced (flow-through cells) during the entire experiment. The choice of static or flow-through conditions in the receptor cell should be made on a compound-by-compound basis, depending on its theoretical absorption properties and the objective of the study. The choice of the test system should be justified in the study report. The amount of penetrated substance in the receptor fluid should not exceed 10% of its saturation level at any time, in order to minimise interference with the free diffusion process that could produce an underestimation of dermal absorption. The substance should be stable in the receptor fluid for the duration of the *in vitro* test and the subsequent analysis.

4.3. Skin preparations

Human skin is the best choice but is not always readily available. Alternatively, pig skin may be used because it shares essential permeation characteristics with human skin. Rat skin is 2 to 10 times more permeable than human skin [Ross et al. 2000] and it is therefore not recommended.
The use of cultured or reconstructed human skin models is under development and those systems are not yet advised for *in vitro* testing on the basis of their insufficient barrier function [Bouwstra et al. 2008].

The following information is required:

- Origin of skin samples used.
- Species: by preference human or pig skin should be used.
- Skin location: abdomen, leg or breast (human skin); abdomen, breast, back, flanks or ears (pig skin).
- Gender and age: although these factors are not believed to be important variables, they should be stated.
- Fresh/frozen skin: when significant biotransformation of the test compound in the skin is expected, freshly excised, viable skin should be used [Diembeck et al. 1999].
- Details on preservation and storage conditions of the skin should be specified (e.g. skin can be stored in aluminium foil at -20°C or lower) [Howes et al. 1996, Bronaugh et al. 1986]. During transport skin samples should be kept at or below 4°C.
- Numbers of skin samples and donors (see also point 4.13.)

Skin samples that may be used are split-thickness (200-500 µm) or full-thickness (500-1000 µm) skin preparations [Sanco/222/2000]. Dermatomed skin is often used. Skin thickness should be measured by an appropriate method, which should be described in the report. The skin samples should be prepared to fit the experimental cell.

- For human skin: split-thickness skin should be the general rule. If for a specific reason, full-thickness is required, this should be justified.
- For pig skin: since it is technically more difficult to obtain intact split-thickness skin, this could justify the use of full-thickness skin.

When epidermal membranes are used for the *in vitro* dermal absorption study, the reason for this should be justified. Epidermal membranes are sometimes quite fragile and some mass balance techniques (e.g. tape stripping) cannot be applied to this model. It must also be mentioned that epidermal membranes may overestimate human *in vivo* skin absorption [Van de Sandt et al. 2000].

The minimum skin area to be covered is considered to be 0.64 cm^2.

4.4. Skin integrity

Barrier integrity is crucial for the experiment, and must therefore be measured. This is achieved by either measuring the penetration of a marker molecule, e.g. tritiated water, caffeine or sucrose, or by physical methods, such as determination of TEWL (Transepidermal Water Loss) or TER (Transcutaneous Electrical Resistance). Data obtained should be reported.

4.5. Skin temperature

Because the rate and extent of skin absorption is temperature-dependent, the skin disc temperature should be maintained constant (32 ± 1°C, corresponding to the normal human skin surface temperature). The method of temperature maintenance should be described in the report.

4.6. Test substance

The relevant physical and chemical data (e.g. MW, log P_{ow}, solubility, stability, and pK_a of the test substance) should be given.

The purity of the test substance should be described and should be comparable to that of the substance in marketed products (see 4.10). It is recognised that synthesis of radio-

labelled (i.e. ^{14}C- or ^{3}H-labelled) substances may result in a slightly different purity and/or impurity profile than that of substances produced by large-scale chemical production. These differences should be reported.

As mentioned under 4.2, the solubility and stability of the test substance in the receptor fluid for the entire test duration should be documented.

4.7. Preparation of the dose and vehicle / formulation

The dose and vehicle / formulation should be representative for the in use condition(s) of the finished cosmetic product. The quantitative composition of every formulation used during the experiment should be given.

For oxidative hair dyes, the relevant combination(s) of hair dye precursor(s), coupler(s) and developer(s) should be tested.

Several concentrations, including the highest concentration of the test substance in a typical formulation should be studied.. These concentrations should be selected in such a way that the range of the linear curve of concentration versus dermal absorption is demonstrated.

The stability of the test substance under the foreseeable conditions of application and usage must be ascertained.

4.8. Dose and volume of test substance

The dose of the test formulation as well as its contact time (exposure) with the skin should resemble use conditions. The amount of the formulation to be applied should be adapted to the consumer use/technical conditions. The values to be used are described in the Notes of Guidance: 2-5 mg/cm^2 for solids and semi-solid preparations, and up to 10 µl/cm^2 for liquids. For oxidative hair dye formulations, 20 mg/cm^2 is applied. Deviations should be explained. The volume of formulation used should be appropriate to spread the sample homogeneously over the skin surface. This depends on the viscosity and lipophilicity of the formulation. Both mass and volume applied should be stated in the test report.

4.9. Study period and sampling

The exposure time and sampling period(s) should be defined in the protocol. The normal exposure time is 24 hours with regular sampling intervals. Longer duration of the study may result in membrane deterioration and requires careful control of membrane integrity. The exposure time should be consistent with the intended use of the cosmetic formulation. E.g. for oxidative hair dye formulations, the time of contact could vary according to the in-market use. The SCCS recommends at least 30 minutes contact in dermal absorption testing. The skin surface will then be rinsed using a procedure mimicking the consumer situation. Sampling of the receptor fluid is continued until e.g. 24 hours.

The frequency of sampling should be chosen adequately to allow the determination of the extent/rate of absorption and the absorption profile. In order to estimate absorption kinetics, samples should be obtained from at least 6 post-application time points, including one early time point (30 minutes). For rinse-off products, measurements after rinsing have to be taken. The full sampling procedure must be described in the report.

4.10. Analytical methods

Appropriate analytical techniques, e.g. liquid scintillation counting, HPLC or GC, should be used. Their validity, sensitivity and detection limits should be documented in the report. When high sensitivity is required, the test substance should, whenever possible, be radio-labelled.

Qualitative or semi-quantitative methods, such as micro-autoradiography, may be useful tools for skin distribution assessments.

4.11. Data collection

The test compound must be determined in the following compartments:

- Product excess on the skin (dislodgeable dose)
- Stratum corneum (e.g. adhesive tape strips)
- Living epidermis (without stratum corneum)
- Dermis
- Receptor fluid

To calculate the mass balance correctly, it is also necessary to measure the amounts of test substance adsorbed to the equipment (included in rinsing solutions and/or compartments).

4.12. Mass balance analysis / recovery

The mass balance of the applied dose must be determined.

The overall recovery of test substance (including metabolites) should be within the range of 85-115%. Lower or higher recovery rates should be investigated and/or explained.

4.13. Variability / validity / reproducibility

The technical ability of the performing laboratory and the validity of the method used should be assessed at regular intervals, at least twice per year, by using reference compounds like caffeine or benzoic acid. These data should be included in the study report [OECD 2004, Van de Sandt et al. 2004].

Factors that affect the variability in the dermal absorption of a test substance are:

- Inter-individual and intra-individual characteristics of the stratum corneum barrier,
- The variation in various parameters, such as skin temperature, skin thickness, vehicle, concentration of applied substance, amount of applied formulation, and exposure duration
- The use of static or flow through cell system
- The uncertainty of the measurement of the test substance.

During a meeting with industry in October 2008, the variability issue was discussed in detail, especially in view of the outcome to be used for further calculations and in view of the number of required skin samples. Based on this discussion, the following decision was made by the SCCS: for a reliable dermal absorption study, 8 skin samples from at least 4 donors should be used.

When studies correspond to all of the basic requirements of the SCCS, the **mean + 1SD** will be used for the calculation of the MoS. The reason for not using the mean *per se* is the frequently observed high variability in the *in vitro* dermal absorption assays. Moreover, the method was validated based upon practical experience only and did not go through the elaborated validation process as we know it today.

In case of significant deviations from the protocol and/or very high variability, the **mean + 2SD** will be used as dermal absorption for the calculation of the margin of safety.

5. CONCLUSION

Dermal absorption can be expressed as an absolute amount [µg/cm^2 of skin surface] and/or as a percentage of the amount of test substance contained in the intended dose applied per square centimetre of skin surface.

In a classical *in vitro* dermal absorption setting, the amounts of penetrated substance(s) found in the receptor fluid are considered to be systemically available. Both the epidermis (except for the stratum corneum) and dermis are considered as a sink, wherefore the amounts found in these tissues are considered as absorbed and are added to those found in the receptor fluid. The amounts that are retained by the stratum corneum at the time of sampling are not considered to be dermally absorbed, and thus they are not expected to contribute to the systemic dose.

The absorption rate and mass balance should be calculated separately for each diffusion cell. Considering skin samples as the main contributor of the variability in results of a dermal absorption study, the mean and SD of the dermal absorption rate should be calculated from at least 8 evaluable results representative of skin from at least 4 donors. All measurements, statistical processing and obtained kinetic curves should be provided.

The SCCS considers the following criteria as critical for the decision if a study fulfils the requirements:

- the availability of 8 evaluable samples originating from 4 donors (for studies performed previous to this decision, 3 donors may be accepted);
- a mass balance of the applied dose which shows to be ≥ 85%;
- a clear mention of the relative standard deviation (RSD or CV) of the measured dermal absorption rate;
- thorough consideration of factors 4.1 to 4.11 as described in the current opinion.

When studies correspond to all of the basic requirements of the SCCS, the **mean + 1SD** will be used for the calculation of the MoS. The reason for not using the mean *per se* is the frequently observed high variability in the *in vitro* dermal absorption assays. Moreover, the method was validated based upon practical experience only and did not go through the elaborated validation process as we know it today.

In case of significant deviations from the protocol and/or very high variability, the **mean + 2SD** will be used as dermal absorption for the calculation of the margin of safety.

In case the results are derived from an inadequate *in vitro* study, **100%** dermal absorption is used. However, in case MW > 500 Da and log P_{ow} is smaller than -1 or higher than 4, the value of **10%** dermal absorption is considered [ECHA 2008].

6. REFERENCES

2000/6/EC - Twenty-fourth Commission Directive 2000/6/EC of 29 February 2000 adapting to technical progress Annexes II, III, VI and VII to Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. *Official Journal L 56, 01/03/2000 p.42.*

Beck H., Benech-Kieffer F., Bracher M., Diembeck W., Dupuis J., Lovell W., Steiling W. Cosmetic ingredients : Guidelines for percutaneous absorption / penetration.COLIPA Guidelines, 1-36 (1995).

Bouwstra J.A., Groenink H.W., Kempenaar J.A., Romeijn S.G., Ponec M. Water distribution and natural moisturizer factor content in human skin equivalents are regulated by environmental relative humidity. Journal of Investigative Dermatology 128(2):378-88 (2008).

Bronaugh R.L., Stewart R., Simon M. Methods for *in vitro* percutaneous absorption studies VII : use of excised human skin. Journal of Pharmaceutical Sciences 75, 1094-1097 (1986).

Diembeck W., Beck H., Benech-Kieffer F., Courtellemont P., Dupuis J., Lovell W., Paye M., Spengler J. and Steiling W. Test guidelines for *in vitro* assessment of dermal absorption and percutaneous penetration of cosmetic ingredients. Food & Chemical Tox., 37, 191-205 (1999).

Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market *Official Journal L 123, 24/4/98 p. 1.*

ECB (European Chemicals Bureau) Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Doc. EUR 20418 EN/1, European Communities (2003).

ECHA 2008. Guidance on information requirements and chemical safety assessment. Chapter R.7c: Endpoint specific guidance. Available through: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7c_en.pdf?vers=20_08_08 (consulted June 2010).

EU B.45 - Skin absorption - *In vitro* method. Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L142, 31 May 2008, 438-443 (2008).

ECETOC Percutaneous absorption. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Monograph No 20, Brussels (1993).

Howes D., Guy R., Hadgraft J., Heylings J., Hoeck U., Kemper F., Maibach H., Marty J-P., Merk H., Parra J., Rekkas D., Rondelli I., Schaefer H., Täuber U. and Verbiese N. Methods for assessing percutaneous absorption, ECVAM Workshop Report n. 13. Alternatives To Laboratory Animals 24, 81-106 (1996).

OECD 427 - OECD Guideline for testing of chemicals - Guideline 427 : Skin absorption: *In vivo* method Organization for Economic Cooperation and Development, Paris, adopted 13 April 2004.

OECD 428 - OECD Guideline for testing of chemicals - Guideline 428 : Skin absorption: *In vitro* method Organization for Economic Cooperation and Development, Paris, adopted 13 April 2004.

OECD Guidance Document for the Conduct of Skin Absorption Studies. Organization for Economic Cooperation and Development (OECD), Environment Directorate, OECD Environmental Health and Safety Publications, Series on Testing and Assessment No. 28, Paris (2004).

Pauwels M. and Rogiers V. How to improve the quality of a European cosmetic ingredients' dossier? Oral presentation given during the Scientific Forum of the COLIPA (The European Cosmetic Toiletry and Perfume Association) General Assembly 2004, Amsterdam, 24/06/2004.

Rogiers V., Pauwels M. (Eds.), 2008. Current Problems in Dermatology 36: Safety Assessment of Cosmetics in Europe. S. Karger, Basel, Switzerland. ISBN 978-3-8055-8655-9.

Ross J.H., Dong M.H. and Krieger M.I. Conservatism in pesticide exposure assessment. Regulatory Toxicology and Pharmacology 31, 53-58 (2000).

Sanco/222/2000 – Guidance Document on Dermal Absorption European Commission, Health and Consumer Protection Directorate-General Doc. Sanco/222/2000 rev 6, of 27 November 2002.

SCCNFP/0167/99, Final: Basic Criteria for the *in vitro* assessment of percutaneous absorption of cosmetic ingredients, *adopted by the SCCNFP during the 8th plenary meeting of 23 June 1999.*

SCCNFP/0690/03, Final: Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation, *adopted by the SCCNFP during the 25th plenary meeting of 20 October 2003*.

SCCNFP/0750/03, Final : Basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients, updated October 2003, *adopted by the SCCNFP during the 25th plenary meeting of 20 October 2003.*

Schaefer H. and Redelmeier T.E. Skin Barrier, Principles of Percutaneous Absorption, Karger, Basel (1996).

US EPA (United States Environmental Protection Agency) Proposed rule for *in vitro* dermal absorption rate testing of certain chemicals of interest to occupational safety and health administration. Federal Register, Volume 64, Number 110, June 9 (1999).

Van de Sandt J.J.M., Meuling W.J.A., Elliott G.R., Cnubben N.H.P. and Hakkert B.C. Comparative *in vitro – in vivo* percutaneous absorption of the pesticide propoxur. Toxicological Sciences 58, 15-22 (2000).

Van de Sandt J.J.M., vanBurgsteden J.A., Carmichael P.L., Dick I., Kenyon S., Korinth G., Larese F., Limasset J.C., Maas W.J.M., Montomoli L., Nielsen J.B., Payan J.-P., Robinson E., Sartorelli P., Schaller K.H., Wilkinson S.C., Williams F.M. In vitro predictions of skin absorption of caffeine, testosterone, and benzoic acid: a multi-centre comparison study. Regul Toxicol Pharmacol 39, 271-281 (2004).

WHO (World Health Organisation) Kielhorn J., Melching-Kollmu□ S., Mangelsdorf I. Dermal Absorption. WHO / IPCS Environmental Health Criteria, Draft February 2005, accessible through http://www.who.int/ipcs/methods/dermal_absorption/en/ (consulted Dec 2005).