- Ingredients of variable composition

A. Ingredients of Mineral origin

Depending on the type of ingredient under consideration and the extent to which it is modified, full identification particulars should be considered in the safety assessment. The following are given as examples

* Starting material
* Description of

The preparation process
- physical processing (e.g. destructive distillation)
- chemical modifications
- possible purification

Characteristic elements of the composition
- characteristic components
- toxic components (with percentage)

* Physical and chemical specifications
* Microbiological quality

B. Ingredients of Animal origin

Depending on the type of ingredient under consideration and the extent to which it is modified, full identification particulars should be considered in the safety assessment. The following are given as examples

* Species (bovine, ovine, crustacean, etc.)
* Organs, tissues or liquids (placenta, serum, cartilage, etc.)
* Country of origin[1]
* Description of

The preparation process
- conditions of extraction (solvent, pH, temperature, etc.)
- type of hydrolysis (acid, enzyme, etc.)
- other chemical modifications
- possible purification

Commercial form
- powder product
- product in solution (solvent and concentration)
- freeze-dried, etc.

Characteristic elements of the composition
- characteristic amino-acids
- total nitrogen
- polysaccharides
- molecular mass

* Physico-chemical specifications
* Microbiological quality including viral contamination

[1] Controls on this point must be limited to legitimate control aspects related to health and avoid any discriminating effect as to the use of certain ingredients. (DG XXIV, Unit 01: Legal matters)

* Xenobiotic contamination

C. Ingredients of botanical origin

Depending on the type of ingredient under consideration and the extent to which it is modified, full identification particulars should be considered in the safety assessment. The following are given as examples

* Botanical name and family (Linné system)
* Part of the plant processed
* Description of:

The preparation process

- extraction
- distillation
- destructive distillation (e.g. wood tars)
- possible purification

Commercial form

- powder product
- product in solution (solvent and concentration)

Characteristic elements of the composition

- characteristic components
- toxic components (with percentage)

* Physical and chemical specifications
* Microbiological quality including fungi
* Xenobiotic contamination

D. Special ingredients derived from biotechnology

For special biotechnologically derived ingredients, where a modified microorganism or a potential toxin has not been fully removed, specific data must be available, which can comprise:

* description of organisms

- donor organism
- recipient organism
- modified microorganism

* host pathogenicity
* pathogenicity of the modified organism
* toxicity and, when possible, identity of metabolites (toxins)produced by the organism
* fate of viable organism in the environment - survival - potential for transfer of characteristics to e.g. natural bacteria
* physico-chemical specifications
* microbiological quality
* xenobiotic contamination

E. Commercial addition mixtures

Any ingredient, according to INCI name when available, entering the composition of commercial mixtures supplied as "raw materials" must be given in the qualitative and quantitative formula of the finished product. The following are given as examples:

* main component(s)
* preservatives
* antioxidants
* buffering agents
* solvents
* other additives

2. Transparency of the assessment of the safety for human health of the finished cosmetic product

Each cosmetic finished product is an individual and unique combination of ingredients. The number of finished products is extremely large by comparison with the number of cosmetic ingredients.

In general, the safety evaluation of the finished product can be obtained by ascertaining the toxicity of the cosmetic ingredients (Council Directive 93/35/EEC). Toxicity information on the ingredients should include evaluation of the most relevant toxicological endpoints.

However, in some cases as, for instance, when the metrics used in the finished product are different from the solvents employed in the toxicity studies of the ingredients and are likely to increase considerably the *penetration* or the *irritancy* of some of the ingredients, additional information on finished products may be needed in the interests of better safety assessment.

If there may be potentiation of the toxic effects of the ingredients, or if toxic effects may result from chemical interaction between individual ingredients, specific toxicological information on the finished products should be considered. Conversely, as indicated previously, any claim of decreased absorption or potential hazard of some ingredient, due to the formulation, should be supported by adequate information.

When the combination of ingredients present in the finished product renders highly probable the formation of a new substance of toxicological concern, additional toxicological information on the finished product may be needed.

2.1 Toxicological profile of the ingredients
(Dir. 93/35/EEC Art. 7a(1)(d))

The safety assessor must take account of all the toxicological data available for each ingredient in the final product, including those of (natural) biological origin.
The data sources should be indicated in each case:

Toxicological data:
- should be available in an appropriate form in the safety assessment,
- may be obtained:

* from tests on animals or recognised/validated alternative test methods. Whenever data on clinical human observations are available, they are to be included;
* from specific toxicological studies or from studies conducted for other regulatory purposes;

* from the raw material suppliers and supplemented by data available to the person responsible for safety assessment through databases or published literature;

– must permit determination of the possible toxic effect(s), including the allergenic potential of all ingredients, including those of biological origin.

2.2 Assessment of the safety of the finished product

Details of the scientific reasoning adopted by the safety assessor must be set out in the safety assessment. This should cover all intended and likely routes of human exposure during use.

All toxicological data available on the formulation and its ingredients, both favourable and unfavourable, are taken into account, including an assessment of the potential for chemical or biological interaction of/in the formulated product.

The safety assessor must clearly set out the specific reasons for his conclusions taking into account the acceptability of the inclusion in the formulation of particular ingredients which may have a low safety threshold.

2.3 Qualifications of the safety assessor.

(Directive 93/35/EEC Art. 7a, (1) (e))

The curriculum vitae of the safety assessor referred to in the Directive must be included in the dossier.

The safety assessor may be an external consultant. If the safety assessors are employed by the manufacturer, they must have no connection with production or marketing.. As well as having the requisite training, they must also provide evidence of relevant experience in the fields of toxicology.

3. Fragrances

According to the Code of Practice of the fragrance industry:

"Fragrance manufacturers should provide customers with all available information to ensure that fragrance materials are used in accordance with standards of good practice"

In its guidelines for communicating the IFRA status of a fragrance compound[2], IFRA (International Fragrance Association) notably recommends considering:
– *"a statement that the fragrance complies with the IFRA guidelines for the application mentioned and concentration used",*
– *" a reference to the bases of the IFRA guidelines, RIFM (Research Institute for Fragrance Materials) data and other available sources"....*

Without questioning the principle of intellectual property underlying the derogation concerning the qualitative and quantitative formula of fragrance compounds [dir. 93/35/EEC, art. 7a, 1(a)], **several measures should be considered with a view to**

[2] A fragrance compound is a blend of fragrance ingredients representing a specific formula.

provide something more than a safety guarantee of a purely legal nature and to inform the person responsible for safety with regard to human health.

3.1 Certificate of conformity

The existing certificate of conformity with IFRA standards attached to a fragrance compound should be systematically supplemented by

* a semi-quantitative declaration which also highlights those fragrance ingredients which have been subject to restrictions in the IFRA code of practice and, in a more general way, those which have an established potential to cause contact sensitisation and (or) phototoxic reactions (e.g.. F.I. listed in the European Standard Fragrance Mix)

e.g. Essential oil from the bark of *Cinnamonum zeylanicum* < 1%
Evernia prunastri concrete (oak moss extract) < 3%
Methyl heptine carbonate < 0,01%
Geraniol ....%
Eugenol .... %
Amyl cinnamal ....%
Isoeugenol.....%
Hydroxycitronellal....%
Cinnamal.....%
Cinnamyl alcohol.....%

* an indication of the cosmetic product types in which it may be used

While safeguarding the formula's confidentiality, the safety assessment should be confirmed for the fragrance compound considered as a whole, and the data and the scientific reasoning should be included in the conformity certificate.

3.2 Safety assessment of perfumed cosmetics

The exact concentration of the perfume composition (= fragrance compound) in the cosmetic product should be indicated [art. 7a,1,(a) Dir. 93/35/EEC].

In the safety assessment of the cosmetic product for human health (art 7a, I (d), Dir. 93/35/EEC),

* reference should be made to semi-quantitative formula of the fragrance compound naming the fragrance ingredients declared in the certificate of conformity (see 3.1 above) and consideration should be taken to their toxic potential.
* reference should be made to the safety assessment of the fragrance compound considered as a whole.

ANNEX 8 – GUIDELINES ON MICROBIOLOGICAL QUALITY OF THE FINISHED COSMETIC PRODUCT *

1. Preamble.

Skin and mucous membranes are normally protected from microbial attack by a natural mechanical barrier and defence mechanisms. However, protective integuments may be damaged and slight trauma may be caused by the action of some cosmetics that may enhance microbial infection. These situations may be of particular concern when cosmetics are used in the eye area or on mucous membranes or on damaged skin and when used by children under 3 years, elderly people and people showing compromised immune responses. These are the reasons to define two separate categories of cosmetic products in the microbiological quality control limits.

Although a very low number of cases of contamination in cosmetics leading to microbial infections have been reported, it is likely that under-reported clinical microbiological problems (for instance infectious folliculites) associated to the use of contaminated cosmetics are recognised by several dermatologists (to be reported in a separate document). On the other hand microbial contamination may spoil cosmetic products or reduce the intended quality. These statements make it necessary to carry out routine microbiological control of cosmetics, in order to ensure their quality and the safety for customers to use.

2. Categories of cosmetics in microbiological quality control.

In relation with the microbiological quality control, two categories of cosmetics are defined.

Category 1: Products specifically intended for children under 3 years, eye area and mucous membranes.

Category 2: Other products.

3. Quantitative limits.

The limit for cosmetics classified in Category 1 is: total viable count for aerobic mesophyllic micro-organisms not more than 10^2 cfu/g or ml in 0.5 g or ml of the product. The limit for cosmetics classified in Category 2 is: total viable count for aerobic mesophyllic micro-organisms not more than 10^3 cfu/g or ml in 0.1 g or ml of the product.

* Adopted by the SCCFNP at its plenary meeting 23rd September, 1998 (SCCNFP/0004/98 Final).

4. Qualitative limits.

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Candida albicans* are considered the main potential pathogens in cosmetic products. These specified potential pathogens must not be detectable in 0.5 g or ml of the cosmetic product in cosmetics of Category 1 and in 0.1 g or ml in cosmetics of Category 2.

5. Product preservation.

Microbial contaminants have two origins: during production and filling, and during the use of the cosmetic by the customer. From the moment in which the cosmetic unit is opened until the consumer finishes the product, there is a permanent, variable and additive microbial contamination of the cosmetic caused by the domestic environment and the consumer's body (hands and body skin). The reasons for the need of microbial preservation in cosmetics are the following:

5.1. To ensure the microbial safety of cosmetics for customers to use.
5.2. To maintain the quality and specifications intended for the product.
5.3. To confirm hygienic and high-quality handling.

6. The challenge testing

The efficacy of the preservation has to be assessed experimentally during the development process to ensure microbial stability and preservation by challenge testing. Challenge testing is mandatory for all those products that in normal conditions of storage and use, a risk of infection for the consumer or a deterioration of the product exist. The challenge test consists of an artificial contamination of the finished product and a posterior evaluation of the decrease of this contamination to levels ensuring the microbial limits established in products of Category 1 and 2.

The micro-organisms used in the challenge test will be issued from official collection strains from any state in the EU to ensure reproducibility of the test and will be: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans.* Additional bacteria and fungi might be used for additional specific purposes of the challenge testing. The microcidal activity of preservatives or any other compound in the finished cosmetic must be ruled out in the challenge test by dilution, filtration, neutralisers or any other means. The experimental performance of the microbial controls and the challenge tests must be laid down and validated by a microbiologist.

7. Good Manufacturing Practice.

In order to accomplish with the Good Manufacturing Practices and Microbial Quality Management, manufacturers of cosmetics have to define and follow specific cleaning, sanitation and control procedures to keep appropriately clean and free of micro-organisms that could be harmful for the consumers or adverse for the quality of the cosmetics. These proceedings will include procedures to microbiology control raw materials, bulk and finished products, packaging components, personnel, equipment and locals.

ANNEX 9 – GUIDELINES FOR *IN VITRO* METHODS TO ASSESS SKIN CORROSIVITY IN THE SAFETY EVALUATION OF COSMETIC INGREDIENTS OR MIXTURES OF INGREDIENTS*

Terms of Reference

Two *in vitro* methods developed to assess skin corrosivity of chemicals, the "Rat skin Trancutaneous Electrical Resistance (TER)test" and the "EPISKIN test" have been validated by ESAC (ECVAM Scientific Advisory Committee).

The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has been requested by DG III to advise the Commission on the applicability of the methods to the safety assessment of chemicals used as cosmetic ingredients.

1- Background

The European Centre for the Validation of Alternative Methods (ECVAM) has conducted in 1996-1997 a validation study of *in vitro* tests developed to assess skin corrosivity of chemicals. This study was a follow-up to a pre-validation study of tests developed for replacing the *in vivo* Draize skin corrosivity test in rabbits.

The main objectives of the validation study, as defined by the sponsors and the management team before the study began, were :
(a) to identify tests capable of discriminating corrosives (C) from non corrosive (NC) for selected groups of chemicals (e.g. organic acids, phenols) and/or all chemicals (single chemical entities only);
(b) to determine whether the tests could identify correctly known R35 (UN packing group I) and R34 (UN packing groups II & III) chemicals.

2- Organisation of the study

The study was coordinated from ECVAM. A Management Team (MT) was constituted by four representatives of « lead laboratories », each of them being responsible for one of the four tests being evaluated.

The tests selected for inclusion in the validation study were the rat transcutaneous electrical resistance (TER) test, Corrositex™, the Skin^{2TM} ZK1350 corrosivity test, and Episkin™. Each test was conducted in three different laboratories, according to principles, criteria and procedures previously defined by ECVAM. Prediction models for each of the four tests were defined in the test protocols.

* Adopted by the plenary meeting of the SCCNFP of 25 November 1998 (SCCNFP/0070/98 Final)

Coordination /MT /Laboratories

Sixty chemicals were selected by an independent Chemicals Selection Sub-Committee, and distributed coded to the participating laboratories. These included organic acids (6C/5NC), organic bases (7C/3NC), neutral organics (9NC), phenols (2C/3NC), inorganic acids (6C/1NC), inorganic bases (2C/2NC), inorganic salts (1C/2NC), electrophiles (3C/5NC), and soaps/surfactants (3NC). The selection is fully described in a publication (Ref. 1); the main criterion for including chemicals in the test set was that the corrosivity classifications were based on unequivocal animal data.

The results obtained were analysed by statistician experts. The classifications of the corrosivity potential of the test chemicals, as derived from the *in vitro* data obtained in the three laboratories conducting the test, were compared to the *in vivo* classifications independently assigned to the chemicals before the blind trial, to yield sensitivity, specificity, predictivity and accuracy of the test.

3- Main results

The full details of the validation study have been published (Ref. 2). Two tests, with a good reproductibility within and between test laboratories, proved applicable to the testing of a diverse group of chemicals : the TER test and Episkin.

In the TER test, test materials are applied for 2 to 24 hours to the epidermal surface of skin discs taken from the pelts of humanely killed young rats, and corrosive chemicals are identified by their ability to produce a loss of normal stratum corneum integrity, which is measured as a reduction of the inherent transcutaneous electrical resistance (below a predetermined threshold level).

Episkin is a tri-dimensional human skin model with a reconstructed epidermis and a functional stratum corneum. When utilised in corrosivity testing, application of test chemicals to the surface of the skin for 3, 60 and 240 min, is followed by an assessment of cell viability.

Sensitivity, specificity, predictivity and accuracy in distinguishing corrosive from non corrosive chemicals were very high for both tests: 88, 72, 72, 79 and 83, 80, 77, 81 % respectively for the TER test and Episkin. In addition, Episkin was also able to distinguish between known R35 (UN packing group I) and R34 (UN packing groups II & III) chemicals

4- Opinion of the SCCNFP

ECVAM Scientific Advisory Committee (ESAC), which had been fully informed of the progression of the validation procedure, reviewed the final results and unanimously endorsed a statement that the rat skin TER test is scientifically validated for use as a replacement for the animal test for distinguishing between corrosive and non corrosive chemicals, and that Episkin is scientifically validated as a replacement for the animal test, and that these tests are ready for regulatory acceptance.

Sixty chemicals were used for the validation of these two methodologies; twenty of them are used as cosmetic ingredients, according to the "European inventory and common nomenclature of ingredients employed in cosmetic products" (Ref. 3).

SCCNFP reviewed publications from the validation study and ESAC statements, and propose that these two methods could be applied to the safety assessment of chemicals used as cosmetic ingredients.

A cosmetic ingredient or mixture of ingredients can be corrosive per se. When corrosivity cannot be excluded, testing for irritancy on animals or humans should be preceeded by a corrosivity test using one of these two validated *in vitro* methodologies.

5- References

1- Barratt M.D. & al. Toxicology in Vitro (1998) 12, 471-482

2- Barratt M.D. & al. Toxicology in vitro (1998) 12, 483-524

3- Commission Decision 96/335 EC of 8 May 1996 establishing an inventory and a common nomenclature of ingredients employed in cosmetic products J.O. L 132 of 1 June 1996

ANNEX 10 – GUIDELINES FOR *IN VITRO* METHODS TO ASSESS PERCUTANEOUS ABSORPTION OF COSMETIC INGREDIENTS *

1. Background

In 1995, COLIPA (European Cosmetic, Toiletry and Perfumery Association) presented to the former SCC (Scientific Committee on Cosmetics) Sub-Committee "Alternatives" an industrial view on the *in vitro* assessment of percutaneous absorption / penetration of cosmetic ingredients.

Guidelines for the testing of *in vitro* percutaneous absorption and some different protocols related to the use of excised skin (human, pig and rat) were proposed. Also, a general view on percutaneous absorption / penetration *in vitro / in vivo* correlation was presented based on a set of papers published in the scientific literature. The *in vitro* tests conducted by the cosmetic companies were developed to evaluate the safety of their cosmetic ingredients. They had not been intended for regulatory purposes and they were not subjected to the official validation processes.

The main conclusion of that Sub-Committee was the following (DGXXIV/1874/95): "The most important problem deduced from the documentation submitted is the absence of results and correlation data (in the protocols not in the references attached). The documentation should be implemented with intra- and inter-laboratory results obtained on percutaneous absorption of several strategic compounds (wide spectrum) as well as on correlation of *in vitro / in vivo* data. A more uniform presentation of *in vitro* percutaneous absorption methodology should be considered, taking into account the different protocols presented".

In 1996, a report and recommendations of ECVAM Workshop 13 about Methods for assessing percutaneous absorption was published (ATLA 24, 81-106, 1996).

In the last few years, two OECD Proposals to evaluate percutaneous absorption by *in vivo / in vitro* methods have been presented. COLIPA members have upgraded their initial data submission as requested by the Extended Steering Committee of the OECD but to our knowledge the document has not been finalised by the organisation.

* Adopted by the SCCNFP at the plenary meeting of 20 January 1999 (SCCNFP/0088/98 Final)

2. Position of the Scientific Committee on Cosmetics (SCC)/Scientific Committee on Cosmetic and Non-Food products (SCCNFP)

In the Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation (XXIV/1878/97) the former SCC emphasised that the test protocols used by industry were not subjected to a formal validation test and it recommended that the existing documentation must be supplemented, as regards intra- and inter- laboratory reproducibility, the influence of the vehicle on the release of the cosmetic ingredients and other technical and experimental details. However, the SCCNFP is convinced of the relevance of *in vitro* methods and has since recent years agreed to consider *in vitro* percutaneous absorption data in the evaluation of the safety of several cosmetic ingredients.

3. Submission of COLIPA data on *in vitro/in vivo* dermal absorption/percutaneous penetration (SCCNFP/0073/98)

In November 1998, COLIPA submitted a new document on *in vitro / in vivo* dermal absorption / percutaneous penetration including data and protocols used by several cosmetic companies.

These data refer to the dermal absorption / percutaneous absorption of chemical UV-filters, hair dyes (with rinsing or without rinsing) and several other ingredients.

In the methodologies used, the penetration cell design, the composition of the receptor fluid, the membrane integrity checking and the preparation of the dose of a given substance are described.

Experimental details concerning the application of test substance, reference chemicals, the fluid dynamics, temperature, exposure time, duration of the study, sampling and analytical techniques are also indicated.

Porcine back and flank skin, rat dorsal skin, guinea pig skin and human split-thickness skin have been used for the *in vitro* tests.

Some reference chemicals with a broad range of partition coefficient octanol/water (log P) and with different percutaneous absorption profiles have been evaluated. Benzoic acid, caffeine, estradiol. hydrocortisone, inulin, pentadecanoic acid, salicylic acid, sucrose, thiourea, tritiated water have been tested.

Among others these comparisons have been made:

Pig skin *in vitro* / Human skin *in vivo* (SC stripping)
Pig skin *in vitro* / Rat skin *in vivo*
Human skin *in vitro* / Pig skin *in vitro*

Some intra-assay reproducibility and inter-laboratory comparisons are included in the documentation. Additionally, in this document, information is included about the self-evaluation of each methodology according to the Canadian/US proposal for the Data Submission Form (OECD).

4. Opinion of the SCCNFP

The SCCNFP has reviewed the documentation submitted by COLIPA and agrees with the rationale for using *in vitro* methods to evaluate the dermal absorption / percutaneous penetration of cosmetic ingredients. The data reported in this document indicates the possible usefulness of the *in vitro* methodologies.
However the data provided at the moment are not sufficient to formulate a scientific opinion on how to conduct *in vitro* percutaneous absorption studies and assess the results.

Studies to standardise methodologies for *in vitro* percutaneous absorption for cosmetic ingredients are necessary and the method should be shown to give reproducible and relevant results. It is recommended that independent research institutes should perform or co-ordinate this work.

The minimal requirements needed for the acceptance of *in vitro* percutaneous absorption studies to be evaluated, have been formulated by the SCCNFP, based on the scientific literature and on the experience of the Committee in evaluating the dossiers submitted for inclusion of cosmetic ingredients in the annexes of the Cosmetics Directive 76/768/EEC.

5. Basic Criteria for the *in vitro* assessment of percutaneous absorption of cosmetic ingredients*

5.1. Background

In the "Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation" (SCCNFP/0119/99) adopted by the Scientific Committee on Cosmetic and Non-Food Products intended for Consumers (SCCNFP) Annex 10 reports the opinion adopted by the SCCNFP on the need to formulate the minimal requirements for the acceptance of in vitro percutaneous studies to be evaluated (SCCNFP/0088/98 Final).

In this document the basic criteria for the *in vitro* assessment of percutaneous absorption of cosmetic ingredients, which address the principles and basic elements of such studies are reported.

5.2. General Principles

The purpose of the percutaneous absorption studies of cosmetic ingredients is to obtain quantitative information on the amounts that can enter, under in-use conditions, into the systemic compartment. These quantities can then be taken into consideration to calculate from the NOAEL a safety factor.

The justification of *in vitro* percutaneous absorption studies on isolated skin is based on the fact that the epidermis with the stratum corneum is *in vivo* the principal barrier against the percutaneous absorption of xenobiotics into the body.

* Adopted by the SCCNFP in its Plenary Meeting of 23 June 1999 (SCCNFP/0167/99 Final)

Under *in vivo* conditions, the microcirculatory system (blood and lymph vessels) carries compounds from the epidermis to the dermis into the central compartment. *In vitro* this microcirculation is obliterated. Consequently, under *in vitro* conditions, 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 or such possible *in vitro* retention in the dermis must be taken into account when interpreting the *in vitro* results.

The epidermis renews itself by continuous outward proliferation, differentiation and desquamation. About one layer of corneocytes is shedded 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. Because these processes are not functional *in vitro*, the final epidermal (stratum corneum) levels *in vitro* could be elevated compared with the corresponding *in vivo* levels.

According to these principles, the following rules should be applied for *in vitro* percutaneous absorption studies:

i. Studies should be performed on appropriate standardised skin preparations. The respective choice should be justified in the protocol.

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

iii. When considerable cutaneous metabolism of the ingredients to be tested occurs, advice of a competent biochemist is necessary.

5.3. Principle of the test

These guidelines take into account, according to the present knowledge, only skin preparations of natural origin (not cultured or reconstituted skin).

Every protocol should be preceded by a specific justification of the particular method used and the appropriate references should be mentioned.

The test substance, either as such or in an appropriate solvent or vehicle, thereby yielding the test sample, is applied to the surface of the skin which is positioned between the upper and lower chambers of a penetration cell. This may be either of static or flow-through design. The integrity of the barrier should be checked by an appropriate method. The test sample remains in contact with the skin on the donor side for a defined period of time (leave-on or rinse-off respectively, depending on the intended use conditions). The receptor fluid may be sampled once at the end of the experiment or preferably at various time points before the end so that an absorption profile may be constructed. A justification of the procedure used (static or flow-through conditions) should be provided. The skin and/or fluid samples are analysed by an appropriate method (e.g. scintillation counting, HPLC, GC).

5.4. Description of the method

5.4.1 Penetration cell design

The penetration cell consists of the upper donor and the lower receptor chamber, separated by a skin preparation. The stratum corneum faces the donor chamber. The cells are made from an inert and non-absorbing material (e.g. glass or PTFE - polytetrafluoroethylene)- Temperature control of the receptor fluid, crucial throughout the experiment, must maintain a level comparable to skin surface temperature *in vivo*. The receptor fluid is well-mixed throughout the experiment. The cell design allows multiple sampling without interrupting the experiment.

5.4.2 Receptor fluid

The composition of the receptor fluid is chosen so that it does not limit the extent of penetration of the test substance, i.e. the solubility of the chemical under investigation has to be guaranteed. Saline or buffered saline solution is used for hydrophilic compounds. For lipophilic molecules, serum albumin or other appropriate solubilisers, such as non-ionic surfactants, are added in amounts which do not interfere with membrane integrity. The properties of the receptor fluid should be such that there is no interference with the analytical procedure.

5.4.3 Skin preparations

Human skin would be the obvious choice but is not always readily available. Pig skin is used because it shares essential permeation characteristics with human skin. The use of artificial skin is still under development.

The origin of skin samples must be specified in the respective report in terms of :
- species : human or pig;
- location on the body : in human: abdomen or breast; in pig: additionally the back and flanks;
- sex and age : they are not considered as important variables for this test but should be stated;
- fresh/frozen : fresh skin must be used in case of metabolism studies for absorption.
- details on preservation and storage : skin can be stored at -20°C minimum up to 3 months (conditions should be specified). During transport skin samples should be kept at or below 4°C.

The skin samples which may be used as full-thickness or as split-thickness skin preparations should be prepared to fit the cell.
- Human skin : split-thickness skin should be the general rule. If for a particular reason, full-thickness is required , this should be justified.
- Pig skin : since it is more difficult to obtain intact split-thickness skin, this could justify the use of full-thickness skin.

Skin thickness should be measured by an appropriate method (methods should be mentioned).

5.4.4 Reproducibility/Variability

The variability of percutaneous absorption studies depends on the absolute values of penetration in individual experiments: the lower the penetration rate, the higher the variability. This high variability is due to known intraindividual and interindividual characteristics of the stratum corneum barrier.

Apart from these inherent biological factors, the reproducibility of the method as such should not exceed 30%. The reproducibility of the method used should be assessed at appropriate intervals by including penetrating compounds like caffeine or benzoic acid as reference substances. These data should be included in the study.

With this provision a minimum of a total of six evaluable samples of either human or pig skin from at least 3 donors per experiment/dose is required.

5.4.5 Skin integrity

Barrier integrity is crucial for the experiment, and is therefore checked. This is achieved by either measuring the penetration of a marker molecule, e.g. tritiated water, caffeine or sucrose, for which suitable historical control data are available, or by physical methods like TEWL or TER (Transepidermal Water Loss or Transdermal Electrical Resistance, respectively). Historical data should be reported.

5.4.6 Test substance

Relevant toxicological and physicochemical data (e.g. irritation potential and pH of the actual preparation) and analytical methods and their detection limits are documented for the test substance. In many instances the test substance is radio-labelled to simplify analyses.

5.4.7 Reference substance

The study must include data which demonstrate that *in vitro* data obtained with the study design actually used correspond with known historical *in vitro* or *in vivo* data of compounds with similar properties, especially with respect to the oil/water partition coefficient and the absolute solubility.

5.4.8 Preparation of the dose

The test substance is incorporated at the highest requested concentration into an appropriate vehicle, which should be the prototype of the formulation of the product. The quantitative composition of this vehicle should be specified. The stability of the test substance under the proposed conditions of administration and usage is ascertained.

5.5. Procedure

5.5.1 Application of dose

The dose as well as the contact time (exposure) with the skin are chosen to mimic intended use conditions. The amount of the formulation to be applied is adapted to the consumer use values recommended by COLIPA.

5.5.2 Receptor fluid conditions

The receptor fluid, preferably degassed (e.g. by sonication), is thoroughly stirred at all times or continuously replaced in flow-through cells. The choice of static or flow-through conditions in the receptor cell is made on a compound-by-compound basis, depending on its absorption properties and on the goal of the study. It must be specified in the test report. It has to be ensured that the amount of penetrant in the receptor fluid is less than 10% of its saturation level at any time. This will minimise any interference of the free diffusion process which could lead to underestimation of percutaneous absorption.

5.5.3 Temperature

Because the rate and extent of skin absorption is temperature dependent, the skin temperature is maintained constant (32 ± 1°C = skin surface temperature *in vivo*).

5.5.4 Duration of Study

The exposure time and sampling period(s) are defined in the protocol, the normal study time being a 24 hours period. Longer duration may result in membrane deterioration and requires membrane integrity to be carefully checked. Concerning exposure the period may be shorter, depending on intended use.

5.5.5 Sampling

The frequency of sampling depends on the rate and extent of percutaneous absorption. It must be defined in the report and chosen to allow the extent or rate of absorption and/or the profile to be determined.

5.5.6 Analysis

The mass balance of the applied dose is determined. The receptor fluid and skin washings are analysed and the amounts found in the skin preparation, i.e. its individual layers, and on the skin surface, are determined.
For each skin preparation stratum corneum is removed by adhesive tape stripping (10 to 20 strips) or heat separation. The specific procedure used should be described in the study report. Epidermis and dermis may then be separated prior to analysis.
The overall recovery of test substance (including (bio-)chemical degradation products) should be at least 100 ± 15 %. If lower recoveries of the test substance are obtained, the reasons (binding to proteins, to penetration cell surface and tubing, as well as possible evaporation or loss by chemical reaction) are investigated.

Suitable quantitative analytical procedures are used, e.g. scintillation counting, HPLC or GC. Detailed descriptions on how analytical samples have been obtained must be specified in the report.
Qualitative or semi-quantitative methods such as microautoradiography are useful tools for skin distribution assessments.

5.5.7 Data

The absorption profile is determined up to 24 hours post application with cells of similar barrier integrity. When adequate data are available, the lag time and the absorption rates are calculated.

Normally amounts of the test compound are analysed :
- in the surplus on the skin
- in the stratum corneum (e.g. adhesive tape strips)
- in the epidermis without stratum corneum
- in the upper dermis (depending on the type of skin preparation)
- in the receptor fluid

5.6. Calculation of results

The amounts absorbed are expressed in **[µg/cm2 of skin surface]**. It is only subsequently that they can be expressed as **[percentage of the applied dose]**. They are then transformed into **[mg/kg body weight]** and thus serve for the assessment of a safety factor.

The amounts of penetrated substance(s) found in the receptor fluid are considered to be systemically available. The epidermis (except for the stratum corneum) and dermis are considered as a sink, therefore the amounts found in these tissues are equally considered as absorbed and are added to those in the receptor fluid. The amounts which are retained by the stratum corneum at the time of sampling (usually 24 hours) are not considered to be percutaneously absorbed and thus do not at that time contribute to the systemic dose.

5.7. References

1) Bronaugh R.L., Stewart R.F. and Congdorn E.R. (1982). Toxicol. Appl. Pharmacol., 62, 481-488.

2) Bartek M.J., Labudde J.A. and Maibach H.I. (1972). J. Invest. Dermatol., 58, 114-123.

3) Scott R.C. and Ramsey J.D. (1987). Comparison of the In Vivo and In Vitro Percutaneous Absorption of a Lipophilic Molecule (Cypermethrin, a Pyrethroid Insecticide). Journal of Investigative Dermatology, 89, 142-146.

4) Scott R.C. (1989). Biological Monitoring for Pesticide Exposure : Measurement, Estimation and risk reduction. In ACS Symposium Series 382. Edited by R.G.M. Wang, C.A. Franklin, R.C. Honeycutt, J-C. Reinert, pp. 158-168, Washington, American Chem Society.

5) Grissom R.E. (1990). Prediction of Percutaneous Penetration - Methods, Measurements Modelling. Edited by Scott R.C., Guy R.H. and Hadgraft J, pp 73-83, IBC Technical Services, London.

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

7) Bartnik F.G., Reddy A.K., Klecak G., Zimmermann V., Hostynek JJ. and Künstler K (1987). Percutaneous absorption, Metabolism and Haemolytic Activity of n-Butoxyetha-nol. Fundamental and Applied Toxicology, 8, 59-70.

8) ECETOC Monograph No. 20,1993.

9) COLIPA Guidelines: 'Cosmetic Ingredients: Guidelines for Percutaneous Absorption/penetration', Brussels, Nov. 1995.

10) COLIPA Guidelines: 'Consumer exposure to cosmetic ingredients', BB-97/007, 1997

11) Schaefer. H. and Redelmeier T. E. (1996). Skin barrier. Principles of percutaneous absorption/penetration (Monograph) Karger.

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

ANNEX 11 – GUIDELINES ON THE USE OF HUMAN VOLUNTEERS IN THE TESTING OF POTENTIALLY CUTANEOUS IRRITANT COSMETIC INGREDIENTS OR MIXTURES OF INGREDIENTS*

1- Background

1.1 Emphasis on consumer safety

According to the Council Directive "a cosmetic product put on the market within the Community must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use" (76/768/EEC). In order to achieve this goal of product safety, toxicological data on cosmetic ingredients are needed as outlined in the SCCNFP Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation 2nd Rev. (XXIV/1878/97). Among the data mentioned, also «human data» are cited. However, the document does not specify these in detail. Regarding skin irritation, the SCCNFP considers that at present human testing of cosmetic ingredients or mixtures of ingredients should not be preferred to animal testing.

1.2 Animal tests for assessment of safety to be replaced by alternative methods

In the past, most of the toxicological data mentioned above have been generated by testing on animals. However, according to Council Directive 76/768/EEC, the marketing of cosmetic products containing ingredients or combinations of ingredients tested on animals after 30 June 2000 in order to meet the requirements of this Directive shall be prohibited. The Commission's general policy regarding research on animals supports the development of alternative methods to reduce or replace animal testing when possible.

1.3 Testing of cosmetic ingredients in humans

In this context, the scientific and ethical considerations for testing cosmetic ingredients or mixtures of ingredients in human subjects need to be defined more clearly. The skin irritancy reaction in humans is not an absolute measure and must be related to appropriate controls defining the range of response.

The SCCNFP stresses three points:

1. Since tests in animals or validated alternative methods may be limited regarding their predictive value for exposure of a human population, confirmatory safety tests in humans may be necessary scientifically and ethically, provided that the toxicological profile of an ingredient or a mixture of ingredients based on animal or alternative methods is available and that a high degree of safety is to be expected.