SCCNFP/0119/99 Final
23 June 1999

THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

NOTES OF GUIDANCE FOR TESTING OF COSMETIC INGREDIENTS FOR THEIR SAFETY EVALUATION

(THIRD REVISION)

Adopted by the SCCNFP during the plenary meeting of 23 June 1999

SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

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Acknowledgement

The SCCNFP is indebted to the following scientists who have contributed to the development of the NOTES OF GUIDANCE FOR TESTING OF COSMETIC INGREDIENTS FOR THEIR SAFETY EVALUATION as members of the previous scientific committees since 1978 :

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Nam et ipsa scientia potestas est
For knowledge itself is power

Francis Bacon (1561- 1626) Essays

This document, the "***Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation***" represents a contribution of the members of the Scientific Committee on Cosmetology (SCC) of the Commission of the European Union dedicated to public authorities as well as to cosmetic industry within the scope of interest in the safety evaluation of cosmetic products and their ingredients, as requested by the Directive 76/768 EEC and especially by the Sixth Amendment (93/35/EEC - OJ L 151 of 23.6.93) to this Directive.

The background of the Third Revision 1999 of the "Notes of Guidance" has been the progress in scientific knowledge in general and especially the experiences developed by the former SCC and the present SCCNFP in the field of cosmetology and risk assessment.

In this context the contribution of valuable information which scientists from industry laboratories and the speaker of manufacturers, the European Cosmetic Toiletry and Perfumery Association (COLIPA), submitted, is gratefully acknowledged.

The "***Notes of Guidance***" should not be used as a check list but could be of assistance for those responsible for consumer health protection, in which position whatever.

This document was drawn up in general terms and will require amendments in the future as scientific knowledge and also technical innovation in the cosmetic sector will advance.

The Chairman

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1. INTRODUCTION

Council Directive 76/768/EEC of 27 July 1976, as amended by six Directives, imposes, the following rules related to the safety of cosmetic products:

Art 1: "A cosmetic product means any substance or preparation intended for placing into contact with the various parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and mucous membranes of the oral cavity with a view exclusively or principally to cleaning them, perfuming them or protecting them, in order to keep them in good condition, change their appearance or correct body odours".

Art. 2: "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".

Ingredients are defined as any substances used in cosmetic products. Examples of ingredients are provided by Annexes III, IV, VI, VII to Council Directive 76/768/EEC of 27 July 1976 and its amendments.

Cosmetic products have a history covering thousands of years with the use of many ingredients from plants, animals and mineral sources. Present technology has resulted in the use of many synthetic chemicals as ingredients in cosmetic products .
Present-day use, particularly as toiletries, is extensive and affects most population groups, although the degree and nature may vary within different countries of the European Union.

In practice, cosmetic products have rarely been associated with serious health hazards. However, this does not mean that cosmetics are always safe in use, especially with regard to possible long-term effects. Together with the fact that the products may be used extensively over a large part of the human lifespan, has created a need to ensure, as far as possible, their safety-in-use by controlling the ingredients content and the toxicity.

An original document on "Guidelines for the toxicity testing of cosmetic ingredients" was prepared by the Scientific Committee on Cosmetology in June 1982 (Report EUR 8794).
Two other documents (SPC/803-5/90; XXIV/1878/97) took into account both the experience gained by the SCC-SCCNFP in its past work in evaluating the toxicological profiles of many cosmetic ingredients, as well as the development of scientific knowledge in the field of specific areas of toxicology.

This document - the third revision - takes into account the concept incorporated in the Sixth Amendment (93/35/EEC Directive) to Directive 76/768/EEC and the Commission Directive 97/18/EC of 17th April 1997 (OJ L 114 of 1.5.97) which implies new approaches to improving consumer health protection.
The present notes of guidance (SCCNFP/0119/99) will apply to all cosmetic ingredients for which the producer must perform a safety evaluation to be included in the "*dossier*", as well as new cosmetic ingredients, for inclusion in Annexes IV, VI, and VII of the 76/768/EEC Directive, and to those cosmetic ingredients about which safety concerns have been expressed, bearing in mind their relevant toxicity data already available to the SCCNFP.
These notes of guidance will require further revision in future as scientific knowledge advances.

The relevance of this document also derives from the need to furnish scientific support to the development of the Council Directive 76/768/EEC, represented, at this stage, by its *"Sixth Amendment"* (Council Directive 93/35/EEC of 14 June 1993).

The purpose of this document is to provide guidance for testing cosmetic ingredients and for the safety assessment of the finished product, both to the competent monitoring authorities of the Member States, and to persons responsible for putting cosmetics on the market (manufacturers or importers within the European Union) pursuant to the Sixth Amendment.

These notes of guidance are not a checklist. This means that they can be adapted case by case, depending for instance on the ingredients used, the formulation of the finished product, and the degree and route of consumer application.

2. THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

A Scientific Committee on Cosmetology (SCC) was established on 19th December, 1977 by Commission Decision 78/45/EEC (1978): it has assisted the European Commission in examining the complex scientific and technical problems surrounding the drawing up and amendment of European Union rules governing the composition, manufacture, packaging, and labelling of cosmetic products marketed in EU countries.
In 1997 a new scientific Committee, named Scientific Committee on Cosmetic and Non-Food Products intended for consumers (SCCNFP) has been appointed by the Commission Decision of 23 July 1997 (OJ L 237 of 28-8-97); it is composed by 16 members, which are qualified scientists in the fields of medicine, toxicology, biology, chemistry, and other similar disciplines.

The SCCNFP is consulted by the Commission on any scientific or technical problems arising in the connection with cosmetic products, and, in particular, on substances used in the preparation of cosmetic products and the composition and the conditions of use of such products.

The SCCNFP has also been requested to make it possible to perform the safety evaluation of cosmetic ingredients by

1. **analysing the studies presented to the Commission and developed by the cosmetics industry on potentially hazardous cosmetic ingredients;**
2. **evaluating the most recent scientific literature on different toxicological aspects of relevance for the safety evaluation of the cosmetic ingredients;**
3. **requiring in same cases additional safety testing to examine any new potential hazard connected with a particular ingredient, thus making reassessment of its safety possible.**

The opinions adopted by the Scientific Committee at Commission's request were included in EC-Reports (EUR 7297, 8634, 8794, 10305, 11080, 11139, 11303, 14208). Starting from 1997 they are present in Internet*. They mainly refer to cosmetic ingredients included in Annexes II, III, IV, VI and VII of Council Directive 76/768/EEC.

One of the main actions undertaken by the SCC-SCCNFP has been to recommend a set of guidelines to be taken into consideration by the cosmetics industry in developing adequate studies to be used in the safety evaluation of cosmetic ingredients. The SCC has adopted the following opinions concerning the safety evaluation of cosmetic ingredients:

(a) Notes of Guidance for the toxicity testing of cosmetic ingredients (28 June 1982; EU Report 8794);

* (http://www.europa.eu.int/comm/dg24/health/sc/ncomm6/index_en.htm)

(b) Notes of Guidance for testing of cosmetic ingredients for their safety evaluation (SPC/803/5/90);

(c) Notes of Guidance for testing of cosmetic ingredients for their safety evaluation (DGXXIV/1878/97).

These guidelines recommend as test procedures for the toxicity studies needed to evaluate different toxicological endpoints those reported in Commission Directive 87/302/EEC of 18 November 1987(OJ L 133 of 30-5-88) and in Commission Directive 92/69/EEC of 31 July 1992 (OJ L 383 of 29-12-92) adapting to technical progress the Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances: they represent the basic toxicity testing procedures internationally accepted as being the result of long-term scientific agreement. These procedures include, at present, 27 studies based on *in vivo* animal models and 10 studies based on *in vitro* models (genotoxicity). Moreover, the SCC when evaluating the information dossiers on several cosmetic ingredients proposed for inclusion in Annexes IV, VI, and VII, has accepted all types of testing procedures based on a scientifically justified model and procedure (for instance, *in vitro* studies on percutaneous penetration), or in accordance with OECD Guidelines.

In response to DGXXIV's request to assess the possibility of replacing data obtained on the basis of animal tests with data obtained making use of alternative methods in the safety evaluation of cosmetic ingredients, the SCCFNP established a Working Group (Subcommittee "Guidelines: Alternative Methods") to follow up validation studies co-ordinated by ECVAM (European Centre for the Validation of Alternative Methods) and by other centres, and to study the applicability of validated alternative methods in toxicity testing to evaluating the safety of cosmetic ingredients.
Moreover a mandate for SCCFNP was defined by the Commission and adopted by the plenary section on 20th May 1998 (DGXXIV/1890/98).

1.1 The SCCNFP shall act as a resource of scientific expertise to the European Commission, with regards to the development of alternative methods. To that effect they will regularly meet with representatives from the concerned parties to :

- give pro-active advice on research proposals and on-going studies
- offer, as appropriate, peer reviews of study data.

However, such advice in no way prejudices the right of the SCCNFP to reject data previously discussed, if the scientific standard of the work is considered inappropriate.

- The SCCNFP shall serve as an expert resource, as appropriate, to those services of the Commission charged with the validation of alternative methods. Specifically, they should offer expert guidance in the design and applicability of test methods, the selection of test materials and communication of results as they apply to cosmetic products.
- The SCCNFP shall, on the request of the appropriate Commission services, review data submitted on in vitro methods that have been assessed and validated by the services of the European Commission, or could be considered appropriate for the replacement of methods using animal testing. The SCCNFP shall give an opinion,

and offer guidance as appropriate, on the applicability of such tests in the evaluation of the safety of cosmetic ingredients and products.

- The SCCNFP should encourage the use of appropriate in vitro methods for use in the safety evaluation of cosmetic ingredients and products. To this end, it shall consider data from in vitro tests that are submitted in support of safety dossiers from the Industry, if the scientific design, justification and data presentation of such studies are considered of an acceptable scientific standard.
- Upon request, the SCCNFP shall advice the European Commission on the status of alternatives to animal testing in cosmetics on an on-going basis and in particularly, in accordance with Article 4, 1(1) of the EU Cosmetic Directive 76/768/EEC.

Although this mandate has been developed in relation to cosmetic ingredients, it is envisaged that scientifically sound non-animal methods for safety assessment will have broad application.

1.2. The SCCNFP was requested moreover to continuously update the Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation as stated on page 8 of the 2nd Revision, adopted by the former SCC (Scientific Committee on Cosmetology) on January 16th, 1997.

Due to the almost complete review of the former SCC, and the presence in the SCCNFP of experts on other disciplines, it was decided to include technical modifications to the content of the Notes, such as Guidelines for Human Testing, as it seems appropriate to give advice on this new aspects.

During 1998 SCCNFP received another request by the Commission to elaborate an opinion on Clinical Testing of Cosmetic Finished Products to assess Skin Compatibility, taking into consideration the following items:

- ethical and safety consideration of the cosmetic finished products testing on human volunteers when assessing skin compatibility;
- end-points for which such tests are appropriate and the most robust protocol for such studies.

3. THE SIXTH AMENDMENT (COUNCIL DIRECTIVE 93/35/EEC)

Council Directive 93/35/EEC of 14 June 1993 amended for the sixth time Council Directive 76/768/EEC on the approximation of laws of the Member States relating to cosmetic products. Among several amendments, the revised Article 4 banned the marketing of cosmetic ingredients or their combinations tested on animals after 1 January 1998. The Commission Decision 97/18/EC of 17th April 1997 has postponed the ban to the 30th of June 2000 (Table 1).

According to Council Directive 93/35/EEC, Article 2:

"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, taking into account, in particular, the product's presentation, its labelling, any instructions for its use and disposal as well as any other indication or information provided by the manufacturer or his authorised agent or by any other person responsible for placing the product on the Community market".

Council Directive 76/768/EEC, as amended by Council Directive 93/35/EEC is designed to protect consumer health from possible deleterious effects due to the presence of specific substances or preparations which harm humans because of their intrinsic unsafe properties.

Several mechanisms have been developed under this Directive in order to fulfil its main requirements regarding consumer health protection, namely:

- list of chemicals which must not be contained in finished products (Annex II);
- list of substances which cosmetic products must not contain except under the restrictions and conditions laid down in Annex III;
- lists of authorised substances, which may include colorants, preservatives and ultraviolet filters (Annexes IV, VI, VII).

The amendment of Art.4, subparagraph (I), is also based on the new recital of the Sixth Amendment which states that:
"...assessment of the safety of use of the ingredients employed in cosmetics and of the final product, should take into account the requirement of Directive 86/609/EEC which concerns the protection of animals used for experimental and other scientific purposes".

Table 1

COUNCIL Directive 76/768/EEC ART. 4
1. WITHOUT PREJUDICE TO THEIR GENERAL OBLIGATIONS DERIVING FROM ARTICLE 2, MEMBER STATES SHALL PROHIBIT THE MARKETING OF COSMETIC PRODUCTS CONTAINING:
a. substances Listed in Annex II;
b. substances listed in the first part of Annex III, beyond the limits and outside the conditions laid down;
c. colouring agents other than those listed in Annex IV, Part I. with the exception of cosmetic products containing colouring agents intended solely to colour hair:
d. colouring agents listed in Annex IV, Part 1, used outside the conditions laid down, with the exception of cosmetic products containing colouring agents intended solely to colour hair;
e. preservatives other than those listed in Annex VI, Part 1;
f. preservatives listed in Annex VI, Part 1, beyond the limits and outside the conditions laid down, unless other concentrations are used for specific purposes apparent from the presentation of the product;
g. UV filters other than those listed in Part 1 of Annex VII;
h. UV filters listed in Part 1 of Annex VII, beyond the limits and outside the conditions laid down therein.
i. ingredients or combinations of ingredients tested on animals after 30 June 2000 in order to meet the requirements of this Directive.

Clearly, the legislator wishes to ban animal trials of cosmetic ingredients or combinations of ingredients whose purpose is to identify the toxicity or evaluate the safety of these specific chemicals or their combinations, only when alternative methodologies are available.

The Sixth Amendment, however, states that "*if there has been insufficient progress in developing satisfactory methods to replace animal testing, and in particular in those cases where alternative methods of testing, despite all reasonable endeavours, have not been scientifically validated, the Commission shall, by 1st January 1997, submit draft measures to postpone the date of implementation of this provision*". This requirement is not in conflict with Art.7 of Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes (OJ L 358 of 18.12.86) which states that "*an experiment shall not be performed, if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practically available*".

Should it prove impossible to adopt alternative methodologies as a substitute for animal toxicity testing procedures in the safety evaluation of cosmetic ingredients, the Commission, having consulted the Scientific Committee on Cosmetology, will seek to postpone the date of the implementation of such a ban.

Commission Directive 97/18/EC of 17th April 1997 has postponed the date of 1st January 1998 to 30th June 2000 (Art. 1). Article 2 states that " *if there has been insufficient progress in developing satisfactory methods to replace animal testing, and in particular in those cases where alternative methods of testing, despite all reasonable endeavours, have not been scientifically validated ...., the Commission shall, by 1st January 2000, submit draft measures to postpone the date"*

Alternative methodology means any modification to the present toxicity assay protocols, which are internationally and scientifically approved and based on animal models, so as to introduce a different method of conducting the toxicological studies necessary to assess the safety of ingredients used in the manufacture of finished cosmetic products.
Alternative Methods must offer a level of protection to consumers equivalent to that now offered by toxicological studies performed on animals: this means that the alternative methods must be scientifically validated.

A series of other improvements in the safeguarding of public health were introduced in EC cosmetics law with the adoption of the Sixth Amendment (Council Directive 93/35/EEC). These improvements comprise:

(A) the compilation by the Commission, of an inventory of ingredients used in products, in particular on the basis of information supplied by the industry concerned (Article 5a.1). The same article states that "cosmetic ingredient" shall mean any chemical substance or preparation of synthetic or natural origin, except for perfume and aromatic compositions, used in the composition of cosmetic products. The inventory shall contain information on:

(i) the identity of each ingredient, in particular its chemical name, the CTFA name, the European Pharmacopeia name, the international non-proprietary names recommended by the World Health Organisation, the IUPAC name, the EINECS, CAS and Color Index Numbers, and the common name;

(ii) the usual functions of the ingredient in the final product;

(iii) where appropriate, restrictions and conditions of use and warnings that must be printed on the label. This inventory shall be updated periodically. It is indicative and shall not constitute a list of substances authorised for use in cosmetic products.

(B1) The manufacturer or his agent or the person to whose order a cosmetic product is manufactured or the person responsible for placing an imported cosmetic product on the Community market shall for control purposes keep the following information readily accessible to the competent authorities of the Member State concerned at the address specified on the label in accordance with Article 6(1)(a):

(a) The qualitative and quantitative composition of the product; in the case of perfume compositions and perfumes, the name and code number of the composition and the identity of the supplier.

(b) The physico-chemical and microbiological specifications of the raw materials and the finished product and the purity and microbiological control criteria of the cosmetic product

(c) The method of manufacture complying with the good manufacturing practice laid down by Community law or, failing that, laid down by the law of the Member State concerned; the person responsible for manufacture or first importation into the Community must possess an appropriate levels of professional qualification or experience in accordance with the legislation and practice of the Member State which is the place of manufacture or first importation.

(d) Assessment of the safety for human health of the finished product. To that end the manufacturer shall take into consideration the general toxicological profile of the ingredients its chemical structure and its levels of exposure. Should the same product be manufactured at several places within Community territory, the manufacturer may choose a single place of manufacture where that information will be kept available. In this connection, and when so requested for monitoring purposes, he shall be obliged to indicate the place so chosen to the monitoring authority/authorities concerned.

(e) The name and address of the qualified person or persons responsible for the assessment referred to in (d). That person must hold a diploma as defined in Article I of Council Directive 89/48/EEC in the field of pharmacy, toxicology, dermatology, medicine or a similar discipline.

(f) Existing data on undesirable effects on human health resulting from use of the cosmetic product.

(g) Proof of the effect claimed for the cosmetic product, where justified by the nature of the effect or product.

(B2) The assessment of the safety for human health referred to in Paragraph 1(d) shall be carried out in accordance with the principles of good laboratory practice laid down in Council Directive 87/18/EEC of 18 December 1986 (OJ L 15 of 17.1.87) on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their application for tests on chemical substances.

(B3) The information referred to in Paragraph 1 must be available in the national language or languages of the Member State concerned, or in a language readily understood by the competent authorities.

The authorities requirements for Product Information (I), as in the text cited, are clearly related to the requirement to have prompt access to all information that might be needed “for control purposes” on the technical characteristics and safety of every cosmetic product placed on the market. The (I) is in fact set out so that information is easily accessible for an overall assessment of the safety of the cosmetic product on the basis of all relevant knowledge.

4. TESTING OF INGREDIENTS AND SAFETY ASSESSMENT OF THE FINISHED PRODUCT

Although there are many thousands of different cosmetic products on the market within the EU, they are all derived from a far smaller number of ingredients. This is the rationale for concentrating toxicity testing on ingredients, and particularly those of most concern. This is the basis of the lists of authorised ingredients referred to in the preamble to Council Directive 76/768/EEC currently covering colouring agents, preservatives and UV filters. This approach avoids the costly duplication of studies and the unjustifiable use of animals that would result from the routine testing of products.

Article 2 of Council Directive 76/768/EEC requires that cosmetic products put on the Community market must not cause damage to human health when they are applied under normal and reasonably foreseeable conditions of use. Adequate information should therefore be provided in order to evaluate the safety of the final product. In general this can be derived from knowledge of the toxicity of the ingredients, with no need to test the final product. However, in a few cases, testing of the final product may be necessary (Annex 6). Examples are when the vehicle used results in considerably greater skin penetration than that observed in the toxicity studies on the ingredients or if interaction between ingredients is likely to result in the formation of a new, potentially toxic substance, or when there is a claim of reduced skin penetration or toxicity resulting from the formulation. It is up to the suppliers of new products placed on the Community market to ensure that adequate information can be provided for a safety assessment of the finished product.

5. LISTS OF INGREDIENTS

Through progressive amendments to the Council Directive 76/768/EEC, several lists of ingredients have been established on the basis of the results of the latest scientific and technical research.
These lists include cosmetic ingredients for which already existing and new toxicological data have been evaluated and for which conclusions have been drawn concerning their risk for human health when used in cosmetic products.
For some ingredients only concentrations below certain limits are allowed and the field of application is limited for safety reasons.
All cosmetic ingredients so far analysed by the SCC-SCCNFP have been included in a series of Annexes to Council Directive 76/768/EEC. Annexes IV, VI and VII represent the existing "positive lists" respectively for Colouring agents, Preservatives and UV Filters.
Annex II of Council Directive 76/768/EEC lists all the cosmetic ingredients which may not be used cosmetic products, due to their toxicological properties. Annex III lists substances which cosmetic products must not contain except subject to restrictions and conditions laid down.
The Sixth Amendment provides that the Commission shall compile an inventory of cosmetic ingredients on the basis in particular of information supplied by the industry concerned. Also, the Commission must adopt a common ingredient nomenclature which will be integrated into the inventory. The Inventory must be indicative, updated periodically and must not constitute a list of substances authorised for use in cosmetic products.

On 8th May 1996, the European Commission established an Inventory and a common nomenclature of the ingredients employed in cosmetic products (Commission Decision 96/335/EC - OJ L 132 of 1.6.96).
The Inventory contains information concerning a series of details need to identify correctly each ingredient.

The ingredients included in Section 1 (more than 6.000 entries) are listed in the alphabetical order of their INCI names, and the information provided covers all particulars concerning identity, usual functions and restrictions. The abbreviation INCI stands for International Nomenclature of Cosmetic Ingredients and was adopted by COLIPA (European Cosmetic, Toiletry and Perfumery Association) as a truly international approach.
The following technical identification is also included, where applicable:

* INN (International Non-proprietary Name) name

* Ph. Eur. (European Pharmacopoeia) name
* CAS (Chemical Abstract Service) number
* EINECS (European Inventory of Existing Commercial Chemical Substances) number
* ELINCS (European List of Notified Chemical Substances) number
* Chemical/IUPAC (International Union of Pure and Applied Chemistry) name

Section II, on perfume and aromatic raw materials, includes more than 2.400 entities with the information necessary to describe a chemical substance, i.e., a chemical name, a CAS number and an EINECS number. The function of all ingredients is to perfume and the restrictions on the use of a given ingredient are identified wherever relevant with asterisks.

Progress is being made to improve the Inventory.

The SCCNFP has adopted in its Plenary Meeting of 17 February 1999, the following Status Report on the Inventory of Cosmetic Ingredients (SCCNFP/0098/99 Final):

1) During the 59th Plenary Meeting (19th April 1995) of the former Scientific Committee (SCC), the Committee approved the Inventory of ingredients employed in cosmetic products which was proposed, in spite of its problems, in order to comply with the provisions of the 6th Amendment of the "Cosmetics" Directive 76/768/EEC. Nevertheless the SCC gave its approval under two conditions:
   a) Swiftly improve the inventory on the lines proposed by the Working Party (see Annex I).
   b) Clearly state in the introduction to the Inventory that it would be regularly updated.
2) The objective of the Inventory is to ensure consumer protection and information by an appropriate labelling of the ingredients using a common nomenclature, and to serve as a tool in the Commission's efforts for the protection of the consumer's health.

The following comments on the relevance of the Inventory of cosmetic ingredients may be emphasized:

a) First of all, the word "Inventory" is rather poor to express its role and function, mentioned above, because the Article 5a does not intend to establish just a catalogue of ingredients; on the contrary, each entry must contain information of a given ingredient able to permit a correct chemical identification as well as the ingredient's functions and, where appropriate, any restrictions and conditions of use and warnings. This information is necessary because it is useful for the Health Authorities of the Member States to solve medical problems potentially associated with the use of a cosmetic product. It is impossible for an individual to know the toxicological profile of all possible ingredients which may be included in a cosmetic formulation; in this context, the Inventory of Cosmetic Ingredients should be the essential tool to more easily obtain the information needed to determine a medical decision.
b) Each entry of the Inventory should include a precise identification of the cosmetic ingredient using the following parameters: its chemical name, the CTFA (Cosmetic, Toiletry and Fragrance Association) name, the European Pharmacopoeia name, the international non-proprietary names recommended by the World Health Organisation, the IUPAC name, the EINECS, CAS and Colour Index Numbers, and the Common Name. As a consequence, each entry must

identify only one ingredient and one ingredient must be identified by only one entry.

c) The Commission has adopted as Common Names the former CTFA Names, which were re-named INCI (International Nomenclature Cosmetic Ingredient) Names to indicate their official acceptance at international level. Objective exceptions to this rule were the substitution of the English botanical names by their systematic (Linné) latin names, and the substitution of the US FDA names for certified colors by the names adopted in the Annexes of the Cosmetic Directive (CI Numbers, codified HC hair dyes etc).

3) A Commission's expert has proposed corrections and amendments to the Inventory and has reviewed the information regarding the new ingredients to be incorporated in the Update. Industry has introduced new functions and amended the Inventory accordingly.
4) A draft update concerning existing ingredients was presented by Industry in November 1998. A final Update of the Inventory should include the new ingredients and the modifications proposed for existing ingredients.
5) The Specific Working Party (SWP) "Inventory" has presented working documents on specific issues such as "INCI names of Ethyl Hexyl derivatives", "INCI names of Amphoderivatives", "Nomenclature of ingredients of botanical origin", "Section II of the Inventory on Fragrances" and has discussed these issues with the concerned bodies.

At present the information given for many entries in the Inventory is not adequate and needs to be corrected. In particular, the following six priorities must be incorporated into the 1st update of the Inventory.

- To accomplish the principle: each INCI name should refer to only one specific ingredient.
- To correct the INCI names of Ethyl Hexyl derivatives and to adopt a final decision about Amphoderivatives.
- To solve problems of nomenclature of ingredients of plant and animal origin with more transparency, as approved, particularly by indicating: the part of the plant used and the type of preparation or derivative. In addition the main chemical components and, if appropriate, specific components of potential concern must be included under Chem/IUPAC name.
- To solve problems on chemical identification associated with polymers.
- To solve the problem of hair dyes/cosmetic colorants concerning C.I. identification and restrictions.
- To improve the functions of the ingredient. Examples: Additive, Biological additive, Oral care.

The adoption of these 6 recommendations by the Industry requires:

- To change the statement in the preamble of the Section I: "An INCI name may cover several chemical entities" by "one ingredient one INCI name and one INCI name one ingredient".
- To revise and to modify accordingly the nomenclature conventions referring to the proposed 6 recommendations.

6) The Committee considers that it is indispensable to take into account the suggested modifications of the entries of the Inventory published in 1995, before the adoption of the 1st update of the Inventory.

Annex

INVENTORY SUB-GROUP MEETING
Brussels, 12th December 1996

SCC RECOMMENDATIONS FOR UPDATING THE INVENTORY OF COSMETIC INGREDIENTS

1) Include INCI nomenclature conventions to explain abbreviations and generic names.

2) Include structural formula for well defined chemicals.

3) Specify in the complex extracts (from plants or animal sources) the principal component(s) and other component(s) with specific functions.

4) Apply special case by case consideration for those natural extracts recognised as possibly containing substances known to have toxic potential. Placing into Annex III should be considered.

5) Correct the errors in common names.

6) Include well known ingredients not present in the Inventory but currently in use by the Cosmetic industry.

7) Consider ELINCS as a useful source of new ingredients for updating the Inventory.

8) Delete ingredients not presently used by the Cosmetic industry.

9) Describe more accurately the actual functions of Cosmetic ingredients.

10) If several synonyms exist for a given ingredient, make cross reference to the most widely used (e.g. “Matricaria chamomilla”, See “Chamomilla recutita”; “Acid blue 1”, See “C.I. 42045”).

ADDENDUM

In the light of the revision of the Inventory, some particular problems concerning (1) UV filters and UV absorbers, (2) Preservatives and Antimicrobials, have to be taken into consideration.
According to their use, both categories are submitted to restrictions (positive list Annexes) or not.

Whatever their actual regulatory status, regarding their toxicological profile they must be considered on the same basis.

6. CATEGORIES OF COSMETIC PRODUCTS AND EXPOSURE LEVELS IN USE

The assessment of the safety of a cosmetic product clearly depends on how it used. This is important, since it determines the amount of substance which may be ingested, inhaled or absorbed through the skin or mucous membranes. Consideration of the quantity of ingredients used in the different products is also important, as the following examples may illustrate.

For example, soaps are applied in dilute form and although the area of application may be extensive, the product is rapidly washed off.

Products used on the lips and mouth will be ingested to some extent.

Cosmetics used around the eyes and genital regions may come into contact with the conjunctiva or mucosa respectively, resulting in reactions due to the thin epithelial lining of these areas.

Sunscreens, body lotions or body creams may be applied over a large surface of the body and the ingredients, often at appreciable concentrations, may remain in contact with the skin over several hours. Sunscreens, due to their extensive skin contact, combined with direct exposure to UV radiation for prolonged periods, require a distinct type of safety evaluation (see Annex 2)

Thus before any safety evaluation and risk assessment of a finished product is made, the degree and route of consumer exposure must be ascertained. This has to be done on a case-by-case basis but the following may provide guidance.
In calculating the exposure the following factors at least must be considered.

1. *Class of cosmetic product(s) in which the ingredient may be used.*
2. *Method of application: rubbed-on, sprayed, applied and washed off, etc.*
3. *Concentration of ingredients in product.*
4. *Quantity of product used at each application.*
5. *Frequency of application.*
6. *Total area of skin contact.*
7. *Site of contact (e.g., mucous membrane, sunburnt skin).*
8. *Duration of contact (e.g., rinse-off products)*
9. *Foreseeable misuse which may increase exposure.*
10. *Nature of consumers (e.g., children, people with sensitive skin).*

11. *Quantity likely to enter the body.*
12. *Projected number of consumers.*
13. *Application on skin areas exposed to sunlight.*

The relevant exposure depends upon the toxicological effects under consideration. For example, for skin irritation or phototoxicity the exposure per unit area of skin is important, while for systemic toxicity the exposure per unit of body weight is of more significance.

The exposure route or routes (skin, mucous membranes, ingestion, inhalation, skin exposed to sunlight) must be considered in designing any test programme and in risk analysis. The possibility of secondary exposure by routes other than those resulting from direct application also should be considered, e.g.. inhalation of hairsprays, ingestion of lip products.

Usage of cosmetics products depends on several factors, some of which will vary over time, such as age group, seasonal variations, local habits, fashion trends, disposable income, product innovation.

Because of these changing conditions, it is not possible to indicate in this document specific use levels of cosmetics. They should be defined in a case-by-case approach in the safety evaluation, once the results of testing, as recommended in the guidelines have become available.

In Annexes 4, 5, and 6 some data concerning the level of consumer exposure for specific categories of cosmetic ingredients, are however included.

7. PHYSICAL AND CHEMICAL SPECIFICATIONS

The precise chemical nature of the ingredient and its structural formula, if it is known, should be identified. When available EINECS, ELINCS and CAS numbers should be provided. With regard to ingredients which cannot be identified in terms of their structural formula, sufficient information should be provided on the method of preparation and the material used in their preparation to assess the probable structure and activity of the compound.

The degree of purity should be defined, as well as an identification of the nature of any toxicologically significant impurities that may be present and their concentration. The substances used in toxicity studies should have similar specifications to the substances used in commercial products. Small changes in the nature of impurities can considerably alter the toxicity of substances. In general, therefore, the results of safety studies are relevant only when they refer to the ingredient used or to the product marketed.

It is up to the manufacturer to ensure that no other and no higher amounts of impurities than those chemically defined or technologically unavoidable, which could influence the safety of the finished products, are present in the commercially used material.

Due to the frequent unavailability of chemically pure ingredients, it will be necessary to define the levels of purity, and, in the case of the presence of a toxicologically relevant impurity, to define the maximum admitted concentration of the impurity. The maximum admitted concentration must be based on toxicological values.
With a view to checking the chemical nature of the ingredient and its degree of purity, its physical, chemical and physico-chemical properties should be ascertained and methods should be devised for identification and for qualitative and quantitative control.

8. TOXICITY STUDIES

The determination of toxic potential is the first step in the hazard assessment of an ingredient and consists of a series of toxicity studies, specific to distinct toxicological end points.

SCCNFP stresses that it is aware that toxicity data may be available for new ingredients that are subject to the chemical substances notification procedure (Council Directive 67/548/EEC).

The ***in vitro*** **methodologies** for evaluating the toxic potential of ingredients reported in the literature have not yet been sufficiently validated for use in areas other than the study for mutagenicity/genotoxicity, for pre-screening for severe irritancy, for screening of phototoxicity and for evaluating the percutaneous absorption.
Moreover the *in vitro* methodologies so far available from the toxicological research, have not yet been adequately validated in other areas to be included in regulatory guidelines at this time.

At present, therefore, there are very few alternative methods to the use of *in vivo* studies in most areas*.

In vivo **studies** make it possible to investigate the toxicological profile of a cosmetic ingredient when applied to an animal by a route of exposure (topical, oral or by the inhalation route) similar to that of human exposure. They allow the determination of the no-observed adverse effect levels (NOAEL), and also adverse effects at higher exposure.

The following notes represent information on the need to develop specific toxicity studies and indicate the current methodologies used for the safety evaluation of cosmetics.

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* Within the European Union, Directive 86/609/EEC affirms a few general principles governing the use of animals in toxicity experiments on chemicals. These principles, although at variance with those of earlier rules, have stimulated the design of research strategies and the development of methodologies to ascertain the toxic effects of chemical substances, in agreement with alternative, scientifically valid principles.

Directive 86/609/EEC outlaws all experiments on animals, unless they are carried out with the object of:

- research aimed at preserving the species at issue, or
- essential biomedical purposes, provided that the species employed in experiments represent the only specific ones for attaining the purpose.

This means, in principle, a restriction on animal experimentation in the very scope of toxicity studies and, above all, in those cases where the predictive significance of studies of similar effects on humans is rather scant.

The above-mentioned rule firmly maintains (Art. 7.2.) that "An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practically available".

8.1. ACUTE TOXICITY

Two oral *in vivo* acute toxicity methods recently adopted by OECD (fixed dose method, acute toxic class method) contribute to reducing the number of animals used and the suffering they may incur by comparison with the classical method (OECD 401)

The OECD group of national co-ordinators proposes inclusion in the text of Guideline 401 a declaration addressed to the animal welfare associations to the effect that this method is not recommended and should not be used except when warranted.

In the Annex 1 to these guidelines, the SCCNFP stresses that acute toxicity data only have to be provided when they are already available (for example as a result of compliance with the provisions of the seventh amendment to Directive 67/548/EEC on the notification, classification and labelling of dangerous substances)

8.2 PERCUTANEOUS ABSORPTION

Percutaneous absorption may be defined as the movement of a chemical substance applied to the surface of the skin into the circulatory system.

The percutaneous absorbed dose is the amount of a chemical which is systemically distributed.

If a substance under investigation is found to have penetrated through the stratum corneum into deeper layers of the skin, it should be considered as having been absorbed.

In principle:

- Any scientifically validated method will be acceptable to the SCCNFP.

- Studies in human are the ideal. Contemporary ethical standards may prevent the use of potentially toxic or radiolabelled compounds in such studies. For compounds with known pharmacokinetics, the analysis of plasma or urine of unlabelled compounds may be sufficient to characterise percutaneous absorption.

- *In vitro* studies with animal skin (e.g. porcine or rat) or human skin may also be appropriate.

The experience, gained by the European cosmetics industry in the critical domain of *in vitro* percutaneous absorption studies took concrete shape with the presentation in May 1996 of a draft OECD guideline describing the fundamental principles to be considered and the criteria to be followed in defining the test protocols.

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.

In vitro methods to assess the percutaneous absorption of cosmetic ingredients has been the object of an opinion recently adopted by the SCCNFP (SCCFNP/008/98 Final - January 1999) on 20th January 1999: ANNEX 10 .

SCCFNP has also adopted on 23 June 1999 a document on basic criteria needed to be fulfilled for the acceptance of the *in vitro* percutaneous absorption studies to be evaluated (SCCNFP/0167/99 Final - June 1999).

8.3. SKIN IRRITATION

There are to date no validated alternative methods capable of replacing the OECD 404 *in vivo* skin irritation test.

Following a prevalidation exercise in 1995 on skin corrosion tests (TER, CORROSITEX and SKIN² and EPISKIN), ECVAM (European Centre for the Validation of Alternative Methods) undertook a formal validation study involving four skin corrosion tests and 60 test materials, including 20 cosmetic ingredients.

ECVAM has concluded positively the validation of 2 *in vitro* methods to assess the skin corrosivity potential of different chemicals.
The SCCNFP considers that in the safety evaluation of chemicals intended for use as cosmetic ingredients, when corrosivity potential cannot be excluded, they should be tested by the "*Rat Skin Transcutaneous Electrical Resistance* (TER) Test" or by the "EPISKIN Test", before testing for irritancy on animals or humans.

An opinion has been adopted by the SCCNFP on 25th November 1998 (SCCNFP/0070/98 Final - November 1998).
The Guidelines of the use of TER and EPISKIN methods have been presented by ECVAM to OECD (98/III/COS/21) and it is now ready for approval: ANNEX 9.

8.4 EYE IRRITATION

The international EC/Home Office validation study of alternatives to the Draize eye irritancy test did not achieve the expected objectives but triggered the organisation of an ECVAM workshop on the practical aspects of validation and the preparation of a prevalidation schedule, as well as the planning of the COLIPA study.

Limited results were obtained from a small number of protocols in COLIPA's first international validation phase of alternatives to the Draize test, where prediction models had been prepared for each test.

COLIPA has organised discussions on the second experimental validation phase. Since the analysis of the results of the first phase revealed the variability of the *in vivo* data, COLIPA envisages using specific test materials, authorising a more mechanistic approach.

Currently there are no validated alternative methods capable of replacing the OECD 405 *in vivo* eye irritancy test and COLIPA does not expect significant progress to emerge from the second validation phase before 2000.

However, one might consider encouraging a flexible approach by attempting to evaluate the potential of certain categories of ingredients acting via common mechanisms by comparison with the data available for appropriate control substances.

8.5. SKIN SENSITISATION AND PHOTOSENSITISATION

Concerning skin sensitisation a proposal for developing an *in vitro* test for the detection of the sensitising potential of chemical substances was launched in 1991 (DG XII).
Since then, significant research work has been undertaken to define the mechanistic bases of skin sensitisation. The report of a workshop organised by ECVAM in April 1995 has been published in ATLA, 24, 683-705 (1996).
Definitive results in this domain can only be expected in the medium term or perhaps long term.
No attempts have been made to develop *in vitro* methods for detection of photosensitisation.

8.6. SUBCHRONIC TOXICITY

In the case of the development of ingredients evaluated by the SCCNFP which have specific biological properties, the manufacturer's liability is not confined to compliance with the provisions governing the notification of chemical substances. Evaluation of the systemic risk is a key element in evaluating the safety of new ingredients, even if the fact that they are produced in very small quantities exempts them from complete notification.

The SCCNFP considers that the use of animal experiments to study one or more potential toxic effects [for example subchronic toxicity, oral route] remains a scientific necessity. The SCCNFP is of the opinion that some more appropriate routes of application [dermal application], might offer more relevance to the data. This subject will be evaluated carefully by SCCNFP, during 1999.

8.7. MUTAGENICITY/GENOTOXICITY

Several *in vitro* genotoxicity tests are available. The SCCNFP is of the opinion that the combination of two *in vitro* tests:

– bacterial reverse mutation test (or *in vitro* mammalian cell gene mutation test for specific chemicals, for which a scientific justification must be provided)
– *in vitro* mammalian cell chromosome aberration test

provides in general sufficient evidence of mutagenic and/or genotoxic potential. Depending on the results, other *in vitro* or *in vivo* tests may be required. This approach has been applied for some time by the SCCNFP in evaluating the safety of cosmetic ingredients.

Use of *in vivo* tests is limited to confirmation of a mutagenic activity already observed *in vitro*.

8.8. PHOTOTOXICITY/PHOTOIRRITATION

As reported in the Second Revision of the Notes of Guidance (XXIV/1878/97) adopted by the former SCC, all chemicals which are able to absorb UVA and/or UVB light, may change their molecular configuration and may undergo further biological reaction of toxicological relevance for consumers.

SCCFNP requests on all such chemicals routine testing for phototoxicity. Animal models have not been validated for testing for phototoxicity.

ECVAM in co-operation with COLIPA, has concluded a series of studies representing pre-validation, validation and application to cosmetic ingredients of an *in vitro* method named "*3T3 Neutral Red Uptake Phototoxicity Test* (3T3 NRU PT)" .
The principle of the method is based on a comparison of the cytotoxicity of a chemical when tested in the presence and in the absence of exposure to a non-cytotoxic dose of UVA/visible light .
The predictive value of this method for a potential human photoxic chemical has been demonstrated to be between 95 and 100%.

The method has been demonstrated to be able to identify UV filter chemicals which result non-phototoxic for consumers and which are employed in the cosmetic products.

A guideline for testing UV light adsorbing chemicals by using 3T3 NRU PT test has been presented to OECD.

An opinion has been adopted by the SCCNFP on 25th November 1998, proposing the use of 3T3 NRU PT test as a method for testing UV light absorbing cosmetic ingredients or mixture of ingredients for phototoxic potential (SCCNFP/0069/98 Final - November 1998): ANNEX 3.

8.9. PHOTOMUTAGENICITY / PHOTOGENOTOXICITY

In 1990 the SCC adopted guidelines for testing the photomutagenicity / photogenotoxicity of UV radiation absorbing cosmetic ingredients.

Since 1990, COLIPA has submitted dossiers on UV filters containing photomutagenicity data obtained from different types of tests (see ANNEX 3).

Moreover, COLIPA has organised a round-robin analysis whose results allow the development of compliance criteria suitable for *in vitro* photomutagenicity protocols.

The SCCNFP has recommended that the test protocols used by COLIPA be the subject of a validation study. This recommendation has not been taken up until now because of the difficulty of planning a validation study in the absence of *in vivo* reference data. In the case of photomutagenicity / photogenotoxicity , being known the basic biological mechanism

(alteration of genes, chromosomes, DNA sequences), *in vivo* reference dat are not necessary.

OECD is currently discussing Guidelines for photomutagenicity (March 1999).

8.10. HUMAN DATA

In the Second Revision of the Notes of Guidance (XXIV/1878/97) the former SCC stated that " *for an analysis of potential adverse effects of a cosmetic product or ingredient (e.g. skin irritation, non invasive absorption studies) observations in human subjects should be used if available*".

This opinion has been confirmed recently by SCCNFP, considering the animal testing for skin irritation or not yet validated alternative methods may be limited regarding their predictive value for the exposure of the human population. SCCNFP states that the confirmatory human tests may be necessary scientifically and ethically, providing that the toxicological profile of an ingredient or a mixture of ingredients based on animals and/or alternative methods is available and that a high degree of safety is to be expected (SCCNFP/0003/98 Final November 98).

Therefore an opinion on the use of human volunteers in the testing of potentially cutaneous irritant cosmetic ingredients has been adopted by SCCNFP in its plenary meeting of 25th November 1998 (SCCNFP/0003/98 Final - November 98): ANNEX 11.

SCCNFP has also adopted on 23 June 1999 an opinion concerning the use of human volunteers in compatibility testing of finished cosmetic products (SCCNFP/0068/98 Final - June 1999)

8.11. TOXICOKINETIC STUDIES

Toxicokinetic studies may be required for safety assessment if there is significant absorption. Toxicokinetic studies are also of importance in extrapolating both *in vitro* and *in vivo* animal data to man.

8.12. METABOLISM STUDIES

In some cases the metabolic fate of the cosmetic ingredient, that is absorbed into the biological system of the human body, can have an important bearing of its toxic potential, its disposition in the body and its excretion.

8.13. LONG-TERM TOXICITY STUDIES

The objective of long-term studies is to determine the effects of a cosmetic ingredient in a mammalian species following prolonged and repeated exposure. In these tests, effects which require a long latency period or which are cumulative become manifest (e.g. carcinogenicity, impairment of fertility, reproductive disorders).

8.14. FINISHED PRODUCTS

The in-house experience acquired by the major cosmetics firms is particularly interesting in the domain of *in vitro* testing on finished products. However, no *in vitro* alternative test method has yet been successful in a validation study.

The SCCNFP confirms the view that evaluation of the safety of finished products can in general be based on knowledge of the ingredients' toxicity, provided supplementary information is available in certain cases:

- when the vehicle used in the formulation is different to the solvents used in the toxicity tests and when there is a likelihood of an increase in skin penetration or skin irritation;
- when a new, potentially toxic substance is liable to be created through the combination of ingredients present in the finished product.

Compatibility testing of cosmetic finished products on human volunteers has been recommended by SCCNFP in a recent opinion (SCCNFP/0068/98 Final - June 1999)

9. TEST PROCEDURES (METHODOLOGIES)

Test procedures (guidelines) for the performance of toxicity studies evaluating different toxicological endpoints are those reported in Commission Directive 87/302/EEC (Annex: Part B: Methods for the determination of toxicity) and in Commission Directive 92/69/EEC, adapting to technical progress Council Directive 67/548/EEC.

Tests for assessing photomutagenicity, photoirritancy, photosensitization and skin absorption have not yet been included in these Directives.
OECD Guidelines for testing chemicals for their potential to produce health effects are also suitable for the safety evaluation of cosmetics.

According to the Sixth Amendment (Council Directive 93/35/EEC) "*The assessment of the safety for the human health referred to in Paragraph 1(d) shall be carried out in accordance with the principles of Good Laboratory Practice laid down in Council Directive 87/18/EEC... (Art.7 (a)2) "*.

ANNEX 1 - GENERAL TOXICOLOGICAL REQUIREMENTS FOR COSMETIC INGREDIENTS

A. When requested, the manufacturer shall provide the Commission with the information set out below.

1. ***Acute toxicity***[*]
2. ***Skin absorption;***
3. ***Skin irritation;***
4. ***Mucous membrane irritation;***
5. ***Skin sensitisation;***
6. ***Sub-chronic toxicity;***
7. ***Mutagenicity;***
8. ***Phototoxicity and Photomutagenicity (in case of UV-light absorbing substances);***
9. ***Human data (if available)***

When considerable oral intake can be expected or when the data on skin absorption indicate a considerable penetration of the ingredients through the skin, taking into account the toxicological profile of the substance and its chemical structure, the following further information may be necessary:

10. ***Toxicokinetics;***
11. ***Teratogenicity, Reproduction toxicity, Carcinogenicity, and additional Genotoxicity.***
12. ***Metabolism studies***

There may be instances when it does not appear to be necessary or is not technically possible to provide the information: in such cases scientific justification needs to be given.

According to Art.7 of Council Directive 86/609/EEC(OJ L 358 of 18.12.86) on the protection of animals used for experimental and other scientific purposes an animal study shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonable and practically available.

Safety data can be obtained by means of adequate and acceptable scientific methods, based on documented databases, or can be based on studies conducted in accordance with guidelines reported in Directive 87/302/EEC and in Annex to Directive 92/69/EEC and complying with the principle of Good Laboratory Practice (Directive 87/18/EEC).

When complete studies and the results obtained are submitted, it must be stated that the tests were conducted using a substance with the same physical and chemical characteristics of that to be included in the finished cosmetic product.

[*] Acute toxicity data should only be provided if available (e.g. from the Seventh amendment to Directive 67/548/EEC relating to notification, classification and labelling of dangerous substances).

ANNEX 2 - THE USE OF METHODS ALTERNATIVE TO ANIMAL STUDIES IN THE SAFETY EVALUATION OF COSMETIC INGREDIENTS OR COMBINATIONS OF INGREDIENTS*

1. Preamble

Cosmetic products are regulated in the EU Member States by Council Directive 76/768/EEC; pursuant to article 2:

"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 taking in account in particular, the product's presentation, its labelling, any instructions for its use and disposal as well as any other indication or information provided by the manufacturer or his authorised agent or by any other person responsible for placing the product on the Community market".

The justification of the need to request a series of toxicity tests for a small but not negligible fraction of cosmetic ingredients, represented by those included in Annexes III, IV, VI and VII of Council Directive 76/768/EEC, is supported by the SCC's work since its establishment in 1978 to propose banning a series of cosmetic ingredients from use in finished products which have been included in Annex II of Council Directive 76/768/EEC.

Cosmetic ingredients, moreover, consist of a variety of chemical substances belonging to different chemical classes, whose toxicity potential manifests itself in different toxic mechanisms with regard to different organs and functions, which need to be evaluated by conducting a series of toxicity tests (eye irritation, skin irritation, acute toxicity, genotoxicity, teratogenicity, etc.).

Annex III to Council Directive 76/768/EEC contains cosmetic ingredients which are subject to restrictions and conditions of use on the grounds of their intrinsic toxic potential. Annex IV contains colouring agents, whose use is partly limited to a specific part of the human body because of their potential toxic effect.

Annex VI contains preservatives belonging to different chemical classes: their efficacy in protecting cosmetic products from pathogenic micro-organisms which could affect consumer health depends on their biological reactivity which may be a potential health hazard to consumers.

Annex VII lists UV-filters which, by absorbing UV light, may undergo a change in their chemical structure with possible toxicological implications.

In addition to these groups of cosmetic ingredients, hair dyes may present a potential toxic hazard to consumer health, as documented in several opinions delivered by the SCC.

A new amendment to this Directive was approved in June 1993 (the Sixth Amendment: Council Directive 93/35/EEC) introducing several innovations. These include:

"Member States shall prohibit the marketing of products containing
i) ingredients or combinations of ingredients tested on animals after 1 January 1998" (Art. 4 Council Directive 76/768/EEC as amended by Art(1) of / Council Directive 93/35/EEC).

Moreover, the Sixth Amendment states that "if there has been insufficient progress in developing satisfactory methods to replace animal testing, and in particular in those cases **where alternative methods of testing**, despite all reasonable endeavours, **have not been scientifically validated as offering an equivalent level of protection for the consumers**, taking into account OECD toxicity test guidelines, the Commission shall, by 1 January, 1997 submit draft measures to postpone the date of implementation of this provision . This deadline has been postponed to June 30, 2000 by Commission Directive 97/18/EEC .

Before submitting such measures, the Commission will consult the Scientific Committee for Cosmetology"(Art. 4(1) 93/35/EEC).

2. Alternative methods

2.1. The use of non-animal models as a research tool in toxicity testing has been developed during the last decades mainly to meet the need for better predictions of the potentially toxic effects of various chemicals on human health; it is well known, for instance, that differences in some stages of the metabolic routes of exogenous chemicals do exist between humans and several types of animal models. In the last years, moreover, the public and policymakers have been calling for a reduction of animal experimentation also in the field of toxicity testing.

An alternative methodology means any modification of the official guidelines on conducting toxicological studies for the assessment of potentially toxic effects affecting human health and exerted by chemical substances in general, including cosmetic, as integral parts of the cosmetic products being marketed.
Instead of animals, these alternative methodologies study simpler biological systems, represented by bacterial cell cultures and different mammalian or human cultures or tissues and particular animal organs, or abiotic artificial systems, or computerized analysis programs.

2.2. Several definitions of validity have been discussed and defined (1,2,3,4). Because there are a number of different aspects to validation, the SCC gives top priority to soundly based scientific criteria for validating alternative methods, within the context of the practical use of tests for human safety evaluation.
In essence, the validation exercise is an "*in vitro:in vivo* comparison", whether the *in vivo* data is from man or from animals depends upon the availability of good human data. It seems unlikely that good-quality human data covering a spectrum of cosmetic ingredients with different toxicological protocols will be available. In practice, this means that good quality animal data using currently recommended protocols will be the benchmark for many alternative methods (DGXXIV/1942/95).

Validation is the process by which the reliability and relevance of a procedure are established for a specific purpose (CAAT/ERGATT Workshop, 1990; ECVAM Workshop Report 5, 1995).

According to the OECD, any test guideline proposal, be it an animal study or an alternative test, should: (a) properly address the end-points concerned, (b) have undergone a critical appraisal concerning its scientific justification, its sensitivity and reproducibility, including (where feasible and relevant) a comparative study supporting the validity of the test proposed, (c) allow standardization and (d) not normally require unique equipment or technical experience (OECD, 1993; H.B.W.M.KOETER, 1995).

2.3. The SCCNFP has formulated the following general guidelines on the information that will be required to the assess scientific validity. This is identified under the following five headings:

(1) A Scientific Justification for the Basis of the Alternative Test System(s) Chosen

A fully reasoned explanation should be given for the choice of test protocol(s) along with reasons for ignoring any similar or equivalent methods. This rationale should include an explanation of (i) the biological basis of the test and (ii) the test's relevance to mechanisms of human toxicity and to the circumstances of human exposure. The intended use of the test(s) along with any known limitations must be individually and explicitly pre-defined. This should include reference to existing data on the test(s) and whether the test system is readily and widely available.

(2) A Reasoned Explanation for the Test Chemicals used in the Validation Study

This should make clear the rationale for including the chemicals to be used in the study. The chemicals chosen should include:

(i) examples from each of the different major classes of cosmetic ingredients along with examples of UV-filters, colouring agents, preservatives and hair dyes - because positive lists exist, or will do, for these classes of ingredients;

(ii) chemicals with different toxicological potencies - possibly including some of those listed in Annex II to the Cosmetics Directive;

(iii) chemicals with different toxicological mechanisms (where these are known).

For certain tests the choice of test chemicals may be restricted by the number of chemicals for which good quality *in vivo* data is available. It would be advisable to include several examples of chemicals from current positive lists which are known to be safe (or acceptable) under normal conditions of use - as negative controls.

Because the SCCNFP is concerned with cosmetic ingredient safety, alternative tests for this purpose should be validated with a proportion of chemicals (rule of thumb: over 30%) present in the inventory. Furthermore, it is necessary to include a range of chemicals at the low-end of the potency spectrum of toxicity. Should a validation study be performed

without representatives of any of the major classes of cosmetic ingredients, an explanation should be given as to why they were not included.

(3) Evidence that the Test Methods have been Optimised

Quantitative data with appropriate statistical analysis should normally be provided to show that systematic optimisation studies have been performed. This is normal good scientific practice and experimentation should show (i) that exposure conditions and the test duration have been optimised in several laboratories with respect to the response measured, and (ii) that the data collection intervals and other conditions are optimal for sensitivity, specificity, precision and reproducibility of the assay (see following pages for definitions). An adequate range of doses and number of replicates should be provided to enable statistical analysis. This analysis should lead to an agreed and precisely defined protocol, which will meet the needs of other laboratories participating in the next phase of the work

It is important to note that alternative methods may be optimised to give a quantitative or qualitative result. It seems likely that for pragmatic reasons the latter approach will be adopted in some validation studies. Nonetheless, quantitative dose-response data will be necessary to demonstrate the biological responsiveness and mechanistic relevance of a test, and a simple categorisation of results such as “positive or negative”, “response or non-response” will not be adequate.

(4) Statistical Data on the Performance of the Method in Interlaboratory Trials using a Representative Set Test Compounds (i.e. The Validation Study Data)

This stage of validation may be derived from a sub-step approach where an initial sub-set of chemicals is tested to minimise interlaboratory variation and to verify the adequacy of the protocol (5). While this approach is not obligatory, it might then be followed by testing a full range of the chemicals identified in section 2 above. Whichever approach is taken, the rationale should be explained.

Before commencing the main part of a validation study, the objective of the test should be pre-defined, with an unambiguous statement of criteria for success. The main part of the performance analysis should define the performance characteristics or quality of the test(6). This should include the following quantitative measures of the test in use:

(a) *reproducibility*: i.e. interlaboratory variance, intralaboratory variance and intra-individual variance should be based upon dose-response data from the test system. This is a numerical measure of the robustness of the test system (for example see reference (6)).

(b) *precision*: i.e. will the test correctly identify the potency of the biological effect at a specified dose? Precision is thus a measure of the intrinsic ability of a test method to give a consistent and distinct numerical response to a given concentration of the chemical.

(c) *specificity*: i.e. will the test, within its stated limitations, correctly detect all positive compounds, or are there an unacceptable number of false positives or, worse still for human safety, false negatives? Does the test tend to fail-safe by minimising the number of false negatives?

(d) *sensitivity*: i.e. can the test, when applied as a general screen, reliably detect a positive effect at low exposure concentrations? The test should maximally detect true positives. Demonstrable sensitivity to chemicals with low to moderate toxicological potency will be important for cosmetic ingredient safety evaluation.

These definitions are broadly similar to those used elsewhere (6 and 7) and a combination of measures (c) and (d) above can be considered to indicate the accuracy of a test.

The size of the multilaboratory trial and the number of replicates necessary to demonstrate suitability will depend upon the test method(s) and types of chemicals studied, and should be determined on the basis of statistical analysis of a representative set of results. That means that a very precise and highly reproducible method will require fewer replicates and possibly a smaller number of different laboratories to demonstrate its worth than would a test with a great deal of variability in its results. A calculation of the method's discriminant power in relation to sample size would be advisable.

The overall statistical analysis employed should be justified. This statistical analysis must be applied to results from the full set of test chemicals. If all or any part of the results from a participating laboratory or individual chemical are omitted from the statistical analysis, this must be declared and justified. Selective reporting of results, without full or adequate explanation, would invalidate the scientific basis of the method. Nonetheless, "limited validation" may be a legitimate goal if a test appears to be suitable only for a subgroup of cosmetic ingredients, e.g. surfactants or colouring agents. But if limited validation is to be practically useful, the limits of use must be precisely defined and representatives of all major chemical types within the class should be included in validation studies. It is important to appreciate that the biological basis of the test method and the strength of the link to the underlying mechanism of mammalian toxicity will be a major input to the validity assessment.

It should be noted that the SCCNFP, other scientists and regulatory bodies, will not be able to perform a scientific assessment of the validity of a new method without an opportunity for access to all of the raw data; this enables an independent statistical analysis.

(5) A Comparison of the Test Results with Quantitative Human or Animal Safety Data

This should take the form of a correlation or regression analysis between data from the non-animal method and good quality, quantitative, human data. If, as will often be the case, quantitative human data are not available, the mathematical relationship between non-animal data and quantitative data from animal testing should be analysed. If a good relationship between data from the alternative method and current, good quality, GLP-compliant animal data cannot be shown, i.e. for a complex test such as the Draize eye irritancy test, then a reasoned scientific justification must be provided for why it may be appropriate to relate the results of the alternative method to one of the underlying biological mechanisms which contribute to the overall mechanism of toxicity, for example, thrombus formation, haemorrhage, vascularisation or acute inflammation - rather than eye irritation scores. Any such justification must be based upon data from properly conducted, good quality animal experiments, conducted according to currently recommended protocols.

Retrospective attempts to explain unfavourable results must be open, honest and even-handed to avoid undermining the scientific credibility of a validation study. Any post hoc analysis or selective repetition of tests after an initial analysis of the data must be openly and clearly declared. Ideally, the blinded coding of chemicals should only be broken when all of the results are collated, tabulated and the analysis completed. Each and every step of the statistical analysis should be stated if multiple methods are used.

It is proposed that the relationship between the data derived from animal experiments and alternative methods should be formalised as a mathematical relationship, which, if appropriate, may include a transformation of the *in vitro* data or use of non-parametric regression methods. This should quantitatively represent the degree of agreement between the alternative method and the *in vivo* human or animal data, including a comparison of dose response relationships for each method rather than a simple comparison of categorised data. A direct comparison of the dose-response relationship of individual chemicals in the *in vitro* test with the dose-response for the same chemical in *in vivo* tests is desirable to demonstrate the mechanistic links between the two methods. A statistical analysis of the strength of *in vitro:in vivo* relationships is recommended.

Ultimately, the safety data from validated non-animal alternatives will be integrated into an overall safety assessment, which for the medium-term future will include some tests performed on animals (8). It is the overall safety assessment which will determine the suitability of a chemical for use as a cosmetic ingredient, rather than reliance upon the results of a single or small number of *in vitro* tests. (DGXXIV/1942/95).

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PRESENT DEVELOPMENT AND VALIDATION OF ADEQUATE ALTERNATIVE METHODOLOGIES TO THE USE OF ANIMALS IN SAFETY TESTING OF COSMETICS

Opinion adopted by SCCNFP in its plenary meeting of 23 June 1999 (SCCNFP/0177/99 Final).

1- Preamble

One of the main objectives of Council Directive 76/768/CE of 27 July 1976 on the approximation of the laws of the member states relating to cosmetic products, as stated in its recitals is "the safeguarding of public health". Moreover "cosmetic products must not be harmful under normal or foreseeable conditions of use… taking into account the possibility of danger to zones of the body which are contiguous to the area of application".

These positions have been maintained by means of a set of Commission Directives, and reiterated in a series of Amendments to the Council Directive.

In 1977 the Commission Decision 78/45/EEC of 19 December established a Scientific Committee on Cosmetology (SCC) to assist the Commission in the process of drafting and amending the Community cosmetic laws. The SCC is formed by scientists highly qualified in the fields of medicine, toxicology, biology, chemistry and other similar disciplines.

In 1997 Commission Decision 97/18/EEC of 23 July 1997 reorganized such precious technical assistance to the Commission by establishing a "Scientific Committee on Cosmetic and Non-Food Products intended for Consumers" (SCCNFP) to be consulted in the case laid down by Community legislation, and on questions of particular relevance to consumers' health. The SCCNFP has been requested to produce "scientific advice concerning matters relating to consumers' health in its strict sense".

Art. 4 of Council Directive 76/768/ECC, amended by Council Directive 93/35/EEC, affirms a prohibition of the marketing of cosmetic products containing "ingredients or combinations of ingredients tested on animals after 1 January 1998". The date was later postponed to 30 June 2000 by Commission Directive 97/18/EEC of 17 April 1997.

The Commission shall submit draft measures to postpone the date of implementation of this provision by 1 January 2000. A justification of a possible postponement of the deadline of 30 June 2000, could be an insufficient progress in developing alternative methods to replace animal testing or, also, other cases where alternative methods would not have been scientifically validated as offering an equivalent level of protection to consumers. Before submitting such measures, the Commission shall consult the SCCNFP.

In particular, the Commission has requested the SCCNFP as regards the status of alternative methods for the safety assessment of cosmetic ingredients according to the

current state-of-the-art. Specifically, the Commission has requested the SCCNFP to assess the possibility to replace the data obtained on the basis of animal tests with data obtained making use of alternative methods in the safety evaluation of cosmetic ingredients, and to indicate those end-points for which alternative methods to animal testing are not available yet (Doc. no. 16831 of 11 August 1998).

2- Safety evaluation of cosmetic ingredients

The SCCNFP and previously to it, the SCC, has illustrated in a set of documents the concepts and the criteria of the present procedure of safety evaluation of cosmetic ingredients, based on the experience developed during more than 20 years, by regulating ca. 800 individual cosmetic ingredients of which over 400 have been proposed for a ban, due to their toxic and harmful effects to consumers' health (Report EUR 8794; SPC/803-5/90; XXIV/1878/97; SCCNFP/0119/99 Final).

As stressed several times by the SCCNFP, the primary goal of the safety testing of all cosmetic ingredients presented to the European Commission for their possible inclusion in the technical Annexes of the Cosmetic Directive, is to determine the potential of these ingredients for their harmful effects in an experimental model, so that it makes possible, by extrapolation, to predict the same effect or the absence of harmful effects for consumers.

According to medical science, the safety studies should permit a quantitative determination of the potential for a cosmetic ingredient, or a mixture of cosmetic ingredients to produce local and systemic adverse effects and allow a determination of factors which may influence the nature, severity and possible reversibility of effects (Ref. 1).

Information necessary to the above purposes can be obtained only from carefully designed and well conducted studies. Toxicology testing programmes generally begin with single exposure *in vivo* or *in vitro* studies and progress to evaluate the effects of long-term repeated exposures.

The most used and recognized adequate models for safety testing are those represented by living laboratory animals (mice, rats, rabbits, guinea pigs etc.) which have been the object of millions of experimental studies developed by the toxicological research. In the last twenty years new toxicological systems no longer based on animal models, have been employed by scientists and accepted by national, continental (EU, Ref. 2, 3) and international (OECD, Ref. 4) regulations.

These new test systems are represented by mutagenicity/genotoxicity *in vitro* tests which make use of individual cellular organisms (bacteria, yeast, mammalian cells) or insects. This development has enabled, in many cases, to avoid the use of a large number of animals, as requested by the very expensive and laborious long-term carcinogenicity bioassays (Ref. 1).

After the approval of Sixth Amendment, the SCC and later the SCCNFP have been monitoring the several actions developed by scientific groups, including academics, industrial research and public institutions, to stimulate the progress in the development and validation of alternative methods to the use of animal models in the safety testing of cosmetics. In particular, the SCCNFP has been discussing and evaluating together with ECVAM and COLIPA scientists, the results of the pre-validation and validation studies and the applicability of these results to the safety evaluation of cosmetic ingredients and

cosmetic products. An opinion has been adopted by the SCCNFP on 20 January 1999 during its Plenary Meeting on the use of some alternative methods to animal testing in the safety evaluation of cosmetic ingredients (Ref. 5).

3- Presently validated alternative methods

The following notes are representing the opinion on some alternative methods which could be of some relevant use in the safety evaluation of cosmetic ingredients, and on the state-of-the-art of some in vitro methods which could be validated in the near future.

3-1 Skin Irritation

The present scientific knowledge on the mechanistic basis of skin irritation *in vivo* is still limited, due to the complex set of reactions involved , and in the impossibility to define the key specific and relevant end-points which could be evaluated by an in vitro system (Ref. 6).

However, a pre-validation study is in progress by using human skin models and a pig ear test, under the sponsorship of ECVAM.

In the evaluation of a potential skin irritant effect by a cosmetic ingredient, it is still possible by using a combination of different criteria of evaluation, to identify the corrosivity/non-corrosivity potential of chemical ingredients.

Recently, two alternative *in vitro* methods for skin corrosivity have been validated and demonstrated to be applicable to the procedure for safety testing also in the sector of cosmetic ingredients. A draft new guideline on skin corrosivity testing has been submitted to OECD and to the European Commission (EC) for its inclusion in the Annex V of Council Directive 67/548/EEC. The new *in vitro* methods are represented by the Transcutaneous Electrical Resistance (TER) Test and by the Episkin Test. The SCCNFP has proposed the use of these two methods when corrosivity of cosmetic ingredients must be tested on animals, or when humans cannot be excluded. (Ref. 5)

3-2 Phototoxicity

OECD guidelines or EC guidelines on phototoxicity testing have not been adopted yet for the testing of UV light absorbing substances on animal models. Pre-validation, validation and applicability on cosmetic ingredients, such as the UV filters have been the object of a series of studies and different projects. An *in vitro* model, the 3T3 NEUTRAL RED UPTAKE, Phototoxicity Test has been developed and demonstrated to be valid for the identification of phototoxic UV absorbing chemicals, including cosmetic ingredients (Ref. 7). This method is based on a cell phototoxicity process, observed in a mammalian cell population *in vitro*.

A draft protocol for testing phototoxicity, by employing this new alternative method has been presented to the OECD and to the European Commission for its inclusion in Annex V of Council Directive 67/548/EEC.

The SCCNFP in its Plenary Meeting of January 20 1999 has adopted an opinion which proposes to the EC the use of the “3T3 NRU Phototoxicity Test” as the standard method

for testing the UV light absorbing cosmetic ingredients or mixtures of ingredients for phototoxic potential.(Ref. 5)

3-3 Percutaneous Absorption

OECD guidelines or EC guidelines on safety testing for percutaneous absorption have not been adopted yet. However, some draft measures have been presented to OECD.

The assessment of percutaneous absorption of cosmetic ingredients has primary relevance in the procedure for evaluating the safety of cosmetics for consumers. The SCCNFP has recently reviewed the available scientific literature and data developed by cosmetic industry in this sector of testing and has agreed with the rationale for using *in vitro* methods to evaluate the percutaneous absorption. An opinion has been adopted by SCCNFP during its Plenary Meeting of 20 January 1999 proposing the use of *in vitro* methodologies for the safety testing of cosmetic ingredients. Moreover, due to the lack of a guideline approved by OECD or by the European Commission, the SCCNFP has defined a set of basic criteria for the *in vitro* assessment of percutaneous absorption of cosmetic ingredients, which have been adopted during the Plenary Meeting of 23 June 1999. These basic criteria represent the recommendation put forward by the SCCNFP for all cosmetic industries, in their assessment of the percutaneous absorption (Doc. SCCNFP/0167/99 Final).

3-4 Ocular Irritation

Ocular irritation testing is needed for many cosmetic ingredients applied in particular zones of the consumers' body, especially those which may come into contact with the eye.

Since the approval of Sixth Amendment, several collaborative studies have been developed within the European Union on chemicals of different use or cosmetic ingredients; similar studies have been developed in the USA (finished cosmetic products) and Japan (cosmetic ingredients) (Ref. 8.1 – 8.6).

The results of such extensive studies have revealed that no single test can fully replace the Draize rabbit test; that some *in vitro* tests have a certain level of predictivity and some are promising; that the level of predictivity is improved when combining several and different test systems (batteries) (Ref. 9). Some of these *in vitro* tests combined with Structure Activity Relationships and Physicochemical data could be used to identify potentially non-ocular irritant chemicals, but the need to use *in vivo* tests is still requested.

A document prepared by ECVAM and COLIPA on the current status of the alternatives to eye irritation (Doc. SCCNFP/0174/99) indicates the utility of a "short-time approach optimizing the current strategies and methods, and a long-term approach allowing gaps in knowledge to be filled, so as to increase the current predictivity of the alternative methods, and as a basis for the development of new methods, are being developed and conducted".

Attempts are currently being carried out by COLIPA: (1) to review the validation studies concluded so far, as to extract the maximum bulk of information to help refine the currently available prediction models; (2) to optimise the tier testing strategies as a "reduction alternative" proposed by ECVAM; (3) to propose a research programme to increase the knowledge on the mechanisms of chemically induced eye irritation so as to

develop complementary test methods to the current alternative or to modify these in view of improving their predictive capacity (Ref. 9).

3-5 Skin Sensitization

Skin sensitization is a complex phenomenon which implies a series of biological reactions; skin permeation by the allergen; reaction of the hapten with a skin protein; processing haptenated proteins by epidermal Langerhans cells; migration of Langerhans cells to draining lymph nodes and interaction with T cells; recognition of hapten by specific T cells; etc (Ref. 10).

It should be possible by combining computerized expert systems with appropriate biological *in vitro* systems to identify chemicals able to perform the initial reactions. At present the elucidation of the critical stages of the phenomenon is still under study and considerable research is being undertaken. Recently, a substantial opportunity to refine and reduce animal use in the hazard identification of skin sensitizing cosmetic ingredients has been achieved with the Murine Local Lymph Node Assay (LLNA) (Ref. 11). This aspect is being considered by the SCCNFP.

3-6 Other Toxicological End-points

Besides, apart carcinogenicity, the other fields of toxicology do not seem at present to offer the possibility to substitute animal models with alternative methods not using animals. Due to the same essential basic mechanisms between certain types of carcinogenic substances (genotoxic carcinogens) and mutagenic substances, chemicals that induce mutations in somatic cells *in vitro* should be regarded to as potential carcinogens and hence mutagenicity screens are of value in identifying potential “genotoxic” carcinogens. This is not the case for other fields of toxicology, such as reproductive toxicology, neurotoxicity, teratogenicity, sub-chronic toxicology, etc. The scientific knowledge of the basic mechanisms of the different types of toxic events still requires development of long-term planning or research into basic and cellular events underlying toxicity.

3-7 The use of human volunteers in the safety evaluation of cosmetic ingredients and finished cosmetic products

In a recent opinion, the SCCNFP has stressed the concept that the tests on animals for skin irritation or (not yet) validated alternative methods may be limited regarding their predictive value for exposure of human population. The SCCNFP states that confirmatory tests on humans may be needed scientifically and ethically, provided that the toxicological profile of an ingredient, or a mixture of ingredients, or a finished cosmetic product based on animal or alternative methods is available and that a degree of safety is to be expected (SCCNFP/0003/98). This opinion also stresses the concept that confirmatory skin tolerance tests of cosmetics in human should not be preferred to animal testing; that the safety testing of cosmetics on humans may not be considered an alternative method to the use of animals; and that the use of human volunteers in the safety evaluation of cosmetics is subjected to ethical concern.

The SCCNFP has recently approved Guidelines on the use of human volunteers in the testing of potentially cutaneous irritant cosmetic ingredients or mixtures of ingredients (SCCNFP/0003/98) and Guidelines on the use of human volunteers in compatibility testing of finished cosmetic products (SCCNFP/0068/98 Final).

4- **Opinion of the SCCNFP**

On the basis of the scientific literature, after the evaluation of different research programmes conducted by cosmetic industries, the European Commission (ECVAM) and other Institutions, and considering the results obtained during the period 1993-1999) on the development and validation of alternative methodologies to the use of animals in the safety testing of cosmetic ingredients, the SCCNFP expresses the following opinion to the European Commission.

1) There has been a considerable effort in the technical and scientific fields of the safety testing of chemicals in general and cosmetics in particular, to develop and validate alternative methodologies to the use of animal models which could offer to the consumers an adequate and acceptable level of protection;

2) Due to the complexity of biological mechanisms that represent the basis for the occurrence of toxic events in human organism, a significant effort of scientific research is needed to understand all different steps of the aforementioned mechanisms and their molecular events. A set of research programs have been planned in the sectors of ocular irritation, skin irritation, skin sensitization, neurotoxicity, etc. The results of such researches will considerably influence scientific knowledge on several toxic events which, on turn, will allow the identification of more rigorous and rational criteria and systems to be applied in the safety evaluation of cosmetics, by possibly reducing the need of laboratory animals (Ref. 12);

3) At present, the SCCNFP has identified with the help of the contribute made by ECVAM in this field of activity, the following alternative methods that can be used for the safety testing of cosmetics:

   a) *In Vitro* Methods to assess skin corrosivity in the safety evaluation of cosmetic ingredients or mixtures of ingredients (SCCNFP/0070/98 Final);

   b) *In Vitro* Methods to assess phototoxicity in the safety evaluation of cosmetic ingredients or mixtures of ingredients (SCCNFP/0069/98 Final);

   c) *In Vitro* Methods to assess percutaneous absorption of cosmetic ingredients (SCCNFP/0088/98 Final).

4) Moreover, the SCCNFP has defined the "Basic criteria for the *in vitro* assessment of percutaneous absorption of cosmetic ingredients" (SCCNFP/0169/99 Final) in order to provide the cosmetic industry with a set of recommendations for an adequate protocol for applying the *in vitro* methods in the studies of percutaneous absorption.

5) The SCCNFP has also produced a set of guidelines on the use of human volunteers in the testing of potentially cutaneous irritant cosmetic ingredients or mixtures of ingredients (SCCNFP/0003/98 Final) and in skin compatibility testing of finished products (SCCNFP/0068/98 Final) in order to provide recommendations on the use of human volunteers in the safety evaluation of cosmetics, taking into account scientific and ethical aspects of the problem;

6) The SCCNFP will be monitoring on a regular basis, scientific progress in the development and validation of alternative methods, and it will also evaluate their

applicability to the safety testing of cosmetics, as well as immediately report its opinion to the Commission.

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ANNEX 3 - GUIDELINES FOR THE *IN VITRO* ASSESSMENT OF THE PHOTOTOXIC POTENTIAL OF UV-FILTERS

1- Introduction

All compounds used as sunscreens are, by their nature, chemicals that are able to absorb UVA and/or UVB light. The range of wavelengths which are absorbed by a given compound is termed its absorption spectrum.

As a consequence of such light absorption a chemical may change its molecular configuration, or may be transformed into a different chemical molecule. The resulting molecule may undergo further biological reaction of toxicological relevance different from those displayed by the original molecule, hence the need to investigate specific phototoxic effects. These relate particularly to photoirritancy, photosensitization and photomutagenicity.

Testing for photoirritancy. photomutagenicity and photosensitization will routinely be required on all such compounds.

The following draft guidelines consider the need for testing of sunscreen agents for photomutagenic and phototoxic potential, that is screening for mutagenic and irritation properties under the influence of simulated solar radiation; guidance on the methodologies to use is given.

2- Testing for Photomutagenicity

Introduction

Sunscreen agents should routinely be tested for their potential to induce gene mutation and chromosome aberrations *in vitro* both in the presence and absence of a metabolic activation system. In addition studies to investigate the potential of such agents to exhibit photomutagenic properties will normally be required. However, if evidence can be provided using adequate methods to demonstrate that the compound exhibits complete stability after 10 hours exposure to simulated solar radiation, such photomutagenicity testing may not be required.

Outline of test method

Test substance

The sunscreen agent must be characterised by its absorption spectrum in an appropriate solvent.

Test systems to be used

Both a bacterial assay for gene mutation and an in vitro test for chromosome aberrations in mammalian cells should be performed in the presence of UV radiation. Further testing may be required, depending on the results obtained.

Test conditions

Light source

The test system should be exposed to radiation produced by a solar simulator lamp. The wavelength spectrum of the lamp must be indicated; it should cover both UVA and UVB radiation

Radiation doses

The doses of the simulated solar radiation and the concentration of the sunscreen agent used should be defined in such a way as to permit an adequate evaluation of the potential of the agent to induce mutagenic effects in the presence of light. The rationale for the selection of doses should be given in the test report.

Metabolic activation

Although there exists some information on the possible synergistic effect between metabolic activation and light, present scientific knowledge does not allow the definition of standard conditions for testing the effect of light on a chemical in the presence of a metabolic activation system. The evaluation of the effect of radiation in the presence of an exogenous metabolic activation system is thus not recommended at present.

Positive control

It is suggested that 8-methoxypsoralen be used as a positive control, with effects investigated both in the presence and absence of simulated solar radiation.

Protocol

Regarding general aspects of these mutagenicity studies, these should as far as possible conform to the guidelines given in Directive 92/69/EEC.

3- <u>Testing for Phototoxicity</u>

Introduction

Recent validation and application studies have demonstrated the validity and the relevance of the *in vitro* method of 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT) for the identification of phototoxic and non-phototoxic UV light absorbing chemicals employed as cosmetic ingredients.
Proposal for guideline on the conduct of this test has been presented to OECD for final regulatory acceptance.

SCCNFP recommends the use of this *in vitro* method for the definition of the toxicological profile of all UV light absorbing chemical and especially for those cosmetic ingredients to be used as UV filter included in the Annex VII of Directive 76/786/EEC.
The following opinion of the SCCNFP has been adopted on 25th November 1998.

IN VITRO METHODS TO ASSESS PHOTOTOXICITY IN THE SAFETY EVALUATION OF COSMETIC INGREDIENTS OR MIXTURES OF INGREDIENTS*

Terms of reference

DG III requests the opinion of the Scientific Committee on Cosmetic and Non-Food Products (SCCNFP) as to the status of alternative methods for the safety assessment of cosmetic ingredients according to the current state of the art. Specifically DG III requests that the SCCNFP assesses the possibility of replacing data obtained on the basis of animal tests by data obtained making use of alternative methods in the safety evaluation of cosmetic ingredients (XXIV/1890/98)

1- Background

UV-absorbing chemicals are employed as ingredients of various cosmetic products. Guidelines for the safety testing of cosmetics require a test for photo-irritation potential of this type of compounds. Testing usually is done on animals, although an accepted protocol to test *in vivo* for photo-irritation potential does not exist.

2- Different phases in the study

2-1. In a first phase in 1992-1993, a joint EU/COLIPA prevalidation study was designed to identify *in vitro* test procedures for a validation trial under blind conditions. Twenty chemicals with known phototoxicity properties were selected according to scientific criteria by an independent COLIPA task force of experts. The chemicals underwent different tests e.g. photohaemolysis test, histidine oxidation test, Candida albicans test, SOLATEX PI®, Skin2®, Testskin® and the 3T3 mouse fibroblast test. It came out that the 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT) with mouse fibroblasts using a sun simulator UV source (UVA 5J / cm^2) was giving better overall correlation to *in vivo* data than results from any of the other tests.

2-2. At ZEBET a prediction model for the 3T3 NRU PT test was developed, which took the IC-50 values from cytotoxicity dose-concentration curves in the presence and absence of exposure to UV-light into account. A photo-irritation factor (PIF) was calculated which is the ratio of IC_{50} (-UV) / IC_{50} (+UV).

Discriminant analysis showed that a PIF of 5.0 provided the best prediction to discriminate between phototoxic and non-phototoxic chemicals. (Spielmann et al.1994b, 1995).

* Adopted by the plenary meeting of the SCCNFP of 25th November 1998 (SCCNFP/0069/98 Final)

2-3. In the second phase in 1994-1995, the formal validation trial, the most promising *in vitro* phototoxicity tests were validated using 30 carefully selected chemicals. 11 Laboratories were involved in a bind trial. This study was conducted jointly by ECVAM and COLIPA. The test chemicals were selected according to the recommendations of an ECVAM workshop on phototoxicity testing (Spielmann et al. 1994a). The test chemicals were selected according to their phototoxic properties in humans. The aim of the study was to correctly predict the phototoxic potential of chemicals applied systematically or topically in humans. Besides the assessment of the phototoxic potential by PIF using a cut-off value of 5.0, the mean photo effect (MPE), which takes into account the slope of the concentration response curves for cytotoxicity, with a cut-off value of 0.1 was also used. The latter model was published independently from the validation study (Holzhütter 1997).

2-4. The results of the 3T3 NRU PT test were reproducible and correlated well with the *in vivo* data. Therefore, in 1997, the ECVAM Scientific Advisory Committee (ESAC) and in 1998 DG III and DG XI of the European Commission concluded from the formal validation study under blind conditions "that the 3T3 NRU PT is a well validated test and ready to be considered for regulatory acceptance" (Anon. 1998).

2-5. In 1996, the former Scientific Committee on Cosmetology (SCC) asked ECVAM to test the UV chemicals from Annex VII of the Directive 76/768/EEC in a blind trial using the 3T3 NRU PT test (XXIV/1878/97). The selection of the filters out of this list was done according to scientific criteria based on reliable *in vivo* data. (Guillot et al. 1985; Kaidbey and Kligman 1980). 8 UV filters were tested which were shown *in vivo* to be non phototoxic. To balance the study, 10 phototoxic and 10 non-phototoxic chemicals were tested under blind conditions in 4 laboratories; a correlation between 95 and 100 % was obtained when PIF or MPE, respectively, were used to predict the phototoxic potential and when concentrations between 0.1 and 10.0 µg/ml were tested. The management and the participants of this study concluded in 1998 (Spielmann et al. 1998 b) that the phototoxic potential of UV filters can be correctly assessed by the 3T3 NRU PT test.

2-6. In 1998, the SCCNFP reviewed carefully the publications from the validation studies, the ESAC statement and the application study of the UV filters. Critical questions were posed to the management team. These were all answered using appropriate scientific criteria.

3- Opinion of the SCCNFP

Taking the results obtained in the prevalidation and formal validation study of the 3T3 NRU PT test and the results of the application study of this test to the UV filters of Annex VII of the Directive 76/768/EEC into account, the SCCNFP proposes the use of the 3T3 NRU PT test as the standard method for testing the UV light absorbing cosmetic ingredients or mixtures of ingredients for phototoxic potential.

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ANNEX 4 - GENERAL SCHEME FOR DETERMINING THE MARGIN OF SAFETY OF HAIR DYES

There are two types of hair dyes

(I) Oxidation Or Permanent Hair Dyes

A hair dye of this type contains at least two components one of which comprises an oxidising agent (hydrogen peroxide generator). The components are mixed 10 min. before application of a maximum amount of 100 ml and this is rinsed off after 30 minutes. The normal frequency of application is unlikely to exceed once a month.

(II) Semi-Permanent Hair Dyes (And Lotions)

This type of hair dye comprises normally only one component and the maximum amount applied is 35 ml which is rinsed off after 20 minutes. The normal frequency of application is unlikely to exceed once a week for lotions and once every 10 to 12 ring shampoo for a semi-permanent product

Margin of Safety

The margin of safety is calculated from a comparison of the relationship between the critical NOAEL observed in the most sensitive species from appropriate repeated-dose animal studies and systemic human exposure to the hair dye in use.
This general approach is not appropriate in those cases where it is prudent to assume that the effect does not have a threshold (e.g. mutagenicity, genotoxic carcinogenicity).
Furthermore other data relevant to health risk assessment, such as irritancy or sensitisation are considered separately.

The percentage or rate of skin absorption is determined by an *in vitro* method. Unless reliable data are available on skin absorption it will be assumed that the entire amount applied to the skin is absorbed. Present data on skin absorption in most cases only allows an estimate of the percentage absorption. For the future it is recommended that the systemic dose be calculated from the absorption rate expressed in mg/cm^2 total absorption during single application or in $mg/cm^2/hr$.

It is accepted that for either type of hair dyes a partition coefficient of 0.1 has to be considered, that represents the amount applied to the scalp (COLIPA 16.01.97 BB-97/007).

CALCULATION OF THE MARGIN OF SAFETY	
Maximum amount of ingredient applied (mg)	**I**
Typical body weight of human (kg)	**60**
Maximum absorption through the skin (%)	**A**
Systemic Exposure Dose (mg/Kg/Bw) SED	$\frac{\mathbf{I \times A}}{\mathbf{60}}$
Margin of Safety	$\frac{\mathbf{NOAEL}}{\mathbf{SED}}$

where NOAEL equals no observed adverse effect level in mg/kg/bw from appropriate oral repeated dose study

Note

This approach assumes that the NOAEL derived from the oral study is the result of 100% absorption through the gastrointestinal tract. Frequently this will be an over-estimation of the amount absorbed and hence systemic exposure may be under-estimated. However on the other hand hair dyes are not applied directly to the skin; moreover only a distinct area of the skin, the scalp, is exposed and not the whole body surface.

ANNEX 5 - GENERAL SCHEME FOR DETERMINING THE MARGIN OF MARGIN OF PRESERVATIVES

In order to assess the margin of safety (SM) when considering the acceptability of the use of a preservative in cosmetic products, it is necessary to estimate total exposure from all types of products and to convert this to a total systemic dose based on knowledge of the skin absorption of the preservative. This value is then compared to the critical NOAEL from oral repeated dose studies in animals.

Estimation of Exposure

Exposure data on Cosmetic Products

Consideration must be given to exposure from all types of cosmetic products for which the preservative is allowed.

COLIPA has provided data on consumer exposure arising from normal and extensive use (COLIPA 16.01.97 BB-97/007). The SCCNFP considered that when assessing exposure the extensive use data should be used. However, it was accepted that this would in itself incorporate a significant safety factor and this would be born in mind when considering the acceptability of a preservative for a given use. The global estimate of exposure based on extensive use scenarios would be extreme values that would not be reached in practice because:

(i) use estimates were based on female usage (this tended to be higher that males)

(ii) it was assumed that all types of cosmetic products were used extensively

(iii) it assumed that the same preservatives were used in all products at the maximum level. This would not be the case in practice since certain preservatives were technically more appropriate for certain types of product.

For purposes of estimating exposure cosmetic products can be divided into 4 main types:

Oral Hygiene Products

Eye Products

Non-rinse off Products

Rinse off Products

For oral hygiene products the exposure of concern is the amount ingested. For a mouthwash, 10% of the amount used was considered a reasonable value, and for toothpaste 17%.

For rinse-off products it was considered reasonable to assume a rinse-off coefficient of 10% ie. 10% retention (and thus available for absorption through the skin).

For hair care products such as hair styling and hairspray products (non-rinse off), shampoos and conditioners etc. a partition coefficient of 10% (90% on hair 10% on scalp) was used.

Exposure estimates arising from extensive use for products in each of these 4 categories are given below. These data are used to calculate "global" exposure to cosmetic products.

ORAL HYGIENE PRODUCTS

Product type	Total amount ingested per application (grams)	Frequency of application per day	Exposure grams/day
Toothpaste	1.40	2	0.48
Mouthwash	10.0	3	3.00
Lipstick	0.01	4	0.04
Total			3.52

EYE PRODUCTS

Product type	Total amount per application (grams)	Frequency of application per day	Exposure Grams/day
eye make-up	0.010	2	0.020
Mascara	0.025	1	0.025
Liner	0.005	1	0.005
Total			0.050

NON-RINSE OFF PRODUCTS

Product type	Total amount per application (grams)	Frequency of application per day	Exposure grams/day
face cream	0.8	2	1.6
General purpose cream	1.2	2	2.4
body lotion	8	1	8.0
Anti-perspirant (roll on)	0.5	1	0.5
hair styling products	5	2	1.0
Total			13.5

RINSE OFF PRODUCTS

Product type	Total amount per application (grams)	Frequency of application per day	Exposure grams/day
make-up remover	2.5	2	0.50
shower gel	5.0	2	0.05
Shampoo	8.0	1	0.08
hair conditioner	14.0	0.28	0.04
Total			0.67

The above data do not refer specifically to sunscreens. However, such products are, in general, only used for up to about 3 weeks a year. It was not considered appropriate to add exposure from sunscreens. Similarly, hair dyes are not listed. These are only infrequently applied (at most once a week for semi-permanent and once a month for permanent hair dyes); exposure to preservatives from such usage is insignificant compared to the other use.

"GLOBAL" EXPOSURE

TOTAL ORAL HYGIENE	3.52 g
TOTAL EYE PRODUCTS	0.05 g
TOTAL NON-RINSE OFF PRODUCTS	13.50 g
TOTAL RINSE-OFF PRODUCTS	0.67 g
TOTAL GLOBAL EXPOSURE TO ALL COSMETIC PRODUCTS	17.74 g

EXPOSURE TO PRESERVATIVES

Assume maximum permitted concentration (C) in all products (g%)

Total exposure from all products (g) $\frac{17.7}{100} \times C$

Total exposure from all products (mg) $17.7 \times 10 \times C$

Calculation of amount absorbed

For oral hygiene products assume 100% of ingested dose is absorbed.

For other products assume skin absorption A% under in use conditions (if no data assume 100% skin absorption).

CALCULATION OF MARGIN OF SAFETY	
Maximum amount of ingredient applied (mg)	**I**
Typical body weight of human (kg)	**60**
Maximum absorption through the skin (%)	**A**
Systemic Exposure Dose (mg/Kg/Bw) SED	$\frac{\mathbf{I \times A}}{\mathbf{60}}$
Margin of Safety	$\frac{\mathbf{NOAEL}}{\mathbf{SED}}$

NOAEL: no observed adverse effect level in mg/kg/bw from appropriate oral repeated dose study.

ANNEX 6 - GENERAL SCHEME FOR DETERMINING THE MARGIN OF SAFETY OF UV FILTERS

1) Ultraviolet filters are used in several sorts of cosmetics; in these guidelines only their use in sunscreens is considered. It is appreciated that some ultraviolet filters are now used all the year round, in cosmetics other than sunscreens. They are used particularly in skin care products.

2) The following assumptions are made.

(a) The amount of formulation typically applied in use is 2.0 mg (formulation)/cm^2 over the entire body surface, taken to be 1.8 m^2 (18 mg/person/day). The formulation is left on the skin surface for 24 hrs.

(b) The concentration used for the calculation is the maximum authorised concentration of the ultraviolet filter.

(c) If no data to the contrary are available, 100% absorption of the ultraviolet filter is assumed to occur.

(d) The nature of the vehicle may alter the amount of ultraviolet filter absorbed, and the formulation eventually chosen may be different from the solvent system used in experimental determination of its percutaneous absorption. Evidence of the effect of the formulation on absorption should be presented. Tests preferably could be carried out in a vehicle typical for a sunscreen formulation.

(e) If information on the percutaneous absorption of the active- ingredient is available, it should be expressed in terms of weight of active ingredient absorbed per unit area (e.g. µg/cm^2); the amount absorbed, in terms of percentage of the amount applied, may then be calculated.

(f) The NOAEL used for calculation is generally derived from a 90 oral day study in the rat but the whole toxicological profile should also be taken into account.

CALCULATION OF MARGIN OF SAFETY	
Amount of formulation applied (mg) Concentration of active ingredient (%) Total amount of active ingredient applied (mg)	**F** **C** **F X C/100 = I**
Typical body weight of human (kg)	**60**
Absorption of a.i. (%) Total amount absorbed (mg)	**A** **I x A** **100**
Systemic Exposure Dose (mg/Kg/bw) SED	**(I x A)/100 x 60**
Margin of Safety	**NOAEL** **SED**

ANNEX 7 - GUIDELINES FOR THE SAFETY ASSESSMENT OF THE FINISHED COSMETIC PRODUCT*

Introduction

Pursuant to the Sixth Amendment to Council Directive 76/768/EEC, the safety assessor has to provide a safety assessment for each cosmetic product put on the market. This assessment has to be at the disposal of the person(s) responsible for the marketing of a given product (manufacturer or importer within the European Union).

This assessment has to be accessible to the competent authorities of the Member States. It should not be limited to a simple certificate for an exclusively legal purpose; it must be transparent and accurately documented.

Addressed to the safety assessor as well as to the competent authorities of the Member States, guidance is provided on the particulars referred to in Article 7 of Directive 76/768/EEC as amended by Directive 93/35/EEC.

The following factors should be taken into consideration:

- identity and toxicological profile of ingredients, complex ingredients including specific fragrance ingredients, present in the fragrance compound,
- information concerning the formulation of the finished product, its route of application and use patterns,
- available toxicological data on the finished product.

As part of the information may not be avalaible or needed it is therefore up to the safety assessor to report and justify the scientific reasoning for approving the formulation.

Therefore the guidance hereafter should not be used as a check list; it should be considered and adapted case by case when assessing the safety of a finished product..

1. Transparency of the ingredient's identity

Terminology

Cosmetic ingredient means:
1. any chemically defined substance with a molecular formula and a structural formula;
2. any complex substance, requiring a definition, corresponding to substances of unknown or variable composition and to biological substances;
3. mixtures of 1 and 2, used in the composition of cosmetic products.

* Adopted by the SCC in the 65th meeting of May 24th, 1996.

1.1 Qualitative and quantitative formula
(Dir. 93/35/EEC Article 7a(1)(a))

Precise identification and description of the ingredients is crucial for a toxicological assessment.

The finished product's formula will be supplemented for each ingredient and for each complex ingredient by a "definition" statement comprising all the particulars not included in the inventory. The definition will be sufficiently precise to identify a given ingredient with regard to its composition and its effects.

Ingredients should be defined in particular in terms of the manufacturing and purification process: chemical synthesis, isolation and purification by chemical processes, or physical, enzymatic, biotechnological or microbiological processing using material of biological origin.

Most biotechnologically derived ingredients are well defined chemicals covered by the general requirements e.g. acids, alcohols, amino acids and a series of excipients, additives and foodstuffs.

The molecular formula and the structural formula of the chemically defined substance should be indicated.

Ingredients should be also characterised by their analytical specifications.

1.2 Physico-chemical and microbiological specifications of ingredients
(Dir. 93/351/EEC Article 7a(1)(b))

Appropriate physico-chemical and microbiological specifications should be defined for each ingredient. Major factors affecting safety for cosmetic purposes must be taken into account.

1.2.1 As regards general problems of identification, ingredients requiring a "*definition*" including any impurities that they contain which are of toxicological significance (e.g.. toxic sub-components, residual solvents, heavy metals, etc.) and the ingredients authorised in the annexes to the Cosmetics Directive should be specified using discriminant analytical techniques such as HPLC, GC/MS, NMR, etc.

1.2.2 Microbiological specifications are essential. For ingredients of biological origin (e.g. derived from plants, animals or other sources), specifications must be adapted with appropriate regard to the source material.

1.3 Examples of complex ingredients

A. Ingredients of mineral origin
B. Ingredients of animal origin
C. Ingredients of botanical origin
D. Special ingredients derived from biotechnology
E. Commercial addition mixtures, including perfumes
- Reaction mixtures

- 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 **[**m**g/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

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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.

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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.

2. Confirmatory tests of ingredients or mixtures of ingredients in humans must be limited to situations where no irreversible damaging effects are to be expected for the volunteers and where the study goal is reasonably achievable with a study population of limited size.
3. The recruitment of human volunteers should be in line with the "World Medical Doctors Association Declaration of Helsinki" and "the Good Clinical Practice for trials on Medicinal Products in the European Community."

2- Procedure of irritancy assessment

The following text outlines the steps of an assessment of the irritancy of an ingredient or mixture of ingredients. While this text focuses on irritancy, it is understood that other aspects of toxicity have to be considered in parallel before performing tests in humans.

2.1 Initial considerations

Available chemical and physico-chemical data and structure-activity relationships making use of computer programs and databases for the prediction of skin irritation potential should be used.

2.2 Evaluation of irritation

Ingredients or mixtures of ingredients should be tested on animals and humans only at non-corrosive concentrations. This decision may be based on pH and acid/alkaline reserve measurements and on in vitro tests for skin corrosivity. At the present, in vitro methods for the assessment of irritancy have not yet been validated.

2.3 Confirmation by human volunteer testing

On the basis of a low irritation potential as proven by animal or future validated in vitro methods, the skin tolerability of an ingredient or a mixture of ingredient can be confirmed by testing in human volunteers. A number of test protocols are available such as open and closed patch tests, single and repeated-exposure tests. They should be chosen on the basis of the relevant use pattern of the ingredient or mixture of ingredients (1).
In the open test, the tested ingredient or mixture of ingredients is applied on the skin without occlusion for time periods between 15 min and 24 h.
In closed patch tests, diluted or undiluted products are applied under occlusive chambers over 24 or 48 hours.

Cumulative or repetitive closed patch tests involve applications on the same test site between 1 and 7 times per week over a period of 1 to 5 weeks. These repetitive tests allow the assessment of cumulative irritation that is missed by single application tests.
Controlled use or repeated open application tests (ROAT) imply the repeated application of an ingredient or a mixture of ingredients closely modelled to the use-situation.
While these tests historically have been assessed by clinical methods, non-invasive bioengineering technology such as measurement of transepidermal water loss or of blood flow may provide higher sensitivity and objectivity of these tests and thereby reduce the exposure and risk to volunteers.

However, neither the above confirmatory tests nor the use of bioengineering methods have been validated according to modern scientific criteria. The SCCNFP recommends the Commission to support further research in this area.

2.4 Consumer market surveillance

The evaluation of irritation of an ingredient or mixture of ingredients is not finished with the introduction of respective cosmetic products on the market, but it should continue by making use of data generated by consumer market surveillance and other sources.

3- Ethical considerations

Confirmatory skin tolerance tests of cosmetic ingredients in humans are subject to ethical concerns. In order to take account of these concerns, to minimise the risk to volunteers and to safeguard their rights, test protocols should be submitted to an acknowledged ethical committee and be in compliance with the followings :

World Medical Association Declaration of Helsinki in its current revision (2). Human testing is to be conducted and monitored under the direction of relevantly trained personnel to ensure the health and well being of volunteer subjects involved in the testing. The health and welfare of the subject has first priority and must be highly protected. Importantly, the human testing that is conducted for chemicals and consumer products is associated with minimal risk as it is conducted:

i) to supplement non-clinical information,
ii) to confirm that exposure will not cause significant harm, and/or,
iii) in a controlled fashion that minimises subject risk (4).

National regulations regarding human studies

Good Clinical Practice for Trials on Medicinal Products in the European Community (3).

The investigator(s) in skin tolerability tests of cosmetic ingredients should fulfil the qualifications as mentioned in the CPMP Working Party on Efficacy of Medicinal Products Note for Guidance on Good Clinical Practice (3).

4- References

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ANNEX 12 – GUIDELINES ON THE USE OF HUMAN VOLUNTEERS IN COMPATIBILITY TESTING OF FINISHED COSMETIC PRODUCTS*

Ethical considerations with respect to human testing of cosmetic products have to include the problem of the first topical contact of a human volunteer with the product to be tested. Guidelines have therefore to be based on the principle that no fortuitous e.g. badly defined contact can be allowed. Strict rules have to be defined.

The following guidelines take into consideration the ethical and practical aspects when human volunteers are involved in studies organised to assess skin and mucous membrane compatibility of cosmetic finished products.

1. Background

1.1 Legal requirements for consumer safety

According to the Council Directive (76/768/EEC), "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". According to the 6th Amendment of the Cosmetics Directive (93/35/EEC), a European dossier must be kept readily accessible for inspection by the competent authorities, containing a toxicological file based on safety assessment of the ingredients and the finished products. However, there is no legal requirement that finished products have to be tested on animals nor on human beings before marketing.

1.2 Side effects caused by cosmetic products

Cosmetic products are developed to be applied to the skin and external mucosa and to be used by the normal population. It must, however, be considered that people suffering from skin disorders or sensitive skin also use cosmetics. Occasionally undesirable side effects, both local and systemic, may occur. Local reactions may be, among others, irritation, contact allergy, allergic contact dermatitis, contact urticaria and sunlight, especially UV light, induced reactions. Skin and mucous membrane irritation are the most frequently observed reactions.

1.3. Description of terms

For the purpose of the document, the following terms are described as indicated:
- Compatibility test: corresponds to a test intended to confirm that there are no harmful effects when applying a cosmetic product for the first time to the human skin or mucous membrane.
- Acceptability test: corresponds to a test intended to confirm the fulfilment of the expectations for a cosmetic product in-use.

* **Adopted by SCCNFP in its Plenary Meeting of 23 June 1999 (SCCNFP/0068/98 Final)**

1.4. General statement

Since tests in animals and alternative methods are of predictive limited value with respect to human exposure, confirmatory compatibility tests of cosmetic finished products in humans may be needed scientifically and ethically, provided that the toxicological profile of their ingredients, based on animal testing and/or the use of alternative methods, is available. A high degree of safety has to be expected. Finished cosmetic products are usually tested in small populations

- to confirm the skin and mucous membrane compatibility of the finished products;
- to assess their cosmetic acceptability.

2. Ethical considerations

2.1 Basic Principles

The basic principles for testing in humans are provided by the following documents :

* World Medical Association Declaration of Helsinki in its current revisions (1964-1975-1983-1989-1996)

* Recommendation N° R(90)3, of the Committee of Ministeries/Council of Europe adopted on 4th February 1990

* Draft Directive on Good Clinical Practice for Trials on Medicinal Products in the European Community

* National Regulations regarding human studies.

2.2. SCCNFP recommendations

According to these basic principles, the SCCNFP recommends the following ones which apply directly to the compatibility testing of cosmetic products:

- Cosmetic compatibility tests on human volunteers cannot be considered as a replacement for animal testing.

- Cosmetic compatibility tests on human volunteers can only be performed to confirm, in a limited number of subjects, that products do not damage skin and mucous membrane, as already expected from other sources.

- The study supervisor must have at his disposal, prior starting any test, the full quantitative formula of the product to be tested, its preclinical safety assessment, its conditions of use and possible warnings.

- Studies should conform to generally accepted scientific principles. They should be based on an adequate knowledge of the potential risks incurred, resulting from laboratory experimentation and/or appropriate knowledge of the scientific literature.

- Research involving human volunteers should not be carried out unless the importance of the objective is in proportion to the inherent risk for the subject.

- Tests involving human volunteers which do not conform to scientific criteria and which are unable to provide exploitable results, are unacceptable even if they do not present any risk for the consenting subjects.

- The interest of the human subject should always prevail over the interest of science and society. Therefore the Investigator should cease as soon as risk is found to outweigh the potential benefit of the study.

- Skin compatibility testing involving human volunteers should be conducted only by technically qualified persons and under the supervision of a clinically competent medical doctor/physician.

- Acceptability tests in consumers do not require review by an ethical committee.

- Compatibility test protocols of cosmetic products possibly posing a risk to volunteers ought to be submitted for consideration and comments to an ethical committee provided that this committee conforms with the laws and regulations of the country in which the study is performed.

- Human volunteers should be adequately informed of the aims, methods used and potential risks of the study and the discomfort they may entail. Free informed written consent is mandatory prior to entering the study.

- Volunteers with any current dermatitis or known past allergic contact dermatitis related to the ingredients of the cosmetic product concerned should be excluded from the panel participating in safety tests.

- Except for specific cosmetic products, especially intended to be used by pregnant women and whose safety has been specially assessed for such employment, pregnant or lactating females should never be included in safety confirmatory tests.

- Children should not be involved with the testing of the compatibility of cosmetic products.

- In selected cases when the inclusion of adolescents (10-16 years*)* is warranted, they should be fully informed of the aims, methods used and potential risks of the study in order to obtain their free personal co-operation. They should personally give a free informed consent in written form. Parents or guardians should also give their consent.

- Study reports have to provide all experimental information in order to allow to understand the rationale of the study and to preserve the accuracy of the results.

3. Test methods in human volunteers for the skin compatibility assessment of finished cosmetic products

- Possible adverse reactions include skin irritation, contact allergy, photomediated reactions, acne, contact urticaria, pigment changes, hair and nail changes, subjective symptoms and various end-points measured by non-invasive techniques (elasticity, skin thickness, wrinkling, roughness etc.). Therefore it is not possible to make a complete list of current testing methods. The design of the test protocol depends on the specific question asked. In each case the reasoning and the scientific background of the test should be given.

- Among the most frequently used tests for finished cosmetic products are skin irritation tests as human repeated insult patch tests, chamber scarification tests, repeated open application tests, and soap chamber tests for detergents, and various other occlusive or open test methods developed to stimulate intended use situations. Irritancy reaction in humans is not an absolute measure and should be related to appropriate controls defining the range of response.

- In some test methods the skin of the volunteers may be preconditioned by various physical and chemical factors before exposure to the cosmetic product occurs. The design of tests may vary considerably with respect to the selection of volunteers, exposure time, patch test technique and reading.

- Often visual assessment is applied. Although this type of assessment is subjective, good results can be obtained with trained experimenters.

- Non-invasive bioengineering techniques can be applied in safety assessment to quantify and objectivate the results, to measure even sub-clinical symptoms and, generally speaking, to obtain additional information ; this has not been a common practice so far and further validation of these methods is encouraged by the SCCNFP.

- An important aspect in all human testing with finished cosmetic products is that the result obtained should be regarded as relative to the result of control substances, giving the range of reactivity within the test group. At the basis lays the considerable inter-individual variation between skin responses from different volunteers.

- For specific products, confirmatory safety tests may be performed in the surrounding area of the eye. In such a case, extreme attention should be given with respect to possible local irritations. Such tests should be stopped as soon as a significant adverse effect is observed in anyone of the subjects involved in the study. In such tests only one eye should be investigated per volunteers. The study can only be carried out under the strict supervision of an ophthalmologist.

ANNEX 13 - CLASSIFICATION OF SUBSTANCES

Classification of substances as ingredients of cosmetic products is recommended by the Scientific Committee on Cosmetology on the basis of evaluations of data provided pursuant to the Guidelines on the Safety Assessment of Cosmetic Ingredients. The overriding consideration is that the substances should be safe for consumer use under conditions of intended exposure at the relevant concentrations.

Candidate substances for the positive lists must not be used until a final classification in Group 1 has been made. For substances already in use, the classification may be reconsidered if necessary.

For substances in provisional lists for which there are insufficient data for a final safety assessment, additional information must be adequate and provided within a specified time limit. Otherwise it is concluded that no further use of the substance in cosmetic products should be allowed for the specified purpose.

Group 1:

Substances for which data at the time of assessment support the conclusion that they do not pose a health hazard. They may be used in cosmetic products for the designated purposes and in concentrations not exceeding the limits indicated.

Group 2:

Substances which must not be used in cosmetic products. Substances may be included in this group because either

a) the available data support the conclusion that they constitute a health hazard or

b) the available data do not justify the assumption that their use in cosmetic products can be considered safe.

ANNEX 14 - STANDARD FORMAT OF THE OPINIONS

Executive Summary

1. General data

1.1 Identity of the ingredient
1.2 CAS n°
1.3 Use

2. Terms of reference

3. Toxicological Evaluation & Characterisation (max. two lines per endpoint, no figures)

3.1 Acute toxicity (dermal, oral, i.v., i.p.)
3.2 Chronic/sub-chronic toxicity
3.3 Reproductive toxicity
3.4. Percutaneous absorption
3.5 Irritation and corrosivity
3.6 Allergenicity and sensitisation
3.7 Genotoxicity/carcinogenicity
3.8 ……..

4. Opinion

5. Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

Full Opinion

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

Request for inclusion of ….. in Annex …, part 1 – List of ……. which Cosmetic Products may contain – to Council Directive 76/768/EEC.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is ……. safe for use in cosmetic products?

* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

1.3 Definitions of terms where appropriate

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

Ref. :

2.1.2. Chemical names

Ref. :

2.1.3. Trade names and abbreviations

Ref. :

2.1.4. CAS no.

Ref. :

2.1.5. Structural formula

Ref. :

2.1.6. Empirical formula

Emp. Formula :
Mol weight :

Ref. :

2.1.7. Purity, composition and substance codes

Ref. :

2.1.8. Physical properties

Subst. Code :
Appearance :
Melting point :
Boiling point :
Density :
Rel. vap. dens. :
Vapour Press. :
Log P_{ow} :

Ref. :

2.1.9. Solubility

Ref. :

2.2. Function and uses

Ref. :

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Ref. :

2.3.2. Acute dermal toxicity

Ref. :

2.3.3. Acute inhalation toxicity

Ref. :

2.3.4. Repeated dose oral toxicity

Ref. :

2.3.5. Repeated dose dermal toxicity

Ref. :

2.3.6. Repeated dose inhalation toxicity

Ref. :

2.3.7. Sub-chronic oral toxicity

Ref. :

2.3.8. Sub-chronic dermal toxicity

Ref. :

2.3.9. Sub-chronic inhalation toxicity

Ref. :

2.3.10. Chronic toxicity

Ref. :

2.4. Irritation & corrosivity

2.4.1.	Irritation (skin)

Ref. :

2.4.2.	Irritation (mucous membranes)

Ref. :

2.5.	Sensitisation

Ref. :

2.6.	Teratogenicity

Ref. :

2.6.1.	One-generation reproduction toxicity

Ref. :

2.6.2.	Two-generation reproduction toxicity

Ref. :

2.7.	Toxicokinetics (incl. Percutaneous Absorption)

Ref. :

2.8.	Mutagenicity/Genotoxicity

Ref. :

2.9.	Carcinogenicity

Ref. :

2.10.	Special investigations

Ref. :

2.11.	**Safety evaluation**

Ref. :

CALCULATION OF THE MARGIN OF SAFETY

(Name of substance)

(Class / Group)

Based on a usage volume of X ml, containing at maximum X %

Maximum amount of ingredient applied: I (mg)=
Typical body weight of human: 60 kg
Maximum absorption through the skin: A (%)=
Dermal absorption per treatment: I (mg) x A (%)=
Systemic exposure dose (SED): SED (mg)= I (mg) x A (%) / 60 kg
No observed adverse effect level (mg/kg): NOAEL =
(species, route of application)

Margin of Safety: NOAEL / SED =

2.12.	**Conclusions**

Classification:

Ref. :

2.13.	**References**

3. Opinion of the SCCNFP

4. Other considerations (if any)

5. Minority opinions (if any)