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