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- Testing for Phototoxicity

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) $\frac{17.7 \times C}{100}$

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)/cm² over the entire body surface, taken to be 1.8 m² (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. $\mu g/cm^2$); 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** $\frac{\mathbf{I \times A}}{\mathbf{100}}$
Systemic Exposure Dose (mg/Kg/bw) SED	**(I x A)/100 x 60**
Margin of Safety	$\frac{\mathbf{NOAEL}}{\mathbf{SED}}$

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