Cosmetics Europe
the personal care association

COSMETICS EUROPE:

GUIDELINES FOR THE ASSESSMENT OF SKIN TOLERANCE OF POTENTIALLY IRRITANT COSMETIC INGREDIENTS

1997

CONTENTS

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1. INTRODUCTION

The 6th Amendment to the Cosmetics Directive (93/35/EEC) included a potential ban on the use of animals in the testing of cosmetic products and their ingredients which may, as a result of Commission Directive 97/18/EC, come into effect from June 2000. Knowledge of the skin irritation potential of ingredients is important in the development of cosmetic finished products.

Colipa, the European Cosmetic, Toiletry and Perfumery Association has already developed and published test guidelines for assessment of skin compatibility of cosmetic finished products under controlled conditions in man (Walker et al., 1996). Skin compatibility is defined as the absence of skin irritation under normal conditions of use and reasonably foreseeable misuse, taking into account objective reactions as well as subjective responses such as stinging, burning or itching. Skin irritation is defined as non-immunological local skin inflammation.

These guidelines have been developed to provide information on how and under what circumstances it is possible to assess on humans the skin tolerance of potentially irritant cosmetic ingredients.

The aim of the test(s) is not to "classify" the ingredient for its effect on the skin. The objective of the test(s) is to determine whether or not an ingredient is likely to induce skin irritation when incorporated in finished cosmetic products under normal conditions of use and reasonably foreseeable misuse conditions.

Appropriate measures have to be taken in order to ensure the validity of the test, to define a threshold concentration for irritancy and to make sure that reactions on human skin will remain at an acceptable level, thus avoiding severe reactions.

To meet these requirements, the test procedure has to be defined on a case-by-case basis, taking into account for each ingredient to be tested, its chemical structure and function, its composition in instances where the ingredient is a complex mixture, the quantity, frequency, concentration and the type(s) of formulation(s) in which the ingredient may be used together with adequate vehicle(s) and control(s). A step by step approach with increasing exposure will in most cases be necessary.

The great advantages of assessment in human skin are that testing on animals is not required, extrapolation from other species or *in vitro* models to man is not necessary and criticism of the relevance to man does not arise. Furthermore, subjective effects on the skin may also be evaluated.

Any study involving humans can only be undertaken after a thorough safety review has concluded that the study will involve no significant risk to the volunteers and that it satisfies all other ethical requirements.

2. OBJECTIVE

The objective of this brochure is to outline the process and develop guidelines for the assessment of the skin tolerance of potentially irritant cosmetic ingredients in humans in order to establish the predicted safety to skin of the ingredients when incorporated in cosmetic finished products.

3. ETHICAL REQUIREMENTS

All human studies must be conducted in accordance with the Declaration of Helsinki (1964) and subsequent revisions (World Medical Association, 1989, Council for International Organizations of Medical Sciences and the World Health Organization, 1993). They must be carried out by suitably trained, qualified and experienced personnel.

It is the responsibility of the investigator to take all precautions to avoid the possibility that participants in the study might experience undesirable effects. Consequently, the safety evaluation outlined in chapter 5, (see page 4), is of paramount importance. If any unusual risk to participants is involved the investigator should consider submitting plans for the study to an Ethical Review Committee. Such a Committee will review aspects of a study which may affect the safety and well-being of participants, but responsibility for a study remains with the investigator (Schmitt, 1994).

Ethical requirements which need to be taken into consideration in the planning of studies on humans include:

i) all participants should be informed volunteers selected after application of inclusion/non-inclusion criteria (see Chapter 6.2.2, page 7);
ii) all participants must be made aware of the purpose and nature of the study and of any foreseeable risks involved in participation in the study and must give written informed consent before the study starts;
iii) before any volunteers are exposed to the ingredient, all relevant safety information on the ingredient must be evaluated;
iv) all test procedures must conform to national regulations and, where appropriate, should be approved by an independent Ethical Review Committee;
v) an Ethical Review Committee should include medical, non-medical, appropriate experts and lay members; it should consider the general ethics of the test and verify that the safety and integrity of the participants in the test are protected, taking into account information on the ingredient(s);

vi) all reasonable care should be taken to avoid causing excessive skin reactions or other adverse health effects in the participants during a study;
vii) agreed procedures should be in place in the event of any unexpected/adverse reactions, including appropriate medical cover;
viii) volunteers may be rewarded for their time, inconvenience, etc., but the reward must not be so great that it would persuade them to participate.

4. TESTING APPROACH

Provided the safety and ethical requirements can be met, the skin tolerance for potentially irritant cosmetic ingredients can be assessed by a cautious stepwise testing approach using human volunteers.

Typically, the approach will involve the assessment of the skin irritation threshold under defined conditions of exposure.

The following sections outline:
- the types of tests available;
- how to select the best approach;
- the basic criteria for study initiation, conduct and evaluation.

Whatever the method(s) used, any study involving human volunteers must meet the requirements of responsible human testing which include the following issues:
- a full safety assessment by a suitably qualified and experienced person is necessary for all ingredients to be tested, in order to avoid the risk of induction of skin sensitisation or other adverse health effects;
- the planned study must satisfy ethical requirements;
- the objective of the study has to be well defined;
- the design of the study shall have sufficient power to be able to answer the question(s) set without imposing undue risk to human volunteers;
- data management and data interpretation must be clearly defined;
- the sponsor and the investigator responsible for the conduct of the study must be clearly identified;
- a system for management of adverse effects, should they occur during a study, must be established before the study begins.

5. PRE-REQUISITES BEFORE THE START OF A STUDY INVOLVING HUMAN VOLUNTEERS

A safety assessment of the ingredient(s) under consideration must be carried out.

The safety assessor must:

- carefully consider the chemical structure(s) of the ingredient(s) in order to identify the potential risk(s);
- take into account all the toxicological data available on the ingredient(s) and, where appropriate, on structurally related materials;
- conclude that no significant risk to the volunteers is reasonably foreseeable in the proposed study;
- be a suitably qualified and experienced person who has all the necessary information available;
- conclude that the testing should not adversely affect the health of the volunteers.

When making the safety assessment, the toxicological effects of the ingredients to be considered include:

- corrosivity to skin;
- percutaneous absorption;
- skin sensitisation;
- genotoxicity;
- systemic toxicity.

Under certain circumstances, other toxicological effects (e.g. phototoxicity) may also need to be taken into account.

Information may be derived from many sources including knowledge of structurally related chemicals or, where appropriate, from proven non-animal alternative tests. The information must be adequate to ensure, so far as is possible, that the study may be carried out with minimal risk to the volunteers.

6. GENERAL PROCEDURES

Cosmetic ingredients may be used in a great variety of products over a wide range of concentrations. Therefore, the type of testing and the subsequent safety evaluation need to take into account information about the foreseeable conditions of exposure as well as influences from other ingredients in the intended formulation (e.g. penetration enhancement may be especially relevant).

Severity of irritation response is affected by the concentration of ingredients tested, the duration of exposure, the type of application and the susceptibility of the patch site. A step-wise testing approach is likely to start with conditions expected not to cause effects. The next step would involve increased exposure, (by higher concentration, increased duration or change of type of application) until the objective is achieved.

When the type of application is relevant, the final upper concentration limit should at least reach the in-use concentration. As a pre-requisite, all available data about the class of chemical, experience from similar exposure etc., should be taken into account before starting any study. It should be noted that natural substances like enzymes, aminoacids, plant extracts, animal extracts, etc., are not necessarily harmless to the skin because of their origin.

Some classes of ingredients are often of minor irritant potential but may, under occlusion or at higher concentrations, elicit important irritation effects. The inclusion of control or reference substances in the tests is recommended.

If a vehicle is necessary, it should be relevant to the composition of the finished product in which the ingredient is intended to be used and should be known not to cause adverse skin effects under the test conditions, otherwise it should be tested as a control. The influence of the vehicle should be studied, if appropriate, comparing relevant systems, e.g. hydrophilic and lipophilic.

Evaluation criteria are likely to be similar in all the following tests. Assessment is generally subjective, but may also be objective (Kajs and Gartstein, 1991; Kligman, 1995) for example, transepidermal water loss (TEWL) (Nilsson, 1977; Pinnagoda et al., 1990) and redness intensity (e.g. Westerhof, 1995). See also Appendix 2 which gives details of the test methods and references.

6.1 EXAMPLES OF TYPES OF TESTING ON HUMAN SKIN

a. Single application open epicutaneous test

This test involves the least severe application type and may provide a convenient starting point for the step-wise progression. Furthermore, since the effects on skin are continually under observation, testing can be stopped at any time.

b. Repeat application open epicutaneous test

This test has been proposed to study cumulative effects close to the real use conditions. A decision to proceed with such a test model may depend on the results of a single open application test and the aim of further investigation (e.g. suspected cumulative effects). The frequency of the repeat application test will be decided on a case-by-case basis and may be influenced by information on the intended use of the material with regard to frequency of application, concentration, amount, etc.

c. Single application closed patch test under occlusion or semi-occlusion

A range of protocols is available, for example:
• *4 hour occluded patch test*
This test has been designed to enable classification of substances, based on human data in the context of European Union legislation (York *et al.*, 1996). Consequently, the method may not be found useful for the more general investigation of skin tolerance of potentially irritating cosmetic ingredients for purposes of safety evaluation.

• *24 hour / 48 hour occluded or semi-occluded patch tests*
For comparative assessments of one or more materials simultaneously in the same individual, protocols usually with diluted material have been proposed.

Test substances, diluted or undiluted, are usually applied to the skin of the upper arm or back for periods up to 48 hours. Attention has to be paid to the type of chamber or patch because of its influence on the result (e.g. York *et al.*, 1995; Kim *et al.*, 1987). Readings are performed at 1, 24 and 48 hours after removal of the patches. Parameters are visual effects such as erythema, dryness, oedema, (see appendix 3). Objective evaluations using observation of TEWL and Laser Doppler flowmetry have been described.

d. Repeat application closed patch epicutaneous test under occlusion or semi-occlusion

This test approach may be chosen in order to study cumulative irritation in substances with a low primary irritation potential. Protocols have been proposed with application on the forearm under occlusive or semi-occlusive conditions with varying application times, e.g. 30 minutes/day for two weeks. Evaluation is performed each day after patch removal and before patch replacement, visually assessing, e.g., erythema, dryness and oedema. Objective evaluations using observation of TEWL and Laser Doppler flowmetry have been described.

6.2 SUBJECT SELECTION

6.2.1 NUMBER

An adequate number of volunteers should be recruited to satisfy the objectives of the test and the ethical requirements.

6.2.2 STUDY POPULATION

a. Recruitment of volunteers / informed consent

Volunteers will be selected on the basis of inclusion and non-inclusion criteria. The volunteers must satisfy all the inclusion criteria and not conflict with any of the non-inclusion criteria. The volunteers must be clearly informed, verbally and in writing, regarding the nature of the study, the timetable, constraints and possible risks. They must then give their written informed consent before participation in the study is permitted (see Appendix 1).

b. Inclusion criteria

- informed volunteers, where appropriate of relevant age, gender, ethnic origin and health condition
- panellists agree to follow the conditions specified in the Study Information Sheet (see Appendix 1).

c. Non-inclusion criteria

- pregnancy or nursing condition (except where specifically required)
- blemishes, marks (e.g. tattoos, scars, sunburn) on the test site(s) which could interfere with scoring
- medication which may affect skin response or past medical history
- irritated skin on test site(s)
- any active skin disease which may interfere with the aim(s) of the study
- participation in another simultaneous study
- participation in a previous study without an appropriate rest period between studies

d. Withdrawal criteria

Participants will be withdrawn if:

- they do not follow the conditions of the Study Information Sheet;
- they suffer any illness or accident or develop any condition during the study which could affect the outcome of the study;
- they no longer wish to participate in the study.

6.3 TEST MATERIALS

6.3.1 REFERENCE MATERIALS

Where appropriate, reference materials should be used in order to check inter- and intra-laboratory variations as well as inter-seasonal variability (e.g. Agner and Serup, 1989; Basketter *et al.*, 1996).

6.3.2 CONCENTRATION

The concentration(s) of the test and reference materials will be adjusted according to the type of material, the test protocol and the objective of the study so as not to cause severe skin effects. Any dilution vehicle should be known to have an acceptable toxicity profile and generally should not be expected to cause adverse skin effects under the test conditions. However, where such effects are possible, or known, consideration should be given to testing the vehicle as a control.

6.4 TEST METHOD

6.4.1 TEST MATERIAL APPLICATION

Before the first application, participants will report to the test laboratory for expert evaluation and, if appropriate, instrumental assessments of skin condition.

Exposure conditions will be defined in the protocol and will depend on the test type (see Chapter 6.1, page 6).

When appropriate, test materials will be randomized within and between subjects (see, e.g., Van der Valk and Maibach, 1989); skin areas with the highest likelihood of variability of response (e.g. wrist, shoulder) should be avoided.

In repeated patch studies, the patch site must be marked to ensure that successive patches are placed on exactly the same skin position.

See also Appendix 2 which gives details of the test methods and references.

6.4.2 DOSE LEVEL

In patch tests, the measured amount of test material applied to each patch must be sufficient to fill the chamber or saturate the pad without overflowing from it when applied to the skin. Solids should be moistened sufficiently with an appropriate vehicle to ensure good contact with the skin.

6.4.3 REMOVAL OF TEST MATERIAL

When appropriate, test materials will be removed in the laboratory. Test material will be rinsed, (or otherwise gently removed), from application sites without rubbing (to avoid cross-contamination).

6.4.4 EVALUATIONS

a. Application site assessment

Treatment sites will be assessed before the first application of test material (baseline) and after treatment at times defined in the protocol. For patch tests, there must be a specified period of time (e.g. 30 minutes) after patch removal before assessment. For repeated application studies, should any test sample elicit the threshold response defined in the protocol, it will not be re-applied on that participant for the remainder of the study.

(i) Visual assessment of skin irritation
Reactions at the test sites should be scored throughout the test by the same experienced assessor who made the baseline assessment and under the same lighting source, following a pre-defined scoring scale. An example of an established scoring scale is given in Appendix 3.

(ii) Instrumental measurements of skin irritation
Where appropriate, instrumental evaluations will be made following an acclimatisation period in an environmentally-conditioned room (e.g. below 22°C, constant relative humidity). Calibration of instruments will be checked regularly and the instruments used as described in scientific literature or in the manufacturer's instructions. Such methods permit objective assessments and have been reviewed recently (Kajs and Gartstein, 1991; Westerhof, 1995).

b. Statistical analysis
When relevant, the type(s) of statistical analysis (parametric, non-parametric) to be used must be valid and specified in the protocol, which should also advise, in the case of repeated application tests, on the handling of, (or lack of), data for sites which were not re-treated due to the level of response following the previous application.

c. Data interpretation
Whilst this activity will always be on a case-by-case basis and will depend on the nature and type of study, the most common approach will be to compare the results obtained for the test materials with those of suitable positive and/or negative controls, or with similar materials.

7. STUDY REPORT

The final study report should include, but not be restricted to, information appropriate to the following headings.
- Summary/abstract.
- Type and Objective of the study.
- Number of participants and selection criteria for inclusion/non-inclusion.
- Test material(s) and test procedures used.
- Procedure(s) for assessment of effects.
- Results
  - Number of participants starting and completing the study, including an explanation of any withdrawals during the study.
  - Presentation of data on test and reference material(s) used.
  - Presentation of subjective comments by participants.
  - Description (and justification, where appropriate) of any statistical procedures used.
- Conclusions.
- Appendices
  - Copy of study protocol.
  - Identification of study investigators.
  - Copy of Study Information Sheet and written informed consent.

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APPENDIX 1

EXAMPLE

INFORMED CONSENT FOR PARTICIPATION IN A STUDY

We are inviting you to take part in a test in which different test materials, all of which are ingredients of cosmetic products (e.g. soaps, shampoos, decorative cosmetics) will be placed on your skin. We will not specifically identify the test materials to you. All the test materials have been reviewed by a safety expert to ensure that the existing information on the materials justifies human exposure.

Purpose

We wish to find out if any of the test materials cause adverse effects on your skin. The results of the study will provide useful information for evaluating the safety of the materials for their intended use. The tests will help in the development of products which can be used safely by the general public.

Eligibility

If you would like to participate, we will first ask you to complete a questionnaire about your medical history, allergies, skin problems, any current medication and previous participation in similar skin tests. It is possible that, based on your answers to these questions, you may not be able to take part in the tests.

Test Procedure

All tests are conducted by experienced company employees.

[DESCRIPTION OF PARTICULAR TEST TO BE UNDERTAKEN, i.e. STUDY INFORMATION SHEET]

Leaving the study

You are free to withdraw your consent and discontinue participation in the study at any time.

Risks and discomfort

You may experience some skin irritation during the course of the study, similar to a mild sunburn. The area of skin exposed to the test material may become pink or red, and temporarily burn or itch or become dry. The most severe reaction anticipated would be redness, possibly, in the case of patch tests, accompanied by localised swelling. No permanent effects are anticipated.

EXAMPLE

CONSENT AGREEMENT

I hereby consent to take part in the experimental study which has been described to me and which will be supervised by [Dr/Mr/Ms. . . . .]. I understand that the study may involve some risk of adverse skin effects. This, and my part in the study, have been fully explained to me and I have had complete freedom to ask any questions about the study.

I understand that I am free to withdraw my consent to take part in the study and discontinue participation at any time. I also agree to inform the investigator of any changes in my health status or medication which might occur during the course of the study.

I will be able to ask for further information concerning the study, or report adverse effects, at any time by telephoning the investigator on [telephone number].

I agree that the data recorded during the study can be submitted to computerised treatment by the investigator, but understand that any information which can be identified with me will be kept confidential with the study records.

I have read and signed this consent statement with full knowledge of the facts.

. . . .
Signature of volunteer

. . . .
Printed name of volunteer

. . ./ . . . /. . . .
Date

APPENDIX 2

The references given demonstrate conditions, applications and examples of use of the techniques. Whilst some of the references concern finished products, they are equally applicable to the testing of cosmetic ingredients. Exposure times and concentrations are presented only as illustrations.

A. SINGLE APPLICATION OPEN EPICUTANEOUS TEST

Devices:	glass sticks, swab or cellulose paper
Test site:	inner forearm
Frequency of application:	once (for solid or viscous materials); for liquid materials, repeated applications every 30 seconds may be made to maintain exposure of the application site
Duration of treatment:	30 minutes (or 15 - 60 minutes depending on the aim of the study)
Removal of test substance:	by rinsing or gentle swabbing
Time(s) of assessment:	immediately record time when effect(s) first occur(s) immediately after completion of application period and again after 24 & 48 hours
Parameters:	erythema, oedema, scaling, adverse sensation(s) noted by participant
Grading:	zero, weak, moderate, strong
Evaluation:	number of subjects with effect(s) within defined time period, or occurrence of first response

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B. REPEATED APPLICATION OPEN EPICUTANEOUS TEST

Devices:	glass sticks, swab or cellulose paper
Test site:	elbow flex skin
Frequency of application:	twice per day
Amount of substance:	0.1ml
Duration of treatment:	one week
Removal of test substance:	by rinsing or gentle swabbing
Time(s) of assessment:	once per day before second application
Parameters:	erythema, oedema, scaling, adverse sensation(s) noted by participant
Grading:	zero, weak, moderate, strong
Evaluation:	number of subjects with effect(s) within defined time period, or occurrence of first response; type of reaction

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C. SINGLE APPLICATION CLOSED PATCH EPICUTANEOUS TEST UNDER OCCLUSION OR SEMI-OCCLUSION

Devices:	OCCLUSIVE - large Finn Chambers, Hill Top or Leukotest (BDF) SEMI-OCCLUSIVE - Webril cotton and Blenderm tape
Test site:	upper arm or back
Frequency of application:	once
Amount of substance:	0.07 to 0.1ml
Duration of treatment:	24 hours (or any period from 30 minutes to 48 hours)
Removal of test substance:	by rinsing or gentle swabbing
Time(s) of assessment:	1 hour after patch removal, then after 24 & 48 hours (72 hours)
Parameters:	erythema, oedema, scaling, adverse sensation(s) noted by participant
Grading:	zero, weak, moderate, strong, very strong
Evaluation:	number of subjects with effect(s) within defined time period, or mean of score values after 3 readings, or area under curve for each parameter

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D. REPEATED APPLICATION CLOSED PATCH EPICUTANEOUS TEST UNDER OCCLUSION OR SEMI-OCCLUSION

Devices:	OCCLUSIVE - large Finn Chambers, Hill Top or Leukotest (BDF) SEMI-OCCLUSIVE - Webril cotton and Blenderm tape
Test site:	upper arm or back
Frequency of application:	once per day
Amount of substance:	0.1ml per application
Duration of treatment:	day 1 : 24 hours day 2-day 5 : 6 hours each day
Removal of test substance:	by rinsing or gentle swabbing
Time of assessment:	on day 8
Parameters:	erythema, oedema, scaling, fissures, adverse sensation(s) noted by participant
Grading:	zero, weak, moderate, strong, very strong for fissures only - zero, weak, moderate, strong
Evaluation:	mean of score values

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APPENDIX 3

EXAMPLE OF SCORING SCALE

ERYTHEMA

0 = no evidence of erythema
0.5 = minimal or doubtful erythema
1 = slight redness, spotty and diffuse
2 = moderate, uniform redness
3 = strong uniform redness
4 = fiery redness

DRYNESS (SCALING)

0 = no evidence of scaling
0.5 = dry without scaling; appears smooth and taut
1 = fine/mild scaling
2 = moderate scaling
3 = severe scaling with large flakes

OEDEMA

– = absence of oedema
\+ = presence of oedema

REFERENCE

- Frosch P.J. & Kligman A.M., *The soap chamber test; a new method for assessing the irritancy of soaps*, J. Am. Acad. Dermatol., 1979, 1, pp. 35-41

THE EUROPEAN COSMETIC
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Colipa, the European Cosmetic, Toiletry and Perfumery Association, was established in 1962. Its objectives are:

- To provide expertise and support to a range of working groups dealing in scientific, economic, fiscal, legal, consumer and environmental issues.
- To assist members in complying with European Union legislation affecting cosmetic industry products and operations.
- To act as an industry voice working with both international authorities and organisations. Additionally, Colipa provides a world-wide perspective to its members through its relationships with equivalent organisations in the USA and Japan.
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SCAAT has already taken a series of initatives which will result in the execution of programmes and the generation of data to contribute to the validation of alternative methods. It is now recognised by the authorities as a credible and authoritative voice on this issue.

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