ECETOC Document

No 10

Skin Sensitisation

October 1980

ECETOC

CENTRE D'ECOLOGIE ET DE TOXICOLOGIE
DE L'INDUSTRIE CHIMIQUE EUROPEENNE

EUROPEAN CHEMICAL INDUSTRY ECOLOGY
AND TOXICOLOGY CENTRE

ECETOC DOC-10

SKIN SENSITISATION

PAPER PREPARED BY AN ECETOC TASK FORCE

29 OCTOBER 1980

29.10.80

SKIN SENSITISATION

REPORT OF TASK FORCE

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A. SUMMARY

1. Scope

The TF report is confined to industrial chemicals and excludes formulations for direct consumer use. Throughout this report, the word "chemical" or "substance" does not imply a pure material but refers to the material actually produced and handled, i.e. possibly containing impurities.

2. Test methods

The guinea pig is the chosen animal for examining the potential contact allergenicity of chemicals. An adjuvant-type of test is the preferred test for the detection of such potential. For examining the potential allergenicity, alternative tests may be used provided that the usual response to standard allergens such as 2,4-dinitro-chloro-benzene (DNCB), p-phenylene-diamine, or other potent sensitisers appropriate to the class of substance being tested, is the production of a positive reaction in at least 80% of the animals.

3. Testing in humans

A number of animal tests give a good prediction of sensitisation potential for humans, especially for potent and moderate sensitisers. Therefore primary testing on humans is not necessary.

4. Definition and labelling of potential human skin sensitisers

In the 6th Amendment to the 1967 "Directive on the Classification Packaging and Labelling of Dangerous Substances", it has to be considered whether a chemical should be classified as requiring the R-Phrase "May cause sensitisation by skin contact". It is recommended that : If in an approved test on 20 animals, 3 or more give a positive result on the first epidermal challenge, irrespective of the severity, the R-Phrase will be required. If groups of less than 20 animals are used, but not less than 10, and if 1 or 2 positive responses are obtained on epidermal challenge, the test should be repeated on a number of animals such as to give a total of 20, and a positive result will be defined as previously.

In certain cases, before deciding on the classification and labelling of a substance on the basis of a positive result in an approved adjuvant test, it may be necessary to supplement this result by further tests on sensitisation, or skin penetration, relevant to the physico-chemical properties of the substance.

It is emphasised that testing and labelling indicate sensitisation potential but not risk. Thus chemicals classified as potential human sensitisers are, and will continue to be, used in the industry and by its customers with due precautions.

B. TERMS OF REFERENCE AND MEMBERSHIP

1. The terms of reference for the TF were defined, at the request of the ECETOC Scientific Committee, by Drs J. BACKSTRÖM and K.W. JAGER, as follows :

   a) to give a state of the art report on animal tests for skin contact sensitisation

   b) to discuss the correlation between animal and human tests, i.e. the predictability of animal sensitisation tests and criteria for sensitising potential for man based on results from animal experimentation

   c) to give priority to some test methods of choice

   d) to discuss if, and if so to what extent and in which ways, animal experimental data on sensitisation can be used for classification (and eventual labelling) of a chemical as a sensitiser in man. Especially important is the question whether animal data only, without human clinical experience, are sufficient for classification and labelling.

   e) to suggest how results from animal skin sensitising experiments should be used as a basis for further testing in man, or alternatively form the basis for general precautions etc.

   Although no limitation was specified in the terms of reference, the TF confined its work to industrial chemicals and excluded formulations, for example detergents, soaps, toiletries, designed for direct use by the public.

   There is a fundamental reason for this. Consumer formulations for which potential sensitisation is relevant are those deliberately applied to the skin, or coming into contact with it during normal use. For these formulations the degree of exposure and the number of people exposed are widely different from those of industrial chemicals where relatively few people are exposed and contact is accidental rather than deliberate. The approach to testing will be different in the two cases, and testing schemes valid for

one class should not be automatically taken over for the other. A second reason for omitting formulations is that the product safety problems of for example detergents and soaps are dealt with by the AIS, toiletries by COLIPA etc. Both of these organisations, and ETAD who deal with dyes, were informed of our activity.

2. Membership of the TF

G. CALVIN	-	Invited as observer for AIS.
G.E. DAVIES	-	ICI Central Toxicology Laboratory, U.K.
H.P. GELBKE	-	BASF, Dept. of Industrial Hygiene and Toxicology, Ludwigshaven.
G. KLECAK	-	HOFFMANN-LA ROCHE, Basel.
J. McL PHILP	-	UNILEVER, London.
M. POTOKAR	-	HENKEL, Düsseldorf.
B.J. SIMPSON	-	SHELL, Group Toxicology Div., London.
L. TURNER	-	ECETOC, chairman and secretary of TF.

C. BACKGROUND TO TF WORK

The work originated from an observation by Dr. H.D. WULF of CHEMISCHE-WERKE HÜLS that although tests for skin sensitisation were being incorporated into legislation, especially Annex VII of the European Communities' 6th Amendment, there existed no adequate definition of the phenomenon for legislatory purposes, and no definitive guide to acceptable test methods. The 6th Amendment also requires labelling with an R-Phrase, "May cause sensitisation by skin contact", for substances classified as sensitisers. This lack of a definition and the existence of a large number of proposed test methods, was also a problem for individual chemical companies who had to carry out such testing for the purposes of industrial hygiene and product safety evaluation. The Scientific Committee of ECETOC therefore set up a Task Force with terms of reference as above.

It was known that the OECD group on Short-term Toxicology was considering test methods for skin sensitisation, but they are not expected to provide an interpretation of test results for labelling or industrial hygiene purposes.
A group of members of the British Society for Immunology, including Dr. DAVIES, was also considering test methods for contact sensitisation.

D. INTRODUCTION TO IMMUNOLOGY OF CONTACT DERMATITIS

Allergic contact dermatitis in man is psychologically disturbing, transiently disfiguring and is disabling in terms of attendance at work. Substances in contact with the skin may produce this condition, and testing for their potential to do this is important because although there may be an inherited distribution of genes in man which predisposes towards allergic response, no one is born with an allergy to a particular chemical.

The allergy, or hypersensitivity, can be acquired from contact with chemicals, and can be demonstrated in guinea pigs either as a frank dermatitis (contact dermatitis) or as a discrete local reaction. Most commonly, delayed hypersensitivity in guinea pigs is demonstrated by topical application or intradermal injection of the chemical, giving after pre-treatment a local response greatly in excess of that produced in non-pretreated animals. The response is at its maximum intensity in 24-48 hours, and circulating antibody does not appear to be involved in the reaction. The sequence of events in the animal body is within the concept of allergy, and in immunological terms belongs to the group of allergic reactions classified by Gell et al (1) as "Type IV", characterized as : "Initiated essentially by the reaction of actively allergised lymphocytes, probably of the T (thymus-derived) population responding specifically to the allergen by the release of lymphokines and/or the development of cytotoxicity, without the participation of free antibody". Histologically it is manifested by the infiltration of cells, at the site where the allergen is formed.

Contact dermatitis is an inflammatory skin reaction occurring in contact-sensitive subjects upon epicutaneous application of the chemical (the hapten) to which this subject has been sensitised. Once the person has been sensitised, an eruption can follow ingestion or inhalation of the allergen, but such cases are few. The contact sensitivity skin reaction differs from other type IV inflammatory reactions both macroscopically and histologically. In the reactions considered by the Task Force the allergen is formed in situ by chemical combination of the reactive chemical with tissue proteins. These conjugates are processed by macrophages or Langerhans cells. The stages involved in the induction of experimental contact dermatitis, e.g. in the guinea pig, can most clearly be described as a series of steps starting with a non-sensitized (i.e. previously unexposed) animal, as follows.

1. Sensitisation

   a) Chemical applied to the skin. Some (possibly small) proportion of the chemical penetrates the skin and combines with autologous proteins and is further processed by, probably, the Langerhans cell, a dendritic cell with macrophage-like properties situated in the epidermis.

   b) The antigenic complex is recognized by specific lymphocytes which are then retained in the lymphnode where they proliferate and divide into memory and effector cells.

   c) "Sensitised" effector cells are distributed over the whole organism which becomes sensitised about 5-6 days after the first contact with the hapten.

2. Challenge

   a) Chemical applied to the skin penetrates and combines as before.

   b) Specifically sensitised T-cells react upon a new contact with the antigenic complex by release of pharmacologically-active substances called lymphokines.

   c) Lymphokine activity leads to tissue damage seen as dermatitis.

   The above description is a gross over-simplication and the following additional features need to be taken into account.

   i) the level of sensitisation and the degree of reaction are enhanced if Freund's adjuvant (essentially dead mycobacteria in an oil medium) is injected.

   ii) the degree of reaction observed is really the resultant of a number of factors, including the activity of T-effector cells mediating damage, and of suppressor cells of various types counteracting the activity of T-cells.

E. REVIEW OF STATE OF THE ART OF ANIMAL TESTING

A complete literature review of all possible animal tests for skin sensitisation was considered unnecessary. During years of experience, including close contact with scientific developments in the field, industry specialists have selected a certain number of test methods as being the most reliable and relevant, and it was therefore decided to review only those which were being used by ECETOC members. Two questionnaires (Appendices 1 and 2) were sent to all ECETOC members, both eliciting 14 replies. The detailed replies are held in the ECETOC office, and can be consulted on request. They formed the basis of this section.

Answers to the questionnaires gave the following pattern of use of the various tests :

Test	Nr of companies using test
Magnusson-Kligman (*)	10
Buehler (xx)	5
Optimisation	3
Open Epicutaneous	3
Landsteiner	2
Draize	2
Freund's Complete Adjuvant	1
Stevens	1
Single-injection Adjuvant	1

(*) the only test used by 6 respondents
(xx) one respondent used this with and without adjuvant.

In general, the M&K test was considered the most difficult to perform and to assess, although a high degree of difficulty to assess was also reported for the Draize Test.

Procedures for dealing with highly-coloured substances included : washing-off after challenge; histological assessment; measurement of oedema; intradermal challenge. Insoluble materials were used as suspensions but little precise detail was presented.

Few respondents were able to provide information to the question "Can you give results of comparing substances in any test or tests ?".

F. RECOMMENDED TEST METHODS

1. Testing in humans

The accumulated experience of testing has shown that certain guinea pig tests yield results which give a good correlation with the sensitisation potential of a chemical to humans. This is especially so for potent and moderate sensitisers. The correlation is such that primary tests on humans are not required.

2. Animal tests - discussion

It is widely accepted that the guinea pig is the preferred animal for assessing potential skin sensitisation of a chemical towards humans, and the TF strongly support this view.

Of the numerous tests available, the adjuvant type, e.g. guinea pig maximisation, is the preferred one. The preference is based on practical experience, and it is relevant that this test is being used by 10 out of 14 member companies who replied to the first questionnaire. There are a number of variants of this test.

The sensitivity of a given strain has to be checked with strong and moderate sensitisers at regular intervals, e.g. 6 to 12 months.
In some cases there may be good reasons for choosing a test involving topical application rather than injection. There is no single test method which is adequate and relevant for all industrial chemicals. In choosing a test, factors such as the physical characteristics of the substance (e.g. its ability to penetrate the skin) and its mode of contact with those exposed to it must be considered.

The choice of an alternative to the adjuvant type of test must be governed by criteria which ensure that it is valid. The TF recommend that any test used must be such that the usual response to standard allergens such as 2,4-dinitrochloro-benzene, p-phenylene-diamine (2), or other potent sensitisers appropriate to the class of substance being tested, is the production of a positive reaction in at least 80% of the animals. Such a limitation reduces to a manageable number the choice from the large number of tests which have been proposed. It should also ensure a degree of comparability between the results.

The above recommendations for alternative tests permit a choice of methods suitable for particular classes of substance, and most relevant to actual conditions of exposure. Such a formulation would also allow the easy introduction of new techniques into any legislatory requirement by avoiding a fixed list of permitted methods, but relying instead on validation criteria.

3. Details of test methods

Within the criteria set out in the previous section a number of test methods can be considered (literature references in brackets) :

Adjuvant techniques

Freund's complete adjuvant test (5), (6).
(Magnusson-Kligman) Guinea pig maximisation test (2),(3),(4).
Optimisation test (11), (12).
Single injection test (9).
Split adjuvant test (7), (8).

Other techniques

Buehler test (10)
Open epicutaneous test (5)

4. Testing highly-coloured or insoluble substances

The TF had hoped to be able to recommend commonly used procedures for testing highly-coloured or insoluble substances from a consideration of the questionnaire replies. The diversity of the methods revealed proved to be such that no general conclusion could be drawn. The list of suggested techniques is held in the ECETOC office.

5. A note on non-reproducible test results

The TF thought it useful to point out that a common cause of poorly-reproducible results was the presence of a trace impurity (perhaps in an amount which varied from batch to batch) which was itself a potent sensitiser.

G. DEFINITION AND LABELLING OF A POTENTIAL HUMAN SKIN SENSITISER

For legislatory purposes a potential human skin sensitiser can be defined from the quantitative result of a test recommended as in the previous paragraph. For example, in the 6th Amendment to the 1967 Directive on Classification, Packaging and Labelling of Dangerous Substances it has to be considered whether a chemical should be classified as requiring the R-Phrase "May cause sensitisation by skin contact". It is recommended that : If in an approved test on 20 animals, 3 or more give a positive result on the first epidermal challenge, irrespective of the severity, the R-Phrase will be required. If groups of less than 20 animals are used, but not less than 10, and if 1 or 2 positive responses are obtained on epidermal challenge, the test should be repeated on a number of animals such as to give a total of 20, and a positive result will be defined as previously.

In certain cases, before deciding on the classification and labelling of a substance on the basis of a positive result in an approved adjuvant test, it may be necessary to supplement this result by further tests on sensitisation, or skin penetration, relevant to the physico-chemical properties of the substance.

H. CONDITIONS UNDER WHICH TESTING IS UNNECESSARY

There is no point in carrying out skin sensitisation testing for substances already shown to be corrosive, or to possess extreme local topical or systemic toxicity.
Under these conditions precautions will already have been taken to limit exposure.

I. APPENDICES

1. First questionnaire
2. Second questionnaire

J. LITERATURE REFERENCES

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5. Klecak G. et al, Screening of Fragrance Materials for Allergenicity in Guinea Pig : I, Comparison of Four Testing Methods. J. Soc. Cosmet. Chem., 28, 53-64 (1977).
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7. Maguire H.C., The Bioassay of Contact Allergens in the Guinea Pig. J. Soc. Cosmet. Chem., 24, 151-162 (1973).
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9. Goodwin B.F.J., Crevel R.W.R. and Johnson A.W., Clinical Allergy (1980), in the press.
10. Beuhler E.V. et al, Experimental Skin Sensitisation in the Guinea Pig and Man. Ref. 8, p. 55-66.
11. Maurer Th. et al, Toxicology 15, 163-171 (1980).
12. Maurer Th. et al, Agents and Actions, 5 (2), 174-179, (1975).

SENSITISATION (1st Questionnaire) APPENDIX 1 31.10.80

NAME OF TEST	Is it technically easy (1) or difficult (5) to perform					Is it easy (1) or difficult (5) to assess the reaction					Does the test cause trauma in the animal		Duration of test (days)	Is the test staff intensive		
	1	2	3	4	5	1	2	3	4	5	yes	no		yes	moderate	no

SENSITISATION (2nd Questionnaire)

NAME ..... COMPANY .....

1. In your reply to our first questionnaire were the tests you mentioned applied to end-user formulations (e.g. cosmetics, soaps, etc.) or to bulk chemicals in general, some of which may be used to prepare such formulations ?

   Please tick :

   Bulk chemicals Formulations

2. How do you test highly-coloured substances and insoluble substances ?

3. Can you give results of comparing substances in any test or tests ?