Contact Sensitisation: Classification According to Potency A Commentary

Document No. 43

Brussels, July 2003

ECETOC Document No. 43

European Centre for Ecotoxicology and Toxicology of Chemicals
4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

Contact Sensitisation: Classification According to Potency A Commentary

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has recently published a report (Technical Report No 87; 2003; Appendix 1) that addresses the opportunities that now exist for the classification of contact allergens according to their relative skin sensitising potency. That report also contains recommendations regarding the configuration of classification schemes based upon the use of data deriving from OECD guideline methods for skin sensitisation testing. In parallel with the work of ECETOC, an Expert Working Group on Sensitisation commissioned by the European Chemicals Bureau (ECB) undertook a similar task, and a draft document was issued by the ECB (Appendix). The ECETOC report and the report of the ECB Expert Working Group have in common a desire to refine the way contact allergens are classified based upon (a) an appreciation that such chemicals may differ very substantially with regard to their skin sensitising potency, and (b) the availability now of experimental approaches that allow assessment of relative skin sensitising activity. Where the reports differ is in the details of how such classification may be achieved in practice - and specifically with regard to models based on animal test data for assigning chemical allergens to different potency categories.

We address here the differences as they relate to the use of local lymph node assay (LLNA) data for categorisation of allergenic potential. The reason for focusing only on the LLNA is 2-fold: first, because it is this assay that will most commonly be used to provide data that are suitable for potency ranking and classification, and second because the same general principles apply to results deriving from guinea pig tests.

With respect to the LLNA there exist two broad issues to identify and resolve. The first of these is the number of classes into which contact allergens should be classified, and the second is the specification for assignment of contact allergens to particular categories.

It is helpful to address the latter issue first - how such categories are defined with respect to EC3 values derived from LLNA data. Both Reports recommend the recognition of categories described as *Extreme*, *Strong* and *Moderate*. The difference is that in the ECB Report, the boundary between *Extreme* and *Strong* is an EC3 of 0.2%, and between *Strong* and *Moderate* of 2%. In contrast, in the ECETOC Report, the thresholds between these categories are EC3 values of, respectively, 0.1% and 1%. Although the ECETOC Task Force retains strong support for the thresholds of 0.1% and 1%, it recognises that the 0.2% and 2% values, as recommended in the ECB Report, are also well considered and workable.

However, with regard to the other issue - the number of categories that should be used for classification according to potency - then there is possibly less harmony. The ECETOC Task Force was strongly of the opinion that, in addition to the 3 categories identified above (*Extreme*, *Strong* and *Moderate*), a fourth should be used; this having the descriptor of *Weak*. The proposal made in the ECETOC Report was that chemical allergens with EC3 values of 10% or above should be assigned to this category and described as *Weak*. The reasoning for this was that chemicals with such high EC3 values have only a very limited potential to cause skin sensitisation, even under conditions where the opportunities for exposure are significant. For example Linalool, a contact allergen that under the ECB scheme would be classified as being of *Moderate* potency, is known to induce sensitisation among humans only rarely and following exposure to comparatively high concentrations. Using the ECETOC scheme Linalool would be assigned a *Weak* classification that we believe better reflects what is known of the relative skin sensitising potency of this chemical. The conclusion is that the use in the ECB scheme of a single category for *Moderate* sensitisers that spans EC3 values from 2% to 100% is too broad, and fails to reflect accurately the data available on relative skin sensitising potency.

MEMBERS OF THE TASK FORCE

I. Kimber	Syngenta UK - Maccesfield
D. Basketter	Unilever UK - Sharnbrook
A. Gamer	BASF D - Ludwigshafen
J-L. Garrigue	L'Oréal F - Aulnay-sous-Bois
G.F. Gerberick	Procter & Gamble USA - Cincinnati
C. Newsome	Dow Chemical UK - West Drayton
W. Steiling	Henkel D - Düsseldorf
H-W. Vohr	Bayer D - Wuppertal
F. Carpanini (Secretary)	ECETOC B - Brussels

MEMBERS OF THE SCIENTIFIC COMMITTEE

(Peer Review Committee)

G. Randall (Chairman) Director, Environmental Laboratory	AstraZeneca UK - Brixham
N. Carmichael (Vice-chairman) Head, Toxicology	Bayer CropScience F - Sophia Antipolis
C. Braun Occupational Toxicologist	Akzo Nobel NL - Arnhem
P. Calow Professor of Zoology	University of Sheffield UK - Sheffield
C. d'Hondt Head, Environmental Safety Department	Syngenta CH - Basel
P. Douben Head, SEAC Environmental Protection Department	Unilever UK - Bebington
T. Feijtel Manager, Professional and Regulatory Services	Procter & Gamble B - Brussels
H. Greim Director, Institute of Toxicology and Environmental Hygiene	Technical University Munich D - Munich
C. Money Industrial Hygiene Adviser, Europe	ExxonMobil B - Brussels
A. Sarrif Director, Toxicology Affairs, Europe	DuPont B - Brussels
G. Swaen Head, Occupational Epidemiology Unit	Maastricht University NL - Maastricht
B. van Ravenzwaaya Director, Experimental Toxicology and Ecology	BASF D - Ludwigshafen
H-J. Wiegand Head, Product Safety Department	Degussa D - Düsseldorf

[a] Responsible for primary peer review

Attachment 1

Contact Sensitisation: Classification According to Potency

Technical Report No. 87

ISSN-0773-8072-87
Brussels, April 2003

ECETOC Technical Report 87

European Centre for Ecotoxicology and Toxicology of Chemicals
4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

Contact Sensitisation: Classification According to Potency

CONTENTS

SUMMARY

Contact allergens vary substantially with regard to the relative potency with which they are able to induce skin sensitisation. In the future, considerations of potency will become a significant factor in the classification of skin sensitising chemicals. It is therefore appropriate to establish what is known of potency and thresholds in the induction of skin sensitisation and the elicitation of allergic contact dermatitis, and to identify approaches that might be available for assessment of relative potency for the purposes of categorising chemical allergens. This report was prepared by a Task Force with the remit 'to recommend approaches for the measurement of potency and definition of thresholds for both the induction and elicitation of contact sensitisation'.

The deliberations recorded here build on recommendations made previously by an ECETOC Task Force that considered the conduct of standard skin sensitisation test methods for the purposes of hazard identification and risk assessment (ECETOC, 2000). The emphasis in this present report is also on standard and accepted methods for the assessment of skin sensitisation, and for which OECD guidelines are available: the local lymph node assay (LLNA), the guinea pig maximisation test and the occluded patch test of Buehler. For various reasons, discussed in detail in this report, attention focused primarily on consideration of categorisation of chemical allergens and the identification of thresholds with respect to the induction of skin sensitisation, rather than the elicitation of allergic contact dermatitis.

Conclusions drawn previously by an ECETOC Task Force (ECETOC, 2000) are reflected by recommendations made here. Thus, although the LLNA is the method of choice for the determination of skin sensitisation potency for the purposes of categorisation, if data are already available from appropriate guinea pig tests then their judicious interpretation may provide information of value in determinations of potency and categorisation. Included here are detailed and specific recommendations on how best the results of the three test methods considered can be used for the categorisation of chemical allergens as a function of skin sensitisation potency.

BACKGROUND

Toxicological evaluations are (conceptually at least) conducted in two steps. The first is identification of hazard, and the second assessment of whether that hazard is likely to translate into a health risk. The development of accurate risk assessments demands an appreciation of the likely conditions of exposure (frequency, route, extent and duration of exposure), linked with an understanding of potency. This applies equally to safety assessments for skin sensitisation.

There are methods available for skin sensitisation hazard identification (ECETOC, 2000; Kimber *et al*, 2001; Steiling *et al*, 2001), and this is no longer the major issue for toxicologists. What represents a more important challenge is accurate evaluation of the relative potency of skin sensitising chemicals that can inform the risk assessment process. This is an issue of some significance, as it is believed that skin sensitising chemicals may vary up to ten thousand-fold with respect to their relative sensitising potency.

In common with other forms of allergic diseases, allergic contact dermatitis develops in two phases. In the first of these (the induction phase) a subject is exposed to an amount of the inducing chemical allergen sufficient to provoke a cutaneous immune response of the vigour and quality necessary to result in systemic sensitisation. If the now sensitised subject is exposed subsequently to the same chemical, at the same or at a different skin site, then an accelerated and more aggressive secondary immune response can be elicited which will in turn provoke the cutaneous inflammatory reaction that is recognised clinically as allergic contact dermatitis.

There are clearly dose-response relationships for both the induction and elicitation phases of contact hypersensitivity, and as a consequence it is possible to determine thresholds for the level of chemical exposure below which sensitisation will fail to be induced in a naïve subject, or below which a reaction will fail to be elicited in a previously sensitised subject (Kimber *et al*, 1999). Although such thresholds can be established, it is important to recognise that:

1. Thresholds are determined largely by the potency of the chemical allergen, but can be influenced by the vehicle or formulation in which the chemical is encountered on the skin surface.
2. Thresholds for the induction of sensitisation to a particular chemical will be different from the amount of the same chemical required to elicit a reaction in a previously sensitised subject. The general rule is that higher levels are necessary for the initial acquisition of sensitisation than are required for the elicitation of a reaction in a sensitised individual.
3. Thresholds for sensitisation vary between individuals.
4. Thresholds for elicitation of allergic contact reactions vary between individuals, the extent to which sensitisation has been acquired being an important determinant.

It is appropriate to define what is meant by the term *potency* as it relates to the induction of skin sensitisation and the elicitation of allergic contact dermatitis. In a general sense the word is used as a descriptor for strength, power or vigour - and this is true also in the context of skin sensitisation. Potency, as it relates to either induction of sensitisation or to the elicitation of reactions is considered as a function of the amount of chemical needed to provoke the response of interest. Thus, for the induction phase, potency is described in terms of the amount of chemical necessary to cause the acquisition of sensitisation; clearly, the more potent the chemical, the less that will be needed for the effective development of sensitisation. For the elicitation phase, potency is described in terms of the amount of chemical required to elicit a discernible allergic reaction in a previously sensitised subject. Again, the more potent the chemical, the less that will be required to elicit a reaction, although in this case the extent of individual sensitisation is an important factor (Friedmann, 1996).

It is important to acknowledge that potency is difficult to define in absolute terms and for this reason it is usually relative potency that is determined, i.e. the potency of a chemical relative to a benchmark allergen for which there already exists some information regarding activity.

Finally, it is important to distinguish *potency* as it relates to the activity of sensitising chemicals from the relative *prevalence* of allergic contact dermatitis; the two are frequently confused. One example will serve to illustrate the point. There is no doubt that in Europe and the USA, nickel is a common cause, and in some areas the most common cause, of allergic contact dermatitis. However, the evidence is that nickel is only a relatively weak allergen. The high prevalence of sensitisation to nickel results from the ubiquitous distribution of this metal and the extensive opportunities for exposure.

Against this background, and in the light of the fact that potency will become a significant factor in sensitisation classification in the future, a Task Force was established with the remit:

> To recommend approaches for the measurement of potency and definition of thresholds for both the induction and elicitation of contact sensitisation.

1. INTRODUCTION

The conduct of standard skin sensitisation test methods, for which OECD guidelines are available (the local lymph node assay [OECD, 2002], the guinea pig maximisation test and the occluded patch test of Buehler [OECD, 1992]) has been considered previously by an ECETOC Task Force (ECETOC, 2000; Kimber *et al*, 2001; Steiling *et al*, 2001), and a similar review is unnecessary here. Instead, the focus of this report is on the potential use of these methods for categorisation of skin sensitising chemicals based on potency. To this end, the utility of each of these methods, and how best the data they provide can be used for this purpose, are considered and specific recommendations made. The application of such categorisations in the context of classification and labelling is also addressed and further recommendations identified. Before looking at specific test methods, it is necessary to make four general points.

Distinction between the induction and elicitation phases of allergic contact dermatitis

As indicated in the Background, there is no doubt that thresholds exist for both the induction of sensitisation (the amount of chemical required for the acquisition of skin sensitisation by a previously naïve subject) and the elicitation of an allergic contact dermatitis reaction (the amount of chemical that is necessary to provoke a clinically detectable dermatitic reaction in a previously sensitised individual). However, for three reasons we focus initially, and primarily, upon consideration of categorisation of chemicals and the identification of thresholds with respect to the induction of skin sensitisation. These reasons are as follows:

- From toxicological, occupational and consumer health perspectives it is more important to prevent the induction of sensitisation, than to prevent the elicitation of a reaction in those who are already sensitised. If an accurate assessment of the risk of sensitisation is made, and the appropriate risk management practices implemented, then sensitisation will not be induced and the conditions under which a reaction would be elicited in a sensitised subject become academic.
- Conceptually, the identification of thresholds for the induction of sensitisation is easier than establishing thresholds for the elicitation of reactions. This is due to the fact that the degree to which skin sensitisation has developed influences the amount of chemical required to provoke a challenge reaction. In general terms the higher the level of sensitisation, the smaller the amount of chemical necessary to elicit a dermatitic reaction (Friedmann, 1996; Scott et al, 2002).
- The preferred method for assessment of relative potency for the induction of sensitisation (the local lymph node assay) is not suitable for consideration of elicitation thresholds.

Notwithstanding these considerations, issues relating to elicitation of allergic contact dermatitis will be identified at the end of this report.

The impact of vehicle matrix or formulation on the activity of skin sensitising chemicals in test methods

There is no doubt that the form in which a chemical allergen is encountered at skin surfaces will affect the extent to which sensitisation is acquired and its activity in predictive test methods. Although this is the case for each of the methods that are considered in this report, most detailed information on the influence of vehicle matrix on skin sensitising activity has derived from studies in mice (Cumberbatch *et al*, 1993; Dearman *et al*, 1996; Heylings *et al*, 1996), including the local lymph node assay (Basketter *et al*, 2001a; Ryan *et al*, 2002). Although such vehicle-related effects are of relevance in the context of risk assessment, it is our view that they have little impact on the accuracy of hazard identification when properly conducted standard test methods are used. With regard to the classification on the basis of relative potency, our view is that if such schemes employ sufficiently broad categories, then vehicle effects will again be of little moment.

Classification and inclusion limits

Any classification scheme must employ clearly identified upper and lower limits for inclusion of a chemical in any particular category. The obvious complication is that the relative potency of skin sensitising chemicals (however judged) will be a continuum, rather than progressing in a step-wise fashion. Inevitably therefore there will be chemical allergens that receive separate classifications on the basis of small differences in activity (that fall either side of a predetermined limit value) that are perceived to be of little or no biological relevance. Such apparent anomalies are unavoidable in any classification scheme that seeks to categorise based upon a characteristic or property that displays continuous, rather than discontinuous, variations. While this does not invalidate attempts to classify chemical allergens as a function of potency, the constraint must be acknowledged.

Non-standard test methods

In addition to the specific test methods accepted currently by regulatory authorities, other protocols at varying stages of development have been proposed. Some of these may in the future prove useful for skin sensitisation hazard identification and/or for potency assessment. (Klecak, 1985; Andersen *et al*, 1995; van Och *et al*, 2000; Vohr *et al*, 2000 and Gerberick *et al*, 2002).

2. SKIN SENSITISATION TEST METHODS AND THEIR USE IN CATEGORISATION OF SKIN SENSITISING CHEMICALS ACCORDING TO POTENCY

Considered in this report are standard and accepted methods for the assessment of skin sensitisation and for which OECD guidelines are available; the local lymph node assay (2.1), the guinea pig maximisation test (2.2.1) and the occluded patch test of Buehler (2.2.2).

2.1 The local lymph node assay

The local lymph node assay (LLNA) was developed initially as an alternative approach to hazard identification, and for this purpose it has now been evaluated extensively and validated formally. Detailed considerations of the development, conduct and application of the LLNA are available elsewhere (Basketter *et al*, 2002; Dearman *et al*, 1999; Gerberick *et al*, 2000; Kimber and Basketter, 1992; Kimber *et al*, 1994; 2002).

The LLNA is based upon measurement of lymphocyte proliferative responses that are induced in draining lymph nodes following topical exposure of mice to chemicals. Skin sensitising chemicals are defined as those that, at one or more test concentration, provoke a three-fold or greater increase in lymph node cell (LNC) proliferation compared with concurrent vehicle controls. This is a relevant read-out for the evaluation of skin sensitising potential. The activation and clonal expansion of allergen-responsive T lymphocytes is the pivotal event in the acquisition of skin sensitisation. First principles dictate that the vigour of LNC proliferative responses should be a major factor in determining the extent of sensitisation and this is borne out by experimentation (Kimber and Dearman, 1991). For this reason it was proposed that the LLNA could be used not only for hazard identification, but also for measurement of the relative sensitising potency of contact allergens (ECETOC, 2000; Kimber and Basketter, 1997; Kimber *et al*, 2001). For this purpose an EC3 value is derived; this being the amount of a chemical sensitiser that is required to elicit a 3-fold increase in LNC proliferative activity. In theory it would be possible to express EC3 values in a number of ways; as a percentage or molar value, or as the amount of chemical per unit area of skin. Although it is the last of these that is probably the most relevant scientifically (as it is known that dose per unit area is the critical exposure determinant for skin sensitisation), the consensus view is that in practice, the use of percentage concentrations is the preferred option. The recommendation is that linear interpolation of values either side of the 3-fold stimulation index (SI) on a LLNA dose response curve is the most robust and the most convenient method for the routine calculation of EC3 values (Basketter *et al*, 1999a).

Experience has revealed that the EC3 values derived in this way are robust determinants of relative skin sensitising potency; equivalent results have been obtained in different laboratories, and over time within a single laboratory (Dearman *et al*, 1998; Kimber *et al*, 1995).

Experience has been gained in the use of EC3 values for determination of relative skin sensitising potency. Various related groups of chemicals have been examined including, dinitrohalobenzenes, various aldehydes, and biocides (Basketter *et al*, 1997; 1999b; 2001b; Hilton *et al*, 1998). It has been demonstrated also that estimates of relative skin sensitising potency, measured as a function of derived EC3 values, are relevant for the induction of skin sensitisation in humans. Collaborative studies in the UK and USA were conducted in partnerships between experimental laboratories performing the LLNA and experienced clinical dermatologists. The latter provided a view of the relative induction potency of two series of known human contact allergens. Chemicals were classified according to relative induction potency based on clinical judgement and compared with EC3 values estimated from LLNA dose responses. A close correlation between clinical assessments of potency and EC3 values was reported (Basketter *et al*, 2000; Gerberick *et al*, 2001).

The issue to be addressed here is how best to categorise chemical allergens with respect to relative skin sensitising activity based on derived EC3 values. Our view is that the most sensible and most practical scheme is one in which 4 categories are used and identified with the descriptors: 'extreme', 'strong', 'moderate' and 'weak'. The suggestion is that the scheme should distinguish between contact allergens on the basis of 10-fold variations in potency, as illustrated in Table 1 below:

Table 1: Categorisation of contact allergens on the basis of relative skin sensitisation potency. Recommended scheme using EC3 values derived from the local lymph node assay

Category	EC3 [%]
Extreme	< 0.1
Strong	≥ 0.1 - <1
Moderate	≥ 1 - < 10
Weak	≥ 10 - ≤100

Employing this scheme, a series of contact allergens and non-sensitising chemicals have been categorised on the basis of EC3 values. The results shown in Table 2 provide some examples.

Table 2: Categorisation of chemicals according to skin sensitising potency using the local lymph node assay

Chemical	EC3 [%]	Category
Oxazolone	0.01	Extreme
Diphencyclopropenone	0.05	Extreme
Methyl/chloromethylisothiazolinone	0.05	Extreme
2,4-Dinitrochlorobenzene	0.08	Extreme
Toluene diisocyanate	0.11	Strong
Glutaraldehyde	0.20	Strong
Trimellitic anhydride	0.22	Strong
Phthalic anhydride	0.36	Strong
Formaldehyde	0.40	Strong
Methylisothiazolinone	0.40	Strong
Isoeugenol	1.3	Moderate
Cinnamaldehyde	2.0	Moderate
Diethylmaleate	2.1	Moderate
Phenylacetaldehyde	4.7	Moderate
Methyldibromo glutaronitrile	5.2	Moderate
Tetramethylthiuramdisulfide	6.0	Moderate
4-Chloroaniline	6.5	Moderate
Hexylcinnamaldehyde	8.0	Moderate
2-Mercaptobenzothiazole	9.7	Moderate
Abietic acid	11	Weak
Citral	13	Weak
Eugenol	13	Weak
p-Methylhydrocinnamaldehyde	14	Weak
p-tert-Butyl-α-methyl hydrocinnamaldehyde	19	Weak
Hydroxycitronellal	20	Weak
Cyclamen aldehyde	21	Weak
Linalool	30	Weak
Ethyleneglycol dimethacrylate	35	Weak
Diethanolamine	40	Weak
Isopropyl myristate	44	Weak

2.2 Guniea pig tests

The guinea pig maximisation test (GPMT) developed by Magnusson and Kligman (1970) and the occluded patch test of Buehler (1965) have provided the core of predictive skin sensitisation testing for many years. However, as reviewed previously (ECETOC, 2000), they are not well suited to potency estimation, having been designed specifically for hazard identification. Not least among the issues associated with guinea pig tests is consideration of how to interpret the endpoint assessment (the subjective assessment of challenge-induced skin reactions). In the context of potency measurement, it is necessary to review whether it is appropriate to consider the frequency of positive reactions alone, or to take into account also the intensity of induced reactions. In practice, it appears that stronger sensitisers tend to produce both a high response frequency and stronger individual reactions, whereas weaker sensitisers lead to lower rates of sensitisation associated with lesser grades of skin reaction. Thus, the question may be somewhat academic. As a consequence, it is recommended that for the interpretation of guinea pig tests of any type in terms of potency, only the frequency with which skin sensitisation is induced should be employed as the endpoint.

2.2.1 Guinea pig maximisation test

When (on the basis of a GPMT) a substance classifies as a skin sensitiser (R43) according to current EC criteria, then further categorisation can be considered. However, this is predicated on an assumption that the study was conducted fully in accordance with OECD Guideline 406, or with EC Test Method B6, and on an understanding that due consideration was given to the issues raised regarding proper conduct of the GPMT (Schlede and Eppler, 1995).

Table 3: Categorisation of contact allergens on the basis of relative skin sensitisation potency. A recommended scheme using the guinea pig maximisation test

[%][1]	Incidence (%)	
	=30 - < 60	≥ 60
< 0.1	Strong	Extreme
≥ 0.1 - < 1	Moderate	Strong
≥ 1 - < 10	Weak	Moderate
≥ 10 - ≤ 100	Weak	Weak

[1] Concentration employed for topical exposure during the induction phase.

It should be noted that emphasis is placed here on the induction concentration, since it is this stage of sensitisation that is most susceptible to dose response effects. In the context of the scheme summarised in Table 3, the recommendation is that the amount of chemical used for topical administration during induction, rather than the concentration of chemical used for intradermal injection employed during induction, should be used as the relevant metric. The basis for this recommendation is that the concentration of test chemical used for intradermal injection is frequently determined, and limited, by the addition of Freund's Complete Adjuvant.

Only limited consideration should be given to the challenge concentration; allergic reactions elicited only at very high concentrations will be of lesser concern and may help to clarify borderline cases. Furthermore, it is acknowledged that a certain degree of judgement will be necessary in borderline cases, or where there are additional data clarifying the sensitivity of the GPMT at the test institution. However, where the GPMT data are regarded as sufficient to derive a R43 classification, but are of inadequate quality to permit a more detailed categorisation of potency, then the substance may be judged to be a stronger allergen than is the case. Where the response rate is 100% with evidence that the result may be on the plateau of a dose response curve (such as when the individual animal reaction grades are all high), then this conclusion may be justified.

Table 4 provides examples of how the classification scheme would operate in practice, using data drawn from (Wahlberg and Boman, 1985; Cronin and Basketter, 1994).

Table 4: Categorisation of chemicals according to skin sensitising potency using the GPMT

Substance	Injection induction	Induction patch	Challenge patch	Incidence (%)	Category
Methyl/chloromethylisothiazolinone	1ppm	37.5ppm	15ppm	100	Extreme
2,4-Dinitrochlorobenzene	0.05%	0.5%	0.1%	100	Strong
Cinnamaldehyde	0.2%	2.5%	0.75%	100	Moderate
Formaldehyde	0.5%	5.0%	2.0%	90	Moderate
Citral	0.2%	5.0%	0.5%	50	Weak
Isoeugenol	0.15%	25%	5.0%	100	Weak
2-Mercaptobenzothiazole	1.0%	25%	15%	40	Weak
Hexylcinnamaldehyde	0.5%	50%	10%	60	Weak
Eugenol	0.05%	75%	25%	60	Weak
Hydroxycitronellal	0.5%	100%	50%	60	Weak

2.2.2 The occluded patch test of Buehler

The Buehler guinea pig test has been in use for over 30 years for the hazard identification of skin allergens. However, little guidance is provided in 3.2.7.1 of Annex VI of Directive 67/548/EEC or OECD Guideline 406 (1992) on classification of sensitisers according to potency using this method. The major reason for this lack of guidance is that the design of the Buehler method does not lend itself readily to the categorisation of skin sensitisers. In practice, dose selection for both the induction and elicitation phase of the response is based on the irritant potential of the test chemical. In the Buehler test a net response of 15% incidence, rather than 30% used in the GPMT, is used as the criterion for a chemical being classified as a sensitiser.

Assuming that clear evidence exists that the study has been conducted fully in accordance with OECD Guideline 406, or the requirements of EC Test Method B6, and with due consideration of the issues raised in relation to proper conduct of the Buehler Test (Robinson *et al*, 1990), then as indicated in Table 5 attempts can be made to characterise relative skin sensitising potency.

Table 5: Categorisation of contact allergens on the basis of relative skin sensitisation potency. Recommended scheme using the occluded patch test of Buehler

[%][1]	Incidence (%)	
	=15- < 60	**≥ 60**
< 0.1	Strong	Extreme
≥ 0.1 - < 1.0	Moderate	Strong
≥ 1.0 - < 10	Weak	Moderate
≥ 10 - ≤ 100	Weak	Weak

[1] Concentration employed during induction phase

This model is based on concentrations used for induction, since it is this stage of sensitisation that is most susceptible to dose response effects. However, consideration should be given to the challenge concentration with regard to noting that the optimal challenge dose was used (highest non-irritating dose). In addition, where the data are regarded as sufficient to derive a R43 classification, but are of inadequate quality to permit a more detailed categorisation of potency, the substance may be judged to be a stronger allergen than is the case. Where a response rate of 100% is found with a relatively high induction concentration, with evidence that the result may be on the plateau of a dose response curve (such as when the individual animal reaction grades are all high), then this conclusion may be justified.

Table 6 provides examples of how the classification scheme would operate in practice, using data drawn from Basketter and Gerberick (1996).

Table 6: Categorisation of chemicals according to skin sensitising potency using the occluded patch test of Buehler

Substance	Induction patch [%]	Challenge patch [%]	Incidence	Category
2,4-Dinitrochlorobenzene	0.1	0.1	100	Strong
Methyl/chloromethylisothiazolinone	0.2	0.2	100	Strong
1,3-Dodecane unsaturated sultone	0.35	0.1	87	Strong
Allyisothiocyanate	0.75	0.75	30	Moderate
Tetrachlorosalicylanilide	1	1	80	Moderate
Chloroamine T	2.5	2.5	70	Moderate
Citronellal	2.5	1	45	Weak
Vanillin	2.5	2.5	40	Weak
Cinnamaldehyde	10	1	80	Weak
Benzoyl peroxide	10	10	42	Weak
Ammonium thioglycolate	10	5	35	Weak
Potassium dichromate	10	3	20	Weak
Thioglycerol	14	14	60	Weak
Trimellitic anhydride	25	10	70	Weak
Phthalic anhydride	25	10	30	Weak
Amylcinnamaldehyde	30	10	100	Weak
Hydroxycitronellal	30	10	25	Weak
Hexylcinnamaldehyde	50	5	60	Weak
Benzocaine	50	50	20	Weak
2-Mercaptobenzothiazole	75	75	55	Weak

Categorisation based on lowest induction concentration resulting in a positive response

2.3 Recommendations regarding use of animal models

The guinea pig maximisation test, the Buehler occluded patch test, and the LLNA are internationally accepted methods for the assessment of skin sensitisation hazard, with standard protocols published by OECD (OECD, 1992; OECD, 2002). Each of these methods was reviewed by ECETOC (ECETOC, 2000) with regard to provision of useful information on the relative skin sensitisation potency of a chemical. The conclusions drawn then remain valid:

- Although attempts have been made to reconfigure guinea pig tests for the purposes of deriving dose response relationships, these methods are considered inappropriate for assessment of relative potency.
- However, if results are available from suitable guinea pig tests, then judicious interpretation of the data may provide information of value in assessing relative skin sensitising potency. This option should be explored before other analyses are conducted.
- The LLNA is the recommended method for new assessments of relative potency, and for the investigation of the influence of vehicle or formulation on skin sensitising potency.

It must be emphasised that whichever methods are used, assessment of potency must be evaluated relative to other chemical allergens of known skin sensitising activity. The estimation of likely threshold concentrations is dependent upon the availability of suitable benchmark chemicals of known potency with respect to human responses.

Finally, a comparison of the illustrative data in Tables 2, 4 and 6 reveals that, on the basis of the paradigms proposed, some chemical allergens are assigned different categories according to the test method used. To take one example, isoeugenol is categorised as being of 'moderate' potency using data from the LLNA, but 'weak' on the basis GPMT results. Undoubtedly this is a reflection of the multiple procedural differences between these methods, including for example the routes(s) of exposure, the use of adjuvant, the basis for dose selection, the use of different vehicles and the endpoint measured. Clearly such differences must be borne in mind when applying information on potency categories.

3. POTENCY IN PRACTICE: CLASSIFICATION AND LABELLING

3.1 Substances

Current European legislation (EC, 1992) requires substances to be classified and labelled according to their intrinsic hazard. Substances are classified as skin sensitisers if, in properly conducted tests, at least 30% of animals show a positive response in a GPMT and 15% in a Buehler test. For the LLNA a positive response is defined as the elicitation of a three-fold or greater increase in murine lymph node cell proliferation compared with concurrent vehicle controls. The label on the substance will then carry the St Andrews Cross hazard symbol, the index "i" (Irritant) and the Risk Phrase R43 (May cause sensitisation by skin contact).

In each of these cases the classification and labelling is binary ('yes' or 'no'); the substance is, or is not, a sensitiser. Any differentiation on the basis of potency to induce or elicit sensitisation is not possible within the framework of the current guidelines and legislation. Thus, classification and labelling makes no distinction between a weak sensitiser and a strong sensitiser.

It is recommended that, where available, information on the potency category should be given in the substance safety data sheet (SDS) to assist risk management without the need for creation of new risk phrases.

3.2 Preparations

Current European legislation (EC, 1999) also requires preparations (mixtures of substances) to be classified and labelled on the basis of their intrinsic hazard. Where there are no data on the preparation itself, classification is made using information on the hazards of the component ingredients. If the preparation contains 1% or more of at least one substance, which is itself classified as a sensitiser, then the preparation will be classified as a sensitiser with the same hazard symbol, index and risk phrase as described above for substances. This is the so-called 'default value' for classification of preparations for sensitisation. [Substances officially classified by the EC as skin sensitisers and listed in Annex 1 to the 'Dangerous Substances' Directive (EC. 1992) may have a different default value. In such cases the listed default value must take precedence over the 1% value].

If the preparation contains one such substance, but at a level of between 0.1% and less than 1%, then the preparation does not formally classify as a sensitiser and will not have the symbol, index and risk phrase described above. However, the following phrase must be placed on the label; "Contains (name of substance). May produce an allergic reaction".

As in the case of chemical substances, the classification and labelling of preparations takes no account of the potency of the sensitising ingredient and thus will not distinguish one preparation from another with regard to the potential risk such preparations may pose to users. For cosmetic products in particular, that are not subject to classification and labelling as 'dangerous preparations', lack of potency data will reduce the effectiveness of suitable personal risk estimation and management.

The SDS and the product label inform users of the hazards presented by the substance or preparation. With this information, appropriate actions can be taken to safeguard health and safety. Clearly, the present regulatory system does not address differences in potency, and so cannot provide this additional information. However, the European Chemicals Bureau (ECB), Classification and Labelling Working Party, is able to review on a case by case basis, all relevant data for any toxicological endpoint. If this review warrants a change to the classification and labelling default value then this can be effected via the European Commission. In the case of sensitisation, data on potency is likely to be part of this review in the future.

Determination of potency can therefore contribute to the protection of workers and consumers by defining lower (and in some cases higher) default values for classification and labelling of preparations.

3.2.1. Proposals

On the basis of a robust determination of skin sensitising potency, it is recommended that chemical allergens are separated into 4 categories. Applying this categorisation to the default value principle for classification of preparations, the following revised values are proposed for classification.

Table 7: Default values as threshold concentrations of ingredients requiring classification of preparations as sensitisers

Potency category	Default value [%]
Extreme	0.003
Strong	0.1
Moderate	1.0
Weak	3.0

These limits were selected following extensive and detailed deliberations by the Task Force of all possible options for definition of default values for threshold concentrations of ingredients for the purposes of classification of preparations. The decisions reached were based on the collective scientific judgement of Task Force members, and recognition of a number of key considerations, including the following:

- The limits identified are not based on consideration of particular substances or preparations, but rather represent a distillation of current knowledge and experience.
- The most potent allergens (categorised here as 'extreme'), and of which there are relatively few, are known to induce skin sensitisation in humans at relatively low exposure concentrations. The judgement was that a default value of 0.003% is appropriate for this group.
- A second group of allergens (categorised here as 'strong') were considered to be of sufficient potency that the current default value of 1% is inadequate for effective risk management. It was decided, therefore, that a more conservative default value of 0.1% should be used for this category.

- The current default value of 1.0% is retained for skin sensitisers categorised here as 'moderate'. Many skin sensitisers fall into this category and retention of this default value is considered appropriate.
- It was recognised that some skin sensitisers are of such low potency (categorised here as 'weak') that, even under conditions of extensive exposure, the development of allergic contact dermatitis is rare. However, it was considered inappropriate, and insufficiently conservative, to propose a 10-fold higher default value of 10%. The judgement was, therefore, to continue with the geometric progression and to recommend a default value of 3%.

Using the potency categories identified above in Table 7, the implications for classification of preparations are summarised below in Table 8. In this scheme, preparations containing levels of skin sensitisers below the threshold concentration will not be classified.

Table 8: Implications of the proposed categorisation of skin sensitisation potency on the classification of preparations

Potency category	Threshold concentration	Classification labelling*
Extreme	≥ 0.003 % (≥ 30 ppm)	Symbol Xi, Risk Phrase R43
Strong	≥ 0.1 % (≥ 1000 ppm)	Symbol Xi, Risk Phrase R43
Moderate	≥ 1 % (≥ 10,000 ppm)	Symbol Xi, Risk Phrase R43
Weak	≥ 3% (≥ 30,000 ppm)	Symbol Xi, Risk Phrase R43

* Symbol Xi = St Andrews Cross and descriptive word "Irritant"; Risk Phrase R43, "May cause sensitisation by skin contact"

4. ELICITATION OF ALLERGIC CONTACT DERMATITIS

Dose-response relationships clearly exist for the elicitation phase of allergic contact dermatitis and it is possible to determine a threshold level of exposure below which a reaction will fail to be provoked in a previously sensitised subject. However, the standard and accepted animal models used for the assessment of skin sensitisation, and for which OECD guidelines are available, are of limited utility in providing information on thresholds for elicitation, or for establishing thresholds in humans. As a consequence, the preferred approach is the conduct of studies in humans under clinical supervision. Thresholds for elicitation of allergic contact dermatitis have been established using the two-day diagnostic patch test, as well as with the repeated open application test (ROAT) that in some circumstances provides for a more realistic exposure scenario. Use tests conducted with products that are known to contain a contact allergen can also be employed to identify or confirm the conditions of exposure under which reactions will not be elicited in previously sensitised subjects. Elicitation threshold studies have been conducted for some contact allergens, including formaldehyde (Flyvholm *et al*, 1997), methyl/methylchloromethylisothiazolinone (Pasche and Hunziker, 1989), nickel (Menne and Calvin, 1993) and isoeugenol (Johansen *et al*, 1996). Thresholds for the elicitation of allergic contact dermatitis vary between individuals sensitised to a particular antigen (Flyvholm *et al*, 1997), partly at least due to differences in the extent to which sensitisation has been acquired (Friedmann, 1996). As indicated earlier in the report (Section 1), the most effective strategy to control the elicitation of allergic contact dermatitis, is to prevent in the first place the induction of skin sensitisation.

5. CONCLUSIONS AND RECOMMENDATIONS

1. Potency in the context of allergic contact dermatitis is best defined as the amount of chemical required for the acquisition of skin sensitisation in a previously naïve individual (induction phase), or the amount of chemical necessary to elicit a clinically discernible cutaneous reaction in a previously sensitised subject (elicitation phase).
2. Chemicals differ substantially with regard to the potency with which they are able to induce sensitisation. Chemicals differ also in terms of their ability to provoke elicitation reactions in previously sensitised subjects, although this is determined at least in part by the extent to which the subject is sensitised.
3. It is possible to discern thresholds for both the induction and elicitation phases of allergic contact dermatitis.
4. In considering relative 'potency' it is important to distinguish this from relative 'prevalence'; the former is an intrinsic property of the chemical, the latter being dependent upon both the activity of the chemical and the conditions of exposure.
5. In the context of classification and labelling, it is the consideration of potency with regard to the induction phase of skin sensitisation that is of greatest importance; the emphasis being on the need to prevent the initial acquisition of skin sensitisation.
6. It is possible to derive information of value in establishing estimates of relative skin sensitisation potency (i.e. potency at the induction phase) from standard and accepted test methods for which there are available OECD guidelines (the local lymph node assay [LLNA], the guinea pig maximisation test and the Buehler occluded patch test).
7. Data available from properly conducted guinea pig tests can, if interpreted judiciously, provide information of value in assessment of relative skin sensitisation potency. However, the LLNA is the method recommended for new assessments of relative skin sensitisation potency (and also for investigation of the influence of vehicle or formulation on skin sensitisation potency).
8. For each of the three standard test methods considered in detail here, it has been possible to recommend paradigms for the categorisation of chemical allergens with respect to their relative skin sensitisation potential. In each instance the categories are identified by the following descriptors: 'extreme', 'strong', 'moderate' and 'weak'.
9. For each of these test methods and their proposed categorisation schemes examples are provided using known contact allergens.
10. Recommendations are made regarding default values for preparations. Based on the application of the categorisation schemes mentioned above, default values have been proposed for classification of preparations as sensitisers.

The conclusion drawn is that it is now possible and appropriate to classify chemicals and preparations on the basis of their relative skin sensitisation potency. A robust scheme for implementation of classification according to potency is proposed that is consistent with the current state-of-the-art. In the future, as our understanding of allergenic potency increases further, it may be possible to make a case for further refinements.

BIBLIOGRAPHY

Andersen KE, Volund A, Frankild S. 1995. The guinea pig maximization test with a multiple dose design. *Acta Derm Venereol* 75: 463-469.

Basketter DA, Blaikie L, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, Harvey P, Evans P, White IR, Rycroft RJG. 2000. Use of the local lymph node assay for estimation of relative contact allergenic potency. *Contact Derm* 42: 344-348.

Basketter DA, Dearman RJ, Hilton J, Kimber I. 1997. Dinitrohalobenzenes: evaluation of relative skin sensitization potential using the local lymph node assay. *Contact Derm* 36: 97-100.

Basketter DA, Evans P, Fielder RJ, Gerberick GF, Dearman RJ, Kimber I. 2002. Local lymph node assay - validation and use in practice. *Fd Chem Toxic* 40: 593-598.

Basketter DA, Gerberick GF. 1996. An interlaboratory evaluation of the Buehler test for the identification and classification of skin sensitisers. *Contact Derm* 35: 146-151.

Basketter DA, Gerberick GF, Kimber I. 2001a. Skin sensitization, vehicle effects and the local lymph node assay. *Fd Chem Toxic* 39: 621-627.

Basketter DA, Lea LJ, Dickens A, Briggs D, Pate I, Dearman RJ, Kimber I. 1999a. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *J Appl Toxic* 19: 261-266.

Basketter DA, Rodford R, Kimber I, Smith I, Wahlberg JE. 1999b. Skin sensitization risk assessment: a comparative evaluation of 3 isothiozolinone biocides. *Contact Derm* 40: 150-154.

Basketter DA, Wright ZM, Warbrick EV, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, White IR. 2001b. Human potency predictions for aldehydes using the local lymph node assay. *Contact Derm* 45: 89-94.

Buehler EV. 1965. Delayed contact hypersensitivity in the guinea pig. *Arch Dermatol* 91: 171-177.

Cronin MTD, Basketter DA. 1994. Multivariate QSAR analysis of a skin sensitization database. *SAR and QSAR in Environmental Research* 2: 159-179.

Cumberbatch M, Scott RC, Basketter DA, Scholes EW, Hilton J, Dearman RJ, Kimber I. 1993. Influence of sodium lauryl sulphate on 2,4-dinitrochlorobenzene induced lymph node activation. *Toxicology* 33: 24-30.

Dearman RJ, Basketter DA, Kimber I. 1999. Local lymph node assay - use in hazard and risk assessment. *J Appl Toxic* 19: 299-306.

Dearman RJ, Cumberbatch M, Hilton J, Clowes HM, Fielding I, Heylings JR, Kimber I. 1996. Influence of dibutylphthalate on dermal sensitization to fluorescein isothiocyanate. *Fundam Appl Toxic* 33: 24-30.

Dearman RJ, Hilton J, Evans P, Harvey P, Basketter DA, Kimber I. 1998. Temporal stability of local lymph node assay responses to hexyl cinnamic aldehyde. *J Appl Toxic* 18: 281-284.

ECETOC. 2000. Skin sensitisation testing for the purpose of hazard identification and risk assessment. Monograph No. 29, Brussels.

EC. 1992. Council Directive 92/32/EEC of 30th April 1992 amending for the seventh time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *OJ Eur Comm* L 154 (05.06.92), 1-29.

EC. 1999. Directive 1999/45/EC of the European Parliament and of the Council of 31st May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparation. *OJ Eur Comm* L 200, (30.07.99), 1-68.

Flyvholm MA, Hall BM, Agner T, Tiedemann E, Greenhill P, Vanderveken W, Freeberg FE, Menné T. 1997. Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients. *Contact Derm* 36:26-33

Friedmann PS. 1996. Clinical aspects of allergic contact dermatitis. In: Kimber I and Maurer T, eds. *Toxicology of Contact Hypersensitivity*. Taylor & Francis, London, 26-56.

Gerberick GF, Cruse LW, Ryan CA, Hulette BC, Chaney JG, Skinner RA, Dearman RJ, Kimber I. 2002. Use of a B cell marker (B220) to discriminate between allergens and irritants in the local lymph node assay. *Toxicol Sci* 68: 420-428.

Gerberick GF, Robinson MK, Ryan CA, Dearman RJ, Kimber I, Basketter DA, Wright Z, Marks JG. 2001. Contact allergenic potency: correlation of human and local lymph node assay data. *Am J Contact Derm* 12: 156-161.

Gerberick GF, Ryan CA, Kimber I, Dearman RJ, Lea LJ, Basketter DA. 2000. Local lymph node assay: validation assessment for regulatory purposes. *Am J Contact Derm* 11: 3-18.

Heylings JR, Clowes HM, Cumberbatch M, Dearman RJ, Fielding I, Hilton J, Kimber I. 1996. Sensitization to 2,4-dinitrochlorobenzene: influence of vehicle on absorption and lymph node activation. *Toxicology* 109: 57-65.

Hilton J, Dearman RJ, Harvey P, Evans P, Basketter DA, Kimber I. 1998. Estimation of relative skin sensitizing potency using the local lymph node assay: a comparison of formaldehyde with glutaraldehyde. *Am J Contact Derm* 9: 29-33.

Johansen JD, Andersen KE , Menné T. 1996. Quantitative aspects of isoeugenol contact allergy assessed by use and patch test. *Contact Derm* 34: 414-418.

Kimber I, Basketter DA. 1992. The murine local lymph node assay: a commentary on collaborative trials and new directions. *Fd Chem Toxic* 30: 165-169.

Kimber I, Basketter DA. 1997. Contact sensitization: a new approach to risk assessment. *Human Ecol Risk Assess* 3: 385-395.

Kimber I, Basketter DA, Berthold K, Butler M, Garrigue J-L, Lea L, Newsome C, Roggeband R, Steiling W, Stropp G, Waterman S, Wiemann C. 2001. Skin sensitization testing in potency and risk assessment. *Toxicol Sci* 59: 198-208.

Kimber I, Dearman RJ. 1991. Investigation of lymph node cell proliferation as a possible immunological correlate of contact sensitising potential. *Fd Chem Toxic* 29: 125-129.

Kimber I, Dearman RJ, Basketter DA, Ryan CA, Gerberick GF. 2002. The local lymph node assay: past, present and future. *Contact Derm* 47: 315-328.

Kimber I, Dearman RJ, Scholes EW, Basketter DA. 1994. The local lymph node assay: developments and applications. *Toxicology* 93: 13-31.

Kimber I, Gerberick GF, Basketter DA. 1999. Thresholds in contact sensitization: theoretical and practical considerations. *Fd Chem Toxic* 37: 553-560.

Kimber I, Hilton J, Dearman RJ, Gerberick GF, Ryan CA, Basketter DA, Scholes EW, Loveless SE, Ladics GS, House RV, Guy A. 1995. An international evaluation of the murine local lymph node assay and comparison of modified procedures. *Toxicology* 103: 63-73.

Klecak G. 1985. The Freund's complete adjuvant test. In: Current Problems in Dermatology, Volume 14. Contact Allergy Predictive Tests in Guinea Pigs. Eds: Andersen KE, Maibach HI, Karger, Basel, pp 152-171.

Magnusson B, Kligman AM. 1970. Allergic contact dermatitis in the guinea pig. Identification of contact allergens. Charles C. Thomas, Springfield IL.

Menne T, Calvin G. 1993. Concentration threshold of non-occluded nickel exposure in nickel-sensitive individuals and controls with and without surfactant. Contact Dermatitis 29: 180-184.

OECD. 1992. Guidelines for Testing of Chemicals. No. 406. Skin sensitisation.

OECD. 2002. Guidelines for Testing of Chemicals. No. 429. Skin sensitisation: local lymph node assay.

Pasche F, Hunziker N. 1989. Sensitization to Kathon CG in Geneva and Switzerland. *Contact Derm* 20: 115-119.

Robinson MK, Nusair TL, Fletcher ER, Ritz HL. 1990. A review of the Buehler guinea pig skin sensitization test and its use in a risk assessment process for human skin sensitization. *Fundam Appl Toxicol* 17: 103-119.

Ryan CA, Cruse LW, Skinner RA, Dearman RJ, Kimber I, Gerberick GF. 2002. Examination of a vehicle for use with water soluble materials in the murine local lymph node assay. *Fd Chem Toxic* 40: 1719-1725.

Schlede E, Eppler R. 1995 Testing for skin sensitisation according to the notification procedure for new chemicals. The Magnusson and Kligman test. *Contact Derm* 32: 1-4.

Scott AE, Kashon ML, Yucesoy B, Luster MI, Tinkle SS. 2002. Insights into the quantitative relationship between sensitization and challenge for allergic contact dermatitis reactions. *Toxicol Appl Pharmacol* 183: 66-70.

Steiling W, Basketter D, Berthold K, Butler M, Garrigue JL, Kimber I, Lea L, Newsome C, Roggeband R, Stropp G, Waterman S, Wiemann C. 2001. Skin sensitisation testing - new perspectives and recommendations. *Fd Chem Toxic* 39: 293-301.

Van Och FMM, Slob W, de Jong WH, Vandebriel RJ, van Loveren H. 2000. A quantitative method for assessing the sensitizing potency of low molecular weight chemicals using a local lymph node assay: employment of a regression method that includes determination of the uncertainty margins. *Toxicology* 146: 49-59.

Vohr H-W, Blümel J, Blotz A, Homey B, Ahr HJ. 2000. An intra-laboratory validation of IMDS: discrimination between (photo)allergic and (photo)irritant skin reactions in mice. *Arch Toxicol* 73: 501-509.

Wahlberg JE, Boman A. 1985. A guinea pig maximisation test. In Andersen KE and Maibach HI, eds, *Current Problems in Dermatology, Volume 14. Contact Allergy Predictive Tests in Guinea Pigs*, Karger, Basel, 59-106.

MEMBERS OF THE TASK FORCE

I. Kimber	Syngenta UK – Maccesfield
D. Basketter	Unilever UK – Sharnbrook
A. Gamer	BASF D – Ludwigshafen
J-L. Garrigue	L'Oréal F – Aulnay-sous-Bois
G.F. Gerberick	Procter & Gamble USA – Cincinnati
C. Newsome	Dow Chemical UK – West Drayton
W. Steiling	Henkel D – Düsseldorf
H-W. Vohr	Bayer D – Wuppertal
M. Butler (Secretary)	ECETOC B – Brussels

MEMBERS OF THE SCIENTIFIC COMMITTEE

(Peer Review Committee)

B. Hildebrand (Chairman)	Consultant D – Weinheim
N. Carmichael (Vice-chairman) Head, Toxicology	Bayer CropScience F – Sophia Antipolis
G. Randall (Vice-chairman) Director, Environmental Laboratory	AstraZeneca UK – Brixham
C. d'Hondt Head, Environmental Safety Department	Syngenta CH – Basel
E. Bomhard[a] Industrial Toxicology	Bayer D – Wuppertal
C. Braun Occupational Toxicologist	Akzo Nobel NL – Arnhem
P. Calow Professor of Zoology	University of Sheffield UK – Sheffield
P. Douben Head, SEAC Environmental Protection Department	Unilever UK – Bebington
T. Feijtel Manager, Professional and Regulatory Services	Procter & Gamble B – Brussels
H. Greim Director, Institute of Toxicology and Environmental Hygiene	Technical University Munich D – Munich
C. Money Industrial Hygiene Adviser, Europe	ExxonMobil B – Brussels
A. Sarrif Director, Toxicology Affairs, Europe	DuPont B – Brussels
G. Swaen Head, Occupational Epidemiology Unit	Maastricht University NL – Maastricht
B. van Ravenzwaay[a] Director, Experimental Toxicology and Ecology	BASF D – Ludwigshafen
H-J. Wiegand Head, Product Safety Department	Degussa D – Düsseldorf

[a]Responsible for primary peer review

ECETOC PUBLISHED REPORTS

Monographs

No.	Title
No. 1	Good Laboratory Practice
No. 2	A Contribution to Strategy for Identification and Control of Occupational Carcinogens
No. 3	Risk Assessment of Occupational Chemical Carcinogens
No. 4	Hepatocarcinogenesis in Laboratory Rodents: Relevance for Man
No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology)
No. 6	Acute Toxicity Tests, LD_{50} (LC50) Determinations and Alternatives
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment (Summary)
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man
No. 11	Eye Irritation Testing
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity)
No. 13	DNA and Protein Adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment
No. 14	Skin Sensitisation Testing
No. 15	Skin Irritation
No. 16	Early Indicators of Non-Genotoxic Carcinogenesis
No. 17	Hepatic Peroxisome Proliferation
No. 18	Evaluation of the Neurotoxic Potential of Chemicals
No. 19	Respiratory Allergy
No. 20	Percutaneous Absorption
No. 21	Immunotoxicity: Hazard Identification and Risk Characterisation
No. 22	Evaluation of Chemicals for Oculotoxicity
No. 23	Receptor Mediated Mechanisms in Chemical Carcinogenesis
No. 24	Risk Assessment for Carcinogens
No. 25	Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents
No. 26	Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances
No. 27	Aneuploidy
No. 28	Threshold-Mediated Mutagens - Mutation Research Special Issue
No. 29	Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment
No. 30	Genetic Susceptibility to Environmental Toxicants
No. 31	Guidance on Evaluation of Reproductive Toxicity Data
No. 32	Use of Human Data in Hazard Classification for Irritation and Sensitisation

Technical Reports

No.	Title
No. 1	Assessment of Data on the Effects of Formaldehyde on Humans
No. 2	The Mutagenic and Carcinogenic Potential of Formaldehyde
No. 3	Assessment of Test Methods for Photodegradation of Chemicals in the Environment
No. 4	The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man
No. 5	Toxicity of Ethylene Oxide and its Relevance to Man
No. 6	Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2
No. 7	Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere
No. 8	Biodegradation Testing: An Assessment of the Present Status
No. 9	Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients
No. 10	Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits
No. 11	Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5
No. 12	The Phototransformation of Chemicals in Water: Results of a Ring-Test
No. 13	The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment
No. 14	The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health
No. 15	The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values
No. 16	A Review of Recent Literature on the Toxicology of Benzene
No. 17	The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4
No. 18	Harmonisation of Ready Biodegradability Tests
No. 19	An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment
No. 20	Biodegradation Tests for Poorly-Soluble Compounds
No. 21	Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment
No. 22	Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity
No. 23	Evaluation of the Toxicity of Substances to be Assessed for Biodegradability
No. 24	The EEC 6th Amendment: Prolonged Fish Toxicity Tests
No. 25	Evaluation of Fish Tainting
No. 26	The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride
No. 27	Nitrate and Drinking Water
No. 28	Evaluation of Anaerobic Biodegradation
No. 29	Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns
No. 30	Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available)
No. 31	The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment
No. 32	Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data
No. 33	Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis
No. 34	Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man
No. 35	Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments
No. 36	Biomonitoring of Industrial Effluents
No. 37	Tetrachlorethylene: Assessment of Human Carcinogenic Hazard
No. 38	A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens
No. 39	Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea
No. 40	Hazard Assessment of Chemical Contaminants in Soil

No. 41 Human Exposure to N-Nitrosamines, their Effects and a Risk Assessment for N-Nitrosodiethanolamine in Personal Care Products
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products
No. 43 Emergency Exposure Indices for Industrial Chemicals
No. 44 Biodegradation Kinetics
No. 45 Nickel, Cobalt and Chromium in Consumoducts: Allergic Contact Dermatitis
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals
No. 47 EC 7th Amendment "Toxic to Reproduction": Guidance on Classification
No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition)
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration
No. 50 Estimating Environmental Concentrations of Chemicals using Fate and Exposure Models
No. 51 Environmental Hazard Assessment of Substances
No. 52 Styrene Toxicology Investigation on the Potential for Carcinogenicity
No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment (CAS No. 61789-80-8)
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment
No. 55 Pulmonary Toxicity of Polyalkylene Glycols
No. 56 Aquatic Toxicity Data Evaluation
No. 57 Polypropylene Production and Colorectal Cancer
No. 58 Assessment of Non-Occupational Exposure to Chemicals
No. 59 Testing for Worker Protection
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard
No. 61 Environmental Exposure Assessment
No. 62 Ammonia Emissions to Air in Western Europe
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings
No. 64 The Toxicology of Glycol Ethers and its Relevance to Man
No. 65 Formaldehyde and Human Cancer Risks
No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank
No. 67 The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs
No. 68 Assessment Factors in Human Health Risk Assessment
No. 69 Toxicology of Man-Made Organic Fibres
No. 70 Chronic Neurotoxicity of Solvents
No. 71 Inventory of Critical Reviews on Chemicals (Only available to ECETOC members)
No. 72 Methyl tert-Butyl Ether (MTBE) Health Risk Characterisation
No. 73 The Value of Aquatic Model Ecosystem Studies in Ecotoxicology
No. 74 QSARs in the Assessment of the Environmental Fate and Effects of Chemicals
No. 75 Organophosphorus Pesticides and Long-term Effects on the Nervous System
No. 76 Monitoring and Modelling of Industrial Organic Chemicals, with Particular Reference to Aquatic Risk Assessment
No. 77 Skin and Respiratory Sensitisers: Reference Chemicals Data Bank
No. 78 Skin Sensitisation Testing: Methodological Considerations
No. 79 Exposure Factors Sourcebook for European Populations (with Focus on UK Data)
No. 80 Aquatic Toxicity of Mixtures
No. 81 Human Acute Intoxication from Monochloroacetic Acid: Proposals for Therapy
No. 82 Risk Assessment in Marine Environments
No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union
No. 84 Scientific Principles for Soil Hazard Assessment of Substances
No. 85 Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies
No. 86 Derivation of Assessment Factors for Human Health Risk Assessment

Joint Assessment of Commodity Chemicals (JACC) Reports

No.	Title
No. 1	Melamine
No. 2	1,4-Dioxane
No. 3	Methyl Ethyl Ketone
No. 4	Methylene Chloride
No. 5	Vinylidene Chloride
No. 6	Xylenes
No. 7	Ethylbenzene
No. 8	Methyl Isobutyl Ketone
No. 9	Chlorodifluoromethane
No. 10	Isophorone
No. 11	1,2-Dichloro-1,1-Difluoroethane (HFA-132b)
No. 12	1-Chloro-1,2,2,2-Tetrafluoroethane (HFA-124)
No. 13	1,1-Dichloro-2,2,2-Trifluoroethane (HFA-123)
No. 14	1-Chloro-2,2,2-Trifluoromethane (HFA-133a)
No. 15	1-Fluoro 1,1-Dichloroethane (HFA-141B)
No. 16	Dichlorofluoromethane (HCFC-21)
No. 17	1-Chloro-1,1-Difluoroethane (HFA-142b)
No. 18	Vinyl Acetate
No. 19	Dicyclopentadiene (CAS: 77-73-6)
No. 20	Tris-/Bis-/Mono-(2 ethylhexyl) Phosphate
No. 21	Tris-(2-Butoxyethyl)-Phosphate (CAS:78-51-3)
No. 22	Hydrogen Peroxide (CAS: 7722-84-1)
No. 23	Polycarboxylate Polymers as Used in Detergents
No. 24	Pentafluoroethane (HFC-125) (CAS: 354-33-6)
No. 25	1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0)
No. 26	Linear Polydimethylsiloxanes (CAS No. 63148-62-9)
No. 27	n-Butyl Acrylate (CAS No. 141-32-2)
No. 28	Ethyl Acrylate (CAS No. 140-88-5)
No. 29	1,1-Dichloro-1-Fluoroethane (HCFC-141b) (CAS No. 1717-00-6)
No. 30	Methyl Methacrylate (CAS No. 80-62-6)
No. 31	1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2)
No. 32	Difluoromethane (HFC-32) (CAS No. 75-10-5)
No. 33	1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) (CAS No. 306-83-2)
No. 34	Acrylic Acid (CAS No. 79-10-7)
No. 35	Methacrylic Acid (CAS No. 79-41-4)
No. 36	n-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9)
No. 37	Methyl Acrylate (CAS No. 96-33-3)
No. 38	Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3)
No. 39	Tetrachloroethylene (CAS No. 127-18-4)
No. 40	Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions

Special Reports

No.	Title
No. 8	HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances
No. 9	Styrene Criteria Document
No. 10	Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1)
No. 11	Ecotoxicology of some Inorganic Borates
No. 12	1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0)
No. 13	Occupational Exposure Limits for Hydrocarbon Solvents
No. 14	n-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document
No. 15	Examination of a Proposed Skin Notation Strategy
No. 16	GREAT-ER User Manual

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development
No. 33	Environmental Oestrogens: A Compendium of Test Methods
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals
No. 37	EC Classification of Eye Irritancy
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach
No. 40	Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene
No. 41	Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1
No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction

EUROPEAN COMMISSION
DIRECTORATE GENERAL - JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Unit: Toxicology and Chemical Substances
European Chemicals Bureau

ECBI/81/02 Rev. 3

Ispra, 26 May 2003

Report from the Expert Working Group on Sensitisation
Ispra 4-6 November 2002

The Group was established on the advice of the EU Commission Working Group on Classification and Labelling (Health effects), the CMR Group, and their activities are coordinated by the ECB.

The purpose of establishing this Group is to examine whether or not it is possible to set concentration limits for individual substances for induction and/or elicitation of sensitisation in humans, in context of Directive 67/548/EEC and 1999/45/EC. The Group has adressed questions formulated by the CMR working group.

The Group agreed to focus solely on skin sensitisation.

The first meeting of the Group was 18-19 April 2002 in Ispra (ECBI/13/02 add. 1 rev 2)

A list of nominated participating experts is attached.

Questions forwarded to the Expert Group from the EU Commission Working Group on Classification and Labelling, the CMR Group:

1. Propose how to use the existing methods to grade allergen potency, providing detailed guidance for current predictive test methods (or small modifications thereof).

2. Suggest ways in which the grading may be translated into practical limits for both induction and elicitation.

3. Describe in detail the best approach to the assessment of elicitation thresholds in sensitised human volunteers.

The design of the Local Lymph Node Assay (LLNA) makes it better suited than the guideline guinea pig assays to the assignment of skin sensitisers into specific potency categories. This is because the LLNA focuses on induction of sensitisation only, incorporates a dose response assessment, and has an objective and quantitative endpoint.

EC3 values derived from LLNA dose responses give the amount of chemical sensitiser that is required to elicit a three-fold increase in lymph node cell proliferative activity [1-3]. For this

purpose the amount of sensitiser should be expressed as a percentage (v/w) value. Based on the above, categorisation of contact allergens can be achieved as shown in Table 1.

TABLE 1. Potency categorisation based on LLNA.

Category	**EC3 value [%]**
Extreme	= 0.2
Strong	> 0.2 - = 2
Moderate	>2

When EC3 values are available from more than one study, the lowest value should normally be used. Where an EC3 value is close to the borderline between 2 categories, careful consideration should be given regarding the assignment into a category, including the sources of uncertainty in the data set [4, 5].

Guideline Guinea Pig Maximisation Tests (GPMT) employ a single induction dose regime and therefore the possibilities for potency evaluation are limited. However, the Expert Group considered that a ranking of allergenic potency could sometimes be derived, see Table 2. The Expert Group acknowledged that categorisation would be associated with a large degree of uncertainty except in the case of substances categorised as extreme sensitisers or where the incidence of sensitisation was in the interval 30-60% and the intradermal induction concentration at the same time was greater than 1% (see Table 2). Data from dose response studies would reduce the level of uncertainty [6 -8].

TABLE 2. Potency categorisation based on GPMT.

Intradermal concentration employed during induction phase [%]*	**Incidence of sensitisation 30% - < 60%**	**Incidence of sensitisation = 60%**
= 0.1	Strong	Extreme
> 0.1 - = 1	Moderate	Strong
> 1	Moderate	Moderate

*according to guideline intradermal induction concentration must be the highest concentration causing mild to moderate irritation

The Guideline Buehler test, which is less sensitive than the GPMT, also uses a single induction dose regime and the possibilities for potency evaluations are therefore also limited. In common with the guideline Guinea pig maximisation test the Expert Group considered that a ranking of allergenic potency could sometimes be derived, see Table 3. The Expert Group acknowledged that, as with the GPMT, categorisation would be associated with a large degree of uncertainty except in the case of substances categorised as extreme sensitisers or where the incidence of sensitisation was in the lower group and topical induction concentration was greater than 20 %. Data from dose response studies would reduce the level of uncertainty [7, 9].

TABLE 3. Potency categorisation based on Buehler.

Concentration employed during induction phase [%]*	**Incidence of sensitisation 15% - < 60%**	**Incidence of sensitisation = 60%**
= 0.2	Strong	Extreme
> 0.2 - = 20	Moderate	Strong
> 20	Moderate	Moderate

*according to guideline topical induction concentration must be the highest concentration causing mild but not excessive irritation

It should be noted that where multiple animal data sets lead to different categorisation of the same substance the higher potency category should apply. Human data (clinical, experimental and/or epidemiological) may indicate the need to change the potency categorisation derived from animal experiments. The Expert Group considered that this should normally only be used to a re-categorisation into a higher potency category.

Elicitation thresholds correlate only poorly with induction potency [5]. Variation in elicitation thresholds between individuals is very large and depends on numerous factors of which the sensitising potency of the substance is only one. Other factors affecting elicitation include the duration, extent and site of exposure, status of the skin and degree of specific sensitisation. For this reason, the Expert Group considered that it would be inappropriate to define elicitation thresholds as a function of skin sensitising potency. The Expert Group concludes that the most practical recommendation is that skin sensitisers are listed on the label when they are present at a concentration of 10 ppm or above, without the additional wording used in the current Preparations Directive ('Contains xxx: May cause an allergic reaction.'). This recommendation should allow the large majority of diagnosed sensitised subjects to avoid exposure to the allergen in question in most circumstances. Listing on the label all skin sensitisers present in a preparation at any concentration would lead to analytical difficulties and would result also in information overload for the user. However, the Expert Group recommends also that *extreme* skin sensitisers should be listed on the label when present in a preparation at a concentration of 1 ppm or greater.

To be fully consistent with the definition of elicitation threshold adopted by the Expert Group at the meeting held in April 2002: 'the threshold for elicitation can be defined as the highest level of exposure that fails to elicit an allergic reaction in a previously sensitised subject', all skin sensitisers at 1 ppm or above would need to be listed on the label. However, for the reasons mentioned above this approach is impractical and therefore not recommended.

For proper implementation of the above recommendation it will be necessary to develop a uniform nomenclature for naming the substances to be listed on the label, for example common names used in clinical testing or International Nomenclature of Cosmetic Ingredients (INCI), when available. Until then it is recommended that common names are used.

Conclusion:

The Expert Group agreed that for induction of skin sensitisation, it would be reasonable to assign chemicals into one of 3 different categories according to potency. The majority of skin sensitising chemicals would then fall into the category corresponding to the current default concentration value of 1% for labelling of preparations with R43. An additional 2 categories should be defined for substances with higher potency; these identify strong (>0.1%) and extreme (>0.001%) sensitisers, respectively. With regard to preparations, moderate and strong skin sensitisers should be listed on the label when present in a concentration of 10 ppm or greater, and extreme skin sensitisers when in a concentration of 1 ppm or greater.

Question forwarded to the Expert Group from the CMR Group:

4. For all of the above, provide a couple of worked examples using well-known human skin sensitisers.

The Expert Group summarised in Table 4 examples of chemicals categorised according to the methods.

TABLE 4. Examples of some substances categorised due to their potency as derived from the different methods discussed and human experience.

Substance	LLNA		GPMT		Buehler		Human
	EC3 value (%)[1]	Category	Ind/incidence[2]	Category	Ind/incidence[3]	Category	Category[4]
(Chloro)methylisothiazolone	0.05	Extreme	0.0001/100	Extreme	0.2/100	Extreme	Extreme
p-Phenylenediamine	0.06	Extreme	0.25/100	Strong	10/90	Strong	Extreme
2,4-Dinitrochlorobenzene	0.08	Extreme	0.05/100	Extreme	0.1/100	Extreme	Extreme
Formaldehyde	0.4	Strong	0.5/90	Strong	2.0/30	Moderate	Strong
Isoeugenol	1.3	Strong	0.15/100	Strong	ND	ND	Strong
Cinnamal	2.0	Strong	0.2/100	Strong	10/80	Strong	Strong
Methyldibromoglutaronitrile	2.0[5]	Strong	0.1/20[5]	Not classified	5/5[6]	Not classified	Strong[7]
Hexylcinnamal	8.0	Moderate	0.5/60	Strong	50/60	Moderate	Moderate
Eugenol	13	Moderate	0.05/60	Extreme	75/0	Not classified	Moderate
Ethyleneglycoldimethacrylate	35	Moderate	5%/0	Negative	ND	ND	Moderate
2-Mercaptobenzothiazole	9.7	Moderate	1.0/40	Moderate	75/55	Moderate	Moderate

[1] Estimated concentration to cause a 3-fold stimulation – data taken from reference [10].
[2] Intradermal induction concentration [%]/incidence of sensitisation [%]; data taken from references [11, 12].
[3] Topical induction concentration [%]/incidence of sensitisation [%]; data taken from reference [13].
[4] Based on a composite expert judgement encompassing all available information, including references [14, 15].
[5] Data taken from reference [16].
[6] Data currently awaiting publication.
[7] Reference [17] was used to generate this classification.

The data presented in Table 4 merits comment. For the 11 example chemicals chosen, it is evident that each of the standard methods, when interpreted according to the criteria for potency categorisation given above, works well in the majority of cases. Of note is the case of methyldibromoglutaronitrile for which the predictive assays (LLNA and GPMT) suggest

the categorisation should be strong. The judgement of the Expert Group is that the chemical is indeed a strong human skin sensitiser. This is based on the use at relatively low levels as a preservative (typically no more than 400ppm), which has been shown to result in an epidemic of allergic contact dermatitis (reviewed in [17]).

Question forwarded to the Expert Group from the CMR Group:

5. Are the animal test methods evaluated and designed for testing preparations?

No, they were not designed for testing preparations. Guinea pig and murine predictive tests were developed for the identification of chemical sensitisation hazard. In addition, it has been shown that these methods can provide information on the impact of the solvent/vehicle on sensitising potency, but the relevance of such data for human risk assessment has not been formally demonstrated. Further research would be needed to evaluate properly the utility of these methods for the safety assessment of preparations.

Question forwarded to the Expert Group from the CMR Group:

6. *How to interpret a negative test result from an animal sensitisation test performed on a preparation - in general - when the preparation contains a positive ingredient? (Can tests on preparations be used to set specific concentrations limits?).*

Based on the above, a negative test result from a sensitisation test on a preparation cannot be taken as a proof of absence of sensitisation capacity of the preparation.

References

1. Basketter DA, Lea L, Cooper K, Dickens A, Stocks J, Pate I, Dearman RJ and Kimber I (1999) Thresholds for classification as a skin sensitiser in the local lymph node assay: a statistical evaluation. Food and Chem Toxicol., 37; 1167-1174.

2. Basketter DA, Lea L, Cooper K, Dickens A, Briggs D, Pate I, Dearman RJ and Kimber I (1999) A comparison of statistical approaches to derivation of EC3 values from local lymph node assay dose responses. J Appl Toxicol, 19; 261-266.

3. Kimber I and Basketter DA. (1995) Contact senitization: a new approach to risk assessment. Human and Ecological Risk Assessment 3; 385-395.

4. Van Och FMM, Slob W, De Jong WH, Vandebriel RJ, Van Loveren H. (2000) A Quantitative method for assessing the sensitizing potency of low molecular weight chemicals using a local lymph node assay: employment of a regression method that includes determinations of uncertainty margins. Toxicol, 146; 49-59.

5. Scott AE, Kashon ML, Yucesoy B, Luster MI, Tinkle SS. (2002) Insight into quantitative relationships between sensitisation and challenge for allergic contact dermatitis reactions. Toxicol. Appl. Pharmacol., 183; 66-70.

6. Andersen KE, Vølund Aa, Frankild S. (1995) The guinea pig maximization test – with a multiple dose design. Acta Derm Venereol, 75; 463-469.

7. Frankild S, Vølund A, Wahlberg JE, Andersen KE. (2000) Comparison of the sensitivities of the Buehler test and the guinea pig maximization test for predictive testing of contact allergy. Acta Derm Venereol, 80; 256-262.

8. van Och FMM, Vandebriel RJ, Prinsen MK, de Jong WH, Slob W and van Loveren H. (2001) Comparison of dose-responses of contact allergens using the guinea pig maximisation test and the local lymph node assay. Toxicology, 167; 207-215.

9. Chan PD, Baldwin RC, Parson RD, Moss JN, Sterotelli R, Smith JM and Hayes AW. (1983) Kathon biocide: Manifestation of delayed contact dermatitis in guinea pigs is dependent on the concentration for induction and challenge. Journal of Investigative Dermatology, 81; 409-411.

10. Basketter DA and Kimber I (2001) Predictive testing in contact allergy: facts and future. Allergy 56; 937-943.

11. Wahlberg JE and Boman A (1985) Guinea pig maximisation test. In: Contact Allergy Predictive Test in Guinea Pigs. Eds KE Andersen and HI Maibach, Karger Basel; 59-106.

12. Basketter D. A., Scholes E. W. (1992) Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food and Chemical Toxicology, 30; 65-69.

13. Basketter DA and Gerberick GF (1996) Interlaboratory evaluation of the Buehler test. Contact Dermatitis, 35; 146-151.

14. Basketter DA, Blaikie L, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, Harvey P, Evans P, White IR and Rycroft RJG (2000) Use of the local lymph node assay for the estimation of relative contact allergenic potency. Contact Dermatitis, 42; 344-348.

15. Gerberick GF, Robinson MK, Ryan CA, Dearman RJ, Kimber I, Basketter DA, Wright Z and Marks JG. (2001) Contact allergenic potency: Correlation of human and local lymph node assay data. Am J Cont Derm, 12; 156-161.

16. Wahlquist H, Boman A, Montelius J, Wahlberg JE. (1999) Senitizing potential in mice, guinea pig and man of preservative Euxyl K400 and its ingridient methyldibromoglutaronitrile. . Contact Dermatitis, 41; 330-338.

17. Wilkinson JD, Shaw S, Andersen KE, Brandao FM, Bruynzeel DP, Bruze M, Camarasa JM, Diepgen TL, Ducombs G, Frosch PJ, Goossens A, Lachappelle JM, Lahti A, Menne T, Seidenari S, Tosti A, Wahlberg JE. (2002) Monitoring levels of preservative sensitivity in Europe. A 10-year overview (1991-2000). Contact Dermatitis, 46; 207-10.

Expert Working Group on Sensitisation nominated by the EU Commission Working Group on Classification and Labelling (Health Effects)

Klaus Andersen, Professor, MD, PhD
Odense University Hospital
Department of Dermatology
Odense
Denmark

David Basketter, Dr
Principal Scientist
Applied Science and Technology
Safety and Environmental Assurance Centre
Unilever Colworth Laboratory
UK

Anders Boman, Assoc. Professor, PhD
Department of Occupational and Environmental Dermatology
Stockholm County Council, Stockholm
Sweden

Jacques Descotes, Professor
C.H.R.U. Hopital E. Herriot
Lyon
France

Matti Hannuksela, Professor, PhD
South Karelia Central Hospital
Lappeenranta
Finland

Ian Kimber, Professor
Head of Research
Syngenta Central Toxicology Laboratory
Alderley Park
Macclesfield, Cheshire
UK

Carola Lidén, Professor, MD, PhD
Department of Occupational and Environmental Dermatolog
Stockholm County Council and Karolinska Institutet
Stockholm
Sweden

Torkil Menné, Professor, MD, PhD
Department of Dermatology
Gentofte Hospital
Hellerup
Denmark

Dr Henk Van Loveren, Professor
National Institute of Public Health and the Environment
Bilthoven
Netherlands

Participants at the Expert Working Group on Sensitisation meeting in Ispra, 4-6 Nov. 2002

Kristiina Alanko, MD, PhD, dermatologist
(replacing Matti Hannuksela)
Finnish Institute of Occupational Health
Helsiniki
Finland

Klaus Andersen, Professor, MD, PhD
Odense University Hospital
Department of Dermatology
Odense
Denmark

David Basketter, Dr
Principal Scientist
Applied Science and Technology
Safety and Environmental Assurance Centre
Unilever Colworth Laboratory
UK

Anders Boman, Assoc. Professor, PhD
Department of Occupational and
Environmental Dermatology
Stockholm County Council, Stockholm
Sweden

Ian Kimber, Professor
Head of Research
Syngenta Central Toxicology Laboratory
Alderley Park
Macclesfield, Cheshire
UK

Carola Lidén, Professor, MD, PhD
Department of Occupational and
Environmental Dermatology
Stockholm County Council and
Karolinska Institutet
Stockholm
Sweden

Dr Henk Van Loveren, Professor
National Institute of Public Health and the
Environment
Bilthoven
Netherlands

Sally Williams, MD
European Director - Health and Environment
Inco Europe
UK

ECB (Europe an Chemicals Bureau):
Elisabet Berggren
Ingrid Langezaal
Marita Luotamo