Contact Sensitisation:
Classification According to Potency

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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).

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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.

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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.

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- 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.

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