Potency Values from the Local Lymph Node Assay: Application to Classification, Labelling and Risk Assessment

Document No. 46

Brussels, December 2008

ECETOC DOCUMENT NO. 46

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

All rights reserved. No part of this publication may be reproduced, copied, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the copyright holder. Applications to reproduce, store, copy or translate should be made to the Secretary General. ECETOC welcomes such applications. Reference to the document, its title and summary may be copied or abstracted in data retrieval systems without subsequent reference.

The content of this document has been prepared and reviewed by experts on behalf of ECETOC with all possible care and from the available scientific information. It is provided for information only. ECETOC cannot accept any responsibility or liability and does not provide a warranty for any use or interpretation of the material contained in the publication.

Potency Values from the Local Lymph Node Assay: Application to Classification, Labelling and Risk Assessment

CONTENTS

EXECUTIVE SUMMARY

In this ECETOC Document, the use of potency values derived from local lymph node assay (LLNA) data has been considered to address the following terms of reference:

- Determine whether an EC3 potency value can be used as a cut-off criterion for the classification and labelling of both substances and preparations;
- Evaluate LLNA data in risk assessment approaches for skin sensitisation and, by taking into account potency considerations, provide a rationale for using concentration responses and corresponding no-effect concentrations.

The following recommendations have been made:

- Although the Task Force was of the view that skin sensitising chemicals having high EC3 values may represent only relatively low risks for human health, it is not possible currently to define an EC3 value below 100% that would serve as an appropriate threshold for classification and labelling of substances as R43;
- Reviews have been conducted of: 1) previous ECETOC Task Force recommendations for the use of four categories for characterising contact allergens and preparations as a function of skin sensitising potency, and 2) proposals for categorisation according to potency that have been made since then. The conclusion drawn from those analyses was that the most appropriate, science-based scheme for classification of contact allergens according to relative potency is one in which four sub-categories are identified. It was proposed that these categories should be termed ‘extreme’, ‘strong’, ‘moderate’ and ‘weak’ to reflect differing skin sensitisation potency based on derived EC3 values. The recommendations made by the previous Task Force have been endorsed (ECETOC, 2003 a,b);
- Quantitative risk assessment approaches describe the relationship between the calculated exposure to a sensitising chemical and the acceptable exposure level. Because proliferation of cells in draining lymph nodes is related causally and quantitatively to the extent to which skin sensitisation will be acquired (potency), LLNA EC3 values are well suited to, and recommended for, determination of a no expected sensitisation induction level (NESIL) that represents the first step in the quantitative risk assessment process.

These recommendations regarding the use of potency considerations derived from LLNA data effectively move the LLNA from the realm of hazard identification to a key component of the development of accurate risk assessments, which can be used as a sound scientific basis for classification and labelling.

1. INTRODUCTION

Skin sensitisation resulting in allergic contact dermatitis is an important occupational, environmental and consumer health issue. Many hundreds of chemicals have been implicated as contact allergens but there remains an important need to identify and characterise accurately skin sensitising hazards.

In this context, the relative skin sensitising potency of contact allergens is of considerable importance. The relevance of potency derives from an appreciation that contact allergens vary by up to four or five orders of magnitude with respect to the minimum concentration that is required to induce skin sensitisation. For this reason, potency should be considered adequately in a proper risk assessment in order to institute the appropriate degree of protection.

In recent years, ECETOC has made significant efforts in addressing the key aspects of skin sensitisation hazard identification and characterisation, in particular with respect to the design, application and interpretation of methods available for hazard identification and risk assessment. In addition, ECETOC has considered the development of proposals for the classification of contact allergens according to potency. The results of these deliberations are available in previous ECETOC Reports and Monographs (ECETOC, 1999, 2000, 2003a,b).

The same material deriving from the reports of ECETOC Task Forces has appeared in the scientific literature, as follows: Steiling *et al*, 2001; Kimber *et al*, 2001; Kimber *et al*, 2003.

The previous ECETOC Task Forces from which those reports and publications derived reviewed and discussed the use of both test methods employing the guinea pig as the test species (the occluded patch test of Buehler and the guinea pig maximisation test) and also the murine local lymph node assay (LLNA) for skin sensitisation hazard identification and characterisation.

In this report, attention is focused solely on the LLNA, the remit the Task Force addressed being:

- Building on the reports of a previous ECETOC Task Force (ECETOC, 2003a,b) on ‘Contact Sensitisation: Classification According to Potency’ and ‘Ditto: A commentary’, determine whether an EC3 (effective concentration for a stimulation index of 3) potency value derived from the LLNA can be used to provide a cut-off criterion for the classification and labelling of both individual substances and preparations according to the Globally Harmonised System (GHS) and Directives 67/548/EEC and 99/45/EEC, and, if confirmed, develop sub-categories based on the EC3 value.
- Evaluate current use of LLNA data in risk assessment approaches for skin sensitisation and, by taking into account potency considerations, provide a rationale for using concentration responses and corresponding no-effect concentrations.

For the purposes of addressing this remit it is appropriate to review briefly the LLNA and how it is currently employed for the measurement of skin sensitising potency.

1.1 The local lymph node assay

The LLNA was developed in mice as an alternative to previously favoured guinea pig tests for the identification of skin sensitising chemicals. Only a brief summary is required here; detailed information is available in a series of review articles (Basketter *et al*, 1996, 2001a, 2007; Dearman *et al*, 1999; Kimber *et al*, 1994; Kimber *et al*, 2002; Cockshott *et al*, 2006; McGarry, 2007; Gerberick *et al*, 2007).

The murine LLNA was conceived originally as a method for hazard identification. For this application the method was evaluated extensively in the context of both national and international inter-laboratory trials. Subsequently, the LLNA was validated for substances in the USA by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) (Dean *et al*, 2001), and in Europe by the European Centre for the Validation of Alternative Methods (ECVAM) (Balls and Hellsten, 2000). Finally, the LLNA was adopted by the OECD as Test Guideline (TG) 429 (OECD, 2002).

The LLNA seeks to identify contact allergens as a function of events induced during the acquisition of skin sensitisation, and specifically, lymphocyte proliferative responses induced in the regional lymph nodes of mice exposed topically to test chemicals. Detailed surveys of methodological aspects of the LLNA, and of the protocol used in the standard assay, are available elsewhere (Kimber and Basketter, 1992; Gerberick *et al*, 2007). A brief description of the standard assay is as follows. Groups of four mice (of CBA strain) receive topical applications, on the dorsum of both ears, of various concentrations of the test substance or of the same volume of the relevant vehicle control. Treatment is performed daily for three consecutive days. Five days following the initiation of exposure, animals receive an intravenous injection of radio-labelled thymidine after which draining (auricular) lymph nodes are excised and processed for β-scintillation counting. Radioactivity is measured as a function of lymph node cell proliferation induced by the test chemical and expressed as a stimulation index (SI) relative to values obtained with concurrent vehicle controls. As has been reported elsewhere (Kimber *et al*, 2002; McGarry, 2007), modified protocols with alternative endpoints are being developed and evaluated. Attention has focussed largely upon modified versions of the LLNA that incorporate methods for measurement of lymph node activation and lymph node cell turnover that do not require the use of radioisotopic labelling. Promising approaches include those described by Takeyoshi *et al* (2001), Yamashita (2005), Ehling *et al* (2005), Yamano *et al* (2005) and Idehara *et al* (2008), but they still require validation and need to gain acceptance by regulatory authorities.

Substances are classified as being skin sensitisers if, at any test concentration (up to and including 100%), they induce a stimulation index of 3 or more compared with the concurrent vehicle control, along with consideration of dose-response and, where appropriate, statistical significance (OECD 2002, US EPA 2003). Experience with the assay in the context of results obtained with a large number of diverse chemicals is summarised in articles describing the compilation of LLNA databases (Gerberick *et al*, 2004, 2005).

The favoured metric for the classification or categorisation of toxic chemicals is relative potency. This can be considered to reflect the amount of chemical that is required to provoke a certain level of adverse health effect. Relative potency applies to consideration of chemicals that cause skin sensitisation and allergic contact dermatitis. As mentioned previously, chemical allergens may vary significantly, and up to four or five orders of magnitude, with respect to their relative ability to induce skin sensitisation. This means that, in theory, exposure to only very low concentrations of strong contact allergens are required to cause skin sensitisation. In contrast, much higher concentrations of weak contact allergens are needed for sensitisation to develop. Recognition of these differences is of pivotal importance in developing accurate risk assessments.

Attention has therefore focused on the use of the LLNA for measurement of relative skin sensitising potency (Kimber and Basketter, 1996). This application is predicated on an understanding that lymph node cell proliferative responses are causally and quantitatively associated with the effectiveness with which skin sensitisation will be acquired. Consequently, the overall vigour of lymphocyte proliferative responses induced following topical exposure to a chemical allergen is believed to provide a direct correlate of skin sensitising activity or relative potency (Kimber *et al*, 1999).

In practice, the approach taken to determine relative potency is to derive from dose-responses in the LLNA an EC3 value (Kimber and Basketter, 1996; Basketter *et al*, 1999a). This EC3 value is defined as the amount of chemical required to induce in the LLNA, a three-fold increase in lymph node cell proliferation compared with vehicle control values. The units of EC3 can be expressed as the percentage concentration of test chemical required (easily translated into the molar value) or as dose per unit area of skin (Kimber *et al*, 2002).

This approach is now well-established (Basketter *et al,* 2007). Relative potency measurements based on derived EC3 values have proven to be of value with a wide range of chemical classes, and provide one important foundation for current approaches to skin sensitisation risk assessment and subsequent risk management (Kimber and Basketter, 1996; Basketter, 1998; Basketter *et al*, 1999b; Gerberick and Robinson, 2000). Importantly, determinations of relative potency based on EC3 values appear to correlate closely with what is known of the relative ability of contact allergens to cause skin sensitisation among humans (Basketter *et al*, 2000; Gerberick *et al*, 2001a; Griem *et al*, 2003).

Employing this approach, proposals have been made to categorise contact allergens according to their relative skin sensitising potency (Kimber *et al*, 2003; Basketter *et al*, 2005a). The most detailed proposals derived from the work of a previous ECETOC Task Force and were described in an ECETOC Technical Report (2003a), and in a subsequent publication (Kimber *et al*, 2003) deriving from that report. The conclusion then drawn from those analyses was that the most appropriate, science-based scheme for classification of contact allergens according to relative potency is one in which four sub-categories are identified. It was proposed that these categories should be termed '*extreme*', '*strong*', '*moderate*' and '*weak*' to reflect differing skin sensitisation potency based on derived EC3 values. In this scheme '*extreme*' sensitisers were defined as those having an EC3 value of less than 0.1%. On the same basis other categories were defined as follows: '*strong*' = EC3 values of equal to or greater than 0.1% and less than 1%, '*moderate*' = EC3 values of equal to or greater than 1% and less than 10%, and '*weak*' = EC3 values of equal to or greater than 10%. This scheme is summarised in Table 1.

Table 1: Sub-categorisation of contact allergens on the basis of relative skin sensitisation potency. Recommended scheme using EC3 values derived from the local lymph node assay *(from: ECETOC, 2003a,b)*

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

The implication from this categorisation scheme is that all other chemicals – that are inactive in the LLNA and for which an EC3 value cannot be derived – should be classified as non-sensitisers (consistent with the prediction model of the standard LLNA).

Against this background, and within the context of the terms of reference, the Task Force addressed a number of questions:

- The first of these is consideration of the distinction between sensitisers and non-sensitisers. Currently, any substance for which an EC3 value can be derived is classified as a skin sensitiser. All other substances that fail to provoke at any test concentration an SI of 3 or greater, and that are therefore negative in the LLNA (and for which an EC3 value cannot be derived), are classified as non-sensitisers. Thus, in effect any measurable EC3 value of up to and including 100% triggers classification of a chemical as a skin sensitiser. The specific question addressed here is whether, in light of any recent developments (since the ECETOC Technical Report was published in 2003), there is any justification for a change in this

threshold level from an EC3 value of 100%. For instance, is there now reason to believe that an alternative threshold, of say 50% or 75% rather than 100%, may more accurately distinguish between relevant skin sensitising substances that warrant an R43 label (EU, 2006), and those that do not;

- Irrespective of whether or not there exists justification for a change in the threshold for classification of a substance as a non-sensitiser, the second question addressed was whether there is now any reason to consider revision of the previous recommendations summarised in Table 1 regarding the specific EC3 values used for the sub-categorisation of substances according to potency;
- Finally, the third question was to identify what recommendations can now be made with regard to the upcoming classification and labelling of preparations under GHS.

2. HAZARD IDENTIFICATION USING THE LLNA

Since the validation of the LLNA by ICCVAM (Dean *et al*, 2001) and ECVAM (Balls and Hellsten, 2000), and assignment of OECD TG 429 (OECD, 2002), the assay has found wide application, and is increasingly used in preference to other OECD guideline tests, i.e. the Guinea Pig Maximization Test and the occluded patch test of Buehler (OECD, 1992). For instance, under the provisions of REACH, "only in exceptional circumstances" should a guinea pig test be used in preference to the LLNA (EU, 2006) and the use of a standard guinea pig test "will require scientific justification" (EU, 2008). Nevertheless, existing data that derive from adequately performed and documented guideline-based guinea pig tests may be acceptable and preclude the need for further *in vivo* testing (EU, 2008).

Against this background it is relevant here to reflect briefly on the current status of the LLNA in hazard identification. Experience to date indicates that the overall accuracy of the LLNA is high (Kimber *et al*, 2002; Cockshott *et al*, 2006; Gerberick *et al*, 2007), and that in most circumstances this method provides a robust and reliable approach to the identification of skin sensitisation potential.

On this basis, the LLNA merits its position as the preferred method for hazard identification. However, it is important to acknowledge that the LLNA, like any predictive test method, can only produce accurate results within its domain of applicability. Consequently, with increasing use, and in particular with increasing experience with a wider range of chemistry, there will be cases where the LLNA may not always provide the best approach for accurate hazard identification. For instance, recent investigations of some surfactant-like substances and certain fatty alcohols have suggested that results obtained with such chemical substances in the LLNA may be somewhat misleading, and that a guinea pig test might provide a more accurate assessment with respect to the situation in humans (Vohr and Ahr, 2005; Kreiling *et al*, 2008; Mehling *et al*, 2008). It is worth noting that areas of chemistry may exist where approaches other than the LLNA will prove useful. Delineation of applicability domains for conduct of the LLNA is a potential area of further scientific evaluation.

3. HAZARD CHARACTERISATION AND CLASSIFICATION OF SUBSTANCES AND PREPARATIONS ACCORDING TO RELATIVE POTENCY AS DETERMINED USING THE LLNA

When the LLNA is conducted according to the current OECD TG 429 (OECD, 2002), substances are classified as being skin sensitisers if, at any test concentration (up to and including 100%), they induce a stimulation index of 3 or more compared with the concurrent vehicle control, along with consideration of dose-response and, where appropriate, statistical significance (OECD 2002; US EPA 2003). The question was raised whether substances with a high EC3 value would pose a significant risk to human health, warranting classification and labelling as sensitisers. Substances with high EC3 values (arbitrarily defined as >50%) in the LLNA were screened for evidence of skin sensitisation hazard in humans. Of the few substances presented in the literature with EC3 values above 50%, most are also reported to represent a skin sensitisation hazard in humans (Table 2).

Table 2: Chemicals with EC3 values >50% and skin sensitisation in humans

Chemical	EC3 [%]	Human sensitiser
Aniline	89[1]	+[3]
Diethylacetaldehyde	76[1]	-
DMSO	72[1]	-
R(+)-Limonene	68[1]	+[3]
Methylmethacrylate	60 (Acetone); 90 (AOO) [2]	+[2]
Pyridine	72[1]	+[4]

[1] Gerberick *et al*, 2005

[2] Betts *et al*, 2006

[3] Schlede *et al*, 2003; positive evidence in humans could be due to oxidised limonene

[4] ICCVAM, 1999

The available evidence (summarised above) therefore does not support an EC3 value below 100% as the threshold for classification and labelling of a substance as a sensitiser. However, the firm opinion of this Task Force is that EC3 values provide a robust metric for assessment of relative potency for the purposes of risk assessment.

The LLNA is well suited for the estimation of skin sensitising potency (ECETOC, 2003a). However, this information has not yet been used in a regulatory capacity as a basis for classification and labelling of potential sensitisers. Skin sensitisation classification and labelling is currently binary in nature, i.e. substances are considered as sensitising or non-sensitising. Such binary classification does not reflect the fact that contact allergens vary by up to four or five orders of magnitude in terms of their relative skin sensitisation potency as measured by EC3

values. The availability of such potency data would importantly inform the derivation of accurate risk assessments.

Management of risk based on an accurate assessment of the risk is widely recognised as preferable to approaches based solely on consideration of the hazard. Thus, failure to take into account potency in the development of risk management measures, such as classification and labelling, impairs resulting decisions, since it does not make use of all the available information. This approach potentially results in the imposition of disproportionately onerous risk management measures, without any concomitant decrease in the risk to public health. There is a danger that over-emphasis of potential hazards and risks serves to 'devalue the currency' and ultimately results in the authenticity of warnings being questioned and advice being ignored. The same applies here with respect to skin sensitisation hazards. Use of a classification system that implies greater hazard than is actually the case will ultimately be self-defeating and might result in less effective risk management and protection of human health

Aside from the fact that in the standard guinea pig models OECD TG 406 (OECD, 1992) only single concentrations are tested within a given study design, results are dependent upon the severity of elicitation responses. Due to the subjective nature of quantification of elicitation reactions and lower elicitation concentrations, elicitation is considered inappropriate for a reliable categorisation of sensitisation potency. Even if one were to use the epidermal induction concentration employed in guinea pig tests for potency evaluation, this decision is fraught with greater uncertainty than when using the LLNA. However, although the LLNA is better suited for potency estimations than guinea pig assays, if data are already available from appropriate guinea pig tests, their judicious interpretation may provide information of value in determinations of potency and categorisation (ECETOC, 2003a).

Against this background, and within the context of the terms of reference, the Task Force has given additional consideration to the characterisation and classification of substances, particularly using LLNA results.

3.1 Classification and labelling of substances - based on their potency categorisation

Not all contact allergens have the same ability to cause skin sensitisation. For example, methyl methacrylate (MMA) does not carry the same risk for sensitisation as isothiazolinone, despite comparatively higher levels of occupational exposure for MMA (Betts *et al*, 2006). Risk is a function of both exposure and the nature and severity of the hazard, so the intrinsic potential of a substance to behave as an allergen can be understood in terms of its potency.

The literature now contains LLNA results for hundreds of chemicals, and the range of EC3 values spans at least four orders of magnitude (Kimber *et al*, 2003). An overall association between EC3 values and relative potency of chemical allergens in humans has been demonstrated (Basketter *et al*, 2005a; Basketter *et al*, 2001b; Gerberick *et al*, 2001a). This concordance supports the use of categorisation schemes based on EC3 values for the purposes of risk management and classification and labelling. Rather than substances being categorised simply as sensitising or not, sensitisers can be grouped according to their relative potency. This type of information could be used as important and specific hazard data for inclusion in safety data sheets. More potent allergens are managed differently from those substances which are sensitising but whose potency is very low. With such specific information, the accuracy of a product safety assessment could be significantly increased.

A previous ECETOC Task Force proposed four categories of sensitisers based on ranges of potency values (ECETOC, 2003a; Kimber *et al*, 2003). In developing the scheme, a spectrum of chemical allergens was considered, and it became evident that there were substances which demonstrated a very high, or extreme, potency. Likewise, there were substances which are considered to be weak allergens; these substances typically have rare cases of sensitisation reports and may require significant exposure to produce the adverse effect. Between weak and extreme were substances which have clear histories of cases of skin sensitisation in humans, but which can be considered quite differently from either end of the spectrum. These sensitisers were considered to be *moderate* or *strong*. Thus, a scheme (Table 1) was proposed which characterises sensitisers as extreme, strong, moderate, or weak with respective EC3 values differing by an order of magnitude between each category (Kimber *et al*, 2003). Characterisation of substances into these four categories on the basis of 10-fold differences in EC3 values provided good delineation of sensitisers based on clinical experience, while providing potency ranges to facilitate consistent categorisation.

Table 3 shows an example of how such categorisation can be applied to a selected number of chemicals. It can readily be seen that the indicated substances would fall into these categories and that there is general congruence with evaluations based on weight of evidence, including human experience (Basketter *et al*, 2000).

Table 3: Categorisation of chemicals according to skin sensitisation potency using the local lymph node assay *(updated from ECETOC, 2003a)*

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
Citral	5.7	Moderate
Tetramethylthiuramdisulfide	6.0	Moderate
4-Chloroaniline	6.5	Moderate
Hexylcinnamaldehyde	8.0	Moderate
2-Mercaptobenzothiazole	9.7	Moderate
Abietic acid	11	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

Shortly after this initial proposal was presented by ECETOC, the European Commission recognised the potential merit of a potency-based classification scheme for the management of skin sensitisers. They asked the European Chemical Bureau (ECB) to convene an expert panel and to consider potency characterisation for purposes of classification criteria (Basketter *et al*, 2005a). The convened expert panel proposed a scheme which, in essence, was very similar to that previously advanced by ECETOC, in particular, maintaining a 10-fold difference between categories. The main difference was the suggestion by the ECB panel to merge the *weak* and *moderate* allergens into a single *moderate* category. This resulted in three categories, rather than four, with different thresholds to describe skin sensitisation potential. Thus, extreme and strong categories were maintained while *moderate* potential was extended to include all chemicals with EC3 values in the range of 2% to 100%. The other notable difference between the ECETOC and ECB proposals is that the respective EC3 thresholds for each category were delineated at ≤0.2% (extreme), >0.2% - ≤2% (strong) and >2% (moderate) (Table 4). The rationale for this difference is not substantiated in the relevant publication. It is possible that these differences were driven as much by considerations of prevalence as those of potency. While a welcome advance on binary classification, the ECB scheme would result in a much more conservative approach than the ECETOC proposal and has a serious impact on classification and labelling, particularly, overly conservative labelling of weak sensitisers. Such conservative labelling of weak skin sensitisers, resulting in inappropriate risk management, would in effect 'devalue the currency' of risk labels, as already discussed.

Table 4: Comparison of proposed potency classifications

Potency rating	ECETOC	ECB
	Concentration thresholds (%)	**Concentration thresholds (%)**
Extreme	<0.1	≤0.2
Strong	≥0.1 – <1.0	>0.2 - ≤2.0
Moderate	≥1.0 - <10	>2.0
Weak	≥10	N/A

Other agencies are also evaluating the aspects of EC3 values and potency. Recently, the US NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) organised an expert panel to consider whether the LLNA can reliably be used for potency categorisation. (NICEATM/ICCVAM, 2008). NICEATM considered 170 substances for its assessment and proposed a two-level categorisation scheme (weak and strong). An interim report concluded that there is a significant positive correlation between LLNA potency and human sensitisation threshold also reported by others (Schneider and Akkan, 2004; Basketter *et al*,

2005b), and suggested that an EC3 around 9.4% produced the most accurate delineation between weak versus strong categorisation. However, the panel did not deem the correlation to be sufficiently strong or precise. Therefore, NICEATM concluded that the LLNA should not be considered as a stand-alone test method for predicting sensitisation potency, but could be used as part of a weight-of-evidence evaluation to discriminate between strong and weak sensitisers. The panel also recommended additional analyses to further investigate these relationships. The use of the LLNA for potency assessment is currently being considered by both ICCVAM and OECD.

Under GHS, the binary categorisation of skin sensitisation in the existing legislation remains with a requirement only to indicate whether a substance is a sensitiser (Category 1) or not. However, the need for potency classification resulting in sub-categories has been recognised by the ECB working group (Basketter *et al*, 2005a). The concept is consistent with that proposed by ECETOC (ECETO*C,* 2003a). Also the OECD has submitted a proposal to GHS for sub-categorisation of potency (OECD, 2008).

To date, no regulatory authority has adopted any scheme for potency categorisation for use in classification and labelling. Currently, classification of substances for sensitisation potential is binary; a substance either is, or is not, classified a skin sensitiser (R43). In the case of the LLNA, this classification is driven by lymph node proliferation, regardless of test substance concentration. Thus, a chemical inducing a three-fold or greater increase in lymph node proliferation at a test concentration of 0.5% is classified in the same way as another chemical requiring a 50% concentration to achieve an SI of 3. Reconsideration of potency characterisation by this ECETOC Task Force, particularly in the context of REACH and GHS, affirmed that sub-categorisation according to potency using extreme, strong, moderate, *and* weak (Table 1) would enable optimal management of the risk of substances with the potential to induce skin sensitisation, and to provide improved information on skin sensitisers. Some examples are presented below to illustrate this point.

Having reviewed these different categorisation proposals in the context of risk management, this ECETOC Task Force revisited the 2003 recommendation of four potency categories. The recognition of weak sensitisers as a separate category is important and appropriate for those chemicals with high EC3 values (≥10%) that have only a limited potential to cause skin sensitisation, even under circumstances where exposure is significant. For example, linalool is a contact allergen with an EC3 around 30% (Ryan *et al*, 2000), which would be considered a moderate sensitiser under the ECB scheme, but a weak sensitiser according to ECETOC considerations. In practice, however, linalool is not a significant allergen (Sköld *et al,* 2008; Basketter *et al*, 2002; Rastogi *et al*, 2001; Schnuch *et al*, 2007). As such, the characterisation as *weak* better reflects the relative sensitisation potency for purposes of warning and classification and labelling. The Task Force recommends that this aspect of characterisation should be implemented under GHS.

Two other fragrance ingredients, methylionone and citral, also help to illustrate the value of discriminating moderate from weak sensitisers. Methylionone is a skin sensitiser which has a relatively high EC3 (21.8%), as well as a high human repeat insult patch test (HRIPT) threshold for response (Lapczynski *et al*, 2007), and which is considered to be of low clinical significance (Schnuch *et al*, 2007). Conversely, citral is considered to be an allergen of considerable clinical significance with a report of increased frequency for sensitisation in recent years (Schnuch *et al*, 2007). Reviews conducted under the auspices of RIFM (Research Institute for Fragrance Materials) indicate that relative exposure to methylionone from all personal care products is appreciably greater than that for citral. When last evaluated, the annual use of methylionone was greater than 1,000 tonnes (Lapczynski *et al*, 2007), while that of citral was an order of magnitude less, i.e. at above 100 tonnes (RIFM, personal communication). Both ingredients mentioned are used solely as fragrance ingredients; therefore, the tonnage effectively reflects exposure. The relative potencies as predicted by LLNA EC3 values support the proposal that the sensitisation potential of these two ingredients should be recognised as different from one another. Thus, the EC3 values for methylionone (21.8%; Lapczynski *et al*, 2007) and citral (5.7%; Lalko and Api, 2008) would lead both substances to be classified as moderate sensitisers, according to the ECB scheme, while the ECETOC scheme discriminates between them.

With respect to identification of intrinsic hazard, the designation of both of these chemicals as skin sensitisers (R43) is reasonable and ensures appropriate warnings. Even chemicals with comparatively weak skin sensitisation potential may be able to cause allergy in some individuals under circumstances where there is sufficient and sustained exposure. As presented above, although methylionone is a weak sensitiser (EC3 = 21.8%, i.e. is greater than 10%), substantiated cases of allergy exist consistent with classification as R43. Another example of a relatively weak skin sensitiser is MMA. Investigations using the LLNA have reported EC3 values greater than 60% (Betts *et al*, 2006). Nevertheless, skin allergy to MMA has been observed among dental workers, presumably due to comparatively high levels of exposure (Aalto-Korte *et al*, 2007; Goon *et al*, 2006; Betts *et al*, 2006).

3.2 Classification and labelling of preparations - based on the potency of their individual substances

Under the GHS, a preparation is defined as a mixture composed of two or more substances which do not react. This definition is a similar to the definition of preparations under the EU Dangerous Preparations Directive (EU, 1999).

In line with both the current European regulations prohibiting animal tests with consumer products (e.g. cosmetic products), and the long-term experience of successful risk evaluation based on individual ingredient data, many preparations are not tested in animals. The individual toxicological profiles of the component ingredients are currently used to decide on classification

and labelling of a preparation with regard to skin sensitisation potential (R43). Currently, according to the Dangerous Preparation Directive (EU, 1999) and its amendment (EU, 2006), a level of ≥1% of a skin sensitiser ingredient requires a hazard categorisation of the preparation as a skin sensitiser, irrespective of potency. For a quantity of ≥0.1% but <1%, the skin sensitising substance has to be declared on the label, even when the preparation is not classified as sensitising. From this perspective, the reliability of substance data is essential in the evaluation of preparations.

In 2003, ECETOC applied the four potency categories identified above (weak - moderate - strong - extreme) to propose threshold concentrations of substances (i.e. ingredients) for the classification (R43) of preparations with respect to skin sensitising hazard (Table 5) referenced from ECETOC, 2003a,b).

Table 5: Default values as threshold concentration of ingredients requiring classification of preparations as sensitisers *(from: ECETOC, 2003a,b)*

Potency	Concentration (%)
Extreme	0.003
Strong	0.1
Moderate	1.0
Weak	3.0

In light of new evidence, the cut-off values and their rationales recommended by the previous Task Force (ECETOC, 2003a) were re-examined by the current Task Force. A correlation has been demonstrated between the concentration of a substance required for the acquisition of skin sensitisation in humans and skin sensitisation potency, as measured in the mouse LLNA (Schneider and Akkan 2004; Basketter *et al*, 2005b). For those substances that are considered extreme, skin sensitisation is acquired at relatively low concentrations. The previous Task Force concluded that in such circumstances a default value of 0.003% should trigger classification of a preparation as a sensitiser (R43) (ECETOC, 2003a). A second group of allergens (categorised here as strong) were considered to be of sufficient potency that required a lower value than the current 1% default (ECETOC, 2003a). Therefore, a more conservative default value of 0.1% was decided to 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 for preparations. 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%.

As developed in the previous ECETOC Task Force (ECETOC, 2003a) and published by Kimber *et al*, in 2003, the following scheme of potency-based cut-off values is defined and the following sub-categories are recommended by this Task Force (Table 6).

Table 6: Scheme of potency-based cut-off values

Potency	Sub-category	Concentration limit of sensitising ingredient present in solid and liquid preparation (% w/v)
Extreme	1a	0.003
Strong	1b	0.1
Moderate	1c	1.0
Weak	1d	3.0

This scheme provides guidance for effective characterisation of hazards of preparations and limits the need for additional testing of preparations in animals. Such potency-based ingredient-specific evaluation of the skin sensitisation activity of preparations will provide improved classification and labelling compared with what is currently required by the Dangerous Preparations Directive (EU, 1999).

Applying the above-mentioned process, preparations could be properly assessed for their sensitisation hazard, but without sub-categorisation of their overall potency to induce skin sensitisation as detailed for substances. This limited level of information is generally acceptable because of its similarity to the current safety evaluation of preparations and the reduction of complexity. It is recommended to label and categorise a sensitising preparation based on this process just as category '1', without any sub-categorisation. If specific information on potency is required, the individual preparation has to be tested using the LLNA, or has to be evaluated using the bridging process outlined under 3.4.

Both the dose of a skin sensitiser per area of skin and the substance-specific sensitisation potency are the relevant factors for the induction of skin sensitisation. To enable consideration of the potency of an individual skin sensitising substance for the evaluation of a preparation, the potency-based sub-categorisation (1a-d) should be provided ideally within the (Material) Safety Data Sheet ((M)SDS) of each ingredient. The Task Force suggests that the (M)SDS, which is required by regulation, provides the most appropriate vehicle for provision of such important information.

3.3 Classification and labelling of preparations - based on their direct testing

When reliable and high quality data from appropriate animal studies with preparations are available such preparations should be classified and labelled based on these data. It should be emphasised that, in line with the process of testing substances, all available data on the preparation should be used in a weight-of-evidence approach when deciding on classification. In particular, the composition of such a preparation should be specified.

Adhering to the testing requirements for substances, the evaluation of preparations (e.g. pesticide products) using the LLNA provides similar information on skin sensitisation potency as for substances. With such reliable data, a tested preparation can and should be categorised for skin sensitisation based on the potency.

3.4 Classification and labelling of preparations - based on comparisons with similar preparations

It is also an accepted practice to estimate the skin sensitisation potential of a preparation based on data obtained on a preparation with a similar but relevant composition. For such a 'bridging process', the chemical composition (chemical structure and concentration) of both the untested and 'bridging' preparation should be known.

As mentioned earlier for substances, classification based on potency provides improved consumer/user protection because potency-based classifications can be readily translated into meaningful handling guidance and risk management. The same considerations and benefits apply to preparations, not least because contact with human skin is most commonly with preparations. There is a need, therefore, to develop a paradigm based upon the concentration of an ingredient within a preparation and the sensitising potency of that ingredient.

3.5 Translating potency classification into risk management of preparations

Classification and labelling is the fundamental ground for a proper risk management, the primary goal of which is to protect the user. Such a relationship becomes extremely evident for the classification/labelling of preparations that may directly come into contact with a person during professional use or as consumer goods.

When risks of different magnitudes can be differentiated based on the identified skin sensitisation potency, proper risk management can be used that is adequate and proportionate. In this light, the LLNA read-out of lymphocyte proliferation lends itself to the determination of a preparation's relative sensitising potency, which, in turn, is a clear quantitative descriptor of hazard potential.

In cases where preparations have not been specifically tested, their classification/labelling has to be calculated according to the proposed scheme (Table 6) and based on the % content and sensitisation potency of their ingredients. Once classification and labelling of the preparation are determined, credible risk management practices can be applied that should be recognised as realistic and effective.

As stated above, there is a danger that over-emphasis of potential hazards and risks serves to 'devalue the currency' and ultimately results in the authenticity of warnings being questioned and advice being ignored. Use of a classification system that implies greater hazard than is actually the case will ultimately be self-defeating and might result in less effective risk management and protection of human health.

4. EXPOSURE CONSIDERATIONS

Correct data of human exposure to a substance in both an occupational environment or via consumer products is an essential part of a proper risk assessment. For such estimation, the route of exposure is an important consideration and may be different in an occupational setting to that of consumer use. It is standard risk assessment practice to consider exposure scenarios resulting from intended use or foreseeable misuse but not abuse. In the context of this document the following factors are relevant for a scientifically sound risk assessment of skin sensitisation:

- The frequency and duration of exposure to a contact allergen. An exposure could be an incidental single contact, a series of repeated contacts, or continuous contact. For example for consumer products, the exposure may result from products intended to be left on the skin (leave-on, e.g. skin cream) or rinsed off (e.g. shower gel), residues from fabrics (laundry products) or of incidental skin contact (e.g. household cleaning products);
- The exposure concentration of the chemical. In an occupational setting, exposure may be to the undiluted chemical, whereas exposure via a consumer product depends on the concentration of the substance present in the product;
- The dose per unit area is a key parameter for the induction of sensitisation. Therefore, an estimate of the area of skin exposed and the amount of substance coming into contact with this skin area are crucial for a proper exposure assessment.

Exposure scenarios can be developed that reflect the use of the substance in various applications and from these an estimate of exposure can be defined. However, there will be considerable variation in the exposure between individuals and in many instances it may not be possible to measure the exposure accurately. This scenario is particularly relevant in occupational settings where exposure can be unintentional, e.g. as a result of contamination. Modelling of standard occupational procedures can be used to improve the exposure assessment. For consumer exposure, many companies have their own habits and practices data for particular product types. But there are also a number of published sources of typical exposure data for a large number of cosmetic and household products, such as those from the Cosmetics, Toiletry, and Fragrance Association (CTFA) and in the EU Technical Guidance Document (Loretz *et al*, 2006; Hall *et al*, 2007; AISE/HERA, 2002; EU, 2003). In all cases, it is recommended to take a conservative approach towards exposure assessment.

5. RISK ASSESSMENT AS PERFORMED FOR CONSUMER PRODUCTS

Risk assessment in the context of this document is aimed at preventing the induction of skin sensitisation. Historically, the approach adopted was one of comparative analysis, involving benchmarking of new allergens against other allergens of known potency that are used in similar product types without inducing skin sensitisation. Similarly, substances of known sensitising potency that have historically been associated with outbreaks of allergic contact dermatitis might also influence the decision-making process. More recently, efforts have been made to supplement these benchmarking approaches with a quantitative risk assessment (QRA) for skin sensitisation and thereby provide them with a better scientific basis (Api *et al*, 2008).

Both of these approaches require a thorough understanding of anticipated human exposure and data relating to the relative potency of the substances in terms of their intrinsic ability to induce skin sensitisation. Because proliferation of cells in draining lymph nodes is quantitatively related to the acquisition of skin sensitisation, the development of the LLNA has made it possible to quantify relative potency to a greater extent and more easily than was previously possible with predictive methods, such as the guinea pig maximisation or Buehler tests (Gerberick *et al*, 2005; Basketter *et al*, 2007). Therefore, it should be possible to more accurately differentiate between skin sensitising substances on the basis of potency. In this section, the QRA process for skin sensitisation is outlined as performed for consumer products. It is also described how LLNA EC3 values are used in this context.

5.1 Dose metric for skin sensitisation

It is now recognised that the appropriate dose metric for the induction of skin sensitisation is not the total dose applied but the dose applied per unit area of skin. This concept is best illustrated in a series of human sensitisation studies performed by Friedmann and colleagues using dinitrochlorobenzene (DNCB) as a model allergen (Friedmann *et al*, 1983, 1990; White *et al*, 1986; Rees *et al*, 1990; reviewed in Friedmann, 2007; Kimber *et al*, 2008). In summary, this series of investigations demonstrated that increasing the total dose of DNCB failed to induce a concomitant increase in the incidence of sensitisation, if the dose per unit area was kept constant by increasing proportionately the area of exposed skin. By contrast, when the total dose was kept constant but the dose per unit area was increased by reducing the area of the exposed skin, there was a concomitant increase in the incidence of sensitisation. Similarly, Kligman reported in 1966 that the incidence of sensitisation observed in human volunteers exposed to ammoniated mercury, monobenzyl ether of hydroquinone, nickel sulphate and neomycin sulphate was comparable if the dose per unit area was kept constant, despite increased surface area of exposed skin and, thus, increased total exposure (Kligman, 1966). In addition to the human volunteer studies described above, the relevance of applied dose per unit area of skin has also been illustrated in guinea pigs. Magnusson and Kligman (1970) conducted studies with DNCB, *p*-nitroso-dimethylaniline and

p-phenylenediamine and found that an increase in the surface area of exposed skin by up to two orders of magnitude was without effect on the incidence of sensitisation when the dose of allergen applied per unit area of skin was kept constant.

5.2 Quantitative risk assessment

The key steps of the QRA process are as follows:

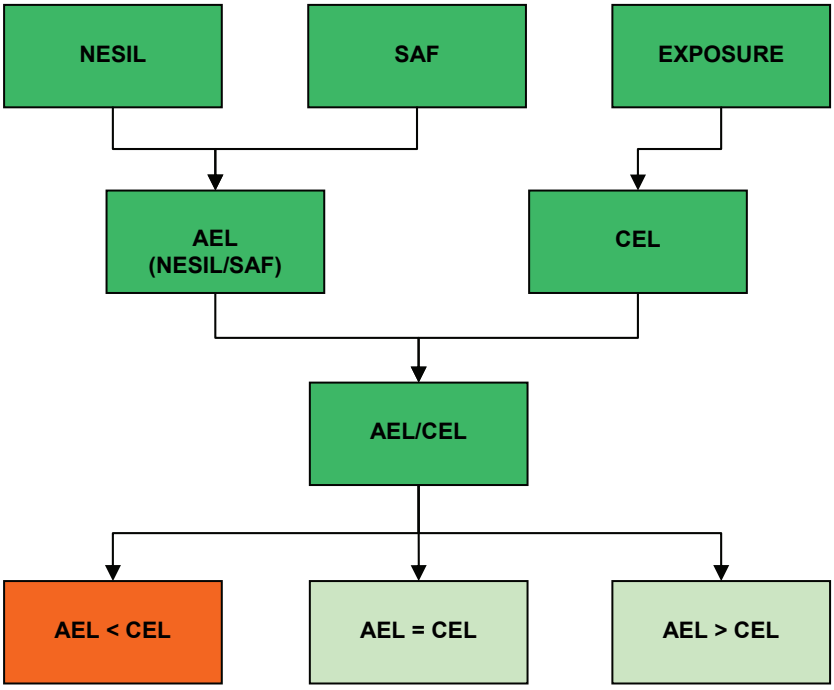
1) Identification of a predicted dose threshold for the induction of skin sensitisation in humans, referred to as the no expected sensitisation induction level (NESIL);

2) The assignment of sensitisation assessment factors (SAFs) that serve to represent uncertainties associated with inter-individual variability, matrix differences, and exposure considerations;

3) Calculation of an acceptable exposure level (AEL) by dividing the NESIL by the product of three SAFs;

4) Comparison of the AEL with the actual exposure level (e.g. a consumer exposure level - CEL) associated with the intended use (Api *et al*, 2008). This is depicted in Figure 1. The two green boxes at the end of this figure refer to acceptable exposure scenarios, whereas the red box denotes unacceptable exposure scenarios.

Figure 1: Key steps of the QRA process *(adapted from Api et al, 2008)*



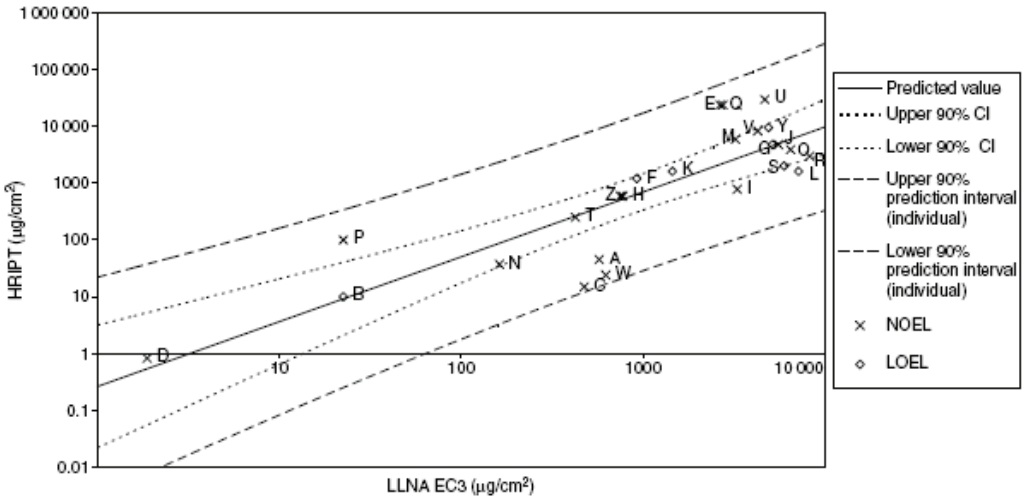
As outlined above, the AEL is determined by dividing the NESIL with the appropriate SAFs. The AEL is subsequently compared with the CEL, and the AEL can either be lower, equal or larger than the CEL. Depending on the outcome, this would have consequences on the risk assessment.

5.2.1 No expected sensitisation induction level

The NESIL (which is sometimes referred to as the no observed effect level or no observed adverse effect level in appropriately designed dermatological investigations) is the starting point for QRA and should represent the threshold for the induction of skin sensitisation (expressed as dose per unit area) in humans. It has been proposed that a weight-of-evidence (WoE) approach should be adopted when identifying a NESIL (Api *et al*, 2008). Such an approach takes into account LLNA dose-response data and existing data from HRIPTs or human maximisation tests (Gerberick *et al*, 2001b).

The LLNA data typically contribute to the QRA process by helping to define the NESIL. For example, Gerberick *et al* (2001b) developed a classification scheme to rank the potency of fragrance allergens based on a WoE from available human data and/or LLNA EC3 values. For each potency class, a conservative default NESIL was identified for the purposes of QRA. More recently, efforts have been made to examine directly the relationship between LLNA EC3 values and thresholds for the induction of skin sensitisation in human. This evaluation requires an understanding of the correlation between EC3 values and human sensitisation thresholds. However, data relating to the latter are scarce, primarily due to ethical considerations associated with human sensitisation testing and the diversity of protocols historically used to generate human data. Nevertheless, historical data do exist and include examples where dose-response data are available from HRIPT and human maximisation tests. Such correlations have recently been investigated (Griem *et al*, 2003; Basketter *et al*, 2005b). In the more recent analysis, Basketter and colleagues undertook a thorough and extensive analysis of existing human predictive assays (e.g. HRIPTs), particularly where dose-response information was available. This analysis identified 26 skin sensitising substances for which the approximate threshold for the induction of skin sensitisation in humans could be identified. These threshold values ranged from 0.83 to 29,525 $\mu g/cm^2$. Similarly, the EC3 values for the same chemicals were obtained and expressed as dose per unit area (range: 2.25 to 8,250 $\mu g/cm^2$). As expected, regression analysis revealed a linear relationship between the two variables (Figure 2). The relationship is not perfect, most likely due to variability in the human data, which were obtained from a number of different laboratories using different protocols over a considerable period of time. But it does substantiate the view that LLNA EC3 values, and therefore potency classes, can be used directly to determine a NESIL as the first step in QRA.

Figure 2: Correlation between EC3 values and human skin sensitisation thresholds *(from Basketter et al, 2005b)* [1]



[1] The compounds corresponding to the letters in this figure are given in Basketter *et al*, 2005b.

Thus, LLNA potency classes can be used as one of the elements of a WoE approach to identify a NESIL. Under certain circumstances, only LLNA data may exist. In such situations, it may be considered appropriate to undertake human repeat insult patch testing to confirm that the predicted NESIL is indeed associated with a clear absence of sensitisation, but not to generate information regarding relative sensitising potency.

5.2.2 Sensitisation assessment factors

Having established a NESIL [in µg/cm²], the QRA process next requires the assignment of appropriate SAFs (which are sometimes referred to as uncertainty factors or sensitisation uncertainty factors). These seek to represent sources of uncertainty associated with inter-individual variability, matrix differences and exposure considerations.

In terms of the inter-individual variability SAF, the general view is that a value of 10 is adequate to represent the variability of the population with respect to variables that contribute to the acquisition of sensitisation (1st SAF). These variables have been reviewed previously and include differences associated with age, gender, ethnicity, genetic factors, sensitive subpopulations and skin barrier function (Felter *et al*, 2002). Some evidence that the value of 10 is appropriate is provided, at least in part, through human sensitisation studies in which a factor of 10 was observed between the lowest dose of DNCB per unit area required to induce sensitisation

(8.8 μg/cm^2 sensitised approximately 8% of the volunteers) and the dose of DNCB found to induce sensitisation in all of the volunteers (71 μg/cm^2) (White *et al*, 1986).

The skin sensitisation QRA framework does not currently embrace a SAF to account for inter-species variability. The rationale for this omission is that for skin sensitisation, the direct quantitative relationship between EC3 values and the human sensitisation thresholds has been elucidated as illustrated above (Figure 2; Basketter *et al,* 2005b), and due to adequate correlation between the two, an inter-species SAF is not warranted.

Exposure to substances in the context of predictive tests for skin sensitisation typically occurs via a relatively simple vehicle. However, consumer exposure to the same substance may occur via relatively complex preparations. The preparation may contain other ingredients that may impact on the ability of a substance to cause skin sensitisation (e.g. due to their irritant properties or increased penetration). Such effects are accounted for by the matrix differences SAF (2nd SAF), which is scaled between 1 and 10, depending upon the degree of difference between the vehicle system used in the predictive test and the product formulation associated with the intended use. Matrix SAFs below 1 could be appropriate if predictive tests were performed under exaggerated conditions like occlusive exposure or in combination with known penetration enhancers. In practice, such test conditions are very rare and beyond the standard protocols available for both human and animal sensitisation tests. Most of the data supporting a matrix difference SAF $\leq$ 10 has been obtained in the LLNA, where the variability in EC3 values has been explored for chemicals that have been tested in different vehicle systems (Lea *et al*, 1999; Warbrick *et al*, 1999, 2000; Wright *et al*, 2001; Lalko *et al*, 2004; reviewed in Basketter *et al*, 2001c; McGarry, 2007).

Qualitative aspects of the exposure associated with intended product use may also impact on the ability of a chemical to induce sensitisation and are represented in the context of QRA by the exposure considerations SAF (3rd SAF). Variables implicit in this SAF include differences in dermal penetration at different anatomical regions (Feldmann and Maibach, 1967), the potential impact of occlusion (Zhai and Maibach, 2001), compromised dermal integrity due to an existing skin disease, and other environmental conditions at the site of exposure such as high humidity and temperature. The exposure considerations SAF is also $\leq$ 10, based upon expert judgement about the potential of these variables to impact on the ability of the chemical to induce sensitisation.

Expert judgement is required when assigning matrix differences and exposure considerations SAFs. Some guidance about the values adopted and the associated rationale may be obtained from published examples of QRA for specific chemicals and product types (Gerberick *et al*, 2001b; Basketter *et al*, 2003, 2007; Jowsey *et al*, 2007; Api *et al*, 2008).

5.2.3 Acceptable exposure level

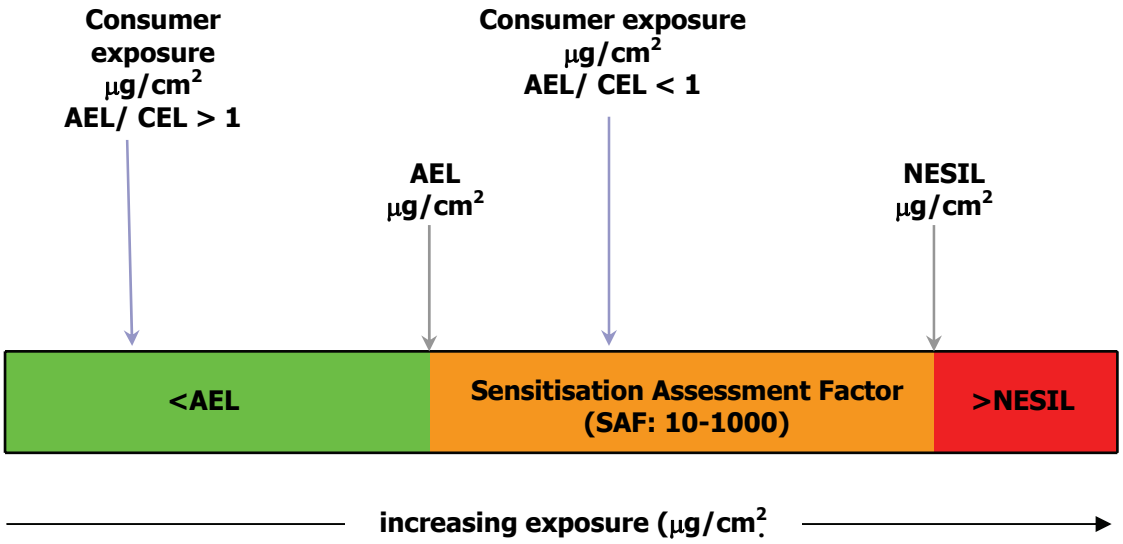
In order to calculate an AEL (sometimes referred to as the reference dose or sensitisation reference dose), the NESIL is divided by the product of the three above-mentioned SAFs. The AEL can be used to determine an appropriate concentration of a sensitising chemical that could be incorporated in a given product type without inducing sensitisation.

5.2.4 Comparison of acceptable exposure level with consumer exposure level

The final stage of the QRA process requires comparison of the calculated AEL with the actual level of exposure to a chemical that will occur through the intended product use (referred to as the consumer exposure level - CEL). Both AEL and CEL should be expressed in terms of dose per unit area. These values are typically compared by dividing AEL by CEL. In order to minimise the risk of inducing skin sensitisation, the AEL/CEL value needs to be equal or greater than 1.

The concepts and terminology used above are illustrated in Figure 3.

Figure 3: Exposure lines *(adapted from Basketter et al, 2003)*



The risk assessment is considered as favourable if the AEL is either larger or equal to the CEL. If consumer exposure is larger than the AEL, the consumer could potentially be exposed to an amount close to the NESIL, and, thus, not have a sufficient margin of safety. The figure illustrates that the anticipated CEL should be clearly in the green zone, meaning below the

defined AEL. As outlined before, this graph would have different scales for different materials and would also vary depending on the use scenarios.

5.3 Published examples of quantitative risk assessment for skin sensitisation

A number of examples of QRA for skin sensitisation have been published. The earliest of these was a study by Gerberick *et al,* who considered the use of cinnamic aldehyde in two hypothetical product use scenarios (Gerberick *et al*, 2001b). This analysis revealed that for a rinse-off shampoo product containing 1000 ppm cinnamic aldehyde, the AEL/CEL value was 12.5. Thus, this use of cinnamic aldehyde was considered to present minimal risk of inducing sensitisation. By contrast, the AEL/CEL for a leave-on eau de toilette product containing the same levels of cinnamic aldehyde was 0.4. The value indicates that the dose of cinnamic aldehyde delivered per unit area of skin (CEL) was greater than the AEL. Such a hypothetical analysis infers that the use of 1000 ppm cinnamic aldehyde in the latter use scenario may present an unacceptable risk of inducing skin sensitisation.

Corea *et al,* also deployed the QRA approach to assess the risk of skin sensitisation associated with exposure to fragrance materials deposited on laundered clothes (Corea *et al*, 2006). For a total of 24 fragrance materials, AEL/CEL was calculated to range from 55 to 17,066. This suggests that the risk to induce fragrance allergy as a result of wearing clothes that have been machine washed with laundry products is extremely low.

More recently, Basketter *et al* (2008) undertook a retrospective QRA on four different preservatives (formaldehyde, MCI/MI, imidazolidinyl urea and 3-iodo-2-propynyl butyl carbamate) in five product types (shampoo, face cream, non-aerosol deodorant, body lotion and lipstick). This analysis illustrated that, for certain preservative/product type combinations, actual exposure through product use resulted in an AEL/CEL value ≤ 1). Thus, whilst preservatives were typically found to present a low risk of inducing sensitisation when used in rinse-off products such as shampoo, there was sometimes a greater risk for face creams and deodorants. This finding is consistent with clinical experience.

It is important to keep in mind that the above examples represent hypothetical or retrospective uses of QRA. The real value of this approach will become apparent when it is used prospectively.

In the context of quantitative risk assessment for skin sensitisation, the question of thresholds of relevant concentrations was raised. Consequently, applying the four categories of sensitisation potency, the concept of dermal sensitisation threshold (DST) was recently developed and presented by Safford (Safford, 2008). This probabilistic approach is grounded on the principles

of the well known threshold of toxicological concern (TTC) concept for systemic toxic effects (e.g. Kroes, *et al*, 2000). A DST can be calculated from the individual skin sensitising potency of a substance. It represents the exposure level below which no appreciable risk of inducing skin sensitisation is expected. This probabilistic approach is possibly not ideal for all individual substances, especially under non-intended exposure conditions. But it may in the future serve as an alternative hazard identification approach, thereby reducing animal testing, without compromising the level of safety in risk assessments.

5.4 Summary

The generation of potency data using the LLNA has permitted the development of quantitative risk assessment approaches (QRA), which supplement and support more traditional benchmarking approaches. QRA describes quantitatively the relationship between the calculated exposure to a sensitising chemical and the acceptable exposure level, determined for specified conditions of use. The approach critically depends on the establishment of a NESIL, based on the correlation between LLNA EC3 values (sensitisation potency classes) and HRIPT data (WoE) demonstrated in recent publications. Where human data do not exist on a specific chemical, LLNA data can also contribute information to benchmark this chemical in relation to existing chemicals with similar properties and applications, which are already in use. Thus, the developed four potency classes based on LLNA EC3 values can form the basis of a strategy to manage the use of skin sensitising chemicals more effectively according to their potency both in the case of traditional benchmarking approaches, as well as with newly developed QRA approaches.

ABBREVIATIONS

AISE	Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien
AEL	Acceptable exposure level
AOO	Acetone/Olive oil (4:1) as a recommended vehicle in the LLNA
CEL	Consumer exposure level
CTFA	Cosmetics, Toiletry and Fragrance Association
DNCB	Dinitrochlorobenzene
EC	European Commission
EC3	Effective concentration for a stimulation index of 3
ECB	European Chemicals Bureau
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Community
EU	European Union
DST	Dermal sensitisation threshold
GHS	Globally Harmonised System of Classification and Labelling of Chemicals
HERA	(AISE) Human and Environmental Risk Assessment (on ingredients of household cleaning products)
HRIPT	Human repeat insult patch test
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
LLNA	Local lymph node assay
MMA	Methyl methacrylate
(M)SDS	(Material) safety data sheet
NESIL	No expected sensitisation induction level
NICEATM	(United States) NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
OECD	Organisation for Economic Co-operation and Development
QRA	Quantitative risk assessment
R43	Risk phrase 43 for the labelling of chemicals and preparations in the EU that "may cause skin sensitisation by skin contact"
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIFM	Research Institute for Fragrance Materials
SAF	Sensitisation assessment factor
SI	Stimulation index
TGD	Technical guidance document
TTC	Threshold of toxicological concern
US EPA	United States Environmental Protection Agency
US NTP	United States National Toxicology Program
WoE	Weight of evidence

GLOSSARY [1]

Allergy: A clinical manifestation of a state of hypersensitivity. Allergy is defined classically as an antigen-specific altered reactivity of the host to antigen.

Antigen: Foreign material which can induce an immune response.

Allergic contact dermatitis: A localised rash or irritation of the skin caused by contact with an allergen.

Benchmarking: Comparing the potency of new allergens with other allergens of known potency that have been used in similar product types without inducing skin sensitisation.

Contact allergen: An antigen capable of inducing allergic reactions in the skin.

Dermal / skin irritation: The production of reversible inflammatory changes in the skin following the application of a substance.

Dermal / skin sensitisation: Chemically induced allergic reaction affected by repeated topical contact to the skin.

Dermal / skin sensitisation threshold: Amount of allergen per square centimetre of skin inducing an immune response.

Dorsum: The back (or, the aspect of an anatomical part corresponding to the back) of animals or humans.

Dose: Total amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub) population.

Dose-response: Relationship between the amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub) population and the change developed in that organism, system, or (sub) population in reaction to the agent.

Draining lymph nodes: Lymph nodes closest to a site where lymphocytes encounter antigen.

EC3 potency value: The amount of chemical required to induce in the LLNA a three-fold increase in lymph node cell proliferation compared with vehicle control values.

[1] The definitions of these generic terms were taken as much as possible from IPCS, 2004.

Exposure: Concentration or amount of a particular agent that reaches a target organism, system, or (sub) population in a specific frequency for a defined duration.

Hazard: Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub) population is exposed to that agent.

Hazard assessment: A process designed to determine the possible adverse effects of an agent or situation to which an organism, system, or (sub) population could be exposed. The process includes hazard identification and hazard characterisation. The process focuses on the hazard, in contrast to risk assessment, where exposure assessment is a distinct additional step.

Hazard characterisation: The qualitative and, wherever possible, quantitative description of the inherent property of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose-response assessment and its attendant uncertainties. Hazard characterisation is the second stage in the process of hazard assessment and the second of four steps in risk assessment.

Hazard identification: The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub) population. Hazard identification is the first stage in hazard assessment and the first of four steps in risk assessment.

Induction (of skin sensitisation): The stimulation of an allergen-specific immune response (i.e. clonal expansion of T lymphocytes) in draining lymph nodes.

Lymph node cell proliferation: Lymphocyte cell division occurring following exposure to a foreign antigen.

Lymphocytes: Cells of bone marrow origin which mature in the thymus and then migrate into blood, lymph and lymphoid tissue. They express antigen receptors and are divided functionally into helper and suppressor/cytotoxic subpopulations.

No observed effect level (NOEL): The maximum tested concentration of a chemical at which no biological effect is found in a toxicological test.

NESIL: No expected sensitisation induction level (sometimes referred to as a no observed effect level (NOEL) or no observed adverse effect level (NOAEL) in skin sensitisation tests.

Prevalence (for skin sensitisation): The ratio of the number of occurrences of a skin sensitisation to the number of exposed people in the population.

Quantitative risk assessment (QRA): A formalised mathematical method for calculating numerical individual or environmental risk level values for comparison with regulatory risk criteria.

R43: Risk phrase 43 for the labelling of chemicals and preparations in the EU that "may cause skin sensitisation by skin contact".

Reference dose: An estimate of a daily exposure to a chemical that is unlikely to cause harmful effects during a lifetime.

Risk assessment: A process intended to calculate or estimate the risk to a given target organism, system, or (sub) population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The risk assessment process includes four steps: hazard identification, hazard characterisation, exposure assessment, and risk characterisation. It is the first component in a risk analysis process.

Sensitisation: Immunological priming resulting from a[n] [immune] response to antigen that may result in allergy upon subsequent exposure to the same related antigen.

Stimulation index (SI): The incorporation of tritiated thymidine into proliferating lymphocytes expressed as disintegrations per minute/lymph node for each experimental group relative to the concurrent vehicle-treated control group.

Topical application / exposure: Application of a substance or preparation to the skin.

Toxicity: Inherent property of an agent to cause an adverse biological effect.

Threshold: Dose or exposure concentration of an agent below which a stated effect is not observed or expected to occur.

Vehicle: Substance (e.g. solvent) in which a test substance is prepared (mixed or solved) for application in a test system.

Weight of evidence: A weight-of-evidence approach considers multiple endpoints like *in vitro*, *in vivo* or human data, as they relate to an overall assessment of whether significant risk of harm exists.

BIBLIOGRAPHY

Aalto-Korte K, Alanko K, Kuuliala O, Jolanki R. 2007. Methacrylate and acrylate allergy in dental personnel. *Contact Dermatitis* 57:324-330.

AISE/HERA. 2002. AISE (Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien): Table of habits and practices for consumer products in Western Europe. Developed with the HERA project in 2002. website: www.heraproject.com.

Api AM, Basketter DA, Cadby PA, Cano MF, Ellis G, Gerberick GF, Griem P, McNamee PM, Ryan CA, Safford R. 2008. Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. *Regul Toxicol Pharmacol* 52:3-23.

Balls M, Hellsten E. 2000. Statement on the validity of the local lymph node assay for skin sensitisation testing. ECVAM Joint Research Centre, European Commission, Ispra, Italy. *ATLA* 28:366-367.

Basketter DA, Gerberick GF, Kimber I, Loveless SE. 1996. The local lymph node assay: a viable alternative to currently accepted skin sensitization tests. *Food Chem Toxicol* 34:985-997.

Basketter DA. 1998. Skin sensitization risk assessment. *Int J Cosmet Sci* 20:141-150.

Basketter DA, Gerberick GF, Kimber, I. 1998. Strategies for identifying false positive responses in predictive skin sensitization tests. *Food and Chemical Toxicology* 36:327-333.

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 Toxicol* 19:261-266.

Basketter DA, Evans P, Gerberick GF, Kimber I. 1999b. Chemical allergy: estimation of potency, thresholds and risk assessment. *Comments Toxicol* 7:79-89.

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 Dermatitis* 42:344-348.

Basketter DA, Gerberick GF, Kimber I. 2001a. Measurement of allergenic potency using the local lymph node assay. *Trends Pharmacol Sci* 22:264-265.

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 Dermatitis* 45:89-94.

Basketter DA, Gerberick GF, Kimber I. 2001c. Skin sensitisation, vehicle effects and the local lymph node assay. *Food Chem Toxicol* 39:621-627.

Basketter DA, Wright ZM, Colson NR, Patlewicz GY, Pease CK. 2002 . Investigation of the skin sensitizing activity of linalool. *Contact Dermatitis* 47:161-164.

Basketter DA, Angelini G, Ingber A, Kern PS, Menné T. 2003. Nickel, chromium and cobalt in consumer products: revisiting safe levels in the new millennium. *Contact Dermatitis* 49:1-7.

Basketter DA, Andersen KE, Liden C, Van Loveren H, Boman A, Kimber I, Alanko K, Berggren E. 2005a. Evaluation of the skin sensitizing potency of chemicals by using the existing methods and considerations for the relevance of elicitation. *Contact Dermatitis* 52:39-43.

Basketter DA, Clapp C, Jefferies D, Safford B, Ryan CA, Gerberick F, Dearman RJ, Kimber I. 2005b. Predictive identification of human skin sensitization thresholds. *Contact Dermatitis* 53:260-267.

Basketter DA, Gerberick GF, Kimber I. 2007. The local lymph node assay and the assessment of relative potency: status of validation. *Contact Dermatitis* 57:70-75.

Basketter DA, Clapp CJ, Safford RJ, Jowsey IR, McNamee P, Ryan CA, Gerberick GF. 2008. Preservatives and skin sensitization quantitative risk assessment. *Contact Dermatitis* 19:20-27.

Betts CJ, Dearman RJ, Heylings JR, Kimber I and Basketter DA. 2006. Skin sensitisation potency of methyl methacrylate in the local lymph node assay: comparisons with guinea-pig data and human experience. *Contact Dermatitis* 55:140-147.

Cockshott A, Evans P, Ryan CA, Gerberick GF, Betts CJ, Dearman RJ, Kimber I, Basketter DA. 2006. The local lymph node assay in practice: a current regulatory perspective. *Human Exp Toxicol* 25:387-394.

Corea NV, Basketter DA, Clapp C, Van Asten A, Marty JP, Pons-Guiraud A, Laverdet C. 2006. Fragrance allergy: assessing the risk from washed fabrics. *Contact Dermatitis* 55:48-53.

Dean JH, Twerdok LE, Tice RR, Sailstad DM, Hattan DG, Stokes WS. 2001. ICCVAM evaluation of the murine local lymph node assay. II. Conclusions and recommendations of an independent scientific peer review panel. *Reg Toxicol Pharmacol* 34:258-273.

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

ECETOC. 1999. Skin sensitisation testing: Methodological considerations. Technical Report No. 78. *European Centre for Ecotoxicology and Toxicology of Chemicals*, Brussels, Belgium.

ECETOC. 2000. Skin sensitisation testing for the purpose of hazard identification and risk assessment. Monograph No. 29. *European Centre for Ecotoxicology and Toxicology of Chemicals*, Brussels, Belgium.

ECETOC. 2003a. Contact sensitisation: Classification according to potency. Technical Report No. 87. *European Centre for Ecotoxicology and Toxicology of Chemicals*, Brussels, Belgium.

ECETOC. 2003b. Contact sensitisation: Classification according to potency: A commentary. Document No. 43. *European Centre for Ecotoxicology and Toxicology of Chemicals*, Brussels, Belgium.

Ehling G, Hecht M, Heusener A, Huesler J, Gamer AO, van Loveren H, Maurer T, Riecke K, Ullmann L, Ulrich P, Vandebriel R, Vohr HW. 2005. An European inter-laboratory validation of alternative endpoints of the murine local lymph node assay: First round / Second round. *Toxicology* 212:60-68 / 69-79.

EU. 1999. Commission Directive 1999/45/EC of the European Parliament and of the Council of 31 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 preparations: (Repealing 88/379/EEC).

EU. 2003. Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances.

EU. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation

(EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

EU. 2008. Guidance on information requirements and safety assessment. Chapter R 7.3. Skin and respiratory sensitisation. ECHA Guidance for the Implementation of REACH. website: reach.jrc.it/docs/guidance_document.

Feldmann RJ, Maibach HI. 1967. Regional variation in percutaneous penetration of 14C cortisol in man. *J Invest Dermatol* 48:181-183.

Felter SP, Robinson MK, Basketter DA, Gerberick GF. 2002. A review of the scientific basis for uncertainty factors for use in quantitative risk assessment for the induction of allergic contact dermatitis. *Contact Dermatitis* 47:257-266.

Friedmann PS, Moss C, Shuster S, Simpson JM. 1983. Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. *Clin Exp Immunol* 53:709-715.

Friedmann PS, Rees J, White SI, Matthews JNS. 1990. Low-dose exposure to antigen induces sub-clinical sensitization. *Clin Exp Immunol* 81:507-509.

Friedmann PS. 2007. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. *Br J Dermatol* 157:1093-1102.

Gerberick GF, Robinson MK. 2000. A skin sensitization risk assessment approach for evaluation of new ingredients and products. *Am J Contact Dermat* 11:65-73.

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

Gerberick GF, Robinson MK, Felter SP, White IR, Basketter DA. 2001b. Understanding fragrance allergy using an exposure-based risk assessment approach. *Contact Dermatitis* 45:333-340.

Gerberick GF, Ryan CA, Kern PS, Dearman RJ, Kimber I, Patlewicz GY, Basketter DA. 2004. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. *Contact Dermatitis* 50:274-288.

Gerberick GF, Ryan CA, Kern PS, Schlatter H, Dearman RJ, Kimber I, Patlewicz GY, Basketter DA. 2005. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. *Dermatitis* 16:157-202.

Gerberick GF, Ryan CA, Dearman RJ, Kimber I. 2007. Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. *Methods* 41:54-60.

Goon AT, Isaksson M, Zimerson E, Goh CL, Bruze M. 2006. Contact allergy to (meth)acrylates in the dental series in southern Sweden: simultaneous positive patch test reaction patterns and possible screening allergens. *Contact Dermatitis* 55:219-226.

Griem P, Goebel C, Scheffler H. 2003. Proposal for a risk assessment methodology for skin sensitization based on sensitization potency data. *Regul Toxicol Pharmacol* 38:269-290.

Hall B, Tozer S, Safford B, Coroama M, Steiling W, Leneveu-Duchemin MC, McNamara C, Gibney M. 2007. European consumer exposure to cosmetic products, a framework for conducting population exposure assessments. *Food Chem Toxicol* 45:2097-2108.

ICCVAM. Interagency Coordinating Committee on the Validation of Alternative Methods. 1999. The Murine Local Lymph Node Assay: A test method for assessing the allergic contact dermatitis potential of chemicals/compounds. The results of an independent peer review evaluation coordinated by the ICCVAM and the NICEATM. NIH Publication No. 99-4494. National Institute of Environmental Health Sciences. website: iccvam.niehs.nih.gov

Idehara K, Yamagishi G, Yamashita K, Ito M. 2008. Characterization and evaluation of a modified local lymph node assay using ATP content as a non-radio isotopic endpoint. *J Pharmacol Toxicol Methods* July-August; 58:1-10.

IPCS. 2004. IPCS Risk Assessment Terminology. *Harmonization Project Document No.1*. World Health Organization, Geneva, Switzerland.

Jowsey IR, Kligman AM, White IR, Goossens A, Basketter DA. 2007. Evidence that two alkyl ester quaternary ammonium compounds lack substantial human skin-sensitizing potential. *Dermatitis* 18:32-39.

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

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

Kimber I, Basketter DA. 1996. Contact sensitisation: a new approach to risk assessment. *Human Ecol Risk Assess* 3:385-395.

Kimber I, Gerberick GF, Basketter DA. 1999. Thresholds in contact sensitization: theoretical and practical applications. *Food Chem Toxicol* 37:553-560.

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, Basketter DA, Ryan CA, Gerberick GF. 2002. The local lymph node assay: Past, present and future. *Contact Dermatitis* 47:315-328.

Kimber I, Basketter DA, Butler M, Gamer A, Garrigue J-L, Gerberick GF, Newsome C, Steiling W, Vohr H-W. 2003. Classification of contact allergens according to potency: Proposals. *Food Chem Toxicol* 41:1799-1809.

Kimber I, Dearman RJ, Basketter DA, Ryan CA, Gerberick GF, McNamee PM, Lalko J, Api AM. 2008. Dose metrics in the acquisition of skin sensitization: thresholds and importance of dose per unit area. *Regul Toxicol Pharmacol* 52:39-45.

Kligman AM. 1966. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. *J Invest Dermatol* 47:375-392.

Kreiling R, Hollnagel HM, Hareng L, Eigler D, Lee MS, Griem P, Dreeßen B, Kleber M, Albrecht A, Garcia C, Wendel A. 2008. Comparison of the skin sensitizing potential of unsaturated compounds as assessed by the murine local lymph node assay (LLNA) and the guinea pig maximization test (GPMT). *Food Chem Toxicol* 46:1896-1904.

Kroes R, Galli C, Munro B, Schilter B, Tran LA, Walker R, Würtzen G. 2000. Threshold of toxicological concern for chemical substances present in the diet: A practical tool for assessing the need for toxicity testing. *Food Chem Toxicol 38:*255-312.

Lalko J, Isola D, Api AM. 2004. Ethanol and diethyl phthalate: Vehicle effects in the local lymph node assay. *Int J Toxicol* 23:171-177.

Lalko J, Api AM. 2008. Citral: Identifying a threshold for induction of dermal sensitization. *Regul Toxicol Pharmacol* 52:62-73.

Lapczynski A, Lalko J, Politano VT, McGinty D, Bhatia S, Letizia CS, Api AM. 2007. Fragrance material review on alpha-iso-methylionone. *Food Chem Toxicol* 45(S1):S280-289.

Lea LJ, Warbrick EV, Dearman RJ, Kimber I, Basketter DA. 1999. The impact of vehicle on assessment of relative skin sensitization potency of 1,4-dihydroquinone in the local lymph node assay. *Am J Contact Dermat* 10:213-218.

Loretz L, Api AM, Barraj L, Burdick J, Davies DeA, Dressler W, Gilberti E, Jarrett G, Mann S, Laurie Pan YH, Re T, Renskers K, Scrafford C, Vater S. 2006. Exposure data for personal care products: Hairspray, spray perfume liquid foundation, shampoo, body wash and solid antiperspirant. *Food Chem Toxicol* 44:2008-2018.

Magnusson B, Kligman, AM. 1970. Allergic contact dermatitis in the guinea pig. CC Thomas, Springfield, IL, USA, pp. 50-56.

McGarry HF. 2007. The murine local lymph node assay: regulatory and potency considerations under REACH. *Toxicology* 238:71-89.

Mehling A, Ball N, Certa H, Eigler D, Esch H, Haux C, Kreiling R, Skirda K, Veenstra G. 2008. Discordant results between the LLNA and guinea pig tests: specificity vs. selectivity. *The Toxicologist* 102:45.

NICEATM / ICCVAM. National Toxicology Program Interagency Centre for the Evaluation of Alternative Toxicological Methods / Interagency Coordinating Committee on the Validation of Alternative Methods. 2008. Validation status of new versions and applications of the Murine Local Lymph Node Assay: A test method for assessing the allergic contact dermatitis potential of chemicals and products. Independent Scientific Peer Review Report. National Institute of Environmental Health Sciences. website: iccvam.niehs.nih.gov

OECD. 1992. Testing Guideline 406. Skin sensitisation. Updated Guideline, adopted 17th July 1992. *Organisation for Economic Co-operation and Development*, Paris, France.

OECD. 2002. Testing Guideline 429. Skin sensitisation: Local lymph node assay. Updated Guideline, adopted 24th April 2002. *Organisation for Economic Co-operation and Development*, Paris, France.

OECD. 2008. Proposal for Revising GHS Chapter 3.4 with respect to strong vs. weak sensitisers: Formal proposal and consequential changes to the GHS. UN/SCEGHS/15/INF.14 draft proposal.

Rastogi SC, Heydorn S, Johansen JD, Basketter DA. 2001. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis* 45:221-225.

Rees JL, Friedmann PS, Matthews JN. 1990. The influence of area of application on sensitization by dinitrochlorobenzene. *Br J Dermatol* 122:29-31.

Ryan CA, Gerberick GF, Cruse LW, Basketter DA, Lea L, Blaikie L, Dearman RJ, Warbrick EV, and Kimber I. 2000. Activity of human contact allergens in the murine local lymph node assay. *Contact Dermatitis* 43:95-102.

Safford RJ. 2008. The dermal sensitisation threshold – a TTC approach for allergic contact dermatitis. *Reg Tox Pharm* 51:155-200.

Schlede E, Aberer W, Fuchs T, Gerner I, Lessmann H, Maurer T. Rossbacher R, Stropp G, Wagner E, Kayser D. 2003. Chemical substances and contact allergy – 244 substances ranked according to allergenic potency. *Toxicology* 193:219-259.

Schneider K, Akkan Z. 2004. Quantitative relationship between the local lymph node assay and human skin sensitization assays. *Reg Toxicol Pharmacol* 39:245-255.

Schnuch A, Uter W, Geier J, Lessmann H, Frosch PJ. 2007. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. *Contact Dermatitis* 57:1-10.

Sköld M, Hagvall L, Karlberg AT. 2008. Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis* 58:9-14.

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.

Steiling W, Vohr HW. 2008. Regulatory Assessment of Skin Sensitisation (Chapter 31). In Roberts MS, Walters KA, eds, *Dermal Absorption and Toxicity Assessment,* Drugs and the Pharmaceutical Sciences; 2nd ed, Vol 177 – Informa Healthcare Inc. New York, NY, USA, pp. 523-535.

Takeyoshi M, Yamasaki K, Yakabe Y, Takatsuki M, Kimber I. 2001. Development of non-radio isotopic endpoint of murine local lymph node assay based on 5-bromo-2'-deoxyuridine (BrdU) incorporation. *Toxicol Lett* Mar 8;119:203-208.

US EPA. 2003. Health Effects Test Guidelines. OPPTS 870.2600 Skin Sensitization.

Vohr HW, Ahr HJ. 2005. The local lymph node assay being too sensitive? *Arch Toxicol* 79:721-728.

Warbrick EV, Dearman RJ, Basketter DA, Kimber I. 1999. Influence of application vehicle on skin sensitization to methylchloroisothiazolinone/methylisothiazolinone: an analysis using the local lymph node assay. *Contact Dermatitis* 41:325-329.

Warbrick EV, Dearman RJ, Basketter DA, Kimber I. 2000. Failure of vehicle to influence local lymph node assay response to benzocaine. *Contact Dermatitis* 42:164-165.

White SI, Friedmann PS, Moss C, Simpson JM. 1986. The effect of altering area of application and dose per unit area on sensitization by DNCB. *Br J Dermatol* 115:663-668.

Wright Z, Basketter DA, Blaikie L, Cooper KJ, Warbrick EV, Dearman RJ, Kimber I. 2001. Vehicle effects on skin sensitizing potency of four chemicals: assessment using the local lymph node assay. *Int J Cos Sci* 23:75-83.

Yamano T, Shimizu M, Noda T. 2005. Quantitative comparison of the results obtained by the multiple-dose guinea pig maximization test and the non-radioactive murine local lymph-node assay for various biocides. *Toxicology* 211:165-75.

Yamashita K, Idehara K, Fukuda, N, Yamagishi, G, Kawada N. 2005. Development of a modified local lymph node assay using ATP measurement as an endpoint. *Alternatives to Animal Testing and Experimentation* 11:136–144.

Zhai H, Maibach HI. 2001. Effects of skin occlusion on percutaneous absorption: an overview. *Skin Pharmacol Appl Skin Physiol* 14:1-10.

MEMBERS OF THE TASK FORCE

S. Loveless (Chairman)	DuPont USA - Newark, DE
A.-M. Api	RIFM USA - Woodcliff Lake, NJ
R. Crevel*	Unilever UK - Sharnbrook
E. Debruyne	Bayer CropScience F - Sophia Antipolis
A. Gamer	BASF AG D - Ludwigshafen
I. Jowsey**	(then) Unilever UK - Sharnbrook
P. Kern	Procter and Gamble Eurocor B - Strombeek-Bever
I. Kimber	University of Manchester UK - Manchester
L. Lea	Givaudan UK - Ashford
P. Lloyd	SC Johnson UK - Camberley
Z. Mehmood	Huntsman B - Everberg
W. Steiling	Henkel KGAA D - Düsseldorf
G. Veenstra +	(then) Shell Chemicals NL - The Hague
M. Woolhiser	Dow Chemical Company USA - Midland, MI
C. Hennes	ECETOC B - Brussels

* as of January 2008 (replacing Ian Jowsey). ** until December 2007 due to job change.

MEMBERS OF THE SCIENTIFIC COMMITTEE

J. Doe (Chairman) Head of Product Safety	Syngenta UK - Bracknell
R. Bars Team Leader, Toxicology Research	Bayer CropScience F - Sophia Antipolis
P. Calow Professor, Department of Environmental, Social and Spatial Change	Roskilde University DK - Roskilde
W. de Wolf Director of Health and Environmental Sciences - Europe	DuPont B - Brussels
D. Farrar* Occupational Health Business Manager	Ineos Chlor UK - Runcorn
A. Flückiger Head of Corporate Health Protection	F. Hoffmann-La Roche CH - Basel
H. Greim Professor, Institute of Toxicology and Environmental Hygiene	Technical University München D - München
F. Lewis Head of Environmental Safety	Syngenta UK - Bracknell
G. Malinverno Governmental and Public Affairs Manager	Solvay B - Brussels
S. Marshall Environmental Science leader	Unilever UK - Sharnbrook
C. Money Industrial Hygiene Adviser	ExxonMobil B - Machelen
D. Owen Regulatory and Science Issues Manager	Shell Chemicals UK - London
M. Pemberton Global Product Integrity Manager	Lucite International UK - Wilmslow
C. Rodriguez Principal Toxicologist	Procter & Gamble Eurocor B - Strombeek-Bever
G. Swaen Epidemiologist	Dow Chemical Company NL - Maastricht

D. Salvito Environmental Scientist	RIFM USA - Woodcliff Lake, NJ
J. Tolls Director of Environmental Safety Assessment	Henkel D - Düsseldorf
S. van der Vies Professor of Biochemistry	Vrije Universiteit Amsterdam NL - Amsterdam
B. van Ravenzwaay * Director, Experimental Toxicology and Ecology	BASF AG D - Ludwigshafen
E. von Keutz Vice President, Head of Toxicology	Bayer Healthcare D - Wuppertal
H.-J. Wiegand Chemical Environment, Safety, Quality Manager	Evonik D - Essen

* Stewards responsible for primary peer review.