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Potency values from the local lymph node assay: Application to classification, labelling and risk assessment

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Dedication: The authors dedicate this paper to Drs. Armin Gamer and Gauke Veenstra. We will miss them as trusted friends and esteemed colleagues.

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ABSTRACT

Hundreds of chemicals are contact allergens but there remains a need to identify and characterise accurately skin sensitising hazards. The purpose of this review was fourfold. First, when using the local lymph node assay (LLNA), consider whether an exposure concentration (EC3 value) lower than 100% can be defined and used as a threshold criterion for classification and labelling. Second, is there any reason to revise the recommendation of a previous ECETOC Task Force regarding specific EC3 values used for sub-categorisation of substances based upon potency? Third, what recommendations can be made regarding classification and labelling of preparations under GHS? Finally, consider how to integrate LLNA data into risk assessment and provide a rationale for using concentration responses and corresponding no-effect concentrations. Although skin sensitising chemicals having high EC3 values may represent only relatively low risks to humans, it is not possible currently to define an EC3 value below 100% that would serve as an appropriate threshold for classification and labelling. The conclusion drawn from reviewing the use of distinct categories for characterising contact allergens was that the most appropriate, science-based classification of contact allergens according to potency is one in which four sub-categories are identified: 'extreme', 'strong', 'moderate' and 'weak'. Since draining lymph node cell proliferation is related causally and quantitatively to potency, LLNA EC3 values are recommended for determination of a no expected sensitisation induction level that represents the first step in quantitative risk assessment.

1. Introduction

Knowledge of the relative skin sensitising potency of contact allergens is of considerable importance for a proper risk warning and risk management. 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 amount of chemical (dose per exposed skin area) that is required to induce skin sensitisation. For this reason, potency should be given due weight in a proper risk assessment in order to institute the appropriate degree of protection and effective risk management.

In recent years, ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) has made significant efforts to address

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the key aspects of skin sensitisation hazard identification and characterisation, in particular with respect to the design, application and interpretation of the methods available for this purpose. 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 (ECETOC, 1999, 2000, 2003a,b) and in the scientific literature (Steiling et al., 2001; Kimber et al., 2001, 2003). These reports and publications reviewed and discussed the use of both principal test methods, i.e. those employing the guinea pig as the test species (the occluded patch test of Buehler and the guinea pig maximisation test) and the murine local lymph node assay (LLNA) for skin sensitisation hazard identification and characterisation.

This paper focuses solely on the LLNA. A key objective was to determine whether an EC3 value (effective concentration for a stimulation index [SI] of 3 in proliferation of lymph node cells) derived from the LLNA can be used to provide a cut-off criterion for the classification and labelling of both individual substances and preparations as a skin sensitiser, according to the Globally Harmonised System (GHS; UN, 2007) and Directives 67/548/EEC (EEC, 1967) and 99/45/EC (EU, 1999). In addition, the current use of LLNA data in risk assessment approaches for skin sensitisation was evaluated and a rationale, which takes into account potency considerations, is proposed for using concentration responses and corresponding no-effect concentrations (ECETOC, 2008).

2. Background

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 presented 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, 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 in the USA for substances by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) (Dean et al., 2001; ICCVAM, 1999), 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. The test concentration causing a threefold increase of lymph node cell proliferation compared to the vehicle control is called the EC3 value (effective concentration).

As reported elsewhere (Kimber et al., 2002; McGarry, 2007), modified protocols with alternative endpoints are being developed and evaluated. Attention has focused 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 et al. (2005), Ehling et al. (2005), Yamano et al. (2005) and Idehara et al. (2008), but in each case there remains a need for validation and acceptance by regulatory authorities. More recently, NICEATM/ICCVAM convened a scientific peer review panel to review three non-radioactive versions of the LLNA (NICEATM/ICCVAM, 2009). The Panel evaluated the validation status of each of the proposed alternative test methods according to established Federal and international criteria. The Panel also commented on draft ICCVAM recommendations regarding the usefulness and limitations of each proposed test method. The Panel agreed that two of the non-radioactive LLNA procedures could be used to identify substances as potential skin sensitisers or non-sensitisers with certain limitations. The Panel supported the third method using flow cytometry, contingent upon demonstration of adequate inter-laboratory reproducibility in at least two other laboratories. ICCVAM will consider the Panel's report and comments from the public and its scientific advisory committee as it develops final recommendations for Federal agencies. Acceptance by Federal agencies of non-radioactive versions of the LLNA should permit more widespread use of the LLNA, which will further reduce and refine animal use compared to the traditional test methods that use guinea pigs. The reduced use of radioactive materials will also provide environmental advantages.

Substances are classified as being skin sensitisers if, at any test concentration (up to and including 100%), they induce a SI 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, which reflects the amount of chemical (dose) 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 vary by up to four or five orders of magnitude with respect to their relative ability to induce skin sensitisation. In theory, exposure to only very low concentrations of strong contact allergens is required to cause skin sensitisation. In contrast, much higher concentrations of weak contact allergens are needed for the induction of skin sensitisation. Recognition of these differences is of pivotal importance in developing accurate risk assessments and recommendations for adequate risk management.

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 proliferation is causally and quantitatively associated with the effectiveness with which skin sensitisation will be acquired. Consequently, the overall vigour of lymphocyte proliferation 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 an EC3 value from dose–responses in the LLNA (Kimber and Basketter, 1996; Basketter et al., 1999a). The units of EC3 can be expressed as the percentage concentration of test chemical required (easily translated into a molar value) or as dose required for induction per unit area of skin (Kimber et al., 2002). This approach is now well established (Basketter et al., 2007). Typically, EC3 values are derived by linear interpolation between one SI value above 3 and one below 3. In cases where all SI values exceed a threefold increase compared to concurrent controls, the EC3 value may be calculated by log-linear interpolation using the two lowest test concentrations, provided the lowest SI value approaches the value of 3 and that a linear dose–response relationship exists (Gerberick et al., 2004).

Relative potency categorisation based on derived EC3 values has proven to be of value with a wide range of chemical classes, and provides 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 from the work of a previous ECETOC Task Force were described in an ECETOC Technical Report (2003a) and in a subsequent publication (Kimber et al., 2003) derived 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*' based on thresholds defined by specific, 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.

The implication of 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, the following questions have been addressed:

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. Thus, in effect any measurable EC3 value, up to and including 100%, triggers classification of a chemical as a skin sensitiser. The specific question addressed 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 50% or 75% rather than 100%, may more accurately distinguish between relevant skin sensitising substances that warrant an R43 *May Cause Sensitisation by Skin Contact* 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 is 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.

A third issue addressed was what recommendations can now be made with regard to the upcoming classification and labelling of preparations under GHS.

The final issue addressed was whether LLNA EC3 values can be recommended for determination of a no expected sensitisation induction level (NESIL), as a first step in a quantitative risk assessment.

Table 1
Sub-categorisation of contact allergens on the basis of relative skin sensitisation potency. (Adapted from ECETOC (2003a,b).)

Category	EC3[a] values (%)
Extreme	<0.1
Strong	⩾0.1 to <1
Moderate	⩾1 to <10
Weak	⩾10 to ⩽100

[a] EC3 values are defined as the amount of chemical required to induce a threefold increase in lymph node cell proliferation compared to vehicle control values.

3. Hazard identification using the LLNA

Since the validation of the LLNA by ICCVAM (ICCVAM/NICEATM, 1999; 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 and recommended in preference to other OECD guideline tests, i.e. the guinea pig maximisation 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 in this species (EU, 2008).

Against this background it is relevant 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.

However, it is important to acknowledge that the LLNA, like any predictive test method, can only produce accurate results within its domain of applicability and when adequately performed. Consequently, with increasing use, and in particular with increasing experience with a wider range of chemistries, 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 such chemical substances may produce somewhat misleading results in the LLNA, 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 other 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.

Very recently, NICEATM/ICCVAM convened a scientific peer review panel to review the applicability of the LLNA for testing pesticide formulations and other products (NICEATM/ICCVAM, 2009). The Panel evaluated the representative data for such test materials according to established Federal and international criteria. The Panel concluded that the LLNA could be used to test any material for allergic contact dermatitis potential, including pesticides and substances such as fragrances and dyes, unless a substance has properties expected to interfere with the conduct or accuracy of the assay. ICCVAM will consider the Panel's report and comments from the public and its scientific advisory committee as it develops final recommendations for Federal agencies. Acceptance by Federal agencies of broader material applications should permit more widespread use of the LLNA, which will further reduce and refine animal use compared to the traditional test methods that use guinea pigs.

4. Hazard characterisation and classification of substances and preparations according to 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).

Given that selected chemicals reported to be skin sensitisers in humans have EC3 values >50% (summarised above), the available evidence 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 the authors 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, currently this information has rarely been used in a regulatory capacity as a basis for classification and labelling of potential sensitisers. The European Scientific Committee on Consumer Products (SCCP) has recently applied the principle of using individual skin sensitisation potency of certain cosmetic ingredients for discrimination of sensitisation hazard (EU, 2007). Skin sensitisation classification and labelling is currently binary in nature, i.e. substances are considered as sensitising or non-sensitising (Directive 67/548/EEC; EEC, 1967). 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 that risk is widely recognised as preferable to approaches based solely on consideration of hazard. Thus, failure to take into account potency in the development of risk management practices, such as classification and labelling, impairs resulting decisions, since it does not make use of all the available information. This current approach potentially results in the imposition of disproportionate risk management measures, without any concomitant decrease in the risk to public health. 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 (i.e. overlabelling) will ultimately be self-defeating and might result in less effective risk management and protection of human health.

In contrast to the LLNA, guinea pig tests are not designed to provide hazard characterisation data of comparable quality. In the standard guinea pig models of OECD TG 406 (OECD, 1992), only single concentrations are tested for induction and elicitation within a given study design, and the overall conclusion (test outcome) is dependent on the number of treatment-related responses. Due to the subjective nature of quantification of skin reactions in dose range-finding studies and lower elicitation concentrations, such test design is considered inaccurate 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, the authors have given additional consideration to the characterisation and classification of substances, particularly using LLNA results.

4.1. Classification and labelling of substances – based on their potency categorisation

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. Not all contact allergens have the same ability to cause skin sensitisation. For example, the weak sensitiser methyl methacrylate (MMA) does not carry the same risk for sensitisation as the extreme sensitiser isothiazolinone, despite comparatively higher levels of occupational exposure for MMA (Betts et al., 2006).

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., 2001b, 2005a; Gerberick et al., 2001a). This concordance supports the use of categorisation schemes based on EC3 values for the purposes of classification and labelling and, in consequence, of risk management. 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 like the (Material) Safety Data Sheet ((M)SDS). More potent allergens are managed differently from those substances which are sensitising but whose potency is very low. With such specific information, the accuracy and value 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 sensitisat-

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

Chemical	EC3 (%)	Human sensitiser
Aniline	89[a]	Yes[c]
Diethylacetaldehyde	76[a]	No
DMSO	72[a]	No
R(+)-Limonene	68[a]	Yes[c,e]
Methylmethacrylate	60 (Acetone); 90 (AOO)[b]	Yes[b]
Pyridine	72[a]	Yes[d]

[a] Gerberick et al. (2005).
[b] Betts et al. (2006).
[c] Schlede et al. (2003).
[d] ICCVAM (1999).
[e] Positive evidence for *R*(+)-limonene in humans could be due to oxidised limonene.

ion reports in humans and may require sustained high level exposure to induce skin sensitisation. 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 four categories (Table 1) 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).

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. 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 (Basketter et al., 2005a). 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 potency. Thus, extreme and strong categories were maintained while *moderate* potential was extended to include all chemicals with EC3 values in the range of 2–100%. The other notable difference between the ECETOC and ECB proposals is that the respective EC3 thresholds for each category were delineated more conservatively (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 on overly conservative labelling of weak sensitisers. Such conservative labelling of weak skin sensitisers, and in particular their impact on mixtures, would result in inappropriate risk management, thereby ‘devaluing the currency’ of risk labels, as already discussed.

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 (ICCVAM, 2008; NICEATM/ICCVAM, 2008). NICEATM considered 170 substances for its assessment and proposed a two-level categorisation scheme (weak and strong). The report concluded that there is a significant positive correlation between LLNA potency and human sensitisation threshold, as also reported by others (Schneider and Akkan, 2004; Basketter et al., 2005b). The report 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.

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 (2003a). Also, the OECD has submitted a proposal to GHS for sub-categorisation of potency (OECD, 2008). To date, however, no regulatory authority has adopted any scheme for potency categorisation for use in classification and labelling.

Currently, classification of substances for sensitisation potential remains 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 threefold or greater increase in lymph node proliferation at a test concentration of 0.5% is classified as a sensitiser in the same way as another chemical that requires a 50% concentration to achieve an SI of 3. Reconsideration of potency characterisation, 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.

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
Methylionene	22	Weak
Linalool	30	Weak
Ethyleneglycol dimethacrylate	35	Weak
Diethanolamine	40	Weak
Isopropyl myristate	44	Weak

Table 4
Comparison of proposed potency classifications.

Potency rating	ECETOC Concentration thresholds (%)	ECB Concentration thresholds (%)
Extreme	<0.1	⩽0.2
Strong	⩾0.1 to <1.0	>0.2 to ⩽2.0
Moderate	⩾1.0 to <10	>2.0
Weak	⩾10	N/A

The recognition of weak sensitisers as a separate category is important and appropriate for those chemicals with high EC3 values ($\geqslant$10%) that have only a limited potential to cause skin sensitisation, even under circumstances where exposure is significant. Two fragrance ingredients, methylionone and citral, illustrate the value of discriminating moderate from weak sensitisers. Methylionone is a skin sensitiser which has a relatively high EC3 value (21.8%), as well as a high threshold for response in a human repeat insult patch test (HRIPT) (Lapczynski et al., 2007), and which is considered to be of low clinical significance (Schnuch et al., 2007). Conversely, citral with an EC3 value of 5.7% (Lalko and Api, 2008) is considered to be an allergen of considerable clinical significance with a report of increased frequency of sensitisation in recent years (Schnuch et al., 2007). Reviews conducted under the auspices of RIFM (Research Institute for Fragrance Materials, Inc.) 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 1000 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 potency of these two ingredients should be recognised as different from one another as by following the scheme of Table 1. Thus, the EC3 values for methylionone (21.8%) and citral (5.7%) 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. greater than 10%), substantiated cases of allergy exist consistent with classification as R43. Another example of a relatively weak skin sensitiser is MMA (methyl methacrylate). 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).

4.2. Classification and labelling of preparations

Under GHS, a preparation is defined as a mixture composed of two or more substances which do not react. This definition is 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 and their concentration in the product are currently used to decide on classification and labelling of a preparation with regard to skin sensitisation potential. According to the former Dangerous Preparation Directive (EU, 1999) and its amendment (EU, 2006), a level of $\geqslant$1% of a skin sensitiser ingredient requires a hazard categorisation of the preparation as a skin sensitisers (R43), irrespective of potency. For a concentration of $\geqslant$0.1%, the skin sensitising substance has to be declared on the label, even when the preparation is not classified as sensitising. Under the current EU GHS and Regulation for classification, labelling and packaging of substances and mixtures (C&L), a mixture has to be classified as a *Category 1* skin sensitiser when it contains a classified ingredient at concentrations $\geqslant$1.0%. When a mixture contains an individual skin sensitising ingredient at $\geqslant$0.1%, the mixture description must state: "Contains Substance X – May produce an allergic reaction" From this perspective, the reliability of substance data is essential in the evaluation of preparations.

4.2.1. Classification and labelling of preparations – based on the potency of their individual constituent substances

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).

In light of new evidence, the cut-off values and their rationales recommended by the previous Task Force (ECETOC, 2003a) were re-examined. 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 if extreme sensitisers are used in preparations, 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 they also required a lower value than the current 1% default. 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 logarithmic 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. (2003), the following scheme of potency-based cut-off values is defined (Table 5).

This scheme provides guidance for effective characterisation of hazards of preparations and limits the need for testing of preparations in animals (where permissible). 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 subcategorisation 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

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

Potency	Concentration limit of sensitising ingredient present in solid and liquid preparation (% w/v)
Extreme	0.003
Strong	0.1
Moderate	1.0
Weak	3.0

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 Section 4.2.3.

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 (M)SDS of each ingredient. The (M)SDS, which is required by regulation, provides the most appropriate vehicle for provision of such important information.

4.2.2. 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 its potency (Table 6).

4.2.3. 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 of similar composition. For such a 'bridging process', the chemical composition (chemical structure and concentration) of both the untested and tested reference 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.

4.3. Translating potency classification into risk management of preparations

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

Table 6
Scheme of potency-based sub-categories for substances and tested preparations.

Potency	Sub-category
Extreme	1a
Strong	1b
Moderate	1c
Weak	1d

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 percent content and sensitisation potency of their individual 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.

5. Exposure considerations

Correct data on human exposure to a substance, both in 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. 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, 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 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 and, depending on the product, its intended dilution during application (e.g. shower gel).
- *The dose per unit area* (a key parameter for the induction of sensitisation (Kimber et al., 2008)). An estimate of the area of skin exposed and the amount of substance coming into contact with this skin area are therefore crucial for a proper exposure assessment.

Exposure scenarios can be described 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, companies often use 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 Personal Care Products Council (formerly known as Cosmetics, Toiletry, and Fragrance Association

(CTFA)) and in the EU Technical Guidance Document (Loretz et al., 2005, 2006, 2008; Hall et al., 2007; AISE/HERA, 2002; EU, 2003). A conservative approach towards exposure assessment is recommended.

6. 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 other 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. Also described is how LLNA EC3 values are used in this context.

6.1. Dose metric for skin sensitisation

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, it has been reported 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.

6.2. 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 use considerations.
(3) Calculation of an acceptable exposure level (AEL) by dividing the NESIL by the product of the 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 comparison is depicted in Fig. 1. The AEL can either be lower, equal or larger than the CEL. The two green boxes at the end of this figure refer to acceptable exposure scenarios, whereas the red box denotes unacceptable exposure scenarios.

6.2.1. No expected sensitisation induction level (NESIL)

The NESIL 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 is a benchmark that is derived from animal or human data, based on no observed effect levels or no observed adverse effect levels. 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

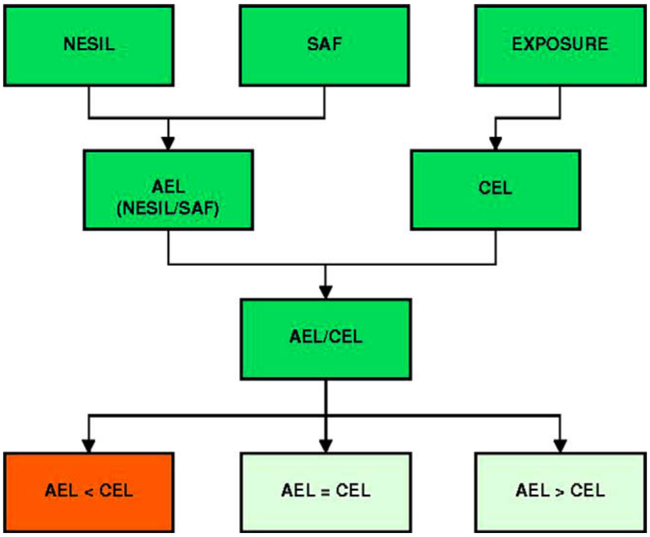

Fig. 1. Key steps of the QRA process. NESIL (no expected sensitisation induction level) is the predicted dose threshold for the induction of skin sensitisation in humans. SAF (sensitisation assessment factors) represent uncertainties associated with inter-individual variability, matrix differences and exposure considerations. AEL (acceptable exposure levels) are calculated by dividing the NESIL by the product of the three SAFs. The AEL is then compared to the CEL (consumer exposure level), which results in acceptable risk if the AEL is equal or larger than the CEL. If the CEL exceeds the AEL, reevaluation of the risk management would be required. (adapted from Api et al. (2008))

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 humans. 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; Api et al., 2009). 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 μg/cm^2. Similarly, the EC3 values for the same chemicals were obtained and expressed as dose per unit area (range 2.25–8250 μg/cm^2). As expected, regression analysis revealed a linear relationship between the two variables (Fig. 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.

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.

6.2.2. Sensitisation assessment factors

Having established a NESIL [in μg/cm^2], 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 use 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 (Fig. 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, which include known penetration enhancers or irritants. 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 $\leqslant 10$ 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 use considerations SAF (3rd SAF). Variables implicit in this

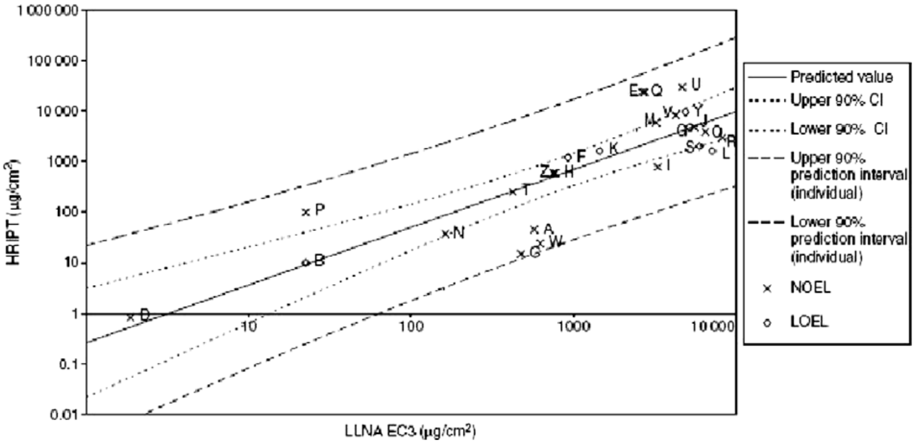

Fig. 2. Correlation between EC3 values and human skin sensitisation thresholds. Linear regression analysis of log human repeated insult patch test (HRIPT) no observed effect levels (NOELs) versus log local lymph node assay (LLNA) EC3. The compounds corresponding to the letters in this figure are given in Basketter et al. (2005b). CI, confidence interval. (from Basketter et al. (2005b))

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 use considerations SAF is also $\leqslant 10$, 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 use considerations SAFs. It is important that the expert judgement is supported by scientific evidence. 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).

6.2.3. 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.

6.2.4. 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 Fig. 3.

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.

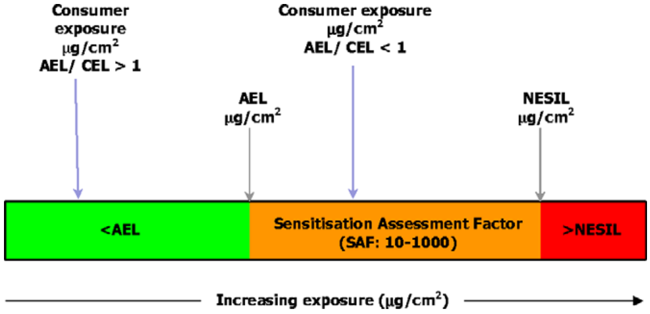

Fig. 3. Exposure lines. An example of a comparison of the CEL, the AEL and the NESIL for a sensitising chemical in a consumer product. Two different consumer exposure scenarios to the chemical are described which lead to an acceptable risk, where the AEL > CEL, or an unacceptable risk if the AEL < CEL. (adapted from Basketter et al. (2003))

6.3. 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 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. A more recent example of the implementation of the QRA method for skin sensitisation of a fragrance ingredient is the application to citral in which the AEL was established for 10 different types of cosmetic, household and personal care product categories (Api and Vey, 2008).

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 range 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 $\leqslant 1$). 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 threshold 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 (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 may not apply to 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 safety.

6.4. Risk assessment 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 four potency classes, which have been developed 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. 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.

7. Overall conclusions

(1) Although skin sensitising chemicals having high EC3 values may represent only relatively low risks to humans, it is not possible currently to define an EC3 value below 100% that would serve as an appropriate threshold for classification and labelling.

(2) After reviewing the use of distinct categories for characterising contact allergens, the most appropriate, science-based classification of contact allergens according to potency is one in which four sub-categories are identified: ‘extreme’, ‘strong’, ‘moderate’ and ‘weak’.

(3) Consistent with a potency related classification of substances, preparations should be classified accordingly under GHS and C&L for mixtures. As preparations other than pesticide formulations are rarely tested experimentally to categorise them in one of the four proposed sub-categories of potency, individual cut-off values based upon ingredient potency impact become relevant, in order to classify properly preparations as Category 1 skin sensitisers under the current GHS and C&L regulation. Such a classification scheme is likely to ensure a better hazard-based handling and management of preparations.

(4) Since draining lymph node cell proliferation is related causally and quantitatively to potency, LLNA EC3 values are recommended for determination of a no expected sensitisation induction level that represents the first step in quantitative risk assessment.

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