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Use of the local lymph node assay for the estimation of relative contact allergenic potency

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The effective toxicological evaluation of skin sensitization demands that potential contact allergens are identified and that the likely risks of sensitization among exposed populations assessed. By definition, chemicals which possess the toxicological property of skin sensitization potentially are capable of causing allergic contact dermatitis (ACD) in humans. However, this hazard is not an all-or-none phenomenon; clear dose-response relationships can be discerned and thresholds identified for both the induction of sensitization and the elicitation of contact dermatitis. Commonly, these parameters are grouped under the heading of potency, determination of which is vital for risk assessment. In the present investigation, the local lymph node assay (LLNA) has been employed to determine the relative potency of a range of 20 chemicals. The parameter used is the estimated concentration required to produce a 3-fold increase in draining lymph-node cell proliferative activity, the EC3 value. These measurements have been compared with an assessment of the human sensitizing potency of the 20 selected chemicals, each being assigned to 1 of 5 classes based on their human sensitizing potency. The EC3 value, derived from LLNA work carried out in acetone/olive oil vehicle, correlated well with the human classification, with the strongest sensitizers having low EC3 values (<0.1%), weaker sensitizers having EC3 values generally in the 1–10% range, and non-sensitizing chemicals having EC3 values in excess of 100%. In conclusion, the derivation of the EC3 for a chemical provides an objective and quantitative estimate of potency that is of considerable utility for skin sensitization risk assessment.

Key words: contact allergy; allergic contact dermatitis; sensitization potential; skin sensitization risk assessment; local lymph node assay; mouse sensitization.

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For both the evaluation of new chemical entities in terms of their intrinsic toxicological properties and for the safety assessment of both new and existing chemicals in novel use situations where skin contact is a possibility, the evaluation of the potential to cause allergic contact dermatitis (ACD) represents an important consideration. Effective evaluation of safety relies not only upon hazard identification, but also demands an accurate assessment of likely risk to human health. The processes involved in skin sensitization risk assessment have been described in some detail elsewhere (1–3). Principally they involve 2 basic elements; firstly, a knowledge of the sensitizing potency of the contact allergen and, secondly, the likely dose, nature, extent and duration of skin exposure. Given that ACD continues to be a frequent cause of disease, it is reasonable to consider in what ways the quality of the safety assessment processes may be improved. Most often, the weak link in the development of a quantitative risk assessment for a contact allergen is objective information regarding its relative potency compared with other skin sensitizers (4, 5). Typically, the considerations of likely exposure do not represent a substantial challenge to the safety assessor, since use patterns for a wide range of products in both a consumer and an occupational setting are well-established (4, 6). Such knowledge incorporates an understanding not only of normal use, but also of anticipated misuse of products. Consequently, the degree of skin exposure can be characterized, although the way the

data are employed and expressed could be improved (7).

However, for assessment of the likely risk such hazards pose to human health, an appreciation is necessary not only of the anticipated exposure but also the intrinsic potency of the chemical allergen. Sensitizing potency in this context is best described as a function of the amount of chemical that is required to induce contact sensitization in a previously naive subject or animal, and on this basis it has been estimated that chemicals vary very significantly in terms of their intrinsic allergenic activity (2, 3). Despite the importance of potency estimation in the development of accurate risk assessments, there has been relatively modest progress in the definition of appropriate experimental models. The standard guinea pig tests, such as the maximization test, have been very successful at hazard identification (8, 9), and there has been some interest in the use of a modified guinea pig maximization test for consideration of relative potency. Of particular note have been the efforts of Andersen et al. (10), who have manipulated the guinea pig maximization test in order to obtain dose-response data. However, the development of a novel predictive assay in the mouse, the local lymph node assay (LLNA) (11–14), provides new opportunities for the objectve and quantitative estimation of skin sensitization potency (15, 16). For the purposes of hazard idenfication, activity in the LLNA is measured as a function of proliferative responses induced in draining lymph nodes by test chemicals, those chemicals that provoke a 3-fold or greater increase in lymph node cell proliferation, compared with vehicle controls, being classified as potential contact allergens. This method has more recently been applied to determination of relative potency, comparisons between chemicals being based on the mathematical derivation of an EC3 value, this being the estimated concentration of chemical necessary to cause a 3-fold increase in proliferative activity. Experience to date with this approach has been encouraging; clear differences between skin sensitizing chemicals can be discerned and such differences appear to correlate with the ability of the materials to induce contact allergy in experimental models and with what is known of sensitizing activity among humans (17, 18). It is this latter correlation that is of greatest moment in evaluating the accuracy of relative potency determinations made using the LLNA and the utility of these in the risk assessment process. For this reason we have, in the present investigations, compared potency rankings derived using the LLNA with clinical assessments of the relative sensitizing activity in humans of a number of chemicals.

Materials and Methods

Estimation of relative skin sensitization potency in man

There are no well-defined or widely applied methods for the determination of relative skin sensitization potency in humans. In consequence, in the present investigation, recourse has been made to the limited but valuable published literature on human predictive testing, both the human maximization test and the human repeat insult patch test (19–23). To this has been added expert judgement based on decades of accumulated experience with clinical allergic contact dermatitis. Using this approach, we have taken a view on some 20 chemicals which have been categorized into 5 discrete classes of sensitizing potency. Class 1 contained the strongest allergens, classes 2 and 3 more moderately and weakly sensitizing chemicals, class 4 chemicals which were judged to be of very limited sensitizing potency (and thus clinically rare allergens) and finally class 5 representing chemicals devoid of skin sensitizing activity. The outcome of these deliberations (which are based on judgement, not on easily defined algorithms) is contained in Table 1. Within each class, the chemicals have simply been arranged in alphabetical order.

Table 1. Human potency estimation

	LLNA EC3 value[a)]	Ref(s)
Human class 1		
(chloro)methylisothiazolinone	0.05%	34
dinitrochlorobenzene (DNCB)	0.08%	26
diphencyclopropenone	0.05%	unpublished
p-phenylenediamine	0.06%	28
Human class 2		
cinnamic aldehyde (cinnamal)	2.0%	36
glutaraldehyde	0.2%	17
isoeugenol	1.3%	26
tetramethylthiuram disulfide	6.0%	14
Human class 3		
citral	13%	36
eugenol	13%	26
hexyl cinnamic aldehyde	8.0%	26, 27
hydroxycitronellal	20%	36
Human class 4		
ethyleneglycol dimethacrylate	35%	14
isopropyl myristate	44%	24
propyl paraben	>50%	14
propylene glycol	non-sensitizing	37
Human class 5		
glycerol	non-sensitizing	24
hexane	non-sensitizing	37
diethyl phthalate	non-sensitizing	24
tween 80	non-sensitizing	38

[a)] EC3 values obtained from tests conducted in AOO vehicle.

LLNA protocol and chemicals tested

The LLNA was conducted as described elsewhere (12, 14, 24). The data reported in the present investigation are derived very largely from existing published studies. Representative references for the sources of data for each of the chemicals discussed in the present work are given in Table 1. However, it should be recognized that the derived EC3 values have been taken from a consideration of all of the laboratories' (sometimes extensive) experience with these chemicals, some of which is currently unpublished.

Potency estimation in the LLNA

The approach to the estimation of relative skin sensitization potency of chemicals in the LLNA has already been outlined previously (15, 25, 26). It involves the mathematical estimation of the dose of chemical necessary to obtain a threefold stimulation of proliferative activity in draining lymph nodes (compared with concurrent vehicle treated controls), and is thus termed the EC3 value. The stability of this measure has already been demonstrated (27), as has its interlaboratory reproducibility (26, 28). Preliminary investigations have also indicated that it might represent a useful correlate of skin sensitization potency in humans (17, 18, 29). In this present work, existing data on 20 chemicals evaluated in the LLNA in a standard vehicle system (acetone/olive oil, 4:1, v/v; AOO) have been used to derive EC3 values (Table 1). The dose-response data used met the quality criteria outlined elsewhere (25). The calculation of the EC3 values was carried out according to the equation

$$EC3 = c + [(3-d)/(b-d)] \times (a-c)\,,$$

where the data points lying immediately above and below the SI value of 3 on the LLNA dose response plot have the co-ordinates (a, b) and (c, d), respectively.

Results

Derived EC3 values for each of the 20 chemicals considered here are displayed in Table 1. For each of the 4 chemicals judged to be potent skin sensitizers in man (Class 1), EC3 values derived from the analysis of LLNA dose response data were $<0.1\%$ (in AOO vehicle). The lowest EC3 values was obtained for (chloro)methylisothiazolinone (0.05%), DNCB (0.08%), p-phenylenediamine (0.06%) and diphencyclopropenone (0.05%), which appeared to possess very similar potency in the LLNA when tested in AOO. 3 of the 4 chemicals assigned to class 2 had EC3 values in the range 1–10%, with glutaraldehyde the most potent with an EC3 of 0.2%, followed by isoeugenol at 1.3%. The 4 chemicals assigned to class 3 were all positive in the LLNA, and had relatively high EC3 values, consistent with their apparently lower, but still significant, skin sensitizing potency in man. Of these, hexyl cinnamic aldehyde was the most potent, with an EC3 of 8%. Class 4 contained the chemicals, such as isopropyl myristate, which would be regarded as extremely weak sensitizers (and consequently rare contact allergens). Materials in this class normally are not regarded as significant skin sensitizers, thus, for example, they would not be classified formally under European regulations as a significant skin sensitizer (30). Consequently, they might not be expected to be positive in the LLNA (13). Nevertheless, 2 substances in fact had calculable, although very high, EC3 values of $\geqslant 35\%$. Finally, the 4 chemicals in class 5 were all regarded as non-sensitizing in humans. All of these chemicals were without activity in the LLNA (despite being tested at very high concentrations), with the consequence that EC3 values would be $>100\%$.

Discussion

Although it has been possible for at least 3 decades to identify chemicals with significant skin sensitization potential using well-defined guinea pig methodologies, this has not led to an obvious reduction in the incidence of the clinical condition, allergic contact dermatitis (31). One reason why this may have occurred is that, in order to undertake proper safety evaluation, it is necessary to have information on the relative potency of a potential skin sensitizer (2, 4, 15). Such information is difficult to obtain from standard guinea pig tests (10, 16, 32). In particular, the endpoint in all guinea pig tests is the frequency of sensitization judged via the subjective assessment of challenge-induced erythema. Furthermore, dose levels for induction and elicitation are based on the irritant potential of the test substance and as a consequence vary widely. However, the development of the LLNA has provided for the 1st time a real opportunity to combine hazard identification with the quantitative estimation of sensitizing potency. Each LLNA involves the production of a dose-response curve using test concentrations which are independent of the irritation potential of the test substance. The endpoint assessed is both objective and quantitative. Simple mathematical interpolation of the LLNA dose-response data can then yield a quantitative estimate of the concentration of test chemical necessary to produce a threshold positive response (15, 16, 25). Importantly, the measure is

stable with time (27) and is reproducible both within (28) and between (26, 28) laboratories. However, it is vital to recognize that it is not an absolute value with direct meaning for man; it does not relate directly to the induction threshold in humans. Rather, it provides information that enables a comparison of the allergenic potency of one potential sensitizer with that of another. Importantly, it does this in the context of the vehicle used for the epicutaneous exposure route employed in the LLNA (33, 34). This represents an important advantage to the risk assessor, in that the derived EC3 values are specific for not only the chemical, but also for the vehicle matrix employed, lending further sophistication to the utility of this information for risk assessment purposes.

The work reported here demonstrates that chemicals which are considered to be of high potency in man, and would thus be regarded as strong allergens (class 1), show a similar activity in the LLNA; the dose necessary for the induction of a significant response is, in relative terms, very low (0.01%–0.08%). Allergens deemed to be of lower potency in man (classes 2 and 3) required substantially higher amounts of allergen to cause the induction of skin sensitization. For class 2 materials (moderate allergens), the EC3 values were generally in the region of 1%, whilst for class 3 (weak allergens) they were in the region of 10%. Lastly, the chemicals which would not be classified as skin sensitizers in a regulatory sense (classes 4 and 5) were without great effect in the LLNA, such that it was either difficult, or indeed impossible, to derive EC3 values for these materials. Class 1, 2 and 3 skin sensitizers also might correspond to the 3 appropriate hazard groups (A, B or C) in COSHH essentials (35). Thus, the potency ranking of these 20 chemicals in the LLNA correlated well with their apparent ability to cause skin sensitization in humans.

In summary, the present investigations reported herein demonstrate that the LLNA can be used to provide quantitative estimates of relative skin sensitizing potency (EC3 values) which correlate closely with what is known of the ability of chemicals to cause skin sensitization in humans. Such information is likely to be of considerable value in the development of soundly based risk assessments for (novel) chemicals which possess the intrinsic capacity to behave as a skin sensitizer.

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