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To cite this article: D. A. Basketter, D. Kan-King-Yu, P. Dierkes & I. R. Jowsey (2007) Does Irritation Potency Contribute to the Skin Sensitization Potency of Contact Allergens?, Cutaneous and Ocular Toxicology, 26:4, 279-286, DOI: [10.1080/15569520701555359](https://doi.org/10.1080/15569520701555359)

To link to this article: <http://dx.doi.org/10.1080/15569520701555359>



Published online: 10 Oct 2008.



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DOES IRRITATION POTENCY CONTRIBUTE TO THE SKIN SENSITIZATION POTENCY OF CONTACT ALLERGENS?

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Chemicals that possess the capacity to cause skin sensitization have long been recognized to be reactive (electrophilic) or at least the precursor of an electrophile. The chemical species (haptens) covalently bound to skin protein then forms the antigen to which the immune system responds, with sufficient exposure ultimately leading to skin sensitization. However, for this process to occur, many have also considered that in addition to haptenation of skin protein, secondary stimuli (danger signals) are also necessary. Such signals might reasonably be expected to derive from keratinocytes and/or Langerhans cells perturbed by the chemical sensitizer. Whether this disturbance comes from the haptenation process itself or from other properties of the chemical is unknown. We hypothesized that chemicals that were stronger sensitizers might appear so, in part, as a consequence not only of greater (pro)electrophilic reactivity, but also if they were more able to produce inflammatory (danger) signals. To assess this, the sensitizing potency of 55 chemicals in the local lymph node assay was compared with their ability to produce pro-inflammatory signal release, measured as a function of their relative skin irritancy in guinea pigs. A limited trend was demonstrated, consistent with the hypothesis, but indicating that either skin irritation is a poor measure of danger signals, or that such signals are perhaps no more than a necessary requirement for the acquisition of skin sensitization rather than a key determinant of the relative potency of a skin sensitizing chemical. In addition, it is possible that irritancy alone does not represent a complete surrogate marker for the ability of a chemical to produce danger signals relevant to the induction of skin sensitization.

Keywords: GPMT; Irritant potency; LLNA; Skin sensitization potency

INTRODUCTION

Current views of the mechanism of both induction and elicitation of skin sensitization indicate that irritancy can act as a significant positive co-factor. During the induction of skin sensitization and based on the original hypothesis put forward by Matzinger (1) this is commonly considered to comprise pro-inflammatory “danger signals,” although the precise relationship between irritancy and danger signals is far from clear (e.g., 2,3). During the elicitation phase of skin sensitization, a substantial body of evidence demonstrates that irritancy can enhance responsiveness, effectively lowering the threshold for response (e.g., 4,5). However, the knowledge

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predates these publications. It was very clear from the development of predictive skin sensitization test methods that irritancy was seen as an important cofactor for ensuring the sensitivity of an assay (e.g., 6,7). Indeed, in regulatory tests such as the guinea pig maximization test, skin irritancy determination is dictated in the regulatory guidelines for this assay (8).

A question related to the aforementioned then is what impact irritancy might have on the perceived relative potency of a skin sensitizer? Put another way, are chemicals that have the intrinsic ability to behave as a skin sensitizer, but with greater irritant capacity, likely to be stronger sensitizers? Some years ago, this was addressed in an investigation that compared 50 chemicals and formulations, using the Draize rabbit skin irritation test and the Buehler guinea pig sensitization test (9). Both of these assays offer only crude estimates of irritation and skin sensitization potency, respectively, but nevertheless indicated a weak relationship. However, with the improved measure of skin sensitization potency offered by the local lymph node assay (LLNA) EC3 value (10) and taking the challenge concentration in the guinea pig maximization test as an index of irritancy, there is now an opportunity to examine any potential relationship with greater rigor.

The topic is of more than academic interest. It seems likely that relative ability to produce pro-inflammatory danger signals will be one of the key properties of skin sensitizing chemicals that determines their relative potency. With the move to adopt *in silico* and *in vitro* approaches to the identification of toxicological properties, there is a real risk that loss of the integrated biological measures provided by *in vivo* methods will entail the loss of aspects such as potency estimation. With this in mind, a paradigm for how to integrate the disparate endpoints of *in silico* and *in vitro* assays for skin sensitization has been described (11). The investigation is intended to help underpin the legitimacy of considering *in vitro* skin irritancy endpoints, for example cytokine release by keratinocytes, as potential inputs in an integrated approach to the estimation of skin sensitisation potency that does not require *in vivo* assays, and that forms part of Unilever's efforts to develop novel ways of delivering consumer safety.

MATERIALS AND METHODS

The historical Unilever database containing records of substances evaluated using the local lymph node assay and the guinea pig maximisation test were examined. Instances where substances had been tested in both methods were identified and instances where both a LLNA EC3 value obtained and a standard guinea pig maximization test (GPMT) conducted according to OECD guidelines (8) were documented. The LLNA EC3 value (10) was used as an index of skin sensitization potency, whereas the challenge concentration in the GPMT was deployed as an index of skin irritation potential. Table 1 presents the 55 records of substances meeting these criteria.

For the benefit of those unfamiliar with the test protocols involved, in brief, the LLNA was conducted as described elsewhere and in accordance with OECD Guideline 429 (12). In brief, the protocol used was as follows: groups of 4 CBA/Ca female mice (7–12 weeks of age) were treated topically on the dorsum of both ears with 25 µl

Table 1 Compilation of potency and irritancy data for 55 substances

Chemical name	LLNA EC3 (%)	GPMT challenge (%)
Chloromethylisothiazolinone/Methyl isothiazolinone	0.009	0.1
Benzoquinone	0.01	2.5
Dinitrochlorobenzene	0.05	0.1
Potassium dichromate	0.08	0.15
p-Phenylenediamine	0.1	0.5
Hydroquinone	0.11	0.5
Dodecylthiosulphonate	0.2	5
Propyl gallate	0.32	5
Methyldodecane sulphonate	0.39	0.25
2-Aminophenol	0.4	5
Chloramine T	0.4	1
Formaldehyde	0.4	2
2-Nitro-p-phenylenediamine	0.4	2.5
Metol	0.8	5
Methyldibromoglutaronitrile	0.9	25
Isoeugenol	1.3	5
2-Hydroxyethylacrylate	1.4	1
Glyoxal	1.4	0.5
Mercaptobenzothiazole	1.7	10
Musk ambrette	1.8	40
Ethylenediamine	2.2	2.5
HC Red No3	2.2	10
Anethole	2.3	10
Benzisothiazolinone	2.3	0.33
Isononanoyl chloride	2.7	2.5
Cinnamic aldehyde	3	0.75
Phenylacetaldehyde	3	5
3-Aminophenol	3.2	5
Methylcinnamal	4.5	10
3,4-Dihydrocoumarin	5.6	50
2-Methoxy-4-methylphenol	5.8	10
Diethyl maleate	5.8	100
Sodium trimethylhexyloxybenzene sulphonate	6.4	2.5
4-Ethyl resorcinol	6.7	100
Dihydroeugenol	6.8	25
Amyl cinnamal	11	2.5
Hexylcinnamal	11	10
Citral	13	0.5
Eugenol	13	25
Abietic acid	15	25
Sodium lauroyl lactylate	15	0.5
Benzoyl benzoate	17	100
Lyrar	17	100
4-Allylanisole	18	10
C15 Azlactone	18	25
Lilial	19	50
Cinnamic alcohol	21	40
Cyclame aldehyde	22	50
Imidazolidinyl urea	24	50
Geraniol	26	10

(Continued)

Table 1 Continued

Chemical name	LLNA EC3 (%)	GPMT challenge (%)
Penicillin G	30	5
Methyleugenol	32	100
Hydroxycitronellal	33	50
Limonene	69	2.5
Aniline	89	100

of test material, or with an equal volume of the vehicle (4:1, Acetone Olive Oil, v/v) alone. Treatment was performed daily for 3 consecutive days. 5 days following the initiation of exposure, all mice were injected via the tail vein with 250 μ l of phosphate buffered saline (PBS) containing 20 μ Ci of tritiated thymidine. Mice were sacrificed 5 h later and the draining lymph nodes excised and pooled for each experimental group. The lymph node cell suspension was washed twice in an excess of PBS and then precipitated with 5% trichloroacetic acid (TCA) at 4°C for 18 h. Pellets were resuspended in TCA and the incorporation of tritiated thymidine measured by β -scintillation counting. The concentration of the chemical required to produce a stimulation of proliferation of 3 compared to the vehicle treated control, the EC3 value, was determined to provide a measure of relative skin sensitizing potential. The EC3 value was calculated by interpolating between 2 points on the stimulation index (SI) axis, one immediately above, and one immediately below, the stimulation value of 3. Where the data points lying immediately above and below the SI value of 3 have the co-ordinates (a,b) and (c,d), respectively, then the EC3 value may be calculated using the equation: $EC3 = c + [(3 - d)/(b - d)](a - c)$. A positive control using hexyl cinnamic aldehyde was performed by the laboratory at routine intervals to ensure test method sensitivity.

The GPMT followed the protocol given originally (6) and detailed in the OECD Guideline (8). In brief, albino Dunkin-Hartley guinea pigs weighing approximately 350 g were used. Preliminary irritation tests were carried out to determine the concentration of the test substances suitable for the induction and challenge phases of each study. For the topical challenge application, the preliminary irritation study used 4 guinea pigs, each treated with a range of concentrations applied under occlusion using 8-mm Finn chambers (Epitest Oy, Finland). Skin reactions were read 24 h and 48 h after patch removal. The maximum concentration producing no reaction was selected as suitable for challenge. This is the concentration reported in Table 1.

The data in Table 1 were subjected to statistical analysis. The % concentration data were transformed into log base 10 and then subjected to simple linear regression and the regression equation, confidence, and prediction intervals calculated. To confirm that this was an appropriate analysis, a Kolmogorov-Smirnov test was undertaken, which confirmed the normality of the residuals (further data on this is not presented). This test was used as it has the advantage of taking an unbiased approach, with no implicit assumptions about the distribution of the datasets.

RESULTS

Table 1 presents the complete data, ordered according to skin sensitization potency as determined by the LLNA EC3 value. The 55 chemicals span a wide range of potency, from an EC3 value of 0.009% to 89% (4 orders of magnitude); skin irritant potential covers 3 orders of magnitude. Inspection of the data shows that for the more potent allergens, the GPMT challenge concentration is no more than 5%, consistent with them possessing some degree of irritancy; the first substance to show no more than a limited level of irritancy is methyldibromoglutaronitrile, where the challenge concentration was 25% and the EC3 value 0.9% (still a relatively strong allergen). At the opposite end of the spectrum, for the allergens of less potency (EC3 value of 3% or more), only 20% had a GPMT challenge concentration of <5%, with several being almost entirely without any evidence of skin irritation potential (i.e., they were tested at 100%).

A simple linear regression was performed on the log data. This shows a linear relationship between the $\log_{10}(\text{EC3})$ and the $\log_{10}(\text{GPMT})$. The slope and the intercept coefficients are significantly different from zero, with p-values less than .0001. The linear equation is:

$$\log(\text{GPMT}) = 0.59\log(\text{EC3}) + 0.50$$

The 95% confidence intervals for the slope (equal to 0.59) and intercept (equal to 0.50) are (0.40, 0.78) and (0.30, 0.69), respectively. The $\log_{10}(\text{EC3})$ explains approximately 42% of the total variability in the $\log_{10}(\text{GPMT})$ ($R^2 = 0.42$) and vice versa. This means that predicting one value from the other using this model would produce very wide confidence intervals.

DISCUSSION

Skin sensitizing chemicals, which have the ability to induce contact allergy in humans, vary enormously in their relative potency. This is well demonstrated by simple inspection of the recently published data on one measure of potency, the LLNA EC3 value (10,13). It also accords with clinical experience (14,15). Knowledge of the potency of a skin sensitizer is therefore an essential prerequisite for risk assessment and risk management. However, there is a concern that future *in vivo* methods that will replace the *in vivo* test could be limited to the most basic level of hazard identification, functioning only in the context of current EU legislation (16). Potential *in vitro* approaches to the identification of skin sensitizing chemicals are well reviewed elsewhere (17–20). They include the use of computer-based identification of chemical structural alerts, peptide binding assays, measurement of keratinocyte responses, and measurement of changes induced in dendritic cells (or equivalents). These may require a degree of “modulation” as a consequence of consideration of epidermal bioavailability (21). However, given that the precise nature of non-animal replacements for skin sensitization remain wholly undefined, then the manner in which data deriving from separate elements of the sensitization induction process might be re-integrated to provide not only hazard but also potency information is far from clear. Nevertheless, one potential paradigm has been published (11). It is in this context

that the current work was initiated because the *in vitro* measures that will contribute to the characterization of potency in such a paradigm remain a matter for some speculation. Bioavailability and peptide reactivity seem probable candidates from a heuristic perspective. It is also possible that the relative ability of a skin sensitizer to trigger the release of danger signals could also contribute. Because danger signals seem likely to correlate well with irritancy, we chose to examine whether more potent allergens were also stronger irritants.

To address this question, the LLNA EC3 value, a measure of skin sensitization potency, for 55 chemicals was compared with the irritant potential of these chemicals, as assessed in the GPMT, the underlying logic being that the challenge concentration in this test, which is the maximum non-irritant concentration under a 24 h occluded application, provides a useful marker of relative irritant threshold/potency. The complete dataset (Table 1) was plotted as a log-log graph (Fig. 1), which demonstrates that there is a trend toward the more irritant materials also being more sensitizing. For example, the well recognized, very strong skin sensitizer 2,4-dinitrochlorobenzene is also one of the strongest irritants examined. It contrasted sharply with weak sensitizers such as cinnamic alcohol and imidazolidinyl urea with high EC3 values and where their maximum non-irritant concentration was 40% and 50%, respectively. However, there were several chemicals that departed from the trend, for example sodium lauroyl lactylate was quite irritant but only weakly sensitizing; methyl dibromogluratnitrile, a relatively potent allergen in contrast, was only of very limited irritancy. Such “discrepancies” are to be expected: at the extreme, chemicals might be highly irritating, but might be entirely without skin sensitizing activity (acetic acid, for example). At a more practical level, different vehicles were used in the assays and this might well be expected to complicate the analysis. Nevertheless, a limited

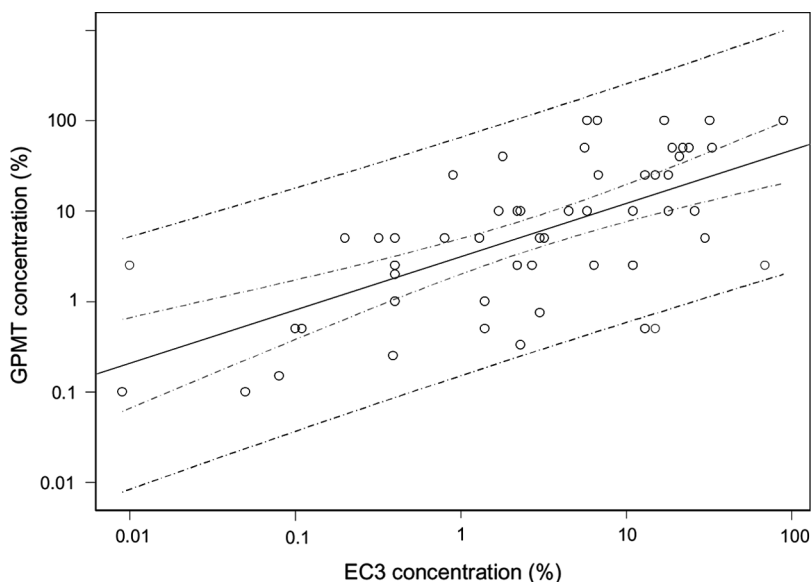


Figure 1 A log-log plot of irritant threshold versus potency (EC3) with the trend line obtained by linear regression and 95% confidence intervals (orange) and prediction intervals (red).

trend was demonstrated, consistent with the hypothesis, but indicating that either skin irritation is a poor measure of danger signals, or more likely that such signals are no more than a necessary prerequisite for the acquisition of skin sensitization rather than a key determinant of the relative potency of a skin sensitizing chemical. Thus, what can be garnered from the material presented is that where a substance has been shown to have other key properties associated with skin sensitization, for example covalent binding to peptide (16,22), then consideration of markers of irritancy, including those obtained from validated *in vitro* assays, might well assist in developing a fuller appreciation of its relatively ability to behave as a skin sensitizer *in vivo*, but only as part of a system that integrates a number of other parameters (11).

This analysis of historical data was undertaken as part of Unilever's ongoing efforts to develop novel ways of delivering consumer safety.

REFERENCES

1. Matzinger P. Friendly and dangerous signals: Is the tissue in control? *Nature Immunology* 2007; 8:11–13.
2. McFadden JP, Basketter DA. Contact allergy, irritancy and danger. *Contact Dermatitis* 2000; 42:123–127.
3. Kimber I, Cumberbatch M, Dearman RJ, Griffiths CE. Danger signals and skin sensitisation. *Br J Dermatol* 2002; 147(3):613–614.
4. Allenby CF, Basketter DA. Minimum eliciting patch test concentrations of cobalt. *Contact Dermatitis* 1989; 20:185–190.
5. Allenby CF, Basketter DA. An arm immersion model of compromised skin. II. Influence on minimal eliciting patch test concentrations of nickel. *Contact Dermatitis* 1993; 28: 129–133.
6. Magnusson B, Kligman AM. *Allergic Contact Dermatitis in the Guinea Pig. Identification of Contact Allergens*; Springfield, Illinois: Charles C Thomas, 1970.
7. Kligman AM. The identification of contact allergens by human assay. III. The maximization test: A procedure for screening and rating contact sensitizers. *Journal of Investigative Dermatology* 1966; 47:393–409.
8. OECD. *Guidelines for Testing of Chemicals. Guideline No. 406. Skin Sensitisation*, Organisation for Economic Cooperation and Development, Paris. 1992.
9. Auton TR, Botham PA, Kimber I. Retrospective appraisal of the relationship between skin irritancy and contact sensitisation potential. *J Tox Environ Health* 1995; 46(2):149–154.
10. Basketter DA, Gerberick GF, Kimber I. The local lymph node assay EC3 value: Status of validation. *Contact Dermatitis* 2007; 57:70–75.
11. Jowsey I, Basketter DA, Westmoreland C, Kimber I. A future approach to measuring relative skin sensitising potency. *J Appl Toxicology* 2006; 26:341–350.
12. OECD. *Guidelines for Testing of Chemicals. Guideline No. 429. The Local Lymph Node Assay*, Organisation for Economic Cooperation and Development, Paris. 2002.
13. Gerberick GF, Ryan CA, Kern PS, Schlatter H, Dearman RJ, Kimber I, Patlewicz G, Basketter DA. Compilation of historical local lymph node assay data for the evaluation of skin sensitization alternatives. *Dermatitis* 2005; 16:157–202.
14. Basketter DA, Blaikie L, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, Harvey P, Evans P, White IR, Rycroft RJG. Use of the local lymph node assay for the estimation of relative contact allergenic potency. *Contact Dermatitis* 2000; 42:344–348.

15. Gerberick GF, Robinson MK, Ryan CA, Dearman RJ, Kimber I, Basketter DA, Wright Z, Marks JG. Contact allergenic potency: correlation of human and local lymph node assay data. *American Journal of Contact Dermatitis* 2001; 12:156–161.
16. EEC Council Directive 92/32/EEC amending for the seventh time Directive 67/548/EEC Off. J Eur Comm 1992; L154:1–29.
17. Aptula AO, Patlewicz G, Roberts DW. Skin sensitization: Reaction mechanistic applicability domains for structure-activity relationships. *Chem Res Toxicol* 2005; 8(9):1420–1426.
18. Divkovic M, Pease CM, Gerberick GF, Basketter DA. Hapten-protein binding: From theory to practical application in the in vitro prediction of skin sensitisation. *Contact Dermatitis* 2005; 53:189–200.
19. Vandebriel RJ, Van Och FM, van Loveren H. In vitro assessment of sensitizing activity of low molecular weight compounds. *Toxicol Appl Pharmacol* 2005; 207(2 Suppl):142–148.
20. Ryan CA, Gerberick GF, Gildea LA, Hulette BC, Betts CJ, Cumberbatch M, Dearman RJ, Kimber I. Interactions of contact allergens with dendritic cells: Opportunities and challenges for the development of novel approaches to hazard assessment. *Toxicol Sci* 2005; 88:4–11.
21. Basketter DA, Casati S, Cronin MTD, Diembeck W, Gerberick GF, Hadgraft J, Kasting G, Marty J P, Nikolaidis E, Patlewicz G, Pease C, Roberts D W, Roggen E, Rovida C, van der Sandt, J. Skin sensitisation and epidermal disposition. *ATLA* 2007; 35:137–154.
22. Gerberick GF, Vassallo JD, Bailey RE, Chaney JG, Morrall SW, Lepoittevin J-P. Development of a peptide reactivity assay for screening contact allergens. *Toxicol Sci* 2004; 81:332–343.