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CONTACT DERMATITIS

Reproducible prediction of contact allergenic potency using the local lymph node assay

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A considerable body of data has been accumulated which demonstrates that the local lymph node assay (LLNA) can provide a valuable estimation of the contact allergenic potency of a substance. This estimate is obtained via interpolation of the LLNA dose–response curve and is expressed as the concentration of the chemical required to evince a 3-fold stimulation of proliferation in lymph nodes draining the site of application compared to the vehicle-treated controls (EC3). It has also been shown that the EC3 estimates are reproducible and are stable over time. In the present work, we have extended this information by a demonstration of the inherent biological variability surrounding EC3 estimation, using data derived (from a single laboratory) from the testing of isoeugenol as a positive control. Isoeugenol gave EC3 values ranging from 0.5 to 2.6% ($n = 29$), with a mean and standard deviation of $1.2 \pm 0.6\%$. Given that EC3 values for a variety of contact allergens range over several orders of magnitude, these results further endorse the utility of EC3 values as a reliable indicator of human contact allergenic potency.

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The local lymph node assay (LLNA) is the only validated alternative to the historical guinea-pig predictive assays for the identification of potentially skin-sensitizing chemicals (1). However, an additional benefit accruing from the LLNA is its ability to provide quantitative measurements of the relative potency of contact allergens via estimation of the concentration of a chemical required to stimulate a threshold positive response, the EC3 value (2–4). EC3 values for a range of substances have been shown to correlate closely with what is understood concerning their allergic potency in man (5–7). Furthermore, evidence of the robustness of EC3 estimations has been derived from studies over time and between laboratories (8–10). Despite this extensive body of data being published, only limited information has been available on the real extent to which an EC3 value for a specific chemical might vary, not least due to heterogeneities in the response system. The largest dataset has been that using hexyl cinnamic aldehyde (10). In the present work, we have combined data for isoeugenol obtained from studies carried out over a 4-year period, during which it had been employed as a routine positive control.

Materials and Methods

Isoeugenol (99%) was provided by Firmenich, Geneva, Switzerland. Each LLNA was carried out as previously described (11, 12) at Burleson Research Technologies Inc., Raleigh, USA, as a positive control. These data were gathered from testing over a period of approximately 4 years. Briefly, groups of 5 CBA female mice were exposed topically on the dorsum of both ears to 25 μl of 0.5, 1.0 and 5.0% isoeugenol in acetone/olive oil 4 : 1, v/v vehicle, or to the same volume of vehicle alone (group size 7–8), daily for 3 consecutive days. 5 days after the initiation of exposure, all mice were injected intravenously via the tail vein with 2 μCi of ^{125}IUDR in 250 μl of phosphate-buffered saline (PBS). 5 h later, mice were killed and the draining auricular lymph nodes were excised for each experimental group. A single-cell suspension of lymph node cells was

prepared by gentle mechanical disaggregation through a 200-mesh stainless-steel gauze. Cells were washed 2× with PBS and precipitated in 5% trichloroacetic acid (TCA) at 4°C. Approximately 12 h later, pellets were resuspended in 1 ml of 5% TCA and transferred to 10 ml of scintillation fluid. Incorporation of ^{125}IUDR was recorded as disintegrations per minute (dpm) per node for each experimental group. In each case, a stimulation index (SI) relative to the concurrent vehicle-treated control value was derived. The estimated concentration of chemical required to induce an SI of 3 relative to concurrent vehicle-treated controls, or EC3 value, was derived by linear interpolation of dose–response data, as described previously (4). The EC3 value was calculated by interpolating between 2 points on the SI axis, one immediately above, and the other immediately below, the SI 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 following equation: $EC3 = c + [(3-d)/(b-d)](a-c)$.

Results and Discussion

The raw data for each experiment, the derived EC3 values and details of the mean and standard deviation are summarized in Table 1. The 29 individual EC3 values for isoeugenol ranged from 0.5 to 2.6%, with a mean value of 1.2% and a standard deviation of 0.6%. This mean value is remarkably close to the previously published figure of 1.3% (5). Furthermore, this set of potency data obtained with isoeugenol supports the often-mentioned perspective that the biological variation associated with the estimation of EC3 values means that any particular EC3 figure can be either halved or doubled; it should be remembered in this context that published EC3 values for a wide range of contact allergens span several orders of magnitude (13). However, to date, the published information that would support this statement has been relatively limited. In reality, only 3 publications present, in any detail, the reproducibility of LLNA-derived EC3 values. The earliest data suggesting the consistency of EC3 values between laboratories was published in 1996 (8). In addition, both interlaboratory and temporal stability of derived EC3 values has been demonstrated for *p*-phenylenediamine (9). For hexyl cinnamic aldehyde, an OECD positive control substance, results have been published which demonstrate the reproducibility of LLNA responses to this material (10). In particular, these data confirmed the temporal stability of hexylcinnamaldehyde (HCA) responses in the LLNA. In addition to these data, examining the impact of vehicle on EC3 values also helps to demonstrate the robustness of this measure (14).

Table 1. Isoeugenol local lymph node assay dataset

Test	SI[1] at each concentration (%)			
	0.5	1.0	5.0	EC3%[2]
1	1.0	1.1	12.4	1.2
2	1.5	2.5	29.8	0.6
3	0.8	1.6	14.1	1.1
4	1.3	2.2	13.1	1.0
5	0.8	2.8	5.6	2.1
6	1.8	2.9	23.2	0.6
7	1.6	1.6	14.7	1.0
8	1.6	1.4	14.7	1.0
9	1.7	1.2	5.0	2.6
10	1.2	4.2	18.4	0.7
11	2.3	1.6	23.6	0.6
12	1.6	2.2	7.5	1.6
13	1.4	1.5	4.9	2.6
14	1.3	3.3	14.7	1.5
15	1.5	3.0	19.2	0.8
16	1.0	1.0	12.8	1.2
17	1.6	2.2	19.0	0.8
18	1.2	1.4	19.3	1.8
19	1.6	4.3	24.4	0.6
20	1.0	1.3	7.5	1.8
21	1.4	1.2	6.7	2.0
22	1.5	2.6	19.2	0.8
23	0.7	2.3	13.8	1.0
24	1.1	1.8	23.2	0.8
25	0.9	6.3	31.0	0.5
26	1.2	3.2	8.7	1.3
27	0.9	1.0	7.2	1.9
28	1.1	1.9	15.3	1.0
29	2.0	1.4	7.6	1.6
Mean EC3 value				1.2

[1]Stimulation index (SI) [mean disintegrations per minute (dpm)/node in the test group divided by mean dpm/node in the concurrent vehicle-treated control group].
[2]Estimated concentration required to cause a SI of 3.

What is the importance of the demonstration of this reproducibility of LLNA EC3 values? In reality, it is the utility of this measure as an indicator of contact allergenic potency that can then be incorporated into quantitative risk assessment that is key. A suite of publications has described how the LLNA predictions of allergen potency match human data (5–7, 15, 16) and how this can then be utilized to assess the human risk arising from specific exposures to that allergen. The fact that transparent quantitative risk assessment of some quality is becoming a reality (17) should greatly assist toxicological safety evaluators to avoid continuing repeats of the 'Dillarstone effect' (18), most recently exemplified by methyldibromo glutaronitrile (19). A critical component of an assured quality risk assessment must be the reliability of the measure of allergenic potency. In the simple dataset presented here, we suggest that such reliability is evident.

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