

The skin sensitization potential of resorcinol: experience with the local lymph node assay

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Resorcinol is a simple aromatic chemical (1,3-benzenediol) that has found widespread use, particularly as a coupler in hair dyes. Clinical experience clearly shows that resorcinol is a (albeit uncommon) skin sensitizer. By contrast, predictive methods, both animal and human, have previously failed to identify resorcinol as such. Here, we describe the outcome of a recent local lymph node assay performed in accordance with Organisation for Economic Co-operation and Development guideline 429, which correctly identified resorcinol as a skin sensitizer. Clear evidence of a dose response was apparent, and an EC3 value of approximately 6% was calculated. This suggests that the skin-sensitizing potency of resorcinol is approximately 2 orders of magnitude lower than that of *p*-phenylenediamine but similar to that of hexyl cinnamic aldehyde. These data show the importance of adherence to test guidelines and aligns the clinical experience with resorcinol with that obtained in predictive animal methods.

Key words: local lymph node assay; potency estimation; resorcinol; skin sensitization. © Blackwell Munksgaard 2007.

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For many years, guinea-pig tests have been the method of choice for the predictive identification of skin-sensitizing chemicals (1, 2). However, the use of these methods for measuring the potency of skin sensitizers has proven more problematic (3–5). Consequently, a newer method, the local lymph node assay (LLNA), which incorporates a dose–response element, has not only improved the basic identification of skin sensitization hazards (6, 7) but also has facilitated the objective quantification of relative sensitization potency (5, 8–10). Quantification of potency is achieved by interpolation of the dose–response curve to estimate the concentration of a chemical necessary to achieve a threefold stimulation of proliferation in lymph nodes draining the site of chemical application – the EC3 value. Occasionally, it may also be appropriate to do a careful extrapolation. These issues have been fully reported elsewhere (9–11).

In a recent publication, the complete data set of LLNA tests where potency estimation has been achieved has been published (12). In this work, it was reported that resorcinol, a coupling chemical widely used in hair dyes (13, 14), was not sensitizing. This outcome was in agreement with guinea-

pig maximization test data (15) and human maximization test data (16) but in contrast to clinical experience, which clearly identifies resorcinol as a (albeit uncommon) contact allergen (13, 14).

In all predictive methods, the sensitivity of the technique under suboptimum conditions can be such that identification of weaker sensitizers is problematic. As such, Organisation for Economic Co-operation and Development (OECD) guideline 429 (17) makes specific recommendations concerning the execution of the LLNA, including the importance of performing routine positive controls. However, the assessment of resorcinol in the LLNA currently reported in the literature (12) predates OECD guideline 429 by some years. Given the apparent discrepancy between the clinical experience and that obtained in the LLNA, we subsequently reassessed the skin-sensitizing potential of resorcinol in the LLNA in accordance with current guidelines (17, 18).

Materials and Methods

The LLNA was conducted as described elsewhere and in accordance with OECD guideline 429 (6). 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 μ l of test material or with an equal volume of the vehicle [4:1 acetone:olive oil (AOO), v/v] alone. Treatment was performed daily for 3 consecutive days. 5 days following the initiation of exposure, all mice were injected through the tail vein with 250 μ l of phosphate-buffered saline (PBS) containing 20 μ Ci of tritiated thymidine. Mice were sacrificed 5 hr later, and the draining lymph nodes were 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 hr. Pellets were resuspended in TCA, and the incorporation of tritiated thymidine was measured by β -scintillation counting. The concentration of the chemical required to produce a stimulation of proliferation of 3 compared with 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, 1 immediately above and 1 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.

Resorcinol (99.9%) was obtained from Vivimed Laboratories Ltd, Hyderabad, India.

Results

The results of the original LLNA performed with resorcinol are presented in Table 1. They have been reported previously (12). This assay was performed some 10 years ago and predates the recommendation to include positive control groups in the LLNA. The maximum concentration tested was 25% (w/v), and the vehicle used was dimethyl

formamide (DMF). While the data are broadly suggestive of a dose response, the maximum SI never exceeded 3. As such, under current guidelines, resorcinol would be designated as a non-sensitizer in the LLNA (at least at concentrations $\leq$ 25% w/v).

The results of a second historical resorcinol LLNA are presented in Table 2. In this case, the maximum concentration tested was 2.5% w/v and the vehicle used was 4:1 AOO. Most striking from these data is the fact that all disintegrations per minute (d.p.m.)/node values obtained with the test material are lower than that obtained with the vehicle control, resulting in SI values of <1 in all cases. Further scrutiny of these data show that the AOO vehicle control values are extremely high. Indeed, a recent publication documenting AOO vehicle control values over time at 4 independent laboratories record the value obtained here (1120 d.p.m./node) as the highest value obtained. These data urge caution in terms of interpretation.

Given the apparent discrepancy between the clinical experience with resorcinol and the results described above, resorcinol was re-evaluated in the LLNA (Table 3). Again, AOO was selected as the vehicle and concentrations of resorcinol $\leq$ 50% w/v were selected. In this case, the vehicle control value (562 d.p.m./node) was much more comparable with the mean values reported previously (19). A very clear dose response was obtained, and the

Table 2. LLNA data for resorcinol (experiment 2)

Concentration (% w/v)	d.p.m./node ^a	Stimulation index ^b
0 (AOO)	1120	1.0
0.1	443	0.4
0.25	250	0.2
0.5	566	0.5
1.0	849	0.8
2.5	1100	1.0

AOO, acetone:olive oil; d.p.m., disintegrations per minute; LLNA, local lymph node assay.

^ad.p.m./lymph node.

^bRatio of d.p.m./node compared with control (0%) value.

Table 3. LLNA data for resorcinol (experiment 3)

Concentration (% w/v)	d.p.m./node ^a	Stimulation index ^b
0 (AOO)	562	1.0
1	407	0.7
5	1231	2.2
10	2948	5.2
25	4743	8.4
50	5827	10.4

AOO, acetone:olive oil; d.p.m., disintegrations per minute; LLNA, local lymph node assay.

^ad.p.m./lymph node.

^bRatio of d.p.m./node compared with control (0%) value.

Table 1. LLNA data for resorcinol (experiment 1)

Concentration (% w/v)	d.p.m./node ^a	Stimulation index ^b
0 (DMF)	314	1.0
5	689	2.2
10	682	2.2
25	853	2.7

DMF, dimethyl formamide; d.p.m., disintegrations per minute; LLNA, local lymph node assay.

^ad.p.m./lymph node.

^bRatio of d.p.m./node compared with control (0%) value.

maximum SI value was 10.4. Thus, in this study, resorcinol was correctly identified as a skin sensitizer, and an EC3 value of 6.3% was calculated.

Finally, we have compiled in Table 4 the data presented in Tables 2 and 3 to obtain a wider dose response for resorcinol. To correct for the unusually high AOO values obtained in experiment 2, we have reanalysed the data using the historical mean AOO d.p.m./node value obtained at the performing laboratory (18). The rationale for this recalculation is discussed in more detail below. The data have then been used to plot a full dose–response curve (Fig. 1) and to calculate the EC3 value, which was 5.5%.

Discussion

The LLNA is proving to be a useful method for the predictive identification of skin sensitization hazard (7, 17, 18), such that it is often the preferred assay, e.g. in the UK (20) and for European Union legislation such as the Registration, Evaluation and Authorisation of Chemicals (21). It was for this purpose, hazard identification, that the LLNA went through formal validation in the USA (6, 7) and in Europe (22). However, it has more recently shown considerable utility for the assessment of the relative skin-sensitizing potency of chemicals (5, 8–10) by means of the calculation of EC3 values, lower values being associated with stronger allergens. Such data can be shown to correlate with what is known of the relative potency of skin sensitizers in humans (23–25). Most importantly, this information can then be incorporated into quantitative risk assessments for novel substances (24–28).

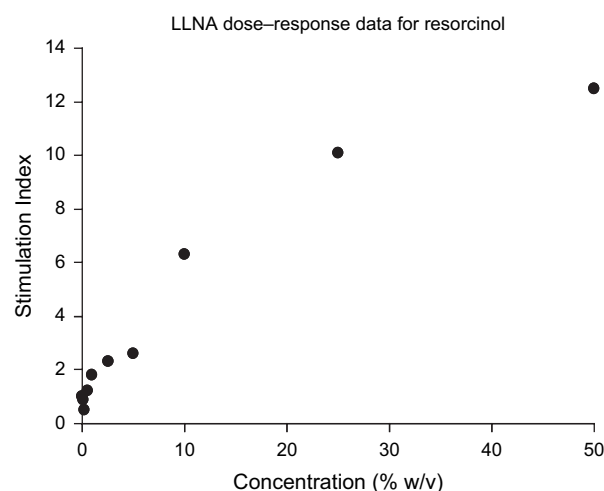


Fig. 1. Combined dose–response data for resorcinol. LLNA, local lymph node assay.

In the present case, the focus is on resorcinol, a simple aromatic chemical used as a coupler in hair dyes, which is associated with a low incidence of cases of allergic contact dermatitis (13, 14). Here we have confirmed that resorcinol has a moderate skin sensitization potential, being positive in the LLNA at relatively high test concentrations. The EC3 value of 5.5% places it as approximately 2 orders of magnitude less potent than the very strong allergen *p*-phenylenediamine and of broadly similar potency to contact allergens such as diethylmaleate (5.8%) and hexyl cinnamic aldehyde (11%) (12).

We have also described our earlier experiences with resorcinol in the LLNA. In the first of these, concentrations of resorcinol $\leq 25\%$ in DMF yielded a maximum SI of only 2.7, thus failing to reach the SI value of 3 required for classification as skin sensitizer. There are a number of possible explanations for this. First, the assay may not have been sufficiently sensitive to identify resorcinol as a skin sensitizer (this study predates the introduction of routine positive controls comprising weak sensitizers to ensure the sensitivity of the test method). The impact of vehicle may also be a contributing factor. Indeed, the EC3 value for methylchloroisothiazolinone/methylisothiazolinone was found to vary by an order of magnitude depending upon the vehicle used (0.0049% in AOO versus 0.048% in propylene glycol) (29). Similarly, significant vehicle effects were observed when determining the EC3 value of a weaker sensitizer, 1,4-dihydroquinone (0.07% in methyl ethyl ketone versus 0.4% in dimethyl sulphoxide) (30). The possibility thus exists that vehicle effects in the original study mean that the observed EC3 value for resorcinol would have been greater than the maximum concentration tested (25%).

Table 4. Corrected^a and combined LLNA data for resorcinol

Concentration (% w/v)	d.p.m./node ^b	Stimulation index ^c
0 (historical mean AOO value)	467	1.0
0.1	443	0.9
0.25	250	0.5
0.5	566	1.2
1.0	849	1.8
2.5	1100	2.3
5.0	1231	2.6
10.0	2948	6.3
25	4743	10.1
50	5827	12.5

AOO, acetone:olive oil; d.p.m., disintegrations per minute; LLNA, local lymph node assay.

^aData have been recalculated using the mean historical AOO control value for the performing laboratory (467 d.p.m./node).

^bd.p.m./lymph node.

^cRatio of d.p.m./node compared with control (0%) value.

In the second LLNA, resorcinol also failed to induce a proliferative response of sufficient magnitude to classify as a skin sensitizer (maximum SI value obtained = 1). Of particular note in this study was the oddity of the vehicle control value (Table 2), which at 1120 d.p.m./node is the highest value ever reported for AOO (18). This study also described a strategy for dealing with anomalous vehicle control values, involving the use of historical data (18). While caution must be exercised and a sound rationale required, for the reasons given, a similar combined analysis was carried out on this occasion, replacing the abnormal vehicle control value in experiments 2 and 3 with the historical mean value of 467 d.p.m./node obtained by the performing laboratory. As expected, this made a large difference for the SI values for experiment 2 but little difference to the SI values for experiment 3 and so produced a very consistent data set (Table 4) with a clear dose–response curve (Fig. 1). It also confirmed the utility of AOO as the first choice of vehicle selection in the OECD guideline 429 for the LLNA (17).

In summary, conduct of the LLNA to the optimum standards identifies resorcinol as a weak skin sensitizer, a conclusion consistent with clinical observation in allergic contact dermatitis. The data presented also exemplify the importance of critical interpretation of LLNA test data.

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