



Extrapolating Local Lymph Node Assay EC3 Values To Estimate Relative Sensitizing Potency

Cindy A. Ryan, Joel G. Chaney, G. Frank Gerberick, Petra S. Kern, Rebecca J. Dearman, Ian Kimber & David A. Basketter

To cite this article: Cindy A. Ryan, Joel G. Chaney, G. Frank Gerberick, Petra S. Kern, Rebecca J. Dearman, Ian Kimber & David A. Basketter (2007) Extrapolating Local Lymph Node Assay EC3 Values To Estimate Relative Sensitizing Potency, Cutaneous and Ocular Toxicology, 26:2, 135-145, DOI: [10.1080/15569520701212258](https://doi.org/10.1080/15569520701212258)

To link to this article: <http://dx.doi.org/10.1080/15569520701212258>



Published online: 10 Oct 2008.



Submit your article to this journal [↗](#)



Article views: 141



View related articles [↗](#)



Citing articles: 17 View citing articles [↗](#)

EXTRAPOLATING LOCAL LYMPH NODE ASSAY EC3 VALUES TO ESTIMATE RELATIVE SENSITIZING POTENCY

Cindy A. Ryan, Joel G. Chaney, and G. Frank Gerberick

The Procter & Gamble Company, Cincinnati, Ohio

Petra S. Kern

Procter & Gamble Eurocor, Temselaan, Strombeek Bever, Belgium

Rebecca J. Dearman and Ian Kimber

Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire

David A. Basketter

Unilever SEAC, Colworth House, Sharnbrook, Bedford

The assessment of the potency of a skin sensitizing chemical is a key starting point for its subsequent risk assessment/management. The Local Lymph Node Assay can provide information on the relative skin sensitizing potency of contact allergens by interpolation from the dose response curve the concentration of a chemical required to elicit a threshold positive response (EC3 value). However, interpolation requires that the dose response curve have at least one stimulation index (SI) value above and one SI value below the threshold value of 3. For instances where all test concentrations result in SI values above 3, there was a need to develop a method that would permit estimation of EC3 values. This has been achieved by log-linear extrapolation using the two lowest test concentrations from the dose response curve. Before applying this approach, it is important that data quality is assessed. The dose response must include concentrations on the linear portion of the curve and, ideally, the SI induced by the lowest dose should approach 3. Judicious use of this approach for extrapolating EC3 values can provide information on a likely potency classification for use in risk assessment and may avoid the need for repeat animal testing.

Keywords: EC3 value; Local lymphnode assay; Potency; Skin sensitization

INTRODUCTION

The murine Local Lymph node Assay (LLNA) is a validated method for the identification of skin sensitization hazard (1) which has been endorsed by the relevant validation authorities in both the United States and Europe (2,3) as a replacement for the traditional guinea pig assays such as the guinea pig maximization test (GPMT) and the Buehler test. While the prospective identification of skin sensitizing

Address correspondence to Cindy A. Ryan, The Procter & Gamble Company, P.O. Box 538707, Cincinnati, Ohio. E-mail: ryan.ca@pg.com

chemicals is an important step in the safety assessment of new ingredients, an understanding of the inherent sensitization potency of the contact allergen, together with the likely extent, duration, and nature of exposure is required to determine if it represents an actual risk for the induction of skin allergy in humans (4). Therefore, a method to obtain objective information regarding a sensitizer's relative potency compared with other skin allergens is needed.

Skin sensitization is a dose response phenomenon; thresholds can be identified for both induction of sensitization and elicitation of contact allergic reactions (5) and allergenic potency can be defined in terms of those threshold values (6). Guinea pig methods like the GPMT and Buehler test are not well suited for estimation of sensitization potency due primarily to the principles of test concentration selection and the experimental design of such studies. However, since the LLNA measures events associated with the induction of sensitization and, by design, provides dose response information, it is considered as the method of choice for the objective and quantitative estimation of skin sensitizing potency (7). Through mathematical evaluation of LLNA dose response data, the concentration of a chemical required to produce a threshold positive response (a 3-fold increase in activity compared with concurrent vehicle controls) can be measured (8). This concentration, referred to as the EC3 (Estimated concentration required for a 3-fold Stimulation Index (SI)) value, can be used to provide an estimation of relative allergenic potency that has been shown to correlate with what is known about the potency of contact allergens in humans (9,10). In addition, the robustness of this parameter has been demonstrated rigorously in terms of inter- and intra-laboratory reproducibility (11–13).

While various statistical approaches have been explored for deriving EC3 values, linear interpolation provides a simple and reliable approach (8). This method of calculating EC3 values requires that the dose response curve have at least one SI value above 3, and one below. However, there are some instances where all test concentrations result in SI values of greater than 3. Therefore, in order to avoid the need for repeat animal testing with different test concentrations and to obtain information on a likely potency classification, a method was developed to estimate EC3 values by log-linear extrapolation using the two lowest test concentrations from the dose response curve (14). However, before applying this approach the quality of the data available must be assessed and confirmed. Here the effect of the dose response curve characteristics on extrapolated EC3 values is examined and the level of confidence one can have for EC3 values estimated via log-linear extrapolation determined.

METHODS

LLNA Data

Historical LLNA data were compiled from multiple sources, which included the published literature and previously unpublished data from several laboratories. The data were derived from studies that were conducted using the LLNA methodology as described in the OECD testing guideline 429 (15). A significant portion of these data has been recently published (16) but that previously published information does not include multiple datasets, which were available for many of the

Table 1 The ECETOC classification scheme for contact allergic potency based on EC3 values

Potency classification	EC3 value (%)
Weak	$\geq 10 - \leq 100$
Moderate	$\geq 1 - < 10$
Strong	$\geq 0.1 - < 1$
Extreme	< 0.1

individual chemicals. A total of 187 data sets representing 86 different chemicals identified as skin sensitizers were selected from the larger database for this current evaluation. Each data set was chosen because it had one SI value less than 3 and at least two SI values greater than 3. Of those data sets 83 had 3 or more data points above SI = 3.

Calculation of EC3 Values

EC3 values were calculated by linear interpolation for 187 data sets according to the equation:

$$EC3 = c + \left[\frac{(3 - d)}{(b - d)} \right] \times (a - c)$$

using the data points lying immediately above and below the SI value of 3 on the LLNA dose response curve that had the (dose, SI) coordinates (a,b) and (c,d), respectively (8).

Extrapolated EC3 (EC3ex) values were calculated on the same data sets using the two SI values greater than 3 with the lowest of the SI values having the lowest % concentration. The point with the higher SI is denoted (a,b) and the point with the lower SI is denoted (c,d). The formula for the extrapolated EC3 value is as follows:

$$EC3ex = 2^{\left\{ \log_2(c) + \frac{(3 - d)}{(b - d)} \times [\log_2(a) - \log_2(c)] \right\}}$$

By log-transformation of the data, extrapolated EC3 values never fall below zero (14).

The allergenic potency class was assigned for each interpolated and extrapolated EC3 value using the ECETOC categorization scheme (17) (Table 1). This classification scheme is based on an order-of-magnitude ranking of potency.

Comparison of EC3 Values Calculated by Linear Interpolation and Log-Linear Extrapolation

Since the selected 187 data sets had at least one SI value less than 3 and at least two SIs above 3, it was possible to calculate both interpolated and extrapolated EC3 values from the same data. This method of comparison was chosen to eliminate any experimental variables, which would have been present if data sets from independent

studies had been used. The correlation between the linear interpolated and log-linear extrapolated EC3 values was determined. 95% Confidence bounds around the diagonal line of equality, were constructed by calculating the difference between the two $\log_{10}$ -transformed EC3 values (interpolated and extrapolated) and finding the 2.5 and 97.5 percentiles.

Assessment of Dose Response Quality

To determine the impact of the quality of dose response curves on extrapolated EC3 values, the 83 data sets with at least 3 data points above $SI = 3$ were used. EC3 values were calculated, $\log_{10}$ -transformed and the difference between the interpolated and extrapolated values was computed. By computing this difference on a $\log_{10}$ scale, a difference of 1 represents the equivalent of 1 potency class. This difference was then compared with a ratio of slopes computed along the dose response. Specifically, the slope of the curve between the second and third data points above $SI = 3$ was computed and divided by the slope of the first and second points above $SI = 3$ to obtain a slope ratio. This ratio was used since values less than one would indicate a slope that is decreasing in the neighborhood of the first three data points above $SI = 3$. A ratio greater than one would indicate a slope that is increasing in this neighborhood. On a typical sigmoid dose-response curve, slope increases near the bottom of the curve, is constant in the middle of the curve, and decreases near the top of the curve, so calculating this ratio would provide information regarding the location on the dose-response curve of the values used for log-linear extrapolation. Correlation analyses were conducted in order to determine whether the slope ratios were significantly related to the differences between the $\log_{10}$ -transformed EC3 values. Spearman's rank correlation coefficient was used since the slope ratios and the $\log_{10}$ -transformed EC3 differences are not normally distributed (18).

Assessment of the Lowest SI Value

To assess the impact of the lowest SI value on extrapolated EC3 values both interpolated and extrapolated EC3s were calculated from the same data set. The EC3 values were $\log_{10}$ -transformed and the difference between the values was computed as described above. This difference was then compared to the lowest SI value > 3 used to calculate the extrapolated EC3 value. The data were also examined by comparing the lowest SI value to the percent difference between the linear interpolated and log-linear extrapolated EC3 values. Spearman correlation analysis was also used to compare the $\log_{10}$ -transformed EC3 differences (both the raw difference and the percentage difference) to the lowest SI values greater than three.

RESULTS

Comparison of EC3 Values Calculated by Linear Interpolation and Log-Linear Extrapolation

EC3 values were calculated by both linear interpolation and log-linear extrapolation for 187 data sets, which had at least one SI value less than 3 and at least

Table 2 Prediction of potency class based on EC3 values calculated by log-linear extrapolation compared with EC3 values calculated by linear interpolation

Prediction	# Predicted/Total (% of total)
Same potency class by both methods	165/187 (88%)
Varied by 1 class	18/187 (10%)
Varied by >1 class	4/187 (2%)

two SI values above 3. Then, potency categories were assigned on the basis of those values using the ECETOC scheme (17) and compared in order to determine the accuracy of the classifications based on log-linear extrapolation relative to those obtained by interpolation. There was agreement in the potency classification for 165 of the 187 data sets (Table 2). When there was disagreement, the categories derived from extrapolated values tended to vary by only a single category (18 of the 187 data sets) and tended to err on the side of caution. That is, the extrapolated EC3 values tended to be smaller and thus the classification was predicted to be more potent than that derived from interpolated values. In comparing EC3 values calculated by linear interpolation and log-linear extrapolation it was noted that when the log-linear extrapolated EC3 were very low (less than 0.001%), the corresponding EC3 value interpolated from the same data set was not nearly as low (Fig. 1). The variability

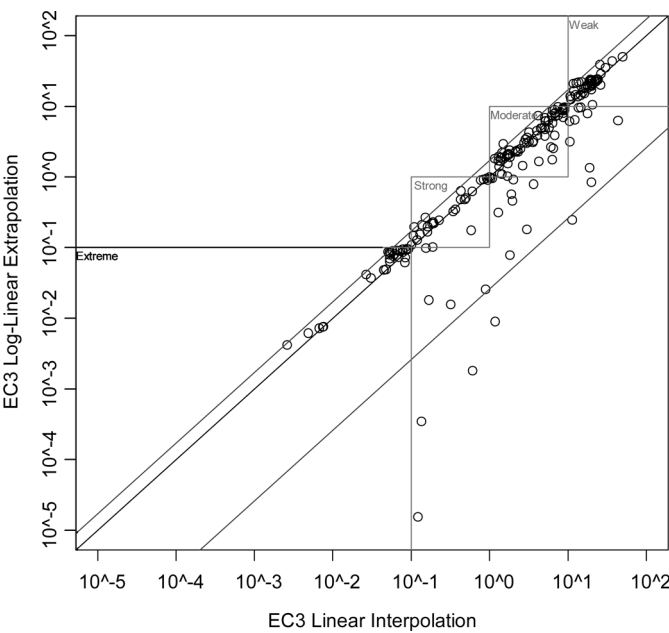


Figure 1 Comparison of EC3 values calculated by linear interpolation and log-linear extrapolation. In this figure, the boxes denote agreement between sensitization categories derived from the two calculation methods. The 95% confidence bounds are shown as diagonal lines. The same potency classification was obtained for 88% of the data set evaluated. For 22% of the data set, when potency classifications did not agree the extrapolated classification tended to be off towards the side of caution, i.e., a stronger classification.

in the difference between the two EC3 values was fairly constant across the range of values. Therefore, setting a 95% confidence interval with a constant width on the $\log_{10}$ scale across the range of data values was reasonable. The confidence bounds, calculated as the difference between the $\log_{10}$ -transformed EC3 values (interpolated and extrapolated) were -0.23 and 1.59 , respectively. This implies that when using extrapolated EC3 values, one can be reasonably confident that the estimated potency classification of a chemical will be equal to or one category stronger than the estimated potency derived through interpolation.

Assessment of Dose Response Quality

The quality of dose response curves and their impact on the accuracy of EC3 values obtained by log-linear extrapolation was assessed by calculating the difference between $\log_{10}$ -transformed EC3 values and then comparing that difference with a ratio of slopes computed along the dose response curve. A difference of 1 on the $\log_{10}$ scale represents the equivalent of 1 potency class. Based on the evaluation of 83 datasets with at least 3 points above $SI = 3$, it was evident that as the slope ratio increases, the difference between the log EC3 values also tended to increase (Fig. 2). The larger EC3 differences (in particular, those that exceed one) occurred when the slope ratio exceeds 2. Slope ratios below 2 tended to be associated with much smaller differences between the EC3 values. The correlation between slope ratios and EC3 differences was somewhat strong and was found to be statistically significant (Spearman's $\rho = 0.52$, $p < 0.001$).

Assessment of the Lowest SI Value

To calculate EC3 values by log-linear extrapolation, two SI values greater than 3 are used, with the lowest of the SI values having the lowest % concentration. To

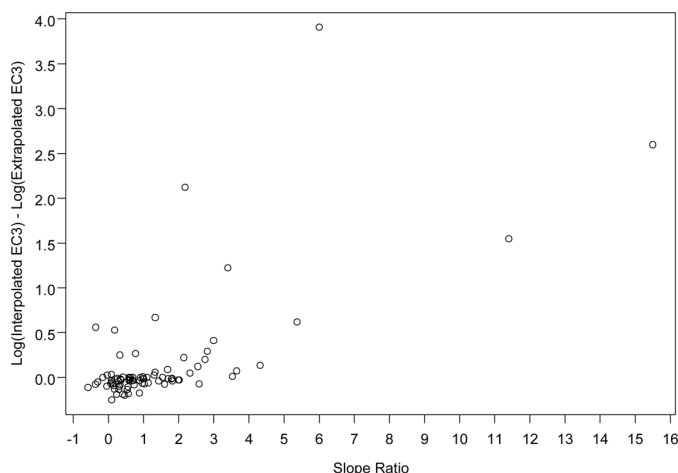


Figure 2 Comparison of the difference between the $\log_{10}$ transformed EC3 values and the ratio of slopes computed along the dose response to assess the impact of dose response curve quality on the accuracy of log-linear extrapolated EC3 values.

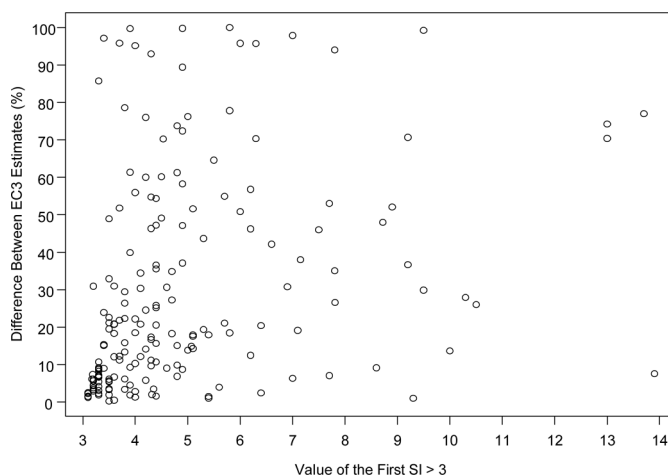


Figure 3 Comparison of the percent difference between the log-linear extrapolated and linear interpolated EC3 values and the lowest SI > 3 to assess the impact of the lowest SI on the accuracy of log-linear extrapolated EC3 values.

determine what effect, if any, the lowest SI value has on the accuracy of log-linear extrapolated EC3 values, both interpolated and extrapolated EC3s were calculated from the same data set. The difference between the $\log_{10}$ -transformed EC3 values was computed and compared with the lowest SI value > 3 used in the extrapolation (data not shown). In general, the accuracy of EC3 values obtained by log-linear extrapolation was found to increase as the lowest SI value used in the calculation approached 3, but the correlation was rather weak and not statistically significant (Spearman's $\rho = -0.08$, $p = 0.304$). However, when the difference between the EC3 values was expressed as a percentage, a comparison with the lowest SI > 3 used to calculate the extrapolated values revealed a statistically significant positive correlation (Spearman's $\rho = 0.47$; $p < 0.001$) (Fig. 3). Based on the statistical significance of this correlation, one may consider taking the lowest SI value into account when assessing the relative accuracy of EC3 values obtained using log-linear extrapolation.

Representative Examples

To illustrate the effect of the slope ratio and lowest SI value > 3 on the accuracy of log-linear extrapolated EC3 values; four representative datasets are provided in Table 3 and Fig. 4. For 3-dimethylaminopropylamine (Fig. 4A, Table 3A) the interpolated EC3 and extrapolated EC3 values were 2.2% and 2.3% respectively, resulting in the same potency classification of moderate. For the extrapolated EC3, the lowest SI value used was close to 3 and the slope ratio was 0.99, indicating that the values used for extrapolation were on the linear portion of the curve. The interpolated and extrapolated EC3 values for isoeugenol were also very similar (Fig. 4B, Table 3B), and the lowest SI value used to derive the extrapolated EC3 was close to 3 and the slope ratio was 1.7. Disagreement between the potency

Table 3 Comparison of EC3 values derived by linear interpolation and log-linear extrapolation

Chemical	Conc. (%)	SI Value	EC3 ¹	EC3 ext ²	Slope ratio ³	Potency category ⁴	Reference
A. 3-Dimethylamino-propylamine	0.5	1.3	2.2	2.3	0.99	Moderate/Moderate	16
	1.0	1.1					
	2.5	3.5 ⁵					
	5.0	7.0					
	10.0	13.9					
B. Isoeugenol	0.5	1.8	1.8	1.9	1.7	Moderate/Moderate	19
	1.0	2.0					
	2.5	3.8 ⁵					
	5.0	5.8					
	10.0	12.6					
C. Phenylacetaldehyde	1.0	0.7	3.0	0.18	3.4	Moderate/Strong	16
	2.5	1.8					
	5.0	7.8 ⁵					
	10.0	8.8					
	25.0	19.0					
D. 1,2-Dibromo-2,4-dicyanobutane	0.5	1.4	0.9	0.03	11.4	Strong/Extreme	16
	1.0	3.4 ⁵					
	2.5	3.5					
	5.0	5.4					

¹EC3 values derived by linear interpolation.²EC3 values derived by log-linear extrapolation.³Slope ratio of the extrapolation EC3 value dose response curve.⁴Potency classification based on the ECETOC categorization scheme. Potency based on interpolated EC3 value/Potency based on extrapolated EC3 value from the same data set.⁵Lowest SI value used to calculate the EC3 values derived by log-linear extrapolation.

classifications based on log-linear extrapolated EC3 values relative to those obtained by interpolation was observed for both phenylacetaldehyde and 1,2-dibromo-2,4-dicyanobutane (Table 3C and 3D). The classifications derived from the extrapolated values were off by one category and were more potent. The dose responses for these two chemicals show that the data used for log-linear extrapolation were located on a flat portion of the curve (Fig. 4C and 4D). Even though the lowest SI value used to derive the extrapolated EC3 for 1,2-dibromo-2,4-dicyanobutane was close to 3, the misclassification was most likely due to the poor quality of the dose response curve, having a slope ratio of 11.4.

DISCUSSION

While many chemicals can be identified as contact sensitizers, it has been estimated that they can vary significantly (and by up to 4 or 5 orders of magnitude) in terms of their intrinsic ability to induce allergic contact dermatitis (7). Such difference in sensitizing activity are referred to as allergenic potency and, in this context, can be defined as the amount of chemical required to induce skin sensitization in a previously unexposed individual. Objective information regarding the relative potency of one allergen compared with other skin sensitizers can be obtained from

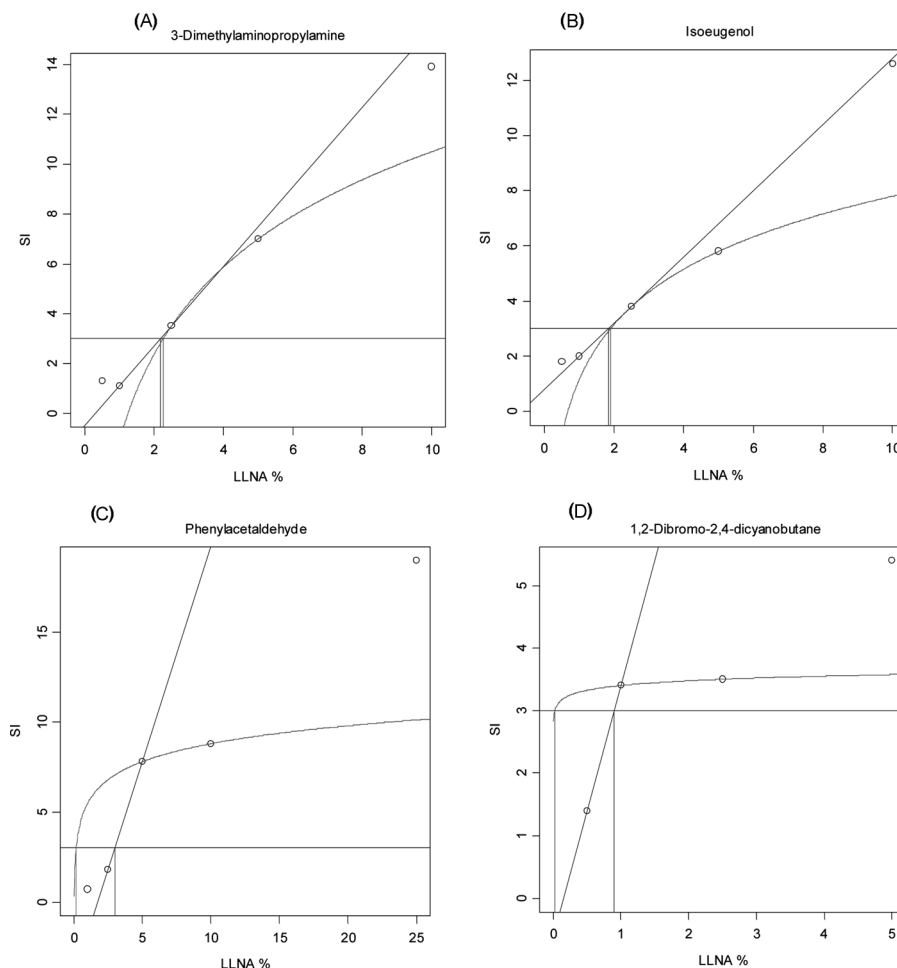


Figure 4 Representative examples of EC3 values calculated by both linear interpolation and log-linear extrapolation. In each graph, the horizontal line denotes $SI = 3$. Lines are also shown to represent fits through the two data points used; the straight line represents the data points used for linear interpolation of the EC3 value and the curved line represents log-linear extrapolation. The data sets for these graphs are given in Table 3. A 3-Dimethylaminopropylamine; B. Isoeugenol; C. Phenylacetaldehyde; D. 1,2-Dibromo-2,4-dicyanobutane. The % concentration tested is shown on the x-axis and the stimulation index (SI) is on the y-axis.

LLNA data and is based on the mathematical estimation of the concentration of chemical necessary to obtain a threshold positive response, the EC3 value (9). Linear interpolation has been shown to provide a simple and reliable method for calculating EC3 values and requires that the LLNA dose response curve contain least one SI value above 3, and one below. A method was developed to estimate EC3 values by log-linear extrapolation when all test concentrations result in SI values greater than 3 in order to avoid the need for repeat animal testing and obtain information on a likely potency classification. With log-transformation of the data, extrapolated EC3 values never fall below zero. To gain insight into the reliability of potency

classifications based on log-linear extrapolated EC3 values, the impact of the quality of the dose response information used to derive those values was explored.

A total of 187 data sets with at least one SI value less than 3 and at least two SI values greater than 3 were used for this evaluation. EC3 values were calculated by two methods namely, linear interpolation and log-linear extrapolation and the resulting values were compared. Use of the same data set to obtain both values eliminated experimental variability, which would have been present if data sets from independent studies had been used. To assess the role of dose response curve quality on the accuracy of log-linear extrapolated EC3 values relative to linear interpolated values, 83 data sets with 3 or more data points above SI = 3 were used.

Overall, potency classification based on log-linear extrapolated EC3 values was reliable and identified the same potency category as derived from linear interpolated EC3 values for 88% of the data set evaluated. When potency classifications derived from extrapolated EC3 values did not agree with those from interpolated EC3 values, the extrapolated classification tended to be off towards the side of caution, i.e., a stronger classification.

Evaluation of these data revealed two aspects of data quality which can affect the accuracy of potency classification derived from extrapolated EC3 values: the location on the dose response curve of the data points used, and the value of the lower SI. The dose response curve location of the extrapolation data points was determined by calculating a ratio of the slopes between the second and third and the first and second points. A higher degree of accuracy was found in the extrapolated values derived from curves with slope ratios below 2, indicating that the use of points which lie on the linear portion of the dose response curve results in more reliable potency class estimations. A significant correlation was also found between the lowest SI value and the accuracy of EC3 values derived by log-linear extrapolation. The reliability of extrapolated values was found to be greater when the lower SI value used in the calculation approached 3. Of these two parameters, it appears that the slope ratio is the more important to consider. Examination of the 83 datasets showed a higher percentage of potency misclassifications when the slope ratio exceeded 2 compared to datasets with slope ratio less than 2 (data not shown).

Clearly, confidence in potency classifications derived from the log-linear extrapolation method is dependent upon the quality of the dose response data. When the data do not permit the use of linear interpolation, calculation of EC3 values by log-linear extrapolation can provide a reliable estimation of sensitization potency class for use in risk assessment and may avoid the need for repeat animal testing.

REFERENCES

1. Kimber I, Dearman RJ, Basketter DA, Ryan CA, Gerberick GF. The local lymph node assay: past, present and future. *Contact Derm* 2002; 47(6):315–328.
2. Dean JH, Twerdok LE, Tice RR, Sailstad DM, Hattan DG, Stokes WS. ICCVAM evaluation of the murine local lymph node assay. Conclusions and recommendations of an independent scientific peer review panel. *Regul Toxicol Pharmacol* 2001; 34(3):258–273.
3. Balls M, Hellsten E. Statement on the validity of the local lymph node assay for skin sensitisation testing. *ATLA* 2000; 28(3):366–367.

4. Gerberick GF, Robinson MK, Felter SP, White IR, Basketter DA. Understanding fragrance allergy using an exposure-based risk assessment approach. *Contact Derm* 2001; 45(6):333–340.
5. Kimber I, Gerberick GF, Basketter DA. Thresholds in contact sensitization: theoretical and practical considerations. *Fd Chem Toxicol* 1999; 37(5):553–560.
6. Kimber I, Basketter DA, Berthold K, Butler M, Garrigue J-L, Lea L, Newsome C, Roggeband R, Steiling W, Stropp G, Waterman S, Wiemann C. Skin sensitization testing in potency and risk assessment. *Tox Sci* 2001; 59(2):198–208.
7. Kimber I, Basketter DA, Butler M, Gamer A, Garrigue J-L, Gerberick GF, Newsome C, Steiling W, Vohr H-W. Classification of contact allergens according to potency: proposals. *Fd Chem Toxicol* 2003; 41(12):1799–1809.
8. Basketter DA, Lea LJ, Dickens A, Briggs D, Pate I, Dearman RJ, Kimber I. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *J Appl Toxicol* 1999; 19(4):261–266.
9. 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 Derm* 2000; 42(6):344–348.
10. 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. *Am J Contact Derm* 2001; 12(3):156–161.
11. Warbrick EV, Dearman RJ, Lea LJ, Basketter DA, Kimber I. Local lymph node assay responses to paraphenylenediamine: intra- and inter-laboratory evaluations. *J Appl Toxicol* 1999; 19(4):255–260.
12. Dearman RJ, Wright ZM, Basketter DA, Ryan CA, Gerberick GF, Kimber I. The suitability of hexyl cinnamic aldehyde as a calibrant for the murine local lymph node assay. *Contact Derm* 2001; 44(6):357–361.
13. Basketter DA, Cadby P. Reproducible prediction of contact allergenic potency using the local lymph node assay. *Contact Derm* 2004; 50(1):15–17.
14. Gerberick GF, Ryan CA, Kern PS, Dearman RJ, Kimber I, Patlewicz GY, Basketter DA. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. *Contact Derm* 2004; 50(5):274–288.
15. OECD. Guidelines for Testing of Chemicals. Guideline No. 429. Skin Sensitization: The Local Lymph Node Assay. Organisation for Economic Cooperation and Development, Paris. 2002.
16. Gerberick GF, Ryan CA, Kern PS, Schlatter H, Dearman RJ, Kimber I, Patlewicz GY, Basketter DA. Compilation of historical local lymph node assay data for evaluation of skin sensitization alternative methods. *Dermatitis* 2005; 16(4):157–202.
17. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Technical Report No. 87, Contact Sensitisation: Classification According to Potency. Brussels, Belgium, April 2003.
18. Snedecor GW, Cochran WG. Statistical Methods. 7th ed; Ames, IA: The Iowa State -University Press, 1980:191–192.
19. Wright ZM, Basketter DA, Blaikie L, Cooper KJ, Warbrick EV, Dearman RJ, Kimber I. Vehicle effects on skin sensitizing potency of four chemicals: assessment using the local lymph node assay. *Int J of Cosmet Sci* 2001; 23(2):75–83.