

The impact of exposure variables on the induction of skin sensitization

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Whereas many investigations of the variables associated with the elicitation of allergic contact dermatitis have been undertaken, to the point where we can begin to predict the likelihood of elicitation occurring in a given situation, the same is not true for the induction of skin sensitization. Studies have demonstrated that increasing dose has an impact; in an experimental setting, a number of variables received attention some decades ago. However, in the work reported here, the relative importance of the frequency and the duration of exposure is highlighted. In an investigation using a human repeated insult patch test, it was demonstrated that reduction of the exposure duration from 48 hr to 5 min decreased the rate of sensitization to 1% *p*-phenylenediamine (PPD) from 54% to 3%. However, in an extended clinical study, it was observed that infrequent but longer duration and higher concentration exposure to PPD was significantly less likely to induce sensitization compared to more frequent, short duration, and lower concentration exposure. Detailed statistical analysis of the results indicated that the most important factor driving the induction of skin sensitization was the number of exposures.

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In recent years, our appreciation of the relative potency of skin sensitizers has increased to a substantial degree in large measure due to the quantitative predictions obtained in the local lymph node assay for a wide range of contact allergens (1–4). Considerable efforts have been undertaken to demonstrate how this information relates to human allergen potency, which, by the measure of the threshold in the human repeated insult patch test (HRIPT), spans at least 4–5 orders of magnitude (5–7). Such knowledge is now actively incorporated into a transparent and quantitative risk assessment (QRA) process (8–10). All this represents positive steps forward in efforts to limit the errors of risk assessment/management, which have led to epidemics of allergic contact dermatitis (ACD) in the past (11, 12). However, QRA requires that certain assumptions are made and degrees of uncertainty accommodated (10). 1 of these areas of uncertainty has already required some modification in order to accommodate risk assessment in relation to mucosal exposure (13). It seems probable that further changes may be nec-

essary as experience is gained with the application of this methodology.

1 area of interest, and where there is still a good deal of uncertainty, concerns our appreciation of the importance of duration and frequency of exposure to a skin sensitizer on the acquisition of contact allergy. Dose, of course, plays a central role, but a careful re-examination of the publication of Professor Kligman in the 1960s pays dividends, as he studied a number of other variables also associated with the induction of skin sensitization (14–16). In addition, Friedmann and co-workers completed key work, notably on the importance of dose/unit area as the fundamental driver of sensitization induction (17). These various aspects have been reviewed recently (18). However, there has been little, if any, information on the impact of frequency of exposure versus duration of exposure on the induction of contact allergy. For example, how many exposures and of what duration are required to cause skin sensitization? Historically, it is clear from the work on the HRIPT that 9 × 24 hr or 48 hr of occluded

exposures would cause sensitization to a range of allergens (19, 20). However, the impact of the omission of occlusion, of shortening the exposure duration, of increasing the number of exposure, and of altering the interval between exposures has not been studied to any degree. It is these aspects that have been examined in the work reported herein.

Materials and Methods

Materials

p-phenylenediamine (PPD) was obtained from Sigma, Gillingham, UK, and incorporated into a hair colourant formulation base at a concentration of 0.96%. This formulation was contained in 1 compartment of a dual compartment sachet, while the second compartment contained the activating peroxide system necessary for dye formation. The sachet was designed to dispense an equal volume of each component so that the final concentration on the head was 0.48% PPD. A commonly used permanent hair dye formulation made by a global hair dye manufacturer and containing approximately 3% PPD was purchased from the marketplace in Bangkok. Diagnostic patch test materials (1% PPD in petrolatum) were applied in Finn chambers[®] (Epitest Oy, Tuusula, Finland) using Scanpor tape (Beiersdorf, Hamburg, Germany).

Human repeated insult patch test

98 healthy volunteers gave fully informed written consent to participate in this study, which had been approved by the local independent ethical review committee (Institutional Review Board). Patches of 1% PPD (>99% purity; Sigma) in pet. were applied on the upper outer arm (deltoid region) for 5 min thrice per week (Monday, Wednesday, Friday, or Tuesday, Thursday, Saturday), for a total of 3 weeks. Patches consisted of 2-cm² non-woven cotton pad backed by 4 cm² of hypoallergenic occlusive tape. After removal of the patch, damp clean gauze was used to wipe residual material from the test site. Challenge, also with 1% PPD in pet., was left in place for 48 hr and read at 30 min after patch removal and again at 96 hr after patch application on a simple 0, 1, 2, 3 scale, where grades 1–3 were regarded as positive. A pet. vehicle control patch was also applied at challenge.

Bangkok study volunteers

Healthy Thai adults not on any systemic medication, aged from 18 to 55 years, who had not dyed during the past 2 years and with no history of

hair dye allergy were recruited from the Bangkok area ($n = 2545$). All the individuals gave fully informed written consent. The study received local ethical approval in Bangkok. All volunteers were screened with a 48-hr patch test with 1% PPD in pet. to ensure that they were not allergic to PPD prior to the start of the study. Individuals were then allocated into test groups to ensure that a balance of age, sex, and social grouping was represented in each. The groups were exposed to hair colourant containing PPD (group 1), exposed to permanent hair dye (group 2) or were unexposed for the duration of the study (group 3).

Bangkok study protocol

The exposure period in this study was 6 months. Group 1 used the hair dye colourant according to the general use instructions, but in an unsupervised manner. Use was intended to be 5 min/day for the first 4 days and then approximately once per week thereafter (still with 5 min/exposure). Group 2 used permanent hair dye (approximately 30–40 min of exposure) once per month (6 exposures in total). Group 3 were unexposed. All the groups were monitored, initially every 2 weeks and then monthly. At the end of this period, following a 3- to 4-week interval, all panellists in each group were retested with 1% PPD as well as with an open test to PPD to assess the extent of the induction of skin sensitization to PPD.

Patch testing

1% PPD in pet. was applied for 48 hr as mentioned above, and reaction sites were read approximately 1 hr after patch removal according to the standard International Contact Dermatitis Research Group (ICDRG) scale (21).

Data analysis

To perform an overall test for equality of the proportions in the 3 groups, a logistic regression model was fitted, with the number sensitized as the response variable and the test group as the predictor variable. This test is asymptotically equivalent to the more familiar chi-squared contingency table test. It is preferred here because there was a requirement to go on to perform multiple comparisons between individual groups. This was achieved using the glimmix procedure in SAS (SAS Institute Inc., Cary, NC, USA) (22), with Tukey multiple comparison adjustment applied to the pair-wise comparisons.

Consumer use data for the colourant group were subsequently analysed to try to determine

the factors affecting likelihood of sensitization. Of the 1107 individuals in this group who were patch tested at the end of the study, a number of individuals had incomplete questionnaire data and were excluded from the detailed analysis (1037 remaining, of whom 71 were sensitized). Exploratory data analysis of the large number of variables measured in the questionnaires was performed. Fisher's exact test was used to analyse nominal variables (e.g. sex). The Cochran–Mantel–Haenszel chi-squared test was used for ordinal variables (e.g. hair length). Wilcoxon's test was used to analyse variables such as the number of sachets used. This is analogous to a *t*-test, but it is based on ranks so that it is not necessary to make distributional assumptions.

Finally, logistic regression with step-wise selection was used to determine the best predictors of sensitization. The objective was not to build a prediction model (the circumstances of the test were rather specific) but to allow exploration of the factors affecting sensitization in a combined framework, thus avoiding the problems associated with multiple comparisons and also allowing for correlation between variables. The factors judged to be affecting sensitization were those that appeared consistently in both the exploratory data analysis and the modelling approach.

Results

Human repeated insult patch test

Skin readings on volunteers with skin reactions at challenge are shown in Table 1. No significant events related to PPD exposure occurred during the study induction phase. At challenge, 3 of 98 (3%) volunteers gave a grade 1 or a grade 2 reaction at 96 hr, consistent with these reactions being due to the induction of a mild state of skin sensitization. 2 individuals gave a grade 1 reaction only at the first scoring time and were judged to be irritant reactions. A single subject was judged to be presensitized based on the very early appearance (effectively 96 hr after the first 5-min induc-

tion application) and on the strength of the reactions (grade 3) at challenge; it should be noted that patch sites were moved during the induction phase for this individual to minimize discomfort.

Bangkok clinical study

In the initial patch test screen, 69 of 2545 individuals gave a positive reaction to 1% PPD in pet. at 48 h and were excluded from the study. This data on prevalence in the adult population have been presented elsewhere (23). The PPD-negative volunteers were allocated into 3 groups. Group 1, exposed to hair dye colourant, of whom 1107 completed the study; group 2, exposed to permanent hair dye, of whom 548 completed the study; and group 3, the unexposed control group, of whom 516 completed the study. Within the exposed groups, a small number of individuals (13 in all) suspected that they might have developed allergy, thus were removed from the study and patch tested immediately. None had significant symptoms and none was PPD patch test positive (data not shown). 292 volunteers, randomly distributed across the groups, did not complete the study. At the regular examination points during the study, the overwhelming majority of individuals exhibited either no scalp reaction or complained only of 'symptoms' typical of those found normally, e.g. scalp itch. At the end of the study, about half of those who were positive to PPD displayed clinical symptoms, ranging from mild to severe itch, erythema, and papules; a single case, associated with group 2, who were exposed to permanent hair dye, presented with a severe allergic reaction.

The results of the occluded 48-hr PPD patch test conducted at the end of the study are presented in Table 2. The highest response occurred in group 1, where 7.2% were judged positive; in contrast, only 1.3% reacted in group 2. To control for the limited ability of a 1% PPD patch test to induce sensitization, the unexposed group 3 was included, where only 0.4% were patch test positive to PPD at the end of the study. Almost all patch test reactions were grade 1. Positive reactions in group 1 significantly greater than group 2 ($P < 0.0001$) or group 3 ($P < 0.0001$); there was no significant difference between groups 2 and 3 ($P = 0.74$).

Table 1. HRIPT results*

Subject	Induction number									Challenge		Result
	1	2	3	4	5	6	7	8	9	48 hr	96 hr	
A	0	0	0	0	0	0	0	0	0	1	1	Sensitized
B	0	0	0	0	0	0	0	0	0	1	0	Irritant
C	0	0	2	2	2	1	0	0	0	3	3	Presensitized
D	0	0	0	0	0	0	0	0	0	1	0	Irritant
E	0	0	0	0	0	0	0	0	0	0	1	Sensitized
F	0	0	0	0	0	0	0	0	0	0	2	Sensitized

HRIPT, human repeated insult patch test.

*Grading scale: 0, no reaction; 1, erythema/induration; 2, erythema/induration with vesicles; and 3, bulla formation.

Table 2. PPD patch test results

Group	1% PPD patch test outcome, n (%)
Group 1	80/1107 (7.2)
Group 2	7/548 (1.3)
Group 3	2/516 (0.4)

PPD, *p*-phenylenediamine.

An open test was also performed at the same time, with scoring being formed as for the patch test. From all groups, those who were patch test positive to PPD, approximately one-third were positive to the open test (data not shown).

In the subsequent analysis of the consumer data associated with those exposed to the hair dye colourant (group 1) where the greatest frequency of sensitization induction was observed, the key variable predicting whether or not a person became sensitized during the test was the number of occasions on which the product was used (Fig. 1). Although this difference is significant ($P = 0.007$), it is not striking, and for most individuals, the number of times the product was used was similar. The likelihood of sensitization was also somewhat increased by covering the head (usually with a towel) (data not shown). The total number of sachets used was not predictive (Fig. 2), and nei-

ther was the average amount of time the product was left on the head (Fig. 3).

Conclusions

There is an expanding body of knowledge associated with our developing understanding of the elicitation of ACD in individuals who are already sensitized. Increasingly, studies of elicitation dose–response relationships become more complex in order to better replicate real-life exposures (24, 25). Such investigations should help considerably in terms of secondary prevention of ACD. However, and as others have indicated, it is more important in our view to avoid the induction of skin sensitization in the first place – primary prevention (26). Experimental investigation of induction dose–response relationships has been limited, not least for ethical reasons, to relatively simple

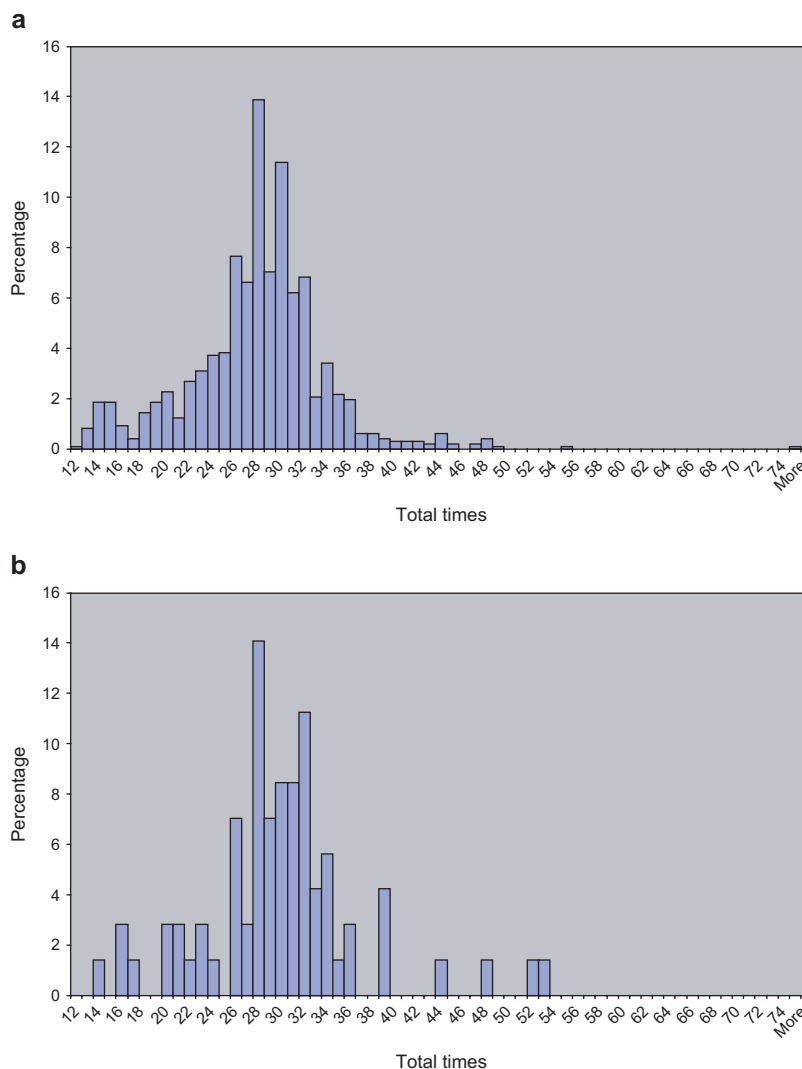


Fig. 1. Number of times test product was used ($P = 0.007$, Wilcoxon). **a** Non-sensitized individuals and **b** sensitized individuals.

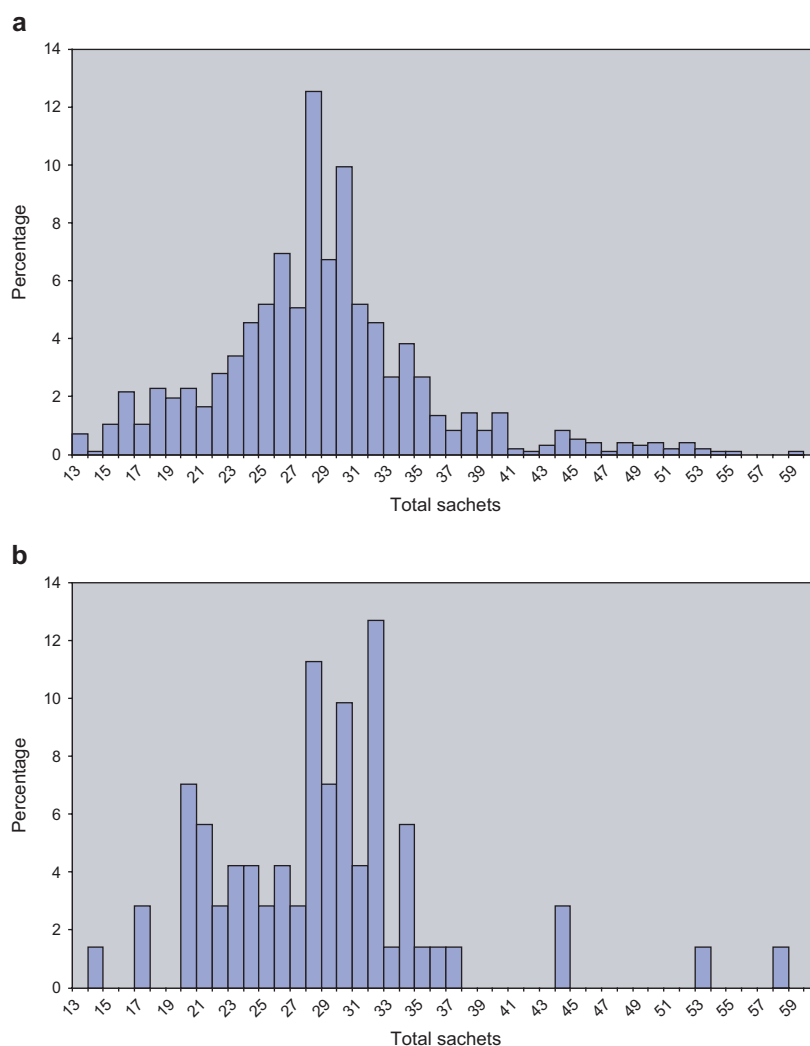


Fig. 2. Total number of sachets used ($P = 0.80$, Wilcoxon). **a** Non-sensitized individuals and **b** sensitized individuals.

types of study. For example, the work of Kligman in the 1960s focused on the development of optimal predictive assays and so drew conclusions largely around how to develop a practicable test protocol. Nevertheless, this work served to illustrate the importance of dose per unit area, of concentration, and of the targeting of individual lymph nodes (14–16, 27). Further studies by Marzulli and Maibach considered the role of induction dose and vehicle (19, 20, 28). A few years later, Friedmann and co-workers confirmed the key aspect of dose per unit area as a driver of the induction of skin sensitization, as well as demonstrating the clear relationship between exposure concentration and the likelihood of the development of sensitization (29–31).

Despite the studies mentioned above, in reality, the understanding of the relationship between real-life exposure and development of skin sensitization is very limited. Where industrial accidents have occurred (32), then, particularly with

strongly sensitizing chemicals, cause and effect can be clearly demonstrated. Then, there exist a number of other occupational exposures, which, by their unique nature, permit at some level and appreciation of exposure-response relationships (33). However, these are relatively uncommon. Nevertheless, the situation contrasts with consumer exposure, where the key determinants of sensitization are poorly understood. Even in situations where an exposure-response relationship has seemed very clear (34), this relationship is between exposure and eczema, the elicitation reaction, not between exposure and the induction of sensitization. At that latter level, knowledge is extremely limited.

In the present work, conducted during the course of a programme to evaluate alternative delivery systems for hair dyes, 2 formulation types were assessed over a 6-month period for their propensity to induce skin sensitization to PPD. The study was carefully controlled from

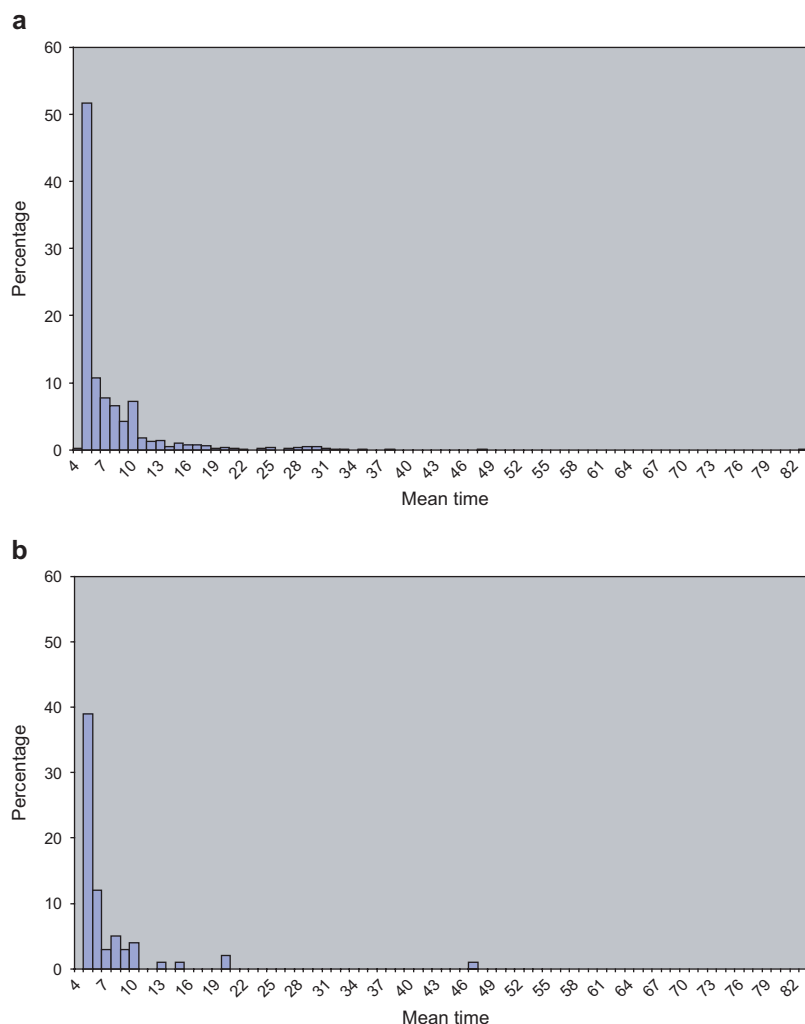


Fig. 3. Mean time for which product was left on the head ($P = 0.31$, Wilcoxon). **a** Non-sensitized individuals and **b** sensitized individuals.

many perspectives but, importantly, allowed the volunteers to use the formulations according to the supplied instructions. Thus, the study was expected to cover both normal consumer use and reasonably foreseeable misuse. Volunteers in the study were PPD negative at the outset, as judged by PPD patch test. The degree of sensitization induced over 6 months was assessed by PPD patch test; to evaluate the extent to which the screening patch alone might be sufficient to sensitize, a control group was employed. Of these, only 2 of 516 (0.4%) were positive at the end of the trial. This is similar to the maximum number thought to be sensitized during diagnostic patch testing (35), but it is also possible that sensitization may have occurred due to a lack in compliance (i.e. use of permanent hair dye that was not detected). Some authors have suggested that a higher number might be sensitized (36), but in our view, this is likely to be a function of the group assessed (normal healthy adults in

the present study versus selected patients with eczema).

Against this very low background, the permanent hair dye user group displayed a sensitization rate of 1.3% (7/548); this dataset has been published elsewhere (23). These group 2 individuals had had 6 exposures (1 per month) to a formulation with an on the head concentration of PPD of approximately 1.5%. Only 1 of the 7 experienced a significant allergic reaction, and this was as a consequence of the sixth exposure. In contrast to group 2, the individuals in group 1, who were exposed to a threefold lower concentration (approximately 0.5% PPD) and for shorter duration (5 versus 30 min), but weekly rather than monthly, displayed a very considerably higher rate of sensitization (7.2%).

The outcome of this study was unexpected. It had been anticipated that the threefold lower concentration and substantially shorter exposure duration would be associated with a lower degree

of sensitization, despite the greater number of exposures (approximately fivefold; Fig. 1). As a consequence, the detailed panellist and use information harvested during the course of the study were interrogated with great care. Were those sensitized in group 1 individuals who tended to overuse the formulation, e.g. by leaving it on the head for a much greater length of time than the recommended 5 min? However, statistical analysis demonstrated that it was in fact largely the number of uses, not the exposure time that was of significance ($P = 0.007$). Although consistent with the results of the HRIPT, the apparent irrelevance of duration of exposure in the 6-month clinical contrasts with observations made on the elicitation of PPD allergy (37). In the current study, the likelihood of sensitization was also increased by covering the head. In contrast, the total number of sachets used was not predictive (Fig. 2), and neither was the average amount of time the product was left on the head (Fig. 3).

In conclusion, it is clear that our appreciation of the variables associated with the acquisition of skin sensitization in the context of real-life exposure is still imperfect. Whereas QRA strategies that aim to ensure that the skin exposure remains below the threshold for the induction of sensitization may be increasingly well developed (9, 10), prediction of the frequency of the induction of skin sensitization in situations where exposure is above threshold remains difficult. The data presented herein add to the limited knowledge and may help guide future risk assessments of this type.

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