

The dose–response relationship between the patch test and ROAT and the potential use for regulatory purposes

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Background: Allergic contact dermatitis is common and can be prevented. The relationship between thresholds for patch tests and the repeated open application test (ROAT) is unclear. It would be desirable if patch test and ROAT data from already sensitized individuals could be used in prevention.

Objectives: The aim was to develop an equation that could predict the response to an allergen in a ROAT based on the dose–response curve derived by patch testing.

Materials/methods: Results from two human experimental elicitation studies with non-volatile allergens, nickel and the preservative methyl-dibromoglutaronitrile (MDBGN), were analysed by logistic dose–response statistics. The relation for volatile compounds was investigated using the results from experiments with the fragrance chemicals hydroxyisohexyl 3-cyclohexene carboxaldehyde and isoeugenol.

Results: For non-volatile compounds, the outcome of a ROAT can be estimated from the patch test by: $ED_{xx}(ROAT) = 0.0296 ED_{xx}(\text{patch test})$. For volatile compounds, the equation predicts that the response in the ROAT is more severe than the patch test response, but it overestimates the response.

Conclusions: This equation may be used for non-volatile compounds other than nickel and MDBGN, after further validation. The relationship between the patch test and the ROAT can be used for prevention, to set safe levels of allergen exposure based on patch test data.

Key words: dose–response; elicitation; patch test; repeated exposure; ROAT. © John Wiley & Sons A/S, 2009.

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Allergic contact dermatitis is a common and preventable disease. It is possible to reduce the incidence of individuals becoming sensitized, as well as morbidity among those who are already sensitized, by reducing allergen exposure. Even though various risk assessment procedures may be used to identify the sensitization potency, allergenic chemicals are present in the environment and consumer products at levels that cause sensitization and allergic contact dermatitis in a considerable number of individuals (1). It is desirable to establish an allergen dose which does not elicit dermatitis in the already sensitized individuals. The dose of allergen required to sensitize an individual is probably

higher than the dose that elicits an allergic reaction in the sensitized individual (2, 3). In order to prevent both new cases of sensitization and chronic or recurrent cases of allergic contact dermatitis, it would be desirable if data from already sensitized individuals could be systematically used in prevention. Two different methods may be used to generate elicitation thresholds for contact allergic reactions: (i) dilution series patch testing, with the allergen in question in sensitized individuals is one possibility; this test is standardized and easy to perform; (ii) repeated open application test (ROAT) (4); this test is not standardized to the same extent (5) and it is time-consuming, but mimics some real-life exposure

situations. A link between these two test methods could be a valuable tool for establishing the elicitation threshold by daily use of a product containing allergens. Several studies have shown a significant relationship between the patch test threshold and the ROAT threshold (6–12), but the amount of allergen required to elicit a reaction for these two study methods is not the same.

The aim of this study was to examine if an equation can be developed, which can be used to predict the response to an allergen in a ROAT based on the dose–response curve derived by patch testing for non-volatile and volatile compounds.

Materials and Methods

Results from the two human experimental elicitation studies of non-volatile compounds were used for analysis. The experiments were performed using the same methodology and two different allergens: a metal (nickel) (13) and a preservative [methylidibromo glutaronitrile (MDBGN)] (14). On the basis of the results from a third study with a fragrance ingredient [hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)] using the same design (15), it was expected that volatile compounds had a different dose–response profile, therefore separate analysis were performed. Further, in order to define ROAT and patch test relation for volatile substances, the literature was searched for additional data, where volatile substances had been tested in a similar design. Dose per unit area was chosen as the dose metric (2, 16–24).

Experimental study design

The dose–response characteristics for the allergen in question in patch testing and a ROAT were simultaneously studied on the allergic individuals. The exact study details are published elsewhere (13–15). The patch tests consisted of a dilution series of 19 doses and one patch containing only the vehicle. The duration of the ROAT was up to 21 days, and the test subjects were exposed twice daily to three different concentrations (the nickel and MDBGN study), and four different concentrations (the HICC study), and a vehicle on different numbered areas on the ventral aspect of the forearms. The doses were chosen for each study so that the dose per application in the ROAT and accumulated 1- and 3-week doses was present in the patch test (Table 1). The dose per application from the ROAT was used for the comparison of the patch test and the ROAT because this value can more easily be translated to in use situations than the accumulated dose from the ROAT. All studies were approved by the local Ethics Committee.

The literature was searched for studies which fulfilled the following criteria: (i) a series dilution patch test and a ROAT performed on the same allergic individuals, (ii) for both test methods, the concentration should be given in $\mu\text{g}/\text{cm}^2$, or it should be possible to calculate the $\mu\text{g}/\text{cm}^2$; for the ROAT, it should be possible to calculate the $\mu\text{g}/\text{cm}^2/\text{application}$, (iii) the duration of the ROAT should be at least up to 3 weeks, (iv) at least five patch test concentrations and at least two ROAT concentrations had to be used, in order to make it possible to estimate dose–response curves, (v) the threshold concentration in the patch test should be defined as the weakest concentration giving a visible reaction in a continuous line of reactions starting from the highest concentration, and (vi) the allergen tested had to be a volatile compound. The search was carried out using Pubmed-Medline, using the search terms: ROAT, use-test, repeated open application test, dose–response, and contact allergy. Furthermore, the references in the identified publications were searched for relevant articles.

Definition of end-points

The patch test threshold was defined as the lowest concentration giving a visible reaction in a continuous line of reactions starting with the highest concentration. For the ROAT, a published scoring scale was used (25). The threshold concentration was defined as the weakest concentration giving a visible reaction, which was also present at the end of week 3 (if the exposure had not been terminated) or the weakest concentration giving a reaction with a score of 5 or above. The dose per application was chosen in the ROAT for comparison between the ROAT and the patch test, because this dose can be translated into relevant use-concentrations.

Development of model for non-volatile compounds

In order to develop a model to convert patch test data (response to $x \mu\text{g}$ allergen/ cm^2) in to ROAT data (response to $x \mu\text{g}$ allergen/ $\text{cm}^2/\text{application}$), three conditions has to be fulfilled. First, a positive correlation between the two test methods should be ascertained. Second, the dose–response curves have to be parallel and third, the conversion factor (F) describing the parallel displacement between the two dose–response curves has to be approximately the same for different allergens (nickel and MDBGN). If all three conditions are fulfilled it will be possible to develop an equation to convert the threshold dose that will elicit a reaction in $xx\%$ of allergic individuals, ED_{xx} for the patch test to the

Table 1. Study design for the dose–response studies with nickel (13), methyl dibromo glutaronitrile (MDBGN) (14) and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (15)

Area in the ROAT (the numbers were randomized for each test subject)	Dose per application ($\mu\text{g}/\text{cm}^2$)	Accumulated number of applications in 1 week	Total accumulated dose after 1 week ($\mu\text{g}/\text{cm}^2$)	Accumulated number of applications in 3 weeks	Total accumulated dose after 3 week ($\mu\text{g}/\text{cm}^2$)
1	0 (vehicle) ^a	14	0	42	0
2	0.0357 ^b	14	0.5	42	1.5
3	0.357 ^b	14	5	42	15
4	3.57 ^b	14	50	42	150
5	35.7 ^c	14	500	42	1500

The dose per application and accumulated dose after 1 and 3 weeks in the repeated open applications test (ROAT). The doses in italic were also present in the patch test.

^aVehicle; Nickel study: 10% ethanol, MDBGN study: 20% ethanol and HICC study: 70% ethanol.

^bIn the nickel study: 0.035, 0.35 and 3.5.

^cThese doses were only present in the HICC study.

corresponding ED_{xx} for the ROAT using the following equation:

$$\text{ED}_{xx}(\text{ROAT}) = F\text{ED}_{xx}(\text{patch test}) \quad (1)$$

Spearman's rank correlation was used to analyse the correlation between the results from the patch test and the ROAT performed on the same patients. The logistic dose–response model has been used earlier to describe the dose–response relation in contact allergy (26–28). A logistic dose–response analysis (29, 30) was used for fitting curves for the patch test and the ROAT, and it was determined if the patch and ROAT curves were parallel. The factor describing the parallel displacement of the dose–response curves on the log-dose axis was then calculated and subsequently a factor common for both nickel and MDBGN was determined.

The patch test and ROAT relation for volatile compounds

To test the dose–response relation, the third study with the fragrance HICC was used. Only one study (6) of isoeugenol from the literature was found to have fulfilled all criteria. Logistic dose–response curves were determined for each study, and the predicted ROAT dose–response curve was estimated by the use of the patch test results, and the curve was compared with the observed ROAT data.

Results

The model

Statistically significant correlations were found between the threshold patch test dose and the ROAT dose for nickel, MDBGN, and HICC (Table 2). The analysis could not be made for isoeugenol, because individual data were not available, but according to the publication (6) there was a significant correlation between the threshold patch test concentration

Table 2. Spearman's rank correlation between the threshold concentrations in the patch test and repeated open applications test (ROAT) for the nickel study (13), the methyl dibromo glutaronitrile (MDBGN) study (14) and for the hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) study (15)

Allergen	Number of patients	Correlation coefficient	<i>P</i> -value
Nickel	18	0.45	0.033
MDBGN	15	0.76	0.0021
HICC	16	0.59	0.011

and the number of days until a positive ROAT. On the basis of the analysis it was decided that the correlation between the patch test and the ROAT was acceptable.

To estimate if the patch test and ROAT dose–response curves were parallel, the logistic model for the probability of a positive response was used:

$$P = \frac{\exp(\alpha + \delta + \beta \ln(x))}{1 + \exp(\alpha + \delta + \beta \ln(x))} \quad (2)$$

where P is the probability of a positive response at the dose x , and α , δ , and β are constants that give the best statistical agreement between the observed and calculated response. The value of δ is per definition 0 for the reference curve (the patch test curve) and it can be shown that the factor, F in Equation (3), which is used for the calculation of ED_{xx} for ROAT from ED_{xx} , for patch test is given by:

$$F = \exp\left(\frac{-\delta}{\beta}\right) \quad (3)$$

The logistic parameters (α and β) were calculated separately for the patch test and ROAT data (Table 3). The values of β for the patch test and the ROAT have to be the same, if the dose–response curves are parallel. However, there is no established statistical test for the equality of the values of β for a given allergen since they are not calculated from independent data. Instead it is possible

Table 3. Analysis of parallelism of the patch test and repeated open applications test (ROAT) dose–response curves and calculation of the separate and combined factor F (describing the displacement between the patch test and the ROAT) for nickel (13) and MDBGN (14)

Allergen	Test	Separate analyses		Analyses of parallel dose–response curves				
		α	β	α	β	δ	$F = \exp(-\delta/\beta)$	F combined
Nickel	Patch	–1.898	0.845	–1.852	0.824	2.811	0.0330	0.0296
	ROAT	0.925	0.793					
MDBGN	Patch	–1.364	0.778	–1.183	0.684	2.483	0.0265	
	ROAT	1.063	0.542					

Table 4. Analysis of parallelism of the patch test and repeated open applications test (ROAT) dose–response curves and calculation of the factors, F (describing the displacement between the patch test and the ROAT) for hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (15) and isoeugenol (6)

Allergen	Test	Separate analyses		Analyses of parallel dose–response curves			
		α	β	α	β	δ	$F = \exp(-\delta/\beta)$
HICC	Patch	–2.971	1.115	–2.897	1.088	2.538	0.0971
	ROAT	–0.349	1.056				
Isoeugenol	Patch	–2.482	0.729	–2.483	0.729	1.573	0.1157
	ROAT	–0.913	0.731				

to compare the paired values of β for different allergens. It is noted from Tables 3 and 4 that β for patch test is largest in three out of the four cases. When the patch test β and the ROAT β (for nickel and MDBGN together and for all four allergens together) is tested, no significant difference between the β 's is found ($P = 0.34$ and $P = 0.19$, respectively, paired t -test). Furthermore, the visual impression (Figs 1–4) is that the curves are parallel, thus the condition of parallel dose–response curves for patch test and ROAT is acceptable.

The factor ($F = \exp(-\delta/\beta)$) required to convert the patch test ED_{xx} to the corresponding ROAT ED_{xx} was calculated for nickel ($=0.0330$) and MDBGN ($=0.0265$). These factors are very close

to one another, and their geometric mean value is $F = 0.0296$ (Table 3).

Therefore, based on the above analysis, it was concluded that all three conditions are fulfilled, and an equation to convert the patch test dose–response data into the ROAT dose–response data was possible by means of the following equation:

$$ED_{xx}(\text{ROAT}) = 0.0296 ED_{xx}(\text{patch test}) \quad (4)$$

In order to check how well the factor predicts the actual ROAT dose–response curves for nickel and MDBGN, dose–response curves were calculated based on Equation (4) and compared with the observed curves (Figs 1 and 2). This illustrates that for nickel and MDBGN the equation can be used with a reliable result, and gives an indication of the possible precision of the method.

The patch test and ROAT relation for volatile compounds

To investigate the patch test and ROAT relationship for volatile compounds, the HICC and isoeugenol studies were used. The logistic parameter values (α and β) were calculated, and the factor F describing the relation between the patch test and the ROAT was calculated for both allergens (Table 4). The factor F was calculated to be 0.0971 for HICC and 0.1157 for isoeugenol. It was decided not to suggest an equation for conversion for volatile compounds because (i) the isoeugenol study was not designed with the purpose of estimating the relation between the patch test and the ROAT, (ii) only two ROAT doses were used in the isoeugenol study, and (iii) fragrance ingredients evaporates with different rates (31). On the basis of the results presented

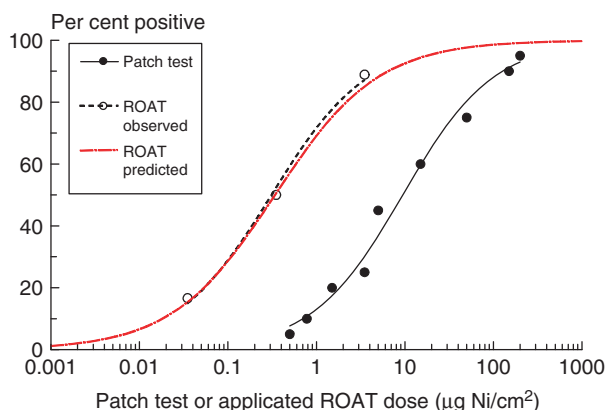


Fig. 1. Comparison of predicted and observed dose–response relations for repeated open applications test (ROAT) of nickel (13). The predicted ROAT dose–response curve (red line) is obtained from the fitted patch test curve (solid black line) by multiplication of the patch test doses by a factor 0.0296, i.e. $ED_{xx}(\text{ROAT}) = 0.0296 ED_{xx}(\text{patch test})$. The observed ROAT dose–response curve is shown as a broken black line.

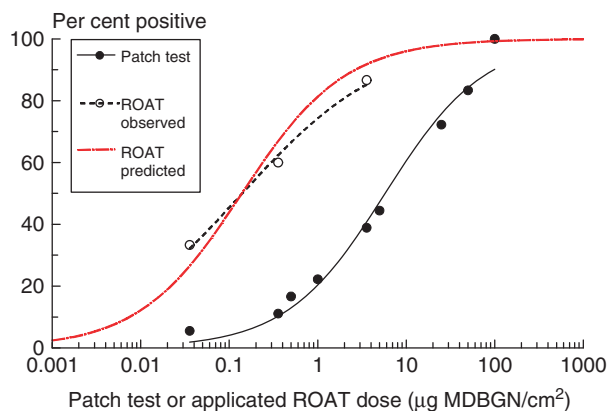


Fig. 2. Comparison of predicted and observed dose–response relations for repeated open applications test (ROAT) of methyldibromo glutaronitrile (MDBGN) (14). The predicted ROAT dose–response curve (red line) is obtained from the fitted patch test curve (solid black line) by multiplication of the patch test doses by a factor 0.0296, i.e. $ED_{xx}(ROAT) = 0.0296 ED_{xx}(\text{patch test})$. The observed ROAT dose–response curve is shown as a broken black line.

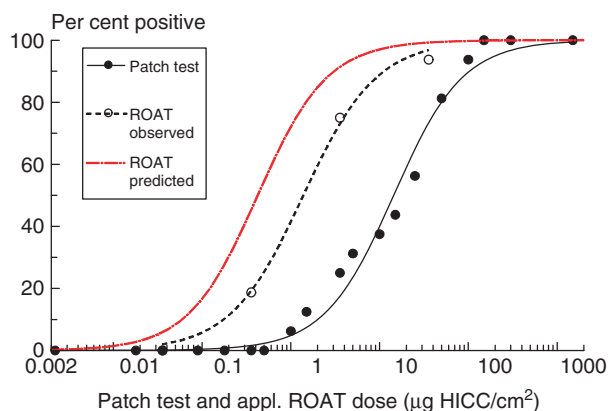


Fig. 3. Comparison of predicted and observed (black broken line) dose–response relations for repeated open applications test (ROAT) of hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (15). The predicted dose–response curve (red line) is obtained from the fitted patch test curve by multiplication of the patch test doses by a factor 0.0296, i.e. $ED_{xx}(ROAT) = 0.0296 ED_{xx}(\text{patch test})$.

here, an equation for volatile compounds could be: $ED_{xx}(ROAT) = 0.1060 ED_{xx}(\text{patch test})$, but further studies with volatile compounds are required.

To compare the difference between volatile and non-volatile compounds, a logistic dose–response analysis of the patch test for each study was performed and used to estimate the ROAT dose–response curve by means of Equation (4) (Figs 3 and 4).

The observed ROAT curves for HICC and isoeugenol are as predicted displaced to the left compared with the patch test dose–response curve, but the formula to predict the ROAT ED_{xx} for non-volatile compounds overestimates the observed response to the ROAT by a factor 3–4 in both

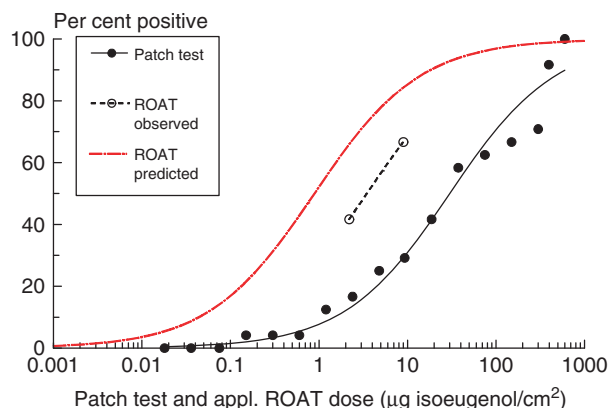


Fig. 4. Comparison of predicted and observed (broken black line) dose–response relations for repeated open applications test (ROAT) of isoeugenol (6). The predicted dose–response curve (red line) is obtained from the fitted patch test curve by multiplication of the patch test doses by a factor 0.0296, i.e. $ED_{xx}(ROAT) = 0.0296 ED_{xx}(\text{patch test})$.

figures, corresponding to the ratio between the factors 0.0971 and 0.1157 relative to 0.0296. Thus the relation between the patch test and the ROAT for volatile compounds is different than for non-volatile compounds.

Discussion

On the basis of the two dose–response studies with nickel and MDBGN, it was possible to develop an equation to translate patch test dose–response data in to ROAT dose–response data. In these two studies, a good correlation between the patch test and the ROAT dose–response data was found. The patch–ROAT transformation equation should not be applied for volatile allergens because it leads to overestimation of ROAT responses meaning that the observed response in the ROAT for the volatile substances is lower than the estimated response. Evaporation of a fragrance chemical from the skin in an open test results in a lower dose over time compared with the non-volatile compounds on which the equation is based. Even though the ROAT for the volatile compounds cannot be predicted from patch test threshold by the equation, it is suggested that the dose per application which elicits a reaction in the ROAT is substantially lower than the dose required to elicit a reaction in the patch test. This may be explained by the accumulation of allergen in the skin from repeated exposure or to the repeated stimulation of the immune system. Other studies have shown that cumulative exposure is an important factor in the elicitation response (32–34) and also for sensitization (35). In a recent review (36) on the dose–response characteristics of contact allergy, it was suggested that the repeated exposure to low

Table 5. Main findings

Main findings	Relevance
The dose ($\mu\text{g}/\text{cm}^2$) per application required to elicit a reaction in a repeated open applications test (ROAT) is lower than the dose required to elicit a reaction in a patch test.	When patch test data are used to determine safe levels of allergen exposure, a negative dose in the patch test is not a safe dose. This is also relevant for the clinical situation.
For non-volatile compounds, the outcome of a ROAT can be expressed by: $\text{ED}_{\text{xx}}(\text{ROAT}) = 0.0296 \text{ ED}_{\text{xx}}(\text{patch test})$.	After validation, regulatory intervention should be undertaken based on patch test dilution series. Safe levels of allergens that will prevent elicitation can be calculated and contact dermatitis be prevented in a majority of cases.

concentrations of allergen will have the same effect as a single high dose application.

The stronger response in the ROAT compared with the patch test threshold is important if human elicitation patch test studies are used to predict the elicitation potential of allergens in final products, because a dose per unit area, which is negative in a patch test, might elicit a reaction when applied repeatedly in an open test. The present data are relevant for risk evaluation of elicitation potential in sensitized individuals related to both consumer products and allergen exposure at the work place.

It would be ideal to establish an independent post-marketing surveillance system based on information of case-reports of allergy towards new substances and epidemiological data of the prevalence of allergy towards different allergens (37). Such surveillance should be followed by a systematic evaluation of the elicitation risk of the allergen in question, and when the safe level of allergen exposure has been established, regulation should be introduced to set the maximum permitted level of exposure. Safe exposure levels based on elicitation data will not only result in secondary and tertiary prevention but also in primary prevention, since the dose required to induce sensitization is probably higher than the dose required to elicit an allergic reaction (2, 3). Regulation of allergen exposure by legislation has proven effective for chromate (38), nickel (39–43), and MDBGN (44–46). So, patch test elicitation data can be used to set safe levels of allergen exposure by applying a conversion factor between the patch test and the ROAT. This factor ends up giving a lower acceptable exposure level for repeated open applications than the threshold for patch testing. For non-volatile compounds, this factor can probably be defined by Equation (4), and for volatile compounds the factor is dependent on the physico-chemical properties of the individual compound. When the safe exposure level of allergen is considered, the choice of vehicle (47, 48), the repeated allergen exposure from, e.g. soaps (49), the exposure site (50), and the possible synergistic or additive effect of mixing several allergens (51–53) in the final product should also be taken into account.

In the studies presented here ethanol–water was used as the vehicle, and this might lower the threshold compared with petrolatum (49, 54), but since the vehicle was the same for the two test methods, it probably did not influence the relationship between the two.

In conclusion (Table 5), the dose per area per application required to elicit a reaction in the ROAT is lower than the dose per area required to elicit a reaction in the patch test. This applies for several allergens. For volatile compounds, the outcome of the ROAT is influenced by evaporation. For non-volatile compounds, the outcome of the ROAT can be expressed by: $\text{ED}_{\text{xx}}(\text{ROAT}) = 0.0296 \text{ ED}_{\text{xx}}(\text{patch test})$. This equation can probably be used for non-volatile compounds other than nickel and MDBGN, but further studies are required to validate the equation. The relation between the patch test and the ROAT can be used in preventative strategies to set safe levels of allergen exposure based on elicitation patch test data.

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