

Nickel allergy: relationship between patch test and repeated open application test thresholds

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Summary

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Key words

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Conflicts of interest

None declared.

Background The frequency of nickel allergy varies between different population groups. Exposure regulation has proven effective in decreasing the frequency. Experimental studies with other allergens have shown a significant relation between patch test reactivity and repeated open application test (ROAT) reactivity.

Objectives This study was aimed at determining the elicitation threshold in nickel-allergic individuals in a patch test and a ROAT, and comparing the threshold from these two test methods.

Methods Twenty nickel-allergic persons were tested with a dilution series of 19 concentrations in a patch test and a dilution series of three concentrations in a ROAT, with duration of up to 21 days. Eighteen persons with no nickel allergy were included as control group for the ROAT.

Results The predicted dose which will elicit a reaction in 10% of allergic individuals was calculated to be $0.78 \mu\text{g nickel cm}^{-2}$ in the patch test. The threshold for the ROAT (in $\mu\text{g nickel cm}^{-2}$ per application) was significantly lower than the threshold for the patch test, while the dose–response for the accumulated ROAT dose at 1 week, 2 weeks and 3 weeks was very similar to the patch test dose–response; indeed, there was no statistically significant difference.

Conclusions For elicitation of nickel allergy the elicitation threshold for the patch test is higher than the elicitation threshold (per application) for the ROAT, but is approximately the same as the accumulated elicitation threshold for the ROAT. This may be important for risk assessment based on dose–response results from allergic patients.

Sensitization to and elicitation of nickel allergy is dependent on the amount of nickel released from metal or metal alloys or, more rarely, exposure to nickel salts. In particular the nickel release from items designed to be in direct and prolonged contact with the skin, such as earrings, has caused a high frequency of sensitization to nickel.^{1–3} A regulation of the nickel release from such items was introduced in Denmark in 1991 and was passed in the European Union in 1994 (implemented in 2000). The regulation introduced a maximum release of nickel of $0.5 \mu\text{g cm}^{-2}$ per week from items designed to be in prolonged contact with the skin, and a maximum content of 0.05% of nickel in piercing studs used during epithelialization.⁴ The regulation has recently been modified, and a maximum release of nickel of $0.2 \mu\text{g cm}^{-2}$ per week from piercing studs has been introduced instead of a maximum content of 0.05% in studs used during epithelialization.^{4,5} The regulation has proven to be effective in decreasing the sensitization rate to nickel.^{6,7}

Several experimental elicitation studies have been performed, investigating the dose–response characteristics of nickel allergy with either patch or use testing (reviewed by Fischer *et al.*⁸). Several studies with other allergens^{9–15} have shown a significant relationship between the elicitation threshold by patch testing and the elicitation threshold by repeated open application tests (ROATs). Such a comparison is of interest, because it gives an idea of how to extrapolate the results from patch test exposure studies into real-life exposure situations. Another study performed by the authors,¹⁶ investigating the influence of dose per area and total dose on the elicitation reaction of nickel allergy, has shown significantly more reactions in the ROAT (in dose per unit area per application) as compared with the same dose per unit area in the patch test. The aim of the present study was to compare the dose–response characteristics for nickel in patch testing and a ROAT performed on the same 20 nickel-allergic individuals simultaneously.

Materials and methods

Test subjects

The inclusion criteria were age above 18 years, and a previous positive standard patch test to nickel, with a positive reaction defined by erythema and homogeneous infiltration in the whole test area. The exclusion criteria were active eczema, systemic immunosuppressive therapy, ultraviolet radiation on the test area within 3 weeks of the testing, pregnancy, breastfeeding and not being able to cooperate. Twenty patients with contact allergy to nickel, demonstrated by a positive patch test (Finn Chamber) to nickel sulphate 5% in petrolatum within the last 6 years, at the Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark, were enrolled in the study after oral and written consent. The test subjects were 18 women and two men aged 19–67 years (mean 44.6).

Control subjects

Inclusion and exclusion criteria were the same as for the test subjects, except for the required negative patch test to nickel sulphate 5% in petrolatum. Eighteen control persons with no nickel allergy were enrolled in the study after oral and written consent. They were responders to an advertisement at the medical faculty, University of Copenhagen. The control subjects were eight women and 10 men aged 18–30 years (mean 23.3).

Study design and materials

The patch test materials ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ in 10% ethanol with 90% water and the control: 10% ethanol with 90% water) for both the patch test and the ROAT were manufactured by the Copenhagen County Hospital Pharmacy, Denmark. Patch testing and ROAT were performed simultaneously. The doses chosen for the patch test dilution series were based on a series of studies previously reviewed,⁸ and especially lower doses were included. Furthermore, the dose per application in the ROAT was represented in the patch test. The accumulated 1-week dose and the accumulated 3-week dose from the ROAT were also represented in the patch test in order to facilitate a comparison of the threshold for the two test methods (Table 1). The placement of the different test concentrations in both tests was randomized and blinded for the investigator and the subjects. After termination of the study the randomization code was broken. The study was performed according to the Helsinki II declaration and the study was approved by the local Ethics Committee (Copenhagen County, KA04032). All subjects gave their written, informed consent.

Patch test

Each test subject was tested on the back with the following dilution series (of $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ in 10% ethanol with 90% water): 200, 150, 50, 25, 15, 5, 3.5, 1.5, 0.78, 0.5, 0.35, 0.2, 0.1,

Table 1 Study design. The dilution series in the patch test and the equivalent exposure using repeated open application test (ROAT)

Patch test dilution series ($\mu\text{g Ni cm}^{-2}$)	Equivalent ROAT exposure ($\mu\text{g Ni cm}^{-2}$)
200	
150	Accumulated 3-week dose (3.5 per application) in the ROAT
50	Accumulated 1-week dose (3.5 per application) in the ROAT
25	
15	Accumulated 3-week dose (0.35 per application) in the ROAT
5	Accumulated 1-week dose (0.35 per application) in the ROAT
3.5	Highest dose per application in the ROAT (3.5)
1.5	Accumulated 3-week dose (0.035 per application) in the ROAT
0.78	
0.5	Accumulated 1-week dose (0.035 per application) in the ROAT
0.35	Middle dose per application in the ROAT (0.35)
0.2	
0.1	
0.05	
0.035	Lowest dose per application in the ROAT (0.035)
0.0125	
0.0063	
0.0031	
0.00156	

Bold: corresponding to the dose per application, and the accumulated 1-week and 3-week doses in the ROAT.

0.05, 0.035, 0.0125, 0.0063, 0.0031 and 0.00156 $\mu\text{g Ni cm}^{-2}$ and one blank containing 10% ethanol with 90% water. Fifteen microlitres of each dilution was applied on a filter paper in a small Finn Chamber of 0.5 cm^2 on Scanpor (Epitest, Tuusula, Finland).

The patch test was applied on day 0, occluded for 2 days and the reactions were read on days 2, 3 or 4 and 7, but only the second reading was used for the statistical calculations. The reading scale for the patch test was based on the International Contact Dermatitis Research Group reading scale, and further developed by Hindsen and Bruze¹⁷ in order to recognize smaller differences in the allergic reactions. The scale used was a modification of this scale to include also weaker reactions. The scale was as follows: 0, no reaction; 1, few papules with no erythema, no infiltration; 2, faint erythema with no infiltration or papules; 3, faint erythema with few papules and no homogeneous infiltration; 4, erythema, homogeneous infiltration; 5, erythema, infiltration and a few papules; 6, erythema, infiltration and papules; 7, erythema, infiltration, papules and a few vesicles; 8, intense erythema, infiltration and vesicles. The threshold concentration was

defined as the weakest concentration giving a visible reaction (minimum score 1) on day 3 or 4 in a continuous line of patch test reactions starting from the highest test concentration ($200 \mu\text{g Ni cm}^{-2}$).

Repeated open application test

Each test subject was tested on four areas on the volar aspect of the forearms on areas of 3×3 cm. Twenty microlitres was applied twice daily using a micropipette (Acura 815, 20 μL ; Buch & Holm, Herlev, Denmark) with a fixed volume, and four numbered bottles. Each number referred to a specific area. The solutions were spread out on the area with the tip of the pipette and allowed to dry by evaporation. The subjects were shown how to use the pipette and were also given oral and written instructions about its use. The areas were tested with a dose per application of 3.5, 0.35 and $0.035 \mu\text{g Ni cm}^{-2}$. These doses per application correspond to an accumulated 1-week dose of 50, 5 and $0.5 \mu\text{g Ni cm}^{-2}$, respectively, and an accumulated 3-week dose of 150, 15 and $1.5 \mu\text{g Ni cm}^{-2}$, respectively. The fourth area was a control and was exposed to 10% ethanol with 90% water. The control subjects were tested on two areas with $0.035 \mu\text{g Ni cm}^{-2}$ and 10% ethanol, respectively. The reactions were read routinely on all subjects at days 2, 3 or 4, 7, 14 and 21 of exposure, and additional readings were made if a reaction occurred. In case of a score of 5 or above (based on the involved area, erythema, number of papules and number of vesicles) the exposure to that area was terminated, and if no reaction occurred, or in case of a score below 5, the exposure was terminated on day 21. The same investigator read the reactions on all the subjects using a ROAT reading scale developed previously.¹⁸ 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.

Statistics

The dose–response relation in the patch test was estimated by fitting a logistic threshold distribution to the observed frequencies of positive response at the increasing doses.

The elicitation dose ED_{xx} which predicts a dose which will elicit a reaction in 1%, 5%, 10%, 25% and 50%, respectively, was calculated and a fitted dose–response curve for the patch test was drawn. The 95% confidence interval (CI) for ED_{xx} was calculated according to Fieller's method.

A comparison of elicitation reactions to the dose per application in the ROAT ($\mu\text{g Ni cm}^{-2}$) and the elicitation reactions to the corresponding dose in the patch test ($\mu\text{g Ni cm}^{-2}$) was performed using McNemar's test for paired binary observations.

Furthermore, the elicitation reactions to the accumulated 1-week dose and the accumulated 3-week dose in the ROAT ($\mu\text{g Ni cm}^{-2}$) were compared with the elicitation reactions to the corresponding doses in the patch test ($\mu\text{g Ni cm}^{-2}$) also using McNemar's test.

A fitted dose–response curve for the patch test and the 1-week accumulated, 2-week accumulated and 3-week accumulated dose–response curves for the ROAT were calculated and drawn.

Results

The patch test

None of the 20 nickel-allergic test subjects had any skin changes to the ethanol control. Nineteen of the nickel-allergic test subjects had a reaction to at least one of the patch test nickel concentrations (Table 2). The lowest threshold concentration observed was $0.5 \mu\text{g Ni cm}^{-2}$ in one test subject, and one test subject did not react to the highest dose ($200 \mu\text{g Ni cm}^{-2}$). In the analysis from the patch test the ED_{50} was $10 \mu\text{g Ni cm}^{-2}$ (95% CI 3.8–27), the ED_{10} was $0.78 \mu\text{g Ni cm}^{-2}$ (95% CI 0.13–2.2) and the ED_1 was $0.048 \mu\text{g Ni cm}^{-2}$ (95% CI 0.0018–0.24) (Table 3). The fitted dose–response curve is shown in Figure 1.

The repeated open application test

None of the (not nickel-allergic) control subjects had any skin changes to the ROAT and none of the (nickel-allergic) test subjects had any skin changes to the ethanol control. Two test subjects were excluded from the ROAT and only participated in the patch test part of the study. One was excluded because

Table 2 Results of patch testing with nickel sulphate in all 20 test subjects (A) and in the 18 test subjects who also performed the repeated open application test (ROAT) (B)

Patch test concentration ($\mu\text{g Ni cm}^{-2}$)	No. test subjects reacting (A)	%	No. test subjects reacting (B)	%
200	19	95	17	94
150	18	90	16	89
50	15	75	14	78
25	12	60	11	61
15	11	55	11	61
5	9	45	8	44
3.5	5	25	4	22
1.5	4	20	3	17
0.78	2	10	2	11
0.5	1	5	1	6
0.35	0	0	0	0
0.2	0	0	0	0
0.1	0	0	0	0
0.05	0	0	0	0
0.035	0	0	0	0
0.0125	0	0	0	0
0.0063	0	0	0	0
0.0031	0	0	0	0
0.00156	0	0	0	0

Bold: corresponding to the dose per application, and the accumulated 1-week and 3-week doses in the ROAT.

Table 3 Predicted doses (ED_{xx}) from the patch test with 95% confidence interval (CI) ($\mu\text{g Ni cm}^{-2}$), and the predicted doses (ED_{xx}) from a review⁸ where the dose-response from eight nickel patch test dose-response studies has been analysed and average predicted doses have been calculated

Probability (%)	Patch test calculated dose-response		Review
	ED_{xx}	95% CI	ED_{xx}
50	10.0	3.8–27	12.8
25	2.8	0.77–7.0	3.6
10	0.78	0.13–2.2	1.04
5	0.33	0.034–1.1	0.44
1	0.048	0.0018–0.24	0.067

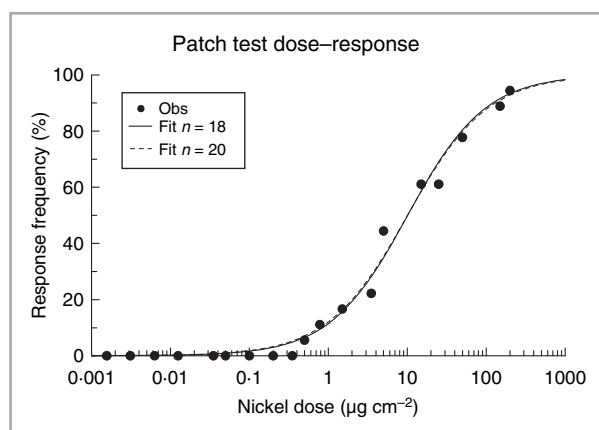


Fig 1. Observed (black dots) and calculated logistic dose-response curve from the patch test for all 20 subjects participating in the patch test (broken line) and for the 18 subjects who also participated in the repeated open application test (solid line).

of eczema on the forearms and the other one because of failure in performing the ROAT correctly. Fifteen of 18 (83%) test subjects had a reaction to the highest concentration ($3.5 \mu\text{g Ni cm}^{-2}$) in the ROAT within the first week, and 16 of 18 (89%) reacted within the 3 weeks of exposure. Ten of 18 (56%) had a reaction within the first week of exposure to $0.35 \mu\text{g Ni cm}^{-2}$, but in one person the reaction disappeared after 3 weeks of exposure, so after 3 weeks nine of 18 (50%)

had a reaction to this concentration. Four of 18 (22%) had a reaction to $0.035 \mu\text{g Ni cm}^{-2}$ within the first week, but again the reaction disappeared in one person during the second week of exposure, so after the 3 weeks three of 18 (17%) had had a reaction (Table 4).

Comparison of the patch test and the repeated open application test

When the dose per application in the ROAT was compared with the same dose in the patch test the following results were seen (Table 5): when $3.5 \mu\text{g Ni cm}^{-2}$ was applied per application in the ROAT 16 of 18 (89%) had a reaction within the 3 weeks. In the patch test four of 18 (22%) reacted to this dose. This difference between the reactivity in the ROAT and the reactivity in the patch test was statistically significant ($P < 0.001$). When $0.35 \mu\text{g Ni cm}^{-2}$ was applied per application in the ROAT nine of 18 (50%) had a reaction within the 3 weeks. In the patch test no one reacted to this dose. This difference between the reactivity in the ROAT and the reactivity in the patch test was also statistically significant ($P = 0.004$). When $0.035 \mu\text{g Ni cm}^{-2}$ was applied per application in the ROAT three of 18 (17%) had a reaction within 3 weeks. In the patch test no one reacted to this dose. This difference was not statistically significant.

When the accumulated 1-week dose and the accumulated 3-week dose in the ROAT were compared with the same dose in the patch test, no significant differences between the response frequencies were found (Table 6). On the fitted curve (Fig. 2) for the patch test ($n = 18$) and the dose-response curves for the ROAT (week 1, 2 and 3, respectively) it is seen that the ROAT curves lie close to the patch test curve.

Discussion

The dose-response relationships for elicitation of allergic contact dermatitis are dependent on a number of factors such as concentration of allergen, frequency of application,¹¹ duration of application,^{19,20} location of exposure,^{21,22} occluded, open or penetrating exposure⁸ and individual degree of sensitivity.^{23,24} In this study we wanted to investigate the patch test dose-response relationship in nickel-sensitive individuals, under standardized conditions, and to investigate the relation

Table 4 The dose per application and the accumulated doses in the repeated open application test (ROAT)

Dose per application ($\mu\text{g Ni cm}^{-2}$)	Accumulated 1-week dose ($\mu\text{g Ni cm}^{-2}$)	No. test subjects with reaction within 1 week	%	Accumulated 3-week dose ($\mu\text{g Ni cm}^{-2}$)	No. test subjects with reaction within 3 weeks	%
3.5	50	15	83	150	16	89
0.35	5	10 ^a	56	15	9 ^a	50
0.035	0.5	4 ^a	22	1.5	3 ^a	17

^aTwo test subjects had a reaction to the ROAT within the first week, but this reaction later disappeared.

Table 5 The response frequency in the patch test compared with the response status in the repeated open application test (ROAT) (the dose per application) after 3 weeks

Dose ($\mu\text{g Ni cm}^{-2}$)	Patch test: response frequency in %	ROAT: response frequency after 3 weeks in %	P-value (McNemar's test)
3.5	22	89	< 0.001
0.35	0	50	0.004
0.035	0	17	0.250

Table 6 The response frequency in the patch test compared with the response status in the repeated open application test (ROAT) (the accumulated dose after 1 and 3 weeks, respectively)

Dose ($\mu\text{g Ni cm}^{-2}$)	Patch test: response frequency in %	ROAT: response frequency in %	P-value (McNemar's test)
0.5	6	22 ^a	0.250
1.5	17	17 ^b	1
5	44	56 ^a	0.727
15	61	50 ^b	0.727
50	78	84 ^a	1
150	89	89 ^b	1

^aAccumulated 1-week dose; ^baccumulated 3-week dose.

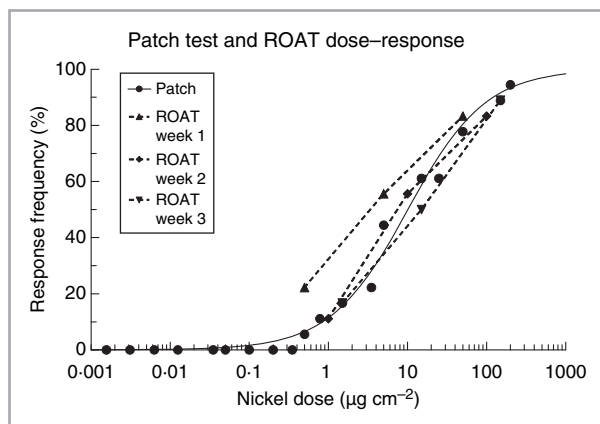


Fig. 2. The fitted dose–response curve for the patch test ($n = 18$) and the accumulated 1-week repeated open application test (ROAT) dose, the accumulated 2-week ROAT dose and the accumulated 3-week ROAT dose.

between the elicitation dose–response between prolonged occluded exposure and the repeated open exposure.

The patch test is an easy test to perform, whereas the ROAT (which more closely mimics many types of real-life exposure) is much more time consuming. If a parallel could be drawn between these two test methods, it would give us an instrument which could be used to translate the thresholds found in

patch testing into ROAT thresholds, and thereby into real-life frequent and unoccluded exposure situations. This instrument could then be used in preventive strategies. What we found in this study was that the ROAT per application response rate was significantly higher for the two highest concentrations compared with the same dose in the patch test. This means that a person might react in a ROAT to a dose per unit area per application which does not give a reaction in a patch test. However, we found that the dose–response for the patch test and the dose–response for the accumulated dose in the ROAT were very close to one another and were not statistically significantly different. This was well illustrated by the curves for both test methods, which showed the similarity between the dose–responses. This means that the dose–response for the ROAT in this study (in accumulated dose per unit area) was approximately the same as the dose–response for the patch test. These results could be an indicator of accumulation of nickel in the skin, and that the immune system might react to the accumulated dose applied per unit area of skin, and not the dose per application. Another explanation could be that the repetitive stimulation of low concentrations of nickel is a stronger stimulus for the immune system than the same concentration applied once and occluded for 48 h, meaning that when the same low concentration is applied repetitively, the immune system may at some point react to this low dose, even though a single 48-h occluded application of this low dose is not strong enough a stimulus for the immune system to react. One study has shown an accumulation of nickel in both fingernails and the skin (measured by tape stripping) after exposure to nickel for 2 weeks, compared with controls with no exposure to nickel.²⁵ Another study has shown that in occupations with a high exposure to nickel, a high level of nickel can be found in the fingernails, compared with occupations with lower or no exposure to nickel, although no increased accumulation of nickel over time could be found.²⁶ One study has investigated the dermal nickel concentration for different occupations after a day of performing normal tasks, and found high cutaneous levels, exceeding $0.35 \mu\text{g Ni cm}^{-2}$ per day in some nickel platers and nickel refinery workers.²⁷ This dose elicited a reaction in 50% of patients in our study, within 3 weeks of exposure. Another study has investigated the nickel concentration on the skin of different workers after 1 or 2 h of daily work, and found mean concentrations on the hands of $0.9 \mu\text{g Ni cm}^{-2}$ in locksmiths, $0.4 \mu\text{g Ni cm}^{-2}$ in carpenters, $0.3 \mu\text{g Ni cm}^{-2}$ in cashiers and $0.03 \mu\text{g Ni cm}^{-2}$ in office workers.²⁸ However, the results are not directly comparable. Knowledge about the exposure, the amount of nickel found on the skin and the solubility of the nickel species measured is needed. A few other published studies have investigated the effect of accumulated exposure to other allergens. In a study investigating the sensitization rate to para-phenylenediamine (PPD) after hair dying with either 1.5% PPD for 30–40 min once a month for 6 months or 0.5% PPD for 5 min once a week for 6 months, it was found that the sensitization rate to PPD was significantly higher in the group exposed more often, for a shorter period (5 min), to the low concentration of PPD, as compared with

fewer times, for a longer period (30 min), with the high concentration, meaning that the more frequent stimulation to a low concentration of PPD was a stronger stimulus for the immune system than the higher concentration applied less frequently.²⁹ Another study found the same response frequency whether the test subjects were exposed to an open test of 0.01% methylidibromo glutaronitrile (MDBGN) four times a day or 0.04% MDBGN once a day. Thus, it was the accumulated dose that was of importance in eliciting a response.³⁰ Similar results were seen in another PPD study, where the percentage of subjects responding after 6 days to 1% PPD, applied for 5 min per day in a ROAT, was comparable with the proportion that responded to a 30-min single exposure patch test with 1% PPD, indicating that it was the accumulated exposure over time which was of importance to the eliciting response.¹⁹ The threshold for formaldehyde in repeated patch testing on the same area is 30 p.p.m., whereas the threshold for a single patch test is 150 p.p.m., which again could indicate that it is the accumulated dose which is essential for the elicitation response (reviewed by Johansen *et al.*³¹).

In conclusion, the ROAT threshold in dose per area per application is significantly lower than the patch test threshold. However, the patch test threshold and the accumulated ROAT threshold are closely related. The results from other experimental studies indicate that this might also apply for other allergens, but further studies with other allergens are needed to elucidate if the patch test elicitation response and the accumulated ROAT response are as closely related as we found in this nickel elicitation study. Studies investigating the accumulation of allergen in the skin would also be important in clarifying the cause of the elicitation response.

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