

Review Article

Experimental nickel elicitation thresholds – a review focusing on occluded nickel exposure

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Nickel (Ni) is the most frequent cause of contact allergy among the female population. This makes it interesting to examine thresholds for elicitation under different conditions. Even though Ni exposure may be open, occluded, penetrating or oral, most dose–response studies in the literature concern single occluded application. The aims of this study were to assess thresholds of response by making a statistical analysis of available dose–response studies with single occluded exposure and comparing the results to thresholds from other modes of exposure. 8 occluded Ni dose–response studies were selected based on statistical considerations. The statistical analysis showed that 5% of a sensitized population react to $0.44 \mu\text{g Ni/cm}^2$ and 10% react to $1.04 \mu\text{g Ni/cm}^2$. In another study with a single open application, 7.8% of sensitized persons responded to a dose $\times 6$ higher than the dose to which 10% reacted in occluded exposure. When combining the exposure to Ni with an irritant, divagating results were found, although the literature shows evidence of an augmented response when combining exposure to an allergen and an irritant. The thresholds of penetrating exposure were found to be lower than the thresholds of single occluded exposure. Comparisons of different kind of exposures across studies are difficult, because of differences in the studies, although a comparison could be made by a study that compares the different exposures within the same individuals at the same time.

Key words: contact dermatitis; elicitation; experimental exposures; nickel; nickel allergy; occluded exposure; open exposure; patch testing; penetrating exposure; threshold. © Blackwell Munksgaard, 2005.

Accepted for publication 20 December 2004

Nickel (Ni) is the most frequent cause of contact allergy among women (1). Recent regulations in the EU area focusing on ear piercing and Ni items designed to be in prolonged skin contact, such as costume jewellery, might change this picture.

In 1991, a Ni skin exposure regulation was passed in Denmark based on elicitation studies on Ni-sensitized individuals. This regulation introduced a maximum on Ni release of $0.5 \mu\text{g Ni/cm}^2/\text{week}$ from certain products. In the EU, the Ni directive was passed in 1994 and laid down the following: Ni may not be used 1) in post assemblies which are inserted into pierced ears and other parts of the body during epithelialization unless such post assemblies have a concentration of Ni less than 0.05%; 2) in products intended to come into direct and prolonged contact with the skin such as earrings, necklaces, bracelets, wristwatches, zippers, etc. if the Ni release is greater than $0.5 \mu\text{g Ni/cm}^2/\text{week}$; 3) in products mentioned in 2, where these have a non-

Ni coating unless such a coating is sufficient to ensure that the rate of Ni release after direct and prolonged contact with the skin will not exceed $0.5 \mu\text{g Ni/cm}^2/\text{week}$ (2). 2 independent studies indicate a significant decline in the frequency of Ni allergy among Danish teenagers after the implementation of the Ni regulation in Denmark (3, 4). 1 study indicates that a previously widely established association between hand eczema and Ni allergy has disappeared (5) although present in another study from the same region in Denmark (6). Nickel allergy is still frequent in many geographical areas of the world and is also an occupational allergen within certain industries, e.g. plating, building, mechanical and electrical work and transportation (7).

Regulation on Ni exposure is an effective tool in preventing sensitization and elicitation of Ni contact dermatitis. Considering the fact that many people in the world population are already sensitized to Ni, it is interesting to examine the

thresholds for elicitation under different conditions. Important factors for the elicitation of contact allergy are hapten concentration, single versus repeated application (8), occlusion versus open application and individual degree of contact allergy (6, 9). The exposure to Ni can be open (temporary skin contact with items/fluids containing Ni), occluded (e.g. hair slides, wrist-watches and jeans buttons), penetrating (e.g. piercing studs) and oral (e.g. food and dental braces). Experimental studies have tried to address the dose–response characteristics of the different types of Ni-exposure conditions. This review will focus on the results of occluded Ni dose–response studies, which will be analysed statistically in order to assess the thresholds of response. Furthermore, the influence of exposure conditions will be examined by a review of the results from Ni-exposure studies performed using methods other than occluded single application.

Experimental Ni Exposure

Experimental open exposure to Ni can be done as a single or repeated application, and to mimic the effect of Ni on irritated skin, the experimental exposure can be done with or without an irritant. Studies have tried to visualize the dose–response characteristics of open and occluded exposure to Ni under these different conditions (10–16).

Occluded Exposure

Reviewing the literature, it is found that there are several dose–response studies made with Ni. However, the studies are often not directly comparable, as materials, substances, methods and details of the study conditions vary, and in some cases, the information given on the said issues are

insufficient or lacking. Occluded exposure to Ni is the most common method in experimental studies used to define the thresholds for elicitation. 13 studies were identified (11–24), and of these, 8 studies (12, 15–18, 20–22) were selected for further analysis based on the following criteria:

- (1) at least 5 dilutions (including vehicle) had been used for the patch test;
- (2) at least 10 patch-tested Ni-sensitized persons;
- (3) the patch-test method was similar to the Finn Chamber method and 2 days of occlusion were used;
- (4) a fair description of the study details was available in the articles chosen.

Criteria (1) and (2) were chosen based on statistical considerations to obtain results that were reliable. The details of the studies are summarized in Table 1. It was assumed that all concentrations were given as the hydrated salt, e.g. $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$. Based on these data, the statistical analysis was performed. 1 study (18) was excluded, because the TRUE TestTM was used. This study, using a vehicle other than that of the Finn Chamber method and known to give more positive reactions to Ni, was clearly displaced to the left when analysing the dose–response curves (results not shown).

Standard logistic regression analysis was used to estimate the dose–response relations, with software based on APL 2000[®], Manugistics Inc., Rockville, MD, USA and validated relative to SAS[®]; version 8.2, Procedure Probit, SAS Institute, Cary, NC, USA. The logistic model describes the probability of positive response P as $P = \exp(a + bx) / [1 + \exp(a + bx)]$, where $x = \log(\text{dose})$. The parameters a and b are

Table 1. Available information of the study details, given in the articles describing occluded nickel dose–response studies, chosen for further analysis

Reference	Number of patients	Number of dilutions	Nickel form	Vehicle	Method and area	Volume
(12)	18	4	NiCl_2 and NiSO_4	Water (5% in petrolatum)	Finn Chamber, assume diameter 8 mm	15 μl
(15)	19	6	NiSO_4	Water	Finn Chamber, diameter 8 mm	15 μl
(16)	25	6	NiSO_4	Water	Finn Chamber, diameter 8 mm	15 μl
(17)	15	5	NiSO_4	Water	Finn Chamber, assume diameter 8 mm	25 μl
(19)	43	12	NiSO_4	Petrolatum	Finn Chamber, assume diameter 8 mm	Assume 15 μl
(20)	53	8	NiSO_4	Water	Finn Chamber, area 79 mm ²	35 μl
(21)	26	10	NiSO_4	Water	Finn Chamber, diameter 8 mm	15 μl
(22)	12	6	NiSO_4	Water (2.5% in petrolatum)	Finn Chamber, diameter 8 mm	16 μl water and 22.7 μl petrolatum

Table 2. Results of logistic regression analyses performed on the data from each of the chosen occluded nickel dose–response studies

	Dose (μg/cm ²)		Agreement		Estimated doses (μg/cm ²)								
Study number	Minimum	Maximum	χ ²	P value	ED _{1%}	ED _{5%}	ED _{10%}	ED _{25%}	ED _{50%}	ED _{75%}	ED _{90%}	ED _{95%}	ED _{99%}
12	0.067	335	0.20	0.91	0.15	0.43	0.70	1.40	2.8	5.7	11.5	18.5	53
17	0.0063	558	0.31	0.96	0.028	0.124	0.24	0.64	1.72	4.6	12.2	24	104
19	0.17	335	24.3	0.0068	0.054	0.36	0.85	3.0	10.8	38	135	320	2148
20	3.9	495	8.57	0.20	0.38	1.37	2.5	5.8	13.5	32	74	132	476
21	0.087	335	13.3	0.10	0.29	1.60	3.5	10.8	34	106	330	715	3956
22	0.23	254	3.18	0.67	0.050	0.30	0.67	2.2	7.2	24	77	173	1030
15	0.087	838	6.74	0.24	0.0175	0.172	0.48	2.2	10.1	46	210	590	5783
16	0.087	838	4.76	0.31	0.165	0.90	1.94	6.0	18.5	57	177	382	2083
Average	0.0063	838	114	<0.0001	0.067	0.44	1.04	3.6	12.8	45	158	370	2440

The estimated doses that predict an allergic reaction to nickel in 1, 5, 10, 25, 50, 75, 90 and 99% of a sensitized population are shown. Furthermore, the analysis based on the data from all the studies resulting in weighted estimated average values, is also shown. Small P values of the χ^2 -statistics indicate lack of agreement between the observed response frequencies and the estimated logistic curves.

determined to give the best agreement between the observed frequencies and the values from the model. The reported χ^2 -tests of agreement between estimated dose–response curves and the observed frequencies require that the latter are independent. This assumption may not be fulfilled, because it is the same subjects who are tested with each dose. These P values should therefore be taken with some reservations. The estimated dose that predicts positive response in 100% of the subjects is calculated as $\text{ED}_{100P} = \exp\{\log[P/(1-P) - a]/b\}$.

Table 2 summarizes the logistic regression analysis, and Fig. 1 shows the estimated dose–response curves for each study. The weighted average curve, representing the data from all studies, is shown in Fig. 2. The dose–response

data differ with a factor of 10 from one another, which is what we would expect from the different study conditions.

The analysis for each study separately show that the estimated elicitation dose ED1%, which represents the concentration to which only a very limited number of sensitized persons will react, is between 0.0175–0.38 $\mu\text{g Ni}/\text{cm}^2$ (weighted average: 0.067 $\mu\text{g Ni}/\text{cm}^2$). The concentration where it is possible that 10% will react is 0.24–3.5 $\mu\text{g Ni}/\text{cm}^2$ (weighted average: 1.04 $\mu\text{g Ni}/\text{cm}^2$). The concentration range to which 25% will react is 0.64–10.8 $\mu\text{g Ni}/\text{cm}^2$ (weighted average: 3.6 $\mu\text{g Ni}/\text{cm}^2$) and the concentration where 50% will react is 1.72–34 $\mu\text{g Ni}/\text{cm}^2$ (weighted average: 12.8 $\mu\text{g Ni}/\text{cm}^2$). Figure 1 shows that the dose–response results from the different studies are remarkably

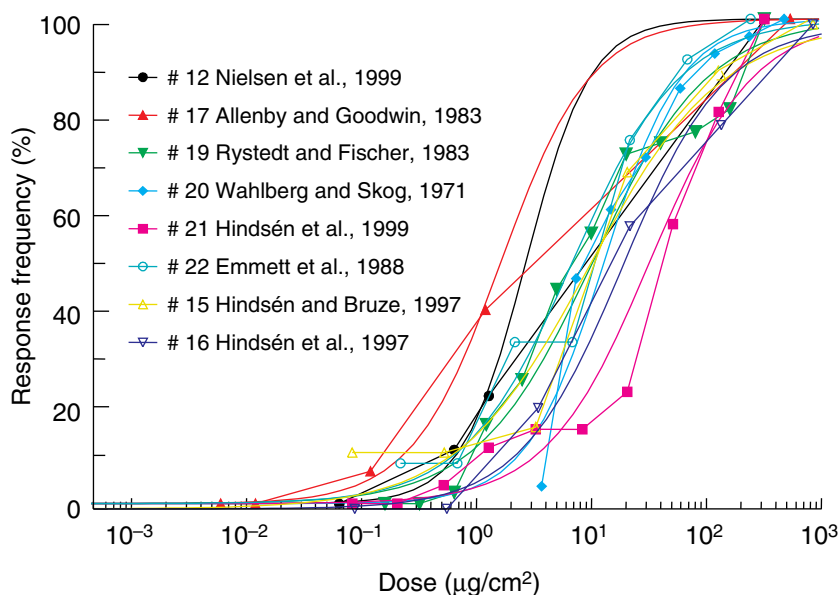


Fig. 1. The resulting observed and adjusted curves from the logistic regression analysis of each chosen occluded nickel dose–response study.

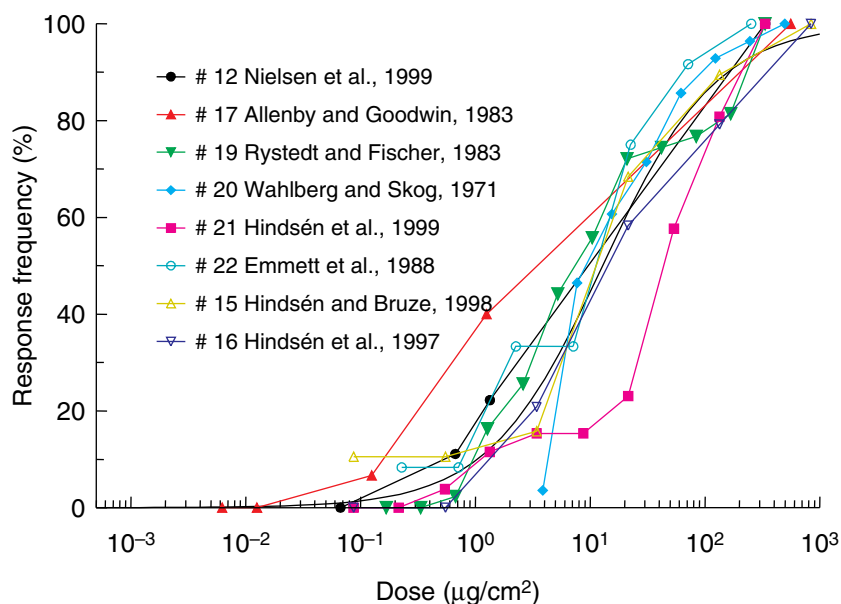


Fig. 2. The resulting weighted, adjusted average curve from all the chosen occluded nickel dose–response studies (black curve) and the observed curves from each of the chosen occluded nickel dose–response studies.

similar and that they have the same (adjusted) s-shaped curve, as also shown in the Ni studies by Hindsén (25) and Andersen et al. (18), and that we also see for other allergens (26–28). The curve from study no. 21 is displaced to the right. This means either that the test subjects were less sensitive to Ni or that the conditions of the study differ from those of the other studies, influencing the response frequency. One explanation could be that the patients presented in this study had a 1–10 years history of a positive patch test to Ni, and may have refrained from contact with Ni, and therefore show a lower reactivity. The curves from study nos. 12 and 17 are displaced to the left. In these 2 studies, the patients were tested with only 5 patches (4 in the low concentration range and 1 in the high concentration range). It is seen that, at the low doses, the available dose–response data from study no. 12 does not diverge from study nos. 19, 20, 22 and 15; however, because only one data point was available for the high dose, this gave a displacement of the adjusted curve, which may not be fully representative of the actual response, if the study group had been tested with more high doses. The observed dose–response curves from study nos. 19 and 21 deviate compared with the adjusted curve. The reason might be that the study groups contain persons with different sensitivity. In study no. 19, the curve can, from a statistical point of view, be explained by the test group being composed of a heterogeneous group where there is a rather large proportion (around 80%) with a higher sensitivity and a smaller proportion

(around 20%) with a lower sensitivity. In study no. 21, the opposite might have been the case, with a group consisting of around 15% very sensitive subjects, while the remaining 85% were less sensitive. Studies have shown that there is both a great inter- and intraindividual difference in Ni sensitivity among Ni-sensitive subjects, and in one study, the sensitivity within 1 person varying by a factor of 250, 2 months apart (21). We also found the results differing between studies as expected from different study conditions and different study populations; however, in all cases, the shape of the dose–response curve was identical and has in one study been found to be constant for groups of Ni-sensitive individuals followed over time with repeated patch testing (21).

In the ordinary patch-test situation (the occluded exposure described above), the measure of exposure is $\mu\text{g Ni/cm}^2$. In the Ni regulation, the limiting exposure to Ni is, apart from the content, the release of Ni from an item/cm²/week. This is based on studies done by Menné et al. (29) and Fischer et al. (30), who addressed the Ni problem by patch testing with different Ni-containing alloys with a known release of Ni/cm²/week. Menné et al. (29) found strong patch-test reactions when testing 267 Ni-sensitive persons with alloys releasing more than 1 $\mu\text{g Ni/cm}^2$ /week and weak reactions (except from 1 alloy) when testing with alloys releasing below 0.5 $\mu\text{g Ni/cm}^2$ /week. Fischer et al. (30) tested 18 Ni-sensitive subjects with different unplated white-gold samples (Ni content 2.5–15%), which had a Ni release of 0.09–0.82 $\mu\text{g Ni/week}$. The subjects were also

Table 3. Studies investigating the elicitation of nickel allergy on different exposures to nickel: open, occluded, single, repeated, with and without an irritant

open	occluded	single	repeated
without an irritant 10) Menné and Calvin 4/51 = 7.8% positive to 6.8 µg Ni/cm ² 18/51 = 35.3% positive to 27.37 µg Ni/cm ²	without an irritant 12) Nielsen et al. increased blood-flow in finger exposed to: 1 st week: 10 ppm NiCl ₂ × 10 minutes 2 nd week: 100 ppm NiCl ₂ × 10 minutes 4/17 = 23.5% follicular reaction on forearm exposed to 1 st week: 10 ppm 2 nd week 100 ppm	without an irritant See Fig. 1	without an irritant 15) Hindsén and Bruze patch test on an area treated with nickel 1 month earlier: 12/20 = 63% positive to 3.43 µg Ni/cm ² (compared to 15% at this concentration when no pre-exposure to nickel (p = 0.08))
11) Christensen and Wall 6/15 = 40% positive to 37.1 µg Ni/cm ² 10/15 = 67% positive to 74.1 µg Ni/cm ² 13/15 = 87% positive to 148.2-222.4 µg Ni/cm ² 14/15 = 93% positive to 296.5 µg Ni/cm ²	with an irritant* 12) Nielsen et al. 8/17 = 47% follicular reaction on arm exposed to: 1 st week: 10 ppm NiCl ₂ 2 nd week: 100 ppm NiCl ₂ (compared to 1/17 = 6% follicular reaction when exposed to vehicle combined with SLS)	with an irritant* 13) Allenby and Basketter An arm immersed daily into SLS until erythema. Following patch test: 2/20 = 10% positive to 0.5 ppm = 0.0049 µg Ni/cm ² (compared to no positives when patch testing at this concentration on normal skin)	16) Hindsén et al. patch test on area treated with nickel 1 month earlier: 2/23 = 8.7% positive to 0.0871 µg Ni/cm ² (compared to 0% at this concentration when no pre-exposure to nickel (p = 0.03))
with an irritant* 10) Menné and Calvin 1/51 = 2% positive to 6.84 µg Ni/cm ² 1/51 = 2% positive to 27.37 µg Ni/cm ²	with an irritant* 14) Agner et al. 4 fold increase of inflammatory response when patch testing with SLS and NiCl ₂ (compared to NiCl ₂ alone) 15) Hindsén and Bruze lowered test-score when pretreating the patch test site with SLS one month prior to exposure to nickel, (compared to the site with no SLS pre-treatment)	with an irritant* No studies	

*the irritant was SLS

tested with 8-rhodium-plated gold discs releasing 0.04–0.54 µg Ni/week. All 18 Ni-sensitive subjects demonstrated at least 1 positive patch-test reaction to white gold, plated or unplated. These data are interesting, because they combine the Ni release of 1 week with the patch test of 2 days occlusion; even though they are not directly comparable to the ordinary patch test situation, it is interesting to note that there is a high response frequency to relatively low weekly Ni release per cm².

Open Exposure

The 2 single open application studies performed (10, 11) gave remarkable similar results (Table 3). Ni applied in a single open application at a dose of 6.84 µg Ni/cm² gave a response in 7.8% of Ni-sensitive individuals. This is a dose that is around ×6 higher than the weighted average for the occluded exposure where 10% of sensitized persons will react. Interestingly, Christensen and Wall (11) found that 24 h after the application of 2.5% Nickel chloride, there were non-significantly more positive reactions to an open test compared with the same concentration in an occluded patch test (even though the dose per cm² was higher in the occluded than in the open test). At the 48-hr reading, this was reversed. The open test is not directly comparable to the patch-test situation unless both the tests are done under standardized conditions on the same subjects.

Exposure to Ni and an Irritant

Real-life exposure to Ni might be on damaged skin or the exposure might be simultaneous with

exposure to an irritant. This can have an impact on the Ni threshold, and several studies have tried to address this by a combination of exposure to an irritant and Ni (Table 3). The method of exposing the patients to Ni and an irritant is very different. In one study (15), the exposure to the irritant was done 1 month earlier followed by patch testing, resulting in a lowered test score at the sodium lauryl sulfate (SLS)-treated site. In 2 studies (10, 14), the exposure to the irritant and Ni was simultaneous, resulting in an increased threshold when open testing with Ni (10) and resulting in a lowered threshold when patch testing with Ni (14). In 2 other studies, the threshold was also lowered when testing in one study (12) with a patch test of SLS followed by 2 weeks of open Ni exposure, and in the other study (13), when exposing the skin daily to SLS until erythema followed by a Ni patch test. There is no systematic difference of the response to a combination of Ni and an irritant according to the type of exposure. However, in a review on the combination of allergens and irritants by Pedersen et al. (31), it is concluded that, in the literature, there is evidence of an augmented allergic response when the exposure to allergen and irritant is combined.

Penetrating Exposure

The high frequency of Ni allergy among the female population has mainly been due to the high Ni release from products intended to be in close contact with the skin – especially earrings. Several studies (32–34) before the implementation of the Ni directive showed a frequency of Ni

Table 4. Studies investigating the sensitization and elicitation of nickel allergy during penetrating exposure (ear piercing)

Penetrating	
Sensitization	Elicitation
Räsänen et al. (35)	Fischer et al. (36)
Investigated 9 persons with symptoms related to ear piercing, 6/9 = 67% had a positive patch test to nickel, the studs the 67% were pierced with had the following nickel-release: (a: in plasma, b: in water)	10 nickel sensitive persons with pierced ears were wearing nickel containing ear-rings with different release of nickel for a maximum of 1 week. (Release in synthetic sweat).
a1: 22.44 µg Ni/cm ² /week	Positive symptoms: itching, redness and dermatitis
b1: 0.46 µg Ni/cm ² /week	4/10 = 40% positive to 0.05 µg Ni/week
a2: 0.15 µg Ni/cm ² /week	3/11 = 27% positive to 2.3 µg Ni/week
b2: 0.02 µg Ni/cm ² /week	4/6 = 67% positive to 7.7 µg Ni/week
a3: 104.59 µg Ni/cm ² /week	6/6 = 100% positive to 15 µg Ni/week
b3: 0.79 µg Ni/cm ² /week	Ingber et al. (37)
a4: 10.06 µg Ni/cm ² /week	25 nickel sensitive persons were ear pierced with piercing-studs with a nickel content of 11.5–12.9%
b4: 0.04 µg Ni/cm ² /week	2/25 = 8% had a transient itch and erythema 48 h after piercing with studs releasing 0.11–0.21 µg Ni/cm ² /week
a5: 0.17 µg Ni/cm ² /week	
b5: 0.01 µg Ni/cm ² /week	
a6: 2.03 µg Ni/cm ² /week	
b6: 0.03 µg Ni/cm ² /week	

allergy among ear-pierced women and children of between 11.1% and 30.8%. In 1 study (33), there was a $\times 4.47$ increased risk of Ni allergy if ear piercing was present, relative to if ear piercing was absent. When piercing is done, the piercing stud is in direct contact with the plasma before epithelialization, and this is a special form of exposure that is different from open or occluded exposure. The results of 3 studies (35–37) done on sensitization and elicitation by ear-piercing studs are presented in Table 4. It is seen in the study by Räsänen et al. (35) that the release of Ni is higher in plasma than in water, and that 2 of 9 persons were sensitized to Ni by the use of ear-piercing studs, releasing as little as 0.15–0.17 $\mu\text{g Ni/cm}^2/\text{week}$. Furthermore, it is seen in the study by Fischer et al. (36) that the lowest concentration of Ni causing elicitation in 40% of the Ni-sensitive individuals is 0.05 $\mu\text{g Ni/week}$, although unfortunately, there is no information about the release per cm^2 . In the study by Ingber et al. (37), 2 of 25, 8% of Ni-sensitive subjects, ear pierced with a certain type of stainless steel ear-piercing stud with a Ni content of 11.5–12.9%, experienced transient itch and erythema for 48 h after the piercing. The ear-piercing studs that had been used for the ear piercing were found to release Ni below the detection limit, whereas the same type of ear-piercing studs that had not yet been used for ear piercing were found to release 0.11–0.21 $\mu\text{g Ni/cm}^2/\text{week}$, indicating that the ear-piercing studs released Ni, while they were in the subjects' ears. Comparing these results to the weighted average for occluded exposure, the doses (in release per cm^2/week) causing sensitization in 20% and elicitation in 8% by penetrating exposure is a dose (in dose per cm^2) that would cause elicitation in less than 5% of the sensitized population by occluded exposure.

Conclusion

The thresholds for elicitation of Ni allergy are dependent on the conditions under which the Ni exposure occurs, e.g. open/closed/penetrating exposure, single or repeated applications, the presence of an irritant or irritated skin, the selection of the study group, the definition of a positive reaction and the vehicle used for testing. The patch-test data presented in this analysis show that the elicitation thresholds determined differ by a factor of 10 between studies. This is a limited variation considering that the studies have been performed in different geographical regions, at different time periods and probably with other differences among the test subjects. According to

the weighted average, 5% of a sensitized population will react to a dose of 0.44 $\mu\text{g Ni/cm}^2$ and 10% of a sensitized population will react to a dose of 1.04 $\mu\text{g Ni/cm}^2$. The elicitation threshold for single open exposure is around $\times 6$ higher than the elicitation threshold for single occluded exposure. The sensitization and elicitation thresholds for penetrating exposure are lower than the elicitation threshold for single occluded exposure.

The thresholds from open testing are not directly comparable to the thresholds from occluded exposure, because the testing is done in different populations. The studies which include an irritant are also not comparable, because the exposures to the irritant are done very differently, and therefore, the results are also diverging. When we look at the results from this review, we see that they are dependent on factors within each study, which makes it difficult to make comparisons of thresholds for open, closed and penetrating exposure across the studies. However, a comparison of the thresholds on different kinds of exposure could be made by a systematic study that compares the threshold for patch testing with, e.g., repeated open exposure within the same individuals at the same time.

Acknowledgement

We thank Aage Vølund, PhD, for assistance with the analysis of the dose–response relations.

References

1. Lidén C, Bruze M, Menné T. Metals. In: *Textbook of Contact Dermatitis*, 3rd edition. Rycroft R J G, Menné T, Frosch P, Lepoittevin J-P (eds) Springer-Verlag: Berlin, 933–979.
2. www.teg.co.uk/teg/nickel/94-27-EC.htm
3. Johansen J D, Menné T, Christophersen F, Kaaber K, Veien N. Changes in the pattern of sensitisation to common contact allergens in Denmark, between 1985–86 and 1997–98, with a special view to the effect of preventive strategies. *Br J Dermatol* 2000; 142: 490–495.
4. Jensen C S, Lisby S, Baadsgaard O, Vølund A, Menné T. Decrease in nickel sensitization in a Danish schoolgirl population with ears pierced after implementation of a nickel-exposure regulation. *Br J Dermatol* 2002; 146: 636–642.
5. Nielsen N H, Linneberg A, Menné T, Madsen F, Frølund L, Dirksen A, Jørgensen T. The association between contact allergy and hand eczema in 2 cross-sectional surveys 8 years apart. *Contact Dermatitis* 2002; 46: 71–77.
6. Bryld L E, Hindsberger C, Kyvik K O, Agner T, Menné T. Risk factors influencing the development of hand eczema in a population based twin sample. *Br J Dermatol* 2003; 149: 1214–1220.
7. Lidén C. Occupational contact dermatitis due to nickel allergy. *Sci Total Environ* 1994; 148: 283–285.
8. Andersen K E, Johansen J D, Bruze M et al. The time-dose–response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001; 170: 166–171.

9. Menné T. Relationship between use test and threshold patch test concentration in patients sensitive to 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (MCI/MI). *Contact Dermatitis* 1991; 24: 375.
10. Menné T, Calvin G. Concentration threshold of non-occluded nickel exposure in nickel-sensitive individuals and controls with and without surfactant. *Contact Dermatitis* 1993; 29: 180–184.
11. Christensen O B, Wall L M. Open, closed and intradermal testing in nickel allergy. *Contact Dermatitis* 1987; 16: 21–26.
12. Nielsen N H, Menné T, Kristiansen J, Christensen J M, Borg L, Poulsen L K. Effects of repeated skin exposure to low nickel concentrations: A model for allergic contact dermatitis to nickel on the hands. *Br J Dermatol* 1999; 141: 676–682.
13. Allenby C F, Basketter D A. An arm immersion model of compromised skin (II). Influence in minimal eliciting patch test concentrations of nickel. *Contact Dermatitis* 1993; 28: 129–133.
14. Agner T, Johansen J D, Overgaard L, Vølund A, Basketter D, Menné T. Combined effects of irritants and allergens. Synergistic effects of nickel and sodium lauryl sulfate in nickel-sensitized individuals. *Contact Dermatitis* 2002; 47: 21–26.
15. Hindsén M, Bruze B. The significance of previous contact dermatitis for elicitation of contact allergy to nickel. *Acta Derm Venereol* 1998; 78: 367–370.
16. Hindsén M, Bruze M, Christensen O B. The significance of previous allergic contact dermatitis for elicitation of delayed hypersensitivity to nickel. *Contact Dermatitis* 1997; 37: 101–106.
17. Allenby C F, Goodwin B F J. Influence of detergent washing powder on minimal eliciting patch test concentrations of nickel and chromium. *Contact Dermatitis* 1983; 9: 491–499.
18. Andersen K E, Lidén C, Hansen J, Vølund A. Dose-response testing with nickel sulphate using the TRUE test[®] in nickel-sensitive individuals. Multiple nickel sulphate patch-test reactions do not cause an angry back. *Br J Dermatol* 1993; 129: 50–56.
19. Rystedt I, Fischer T. Relationship between nickel and cobalt sensitization in hard metal workers. *Contact Dermatitis* 1983; 9: 195–200.
20. Wahlberg J E, Skog E. Nickel allergy and atopy. Threshold of nickel sensitivity and immunoglobulin E determinations. *Br J Dermatol* 1971; 85: 97–104.
21. Hindsén M, Bruze M, Christensen O B. Individual variation in nickel patch test reactivity. *Am J Contact Dermat* 1999; 10: 62–67.
22. Emmett E A, Risby T H, Jiang L, Ng S K, Feinman S. Allergic contact dermatitis to nickel: Bioavailability from consumer products and provocation threshold. *J Am Acad Dermatol* 1988; 19: 314–322.
23. Banfield C C, Basketter D A, Powell S M. Cutaneous reactivity of the hands in nickel-sensitive patients with hand eczema. *Contact Dermatitis* 1998; 38: 316–318.
24. Uter W, Fuchs T, Hausser M, Ippen H. Patch test results with serial dilutions of nickel sulphate (with and without detergent), palladium chloride, and nickel and palladium metal plates. *Contact Dermatitis* 1995; 32: 135–142.
25. Hindsén M. Clinical and experimental studies in nickel allergy. Thesis. *Acta Derm Venereol* 1999; 79: 1–22.
26. Johansen J D. Thesis Contact allergy to fragrances: Clinical and experimental investigation of the fragrance mix and its ingredients. *Contact dermatitis* 2002; 46 (Suppl. 3): 1–31.
27. Bronaugh R L, Roberts C D, McCoy J L. Dose-response relationship in skin sensitization. *Food Chem Toxicol* 1994; 32: 113–117.
28. Johansen J D, Andersen K E, Svedman C et al. Chloroatranol, an extremely potent allergen hidden in perfumes: A dose-response elicitation study. *Contact Dermatitis* 2003; 49: 180–184.
29. Menné T, Brandrup F, Thestrup-Pedersen K, Veien N K, Andersen J R, Yding F, Valeur G. Patch test reactivity to nickel alloys. *Contact Dermatitis* 1987; 16: 255–259.
30. Fischer T, Fregert S, Gruvberger B, Rystedt I. Contact sensitivity to nickel in white gold. *Contact Dermatitis* 1984; 10: 23–24.
31. Pedersen L K, Johansen J D, Held E, Agner T. Augmentation of skin response by exposure to a combination of allergens and irritants – a review. *Contact Dermatitis* 2004; 50: 265–273.
32. Larsson-Stymne B, Wiidstrom L. Ear piercing – a cause of nickel allergy in schoolgirls?. *Contact Dermatitis* 1985; 13: 289–293.
33. Nielsen N H, Menné T. Nickel sensitization and ear piercing in an unselected Danish population. *Contact Dermatitis* 1993; 29: 16–21.
34. Dotterud L K, Falk E S. Metal allergy in north Norwegian schoolchildren and its relationship with ear piercing and atopy. *Contact Dermatitis* 1994; 31: 308–313.
35. Räsänen L, Lehto M, Mustikka-Mäki U P. Sensitization to nickel from stainless steel ear-piercing kits. *Contact Dermatitis* 1993; 28: 292–294.
36. Fischer T, Fregert S, Gruvberger B, Rystedt I. Nickel release from ear piercing kits and earrings. *Contact Dermatitis* 1984; 10: 39–41.
37. Ingber A, Hershko K, Horev L. AISI 316L Stainless steel ear piercing post assembly does not cause dermatitis in nickel-sensitive subjects. *Exog Dermatol* 2003; 2: 195–200.

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