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ORIGINAL ARTICLE

Does clinical testing support the current guidance definition of prolonged contact for nickel allergy?

Rosemary L. Nixon[1] | Claire L. Higgins[1] | Danit Maor[1] | Harini Rajgopal Bala[1] | Alka Lalji[1] | Katherine E. Heim[2]

[1]Occupational Dermatology Research and Education Centre, Skin and Cancer Foundation Inc., Carlton, Victoria, Australia

[2]NiPERA Inc., Durham, North Carolina

Correspondence
Associate Professor Rosemary Nixon, Occupational Dermatology Research and Education Centre, Skin and Cancer Foundation Inc., Level 1, 80 Drummond Street, Carlton, 3053 Victoria, Australia.
Email: rnixon@occderm.asn.au

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Background: The European Chemical Agency (ECHA) definition of prolonged contact was introduced in 2014 and has not been evaluated clinically.

Objectives: To assess whether nickel-sensitized individuals react on patch testing with high nickel-releasing metal discs for short and repetitive periods.

Materials and methods: We patch tested 45 nickel-sensitized individuals double-blind with 2 different types of high nickel-releasing discs for 10, 30 and 60 minutes on 3 occasions over a period of 2 weeks, and for 1 longer period. Discs were tested for nickel release.

Results: Nickel release from both discs significantly exceeded the 0.5 μg Ni/cm^2/week limit of the EU REACH nickel restriction. However, only 1 individual tested had a largely dose-dependent allergic reaction.

Conclusions: The majority of nickel-allergic subjects did not react to nickel discs after 2 hours or after repetitive exposures of up to 30 minutes on 3 occasions over a period of 2 weeks. The length of time needed to cause nickel allergic contact dermatitis in most nickel-allergic individuals is longer than the ECHA guidance definition. Longer test times are needed to define the time required to cause dermatitis in most nickel-allergic individuals. As a limitation, the test conditions did not adequately assess real-life factors such as friction, which is relevant for some uses of nickel.

KEYWORDS

allergic contact dermatitis, contact allergy, nickel allergic contact dermatitis, nickel allergy, nickel directive, nickel regulation, patch test, prolonged contact

1 | INTRODUCTION

Contact allergy to nickel is prevalent worldwide, with up to 17% of women and 3% of men in the general population being nickel-allergic.[1] Although exposure to soluble metal salts may occur in some occupational settings, the high prevalence of nickel sensitization in the general population is primarily attributable to the use of nickel-releasing consumer items that come into direct and prolonged contact with the skin, particularly skin piercings and jewellery. The release of nickel ions is responsible for causing both the induction of nickel sensitization and the elicitation of nickel allergic contact dermatitis (NiACD) in nickel-allergic individuals, which are threshold effects, requiring the release of ions above a specific amount to cause a reaction. Skin piercings and jewellery have historically been considered to be the most common causes of nickel allergy, but it is challenging to estimate their current role in inducing and eliciting NiACD. The nickel release of jewellery and piercings are covered by regulation in the EU, but compliance is uncertain. It is the release of nickel ions at contact–that is, the skin dose–that is responsible for causing induction of nickel allergy or elicitation of NiACD. Both nickel sensitization and NiACD require a specific duration of exposure to nickel-releasing items. This is, in part, attributable to the time needed for corrosion of the material in order to release nickel ions. Several factors contribute

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to variations in the amount of corrosion and the release of constituent ions, including composition, surface area, surface coating, and texture. Time is also needed for absorption of the released nickel ions into the skin. Finally, there is the interaction with the immune system. It is generally accepted that numerous alloys, such as many stainless steels that contain nickel, do not release a sufficient amount of nickel ions to cause either nickel sensitization or NiACD.

In 1991, Denmark instituted a regulation restricting nickel use in consumer products.[2] On the basis of the Danish measure, legislation in the EU was enacted in 1994 as Council Directive 94/27/EC (12th amendment of Directive 76/769/EEC).[3] This legislation (the "Nickel Directive") was aimed at decreasing consumer dermal exposure to nickel-releasing articles intended to come into direct and prolonged skin contact, to prevent new nickel sensitization in all non-nickel-allergic individuals, and to prevent NiACD in the majority of already nickel-sensitized individuals. The Nickel Directive was then incorporated into the EU REACH Regulation in June 2009 as a restriction under entry 27 in Annex XVII.[4] An important aspect of this regulation was the identification of types of article "intended to come into direct and prolonged contact with the skin". Although examples of articles that meet these criteria are provided in the regulation, no specific definition of "prolonged contact" was included. In order for EU member countries to adequately implement this legislation, a definition of "prolonged contact with the skin" was requested from the European Chemicals Agency (ECHA).

In 2013, the ECHA completed an analysis of the available literature,[5] and concluded that "prolonged contact with the skin is defined as contact with the skin of nickel of potentially more than either 10 minutes on three or more occasions within two weeks, or 30 minutes on one or more occasions within two weeks."

This guidance definition of prolonged contact was approved by the European Competent Authorities for REACH and CLP (CARACAL) in April 2014. Very little directly relevant scientific information was available at the time of the ECHA literature review, resulting in these quite conservative assumptions being used to define prolonged skin contact. Our study was designed to explore this definition of prolonged and repetitive skin contact by testing nickel-releasing metallic materials (ie, nickel metal and nickel-plated brass discs) on nickel-sensitized individuals for the varying times included in the definition.

2 | METHODS

2.1 | Study phases

The study was divided into 2 phases, based on the use of 2 different types of material for testing. Each phase contained different participants; that is, no individuals from Phase 1 were included in Phase 2. Phase 1 involved testing nickel metal discs (>99.9% nickel) as high nickel-releasing material, similar to the surface of nickel-plated fashion jewellery, which is a common cause of nickel dermatitis. After Phase 1 results showed a lack of reactivity to the nickel metal discs, Phase 2 was conducted with nickel-plated brass discs that had higher nickel release rates than the nickel metal discs, and were more similar metallurgically to nickel-plated fashion jewellery. The same protocol and patch testing/visit schedule was used for both phases, with the only difference being the type of disc material used for testing.

2.2 | Materials

Nickel metal discs (>99.9% nickel; 7 mm in diameter × 1 mm in thickness; surface area of 38.4 mm^2) were provided by Vale Canada Limited (Toronto, Canada) for the first 20 subjects in the first phase of the study. Nickel-plated brass discs (bright barrel nickel electrodeposit over brass-based metal [70% Cu:30% Zn]; average plating thickness of 110 μm; 7.3 mm in diameter × 1.2 mm in thickness after plating; surface area of 41.9 mm^2) were manufactured and supplied by Hong Kong Productivity Council (HKPC) (Kowloon, Hong Kong) for the next 25 subjects in the second phase of the study.

Nickel metal discs were all of similar size, shape, and appearance, with a smooth finish and slight roughness at edges where they had been sanded to remove sharp edges. Nickel-plated brass discs were all of similar size, shape, and appearance, with a smooth finish all around as a result of plating. A batch of 150 for each disc was provided by the manufacturer. Discs were produced at the same time for each material, so individual discs within each batch would be assumed to have the same metallurgical characteristics.

2.3 | Nickel release testing

Representative discs of both materials used in the study were tested for nickel release with EN 1811:2011 + A1:2015. As testing with this methodology requires expertise and specific equipment that was not available to the study centre conducting the patch testing, laboratories with significant experience in using the EN 1811 protocol were contracted to conduct this testing. Nickel metal discs for Phase 1 were tested by the Assay Office Birmingham (AOB) (coordinated by Dippal Manchanda, MSc, Technical Director of the Birmingham Assay Office, AnchorCert Group, 1 Moreton Street, Birmingham, B1 3AX, United Kingdom), and Phase 2 nickel-plated brass disc EN 1811:2011 + A1:2015 testing was performed by the HKPC (coordinated by C. M. Whittington, CP Eng, Melbourne & Hong Kong, and Dr W. Y. Lo, PhD, Head, Surface Technology, HKPC, Hong Kong). Both laboratories used the following procedures.

Surface areas for all samples were measured with a digital caliper (Absolute Digimatic, Series No. 500, Code No. 500-191U, Model No. CD-6"CP, Serial No. 075834 for AOB [Mitutoyo Corporation, Japan]; Absolute Digimatic electronic digital caliper, Model No. CD-8 in. CSX for HKPC). Test samples were degreased in an anionic surface-active agent (sodium dodecylbenzene sulfate for AOB [MP Biomedicals]; sodium laurylsulfonate for HKPC [BDH Chemicals]) for 2 minutes at room temperature, thoroughly rinsed in deionized water, and then dried with a clean absorbent cloth. After degreasing, items were handled with plastic forceps or clean protective gloves. The test solution representing artificial sweat consisted of deionized water containing 0.5% (m/m) sodium chloride, 0.1% (m/m) lactic acid, 0.1% (m/m) urea, and sodium hydroxide to adjust the pH. The test samples were placed in the test vessel and an amount of test solution was added corresponding to approximately 1 mL per cm^2 sample area, with the sample being totally immersed and left undisturbed in a

thermostatically controlled oven at 30°C ± 2°C for 168 ± 2 hours (1 week) without agitation. Inductively coupled plasma optical emission spectrometry (ICP-OES) [Agilent Model 730ES for AOB (limit of detection [LOD] 0.004 mg/L); Agilent Technologies Model 720 for HKPC (LOD 0.01 ppm)] was used to measure nickel concentrations in the test solutions (with 10 standards and quality controls to ensure accuracy of the equipment). The concentration of nickel released was determined with the following formula: ICP-OES reading of the sample test solution (mg/mL) – ICP-OES reading of the blank (mg/mL). The release per surface area was determined with the following formula: concentration of Ni (μg/mL) × volume of solution (mL)/surface area (cm^2).

The Occupational Dermatology Research and Education Centre (ODREC), Skin and Cancer Foundation (Melbourne, Australia) conducted dimethylglyoxime (DMG) testing on all nickel-containing discs for Phase 1 and Phase 2. Nickel-containing discs were handled indirectly with sterile plastic forceps. DMG testing was performed with the Chemo Nickel Test kit (Chemotechnique Diagnostics, Vellinge, Sweden), which detects free nickel down to a limit of 10 ppm. According to the manufacturer's instructions, a sterile cotton tip was infused with 2 to 3 drops of DMG reagent and passed over the surface of the disc for 30 seconds. The presence of nickel was indicated by the cotton tip turning pink, caused by the DMG forming an insoluble salt with nickel. The disc was then wiped with sterile gauze.

2.4 | Study participants

Nickel-sensitized individuals aged ≥18 years were identified from the ODREC database, having previously been patch tested between January 2013 and March 2017, and found to have positive reactions to nickel. This database comprises all patients patch tested from 1993 to the present day.

As part of their broader patch testing process, individuals had been patch tested with nickel sulfate 5% pet. (Chemotechnique) by the use of allergEAZE Patch Test Chambers (SmartPractice, Phoenix, Arizona), with patches being applied to the back for 48 hours and readings being performed on day (D) 2 and D4 according to the ICDRG criteria. If patch testing had taken place >6 months previously, subjects were patch tested again with nickel sulfate 5% pet. (Chemotechnique) by use of the method described above, to ensure that they were still sensitized.

Further participants were recruited by advertising to staff and patients at the Skin and Cancer Foundation and at a nearby university. Individuals from the public who gave a history of possible nickel allergy were patch tested with nickel sulfate 5% pet. (Chemotechnique) by use of the method described above. Only those with a positive patch test result were then invited to participate in the study.

Eligibility criteria included having a positive patch test reaction to nickel sulfate, lack of current dermatitis, and no ongoing treatment with immunosuppressive medications such as corticosteroids. Pregnant or breastfeeding women were ineligible to participate. Participants were comprehensively informed about the study, and underwent a formal consent process. In particular, they were advised about the likelihood of developing positive patch test reactions as part of the study. Ethics approval for the study was obtained from St Vincent's Hospital Melbourne Human Research Ethics Committee (approval no: HREC-A 047/15), which oversees research ethics at the Skin and Cancer Foundation. Subjects were not paid to participate in the study, but did receive a small payment to cover travel expenses.

TABLE 1 Detailed schedule of visits to the clinic for patch test applications and reading of patch test sites

Study day (visit number)	Activity description
Day 0 (visit 1)	Application of nickel discs (first): 10, 30, 60 min
Day 2 (visit 2)	D2 reading of test sites
Day 4 (visit 3)	D4 reading of test sites Application of discs (second): 10, 30, 60 min
Day 7 (visit 4)	D2 reading of test sites
Day 9 (visit 5)	D4 reading of test sites Application of discs (third): 10, 30, 60 min
Day 11 (visit 6)	D2 reading of test sites
Day 14 (visit 7)	D4 reading of test sites Application of discs (fourth): 30, 60, 120 min
Day 16 (visit 8)	D2 reading of test sites
Day 18 (visit 9)	D4 reading of test sites

The same schedule was used for Phase 1 (nickel metal disc) and Phase 2 (nickel-plated brass disc) subjects.

2.5 | Patch testing with nickel-releasing discs

In both Phase 1 and Phase 2, subjects were patch tested with nickel-releasing discs as per the schedule in Table 1, which shows the timing of visits for patch testing and D2/D4 readings, and the duration of application for each visit. The durations of the short, repeated applications of the metal discs were 10 minutes, 30 minutes, and 60 minutes. These applications occurred on 3 occasions over a period of 2 weeks, in accordance with the repeated exposure section of the ECHA guidance definition of prolonged contact with the skin (10 minutes), and with 2 additional longer time periods (30 minutes and 60 minutes) (Table 1). Single, longer applications of the discs were subsequently performed at the same application sites, lasting for 30 minutes (as per the ECHA guidance definition for prolonged contact), 60 minutes, and 120 minutes, addressing the single-exposure part of the ECHA guidance definition. On D1 of the study period, in all but 1 subject (the first to be tested), patch testing was also performed with the discs for 48 hours on a different area of the back, with readings on D2 and D4, to determine reactivity to the discs.

Between September 2015 and April 2017, a total of 45 subjects were patch tested with either nickel metal discs (in Phase 1) or nickel-plated brass discs (in Phase 2). Further details of the discs are provided in "Materials" above. Subjects were asked not to apply any moisturizer or leave-on products to the back for the duration of the testing. The discs were stored together in sterile plastic containers in a temperature-controlled environment at 22°C. They were handled with sterile plastic forceps.

The same 3 nickel discs were used for each subject across all applications. At the first application, the 3 nickel discs were spot tested with DMG, and then gently wiped with sterile gauze. For each

application, the 3 discs were placed, with plastic forceps, into allergEAZE Patch Test Chambers (SmartPractice), and secured to the upper back of the subject. No pretreatment or cleaning solution was used. The discs had a diameter of 7 mm/7.3 mm and a thickness of 1 mm/1.2 mm, and fitted snugly into the allergEAZE chambers, which measured 8 mm × 8 mm square. The depth of the allergEAZE chambers is not stated in any of the product information; however, it was estimated to be 1 mm, as the discs sat flush with the surface. Three empty allergEAZE Patch Test Chambers were used as negative controls on the contralateral side of the back. The side of the back to which the nickel discs were applied in each subject was determined with an online research randomizer tool (RESEARCH RANDOMIZER, https://www.randomizer.org). The application sites were precisely marked adjacent to the chambers with a surgical marking pen, to ensure that testing at subsequent visits occurred at the same position. The pen markings were reinforced at each visit.

For the short, repeated applications (visits 1, 3, and 5), the 3 nickel discs and the 3 control chambers on the contralateral side were applied in triplicate at time 0 (Figure 1). The first nickel disc and control chamber were removed from the back at 10 minutes, the second disc/control at 30 minutes, and the third disc/control at 60 minutes; an electronic timer was used to ensure precision. For the single, longer application (visit 7), the 3 nickel discs and 3 control chambers were again applied in triplicate at time 0 (Figure 1). On this occasion, the first nickel disc and control chamber were removed at 30 minutes, the second disc/control at 60 minutes, and the third disc/control at 120 minutes. The same nickel discs were retained for each subject and reused for each application at the same site as previously. In between uses, the discs were wiped gently with sterile gauze and stored in sterile plastic containers specific for that individual, with the discs not in contact with each other. They were not washed or treated with any cleaning fluid. They were not reused for another subject.

All patch testing was performed by an ODREC Research Fellow (medical practitioner). Testing was conducted in an air-conditioned environment, with the temperature controlled at 22°C. Readings were performed by the same dermatologist (R.N.) or dermatology trainees who were experienced in interpreting patch tests. The testing was performed in a double-blind manner, with neither the subjects nor the assessors being aware of which side was the test side and which was the control. Only the research fellow applying the discs was aware of which was the test side.

2.6 | Statistical analysis

Descriptive analyses were used to identify baseline patient characteristics and frequencies of reactions. Data were managed in an Excel database (Microsoft Corporation, Redmond, Washington). χ^2 tests were performed to assess statistical differences between groups.

3 | RESULTS

3.1 | Nickel release from test materials

The results for nickel release testing conducted with test method EN1811:2011 + A1:2015 were an average of 6.77 μg Ni/cm^2/week (range 6.04-7.54, n = 4) and 8.44 μg Ni/cm^2/week (range 6.35-10.31, n = 9) for nickel metal and nickel-plated brass patch test discs, respectively. DMG testing performed at the Skin and Cancer Foundation prior to the study gave positive results for all of the nickel metal and nickel-plated brass discs.

3.2 | Demographics of subjects

Of the 270 nickel-positive individuals contacted regarding participation in the study, 20 agreed to participate in testing with nickel metal discs (Phase 1) and 25 agreed to participate in testing with nickel-plated brass discs (Phase 2). Of these 45 participants, 17 were recruited from members of the public, and the remaining 28 were from the ODREC clinical database. In Phase 1, 1 of 20 subjects was recruited from the public, and in phase 2, 16 of 25 subjects were recruited from the public. The nickel-positive subjects recruited for the study showed a range of positive patch test reactions from + to +++, which was comparable

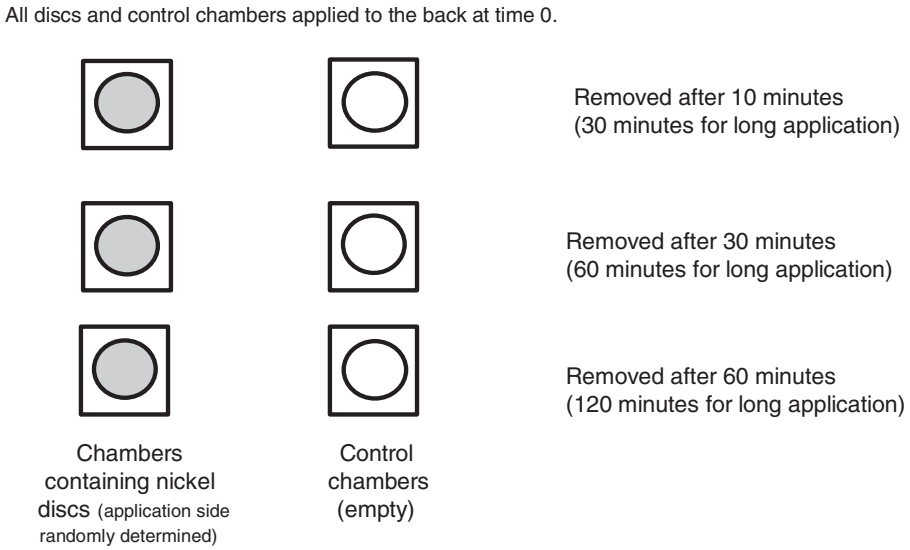

FIGURE 1 Timing of application of nickel and control discs to subjects' backs

TABLE 2 Number of patch test reactions in nickel-positive subjects in the Occupational Dermatology Research and Education Centre clinical database as compared with Phase 1 (nickel metal) and Phase 2 (nickel-plated brass) study subjects

Strength of nickel reaction	Number of nickel-positive patients from the clinic database (percentage of total positive reactions to nickel)	Number of nickel-positive reactions in Phase 1 study subjects (percentage of total Phase 1 subjects)	Number of nickel-positive reactions in Phase 2 study subjects (percentage of total Phase 2 subjects)	Total number of nickel-positive reactions in Phase 1 and Phase 2 study subjects (percentage of total)
+	741 (59.6)	10 (52.6)	12 (48.0)	22 (48.9)
++	442 (35.5)	8 (42.1)	9 (36.0)	17 (37.8)
+++	61 (4.9)	2 (1.1)	4 (16.0)	6 (13.3)
Total	1244[a]	20	25	45

[a] Total in database: 8862.

to the distribution of reactivity in the nickel-positive patients in the ODREC database (Table 2). Patients having a reactivity of only + made up 59.6% of the ODREC clinical database, as compared with 48.9% of the combined Phase 1 and Phase 2 subjects. High reactivity, noted as +++, was found in 4.9% of the clinical database patients as compared with 13.3% of the subjects in Phase 1 and Phase 2 of this study. χ^2 testing confirmed that there were no statistical differences between the patch test reactivity of the clinic database patients and that of the Phase 1 or Phase 2 group (data not shown). There was also no statistical difference between the nickel reactivity of the subjects recruited from the database and the subjects recruited from the public (data not shown).

Of the 20 individuals participating in Phase 1 of the study (patch testing with the nickel metal discs), there were 16 females and 4 males, with a mean age of 48.1 years (age range 20-68 years). Four of 20 had no history of piercings, including 3 of the males. Of these 4 subjects, 1 described problems with a watch band, 1 had orthodontic braces, 1 had a total hip replacement and problems with a belt buckle, and 1 had a negative history for nickel dermatitis but had been found to react positively to nickel sulfate on routine patch testing for the assessment of contact dermatitis.

Of the 25 individuals participating in Phase 2 (patch testing with the nickel-plated brass discs), there were 24 females and 1 male, with a mean age of 40.0 years (age range 21-77 years). All except 1 (the male) had a history of piercings. Of this cohort, 21 of the 25 had a history of skin problems with jewellery, predominantly earrings. Four had no history of skin problems caused by nickel, but had reacted positively to nickel sulfate on routine patch testing for the assessment of contact dermatitis.

3.3 | Reactivity of subjects to nickel discs

For the 48-hour patch tests, at the D4 reading, 16 of 19 Phase 1 subjects had positive patch test reactions to the nickel metal discs, and 22 of 25 Phase 2 subjects had positive patch test reactions to the nickel-plated brass discs (Table 3). Only 1 of the reactions in either Phase 1 or 2 was +++, with most reactions being only +. Overall, the reactions to the nickel metal discs were not significantly different from those to the nickel-plated brass discs (χ^2 test; data not shown).

3.3.1 | Reactivity to nickel metal discs applied for short periods (Phase 1)

There was only 1 participant who had somewhat consistent allergic reactions on testing with the nickel metal disc. The shortest application time to which she reacted was on D11 for the 48-hour reading after their third repetition of 30 minutes of testing (visit 6; see Table 1 for schedule). She did not react to the disc for the 10-minute applications or the repeated 60-minute applications. She had a history of pierced ears at 13 years, application of orthodontic braces at age 15 years, and reactions occurring with cheap jewellery.

Another subject had inconsistent reactivity, with + reactions to the disc on D7, D9 and D11 for the second and third repeated 60-minute applications (visits 4, 5, and 6; Table 1), but no reactivity following the single applications of 30 minutes, 60 minutes, and 120 minutes, or for the fourth application of the discs at all time periods.

A third subject developed doubtful but not positive reactions on D18 for the 96-hour readings of the single 60-minute and 120-minute applications (visit 9; Table 1).

A fourth subject also showed inconsistent reactivity by developing a positive reaction on D18 for the 96-hour reading of the single 60-minute application (visit 9; Table 1) but not for the longer 120-minute exposure time.

There were no positive reactions in the control tests. A fifth subject had a doubtful reaction on D9 for the 120-hour reading after the second repetition of the 30-minute control test application (visit 5; Table 1). Complete results for these participants are shown in the Online Supplement.

TABLE 3 Results of patch testing subjects with nickel metal discs (Phase 1) and nickel-plated brass discs (Phase 2) for 48 hours, with reading performed at day 4 (96 hours) after application

Strength of reaction	Phase 1 (nickel metal discs)	Phase 2 (nickel-plated brass discs)
−1 (negative)	3	3
0 (equivocal)	1	0
+	13	12
++	2	9
+++	0	1
Total	19	25

3.3.2 | Reactivity to nickel-plated brass discs applied for short periods (Phase 2)

There was only 1 subject who had a single positive reaction, and the reactivity was inconsistent. Initially, a +++ reaction to the 48-hour patch test with nickel sulfate and ++ reaction to the nickel-plated brass disc had been observed. A reaction was seen to the third repetition of the 60-minute application of the nickel-plated brass disc, on D11 (visit 6: see Table 1). However, this subject did not react after the subsequent longer single exposures of 30, 60 or 120 minutes, at the same application site that reacted previously. There were no positive reactions to the control tests.

4 | DISCUSSION

4.1 | Nickel release from discs used for testing

Measurements of nickel release for both test materials with the current standardized European test methodology (EN 1811:2011 + A1:2015) showed a nickel release rate (average 6.7 and 8.4 μg/cm^2/week for nickel metal and nickel-plated brass, respectively) above the nickel release limit of 0.5 μg/cm^2/week set by the EU REACH nickel restriction (Annex XVII, entry 27; June 2009) for articles intended for direct and prolonged contact with the skin.[4] Non-compliant, inexpensive fashion/costume jewellery is known to be a frequent cause of NiACD. This type of jewellery is often made of nickel plated onto a base metal,[6] such as the material used for patch testing in Phase 2. Therefore, use of these high nickel-releasing materials is relevant for assessing the definition of prolonged contact.

4.2 | Patch test results

To more accurately determine the definition of prolonged contact in the context of NiACD, patch testing in nickel-sensitized individuals was conducted for varying amounts of time with 2 different nickel-releasing materials (nickel metal and nickel-plated brass). It was surprising that several subjects did not react to the nickel (4/19) or nickel-plated (3/25) discs even at the 48-hour patch testing, despite the nickel release rate being significantly higher than the EU nickel restriction rate limit, as documented by both AOB and HKPC. Because the elicitation threshold varies between individuals, they will react differently to items releasing nickel. These results highlighted that the nickel release rate limit included in the EU nickel restriction may be considered to be conservative, as items releasing 10 times more nickel than the limit may not elicit allergic reactions in all nickel-allergic individuals even after 48 hours of exposure.

Because the elicitation threshold varies between individuals, individuals will react differently to items releasing nickel. As a result, the EU nickel restriction aim was to "protect the majority, but not all, of those sensitized".[7] This is because "the available data suggests that whilst the release rate of 0.5 μg Ni/cm^2/wk after direct and prolonged contact is sufficient to protect against elicitation of an effect in a substantial part of the population, complete protection for the most sensitive sensitised persons may only be achieved at levels that could be an order of magnitude lower".[8] The percentage of nickel-sensitized individuals protected by the number can be estimated from the number of individuals with clinical reactivity in the study by Menné et al,[9] which is the primary source of the EU nickel restriction (and the Danish regulation) release rate limit of 0.5 μg Ni/cm^2/wk. The materials with a release rate lower than this caused clinical reactivity in 3% (stainless steel), 11% (white gold) and 23% (nickel tin) of the nickel-sensitized individuals in the study. The EU restriction could thus be hypothesized to protect an approximate minimum of 77% of nickel-sensitized individuals.

Only 1 study subject showed somewhat consistent reactivity to either of the high nickel-releasing materials for the shorter time periods tested. This subject showed a reaction to the nickel metal discs at 48 hours after the third repetition of the 60-minute application. However, there was a doubtful reaction at 120 hours. Subsequent testing at the single exposure times of 30, 60 and 120 minutes all showed allergic reactivity. It should be noted that, although these exposures were single applications, they were applied at the sites of the previous exposures. As a result, the single applications essentially constituted a fourth repeated exposure in a 2-week period. This previous exposure to the same site probably primed them for allergic reactivity, as would be expected with repeated exposures, but may not be representative for single repetition exposures at those sites. Therefore, the results for the single repetition overestimate reactivity at locations not previously exposed. It is interesting that only the 48-hour reading was positive (but weak) for the 60-minute third repetition, with the 120-hour reading being equivocal, and all other tested times not showing a reaction.

The inconsistent reactions in the other 4 subjects did not correlate with increasing exposure times, and did not show a dose-response relationship, and so are not considered to be reliable or predictive of the time needed to develop NiACD. There are limitations to the use of patch testing to predict reactivity, because it is does not simulate some types of real-life handling situation, which include factors such as friction. Erfani et al[10] found that the highest amount of nickel was deposited by pure nickel metal after repeated touch testing, although no nickel-plated materials were tested for comparison. Although touch testing does create more friction than patch testing, possibly disrupting the surface layer of oxidic nickel more than patch testing, occlusion is also known to create conditions that increase corrosion, owing to contact with sweat.[11] In addition, the allergen dose is concentrated and focused on a single area of skin. Different uses are mimicked by each of these types of testing, with items such as watches and some types of jewellery being more satisfactorily represented by prolonged, occluded exposure.

4.3 | Patch test variability

As noted in Table 3, there is variability in the degree of sensitivity to nickel, as shown by only a minority of nickel-positive clinic attendees and the study group having +++ reactions on patch testing. Patch test variability for nickel sulfate has been previously shown within the same individual at different times.[12] Emmett et al[13] reported that the provocation threshold (the lowest amount of nickel causing a reaction) varied from 240 μg Ni/cm^2 (2.5% $NiSO_4$) to 1 μg Ni/cm^2 (0.01% $NiSO_4$) when tested in petrolatum for the study group of

12 nickel-sensitive volunteers. A review by Fischer et al,[14] which conducted statistical analysis of the dose-response studies of single occluded exposures to nickel sulfate, showed that only 5% of people reacted to doses per area of 0.44 μg Ni/cm^2, whereas 10% reacted to 1.04 μg Ni/cm^2. Another study by Fischer et al[15] showed that, although the elicitation threshold for patch testing was higher than that for the repeated open application test (ROAT), the accumulation elicitation threshold for the ROAT was approximately the same as the patch test threshold. It is important to note that these studies were investigating soluble nickel, with 100% availability of the nickel ions at the time of application, and not nickel metal materials, for which time is required for release. As shown by Bruze,[16] with a shorter time, higher doses are needed and fewer people react. The threshold is therefore linked specifically to the duration of exposure, and cannot be extrapolated to much shorter exposure times. Thus, it is likely that only highly sensitized subjects will react after relatively short exposures to nickel metal. This is supported by our findings that only 1 subject who had a +++ nickel sulfate 48-hour patch test reaction reacted to the third repetition of 30 minutes but not to any 10-minute exposures. This does not, however, explain why this same subject did not react to the third repetition of the 60-minute exposure.

Given that only 1 subject of the 45 tested with either high nickel-releasing material reacted in an even moderately consistent manner, it has been shown that the shorter times tested were not sufficiently long enough to cause NiACD in most of the nickel-sensitized individuals with the materials tested. This is consistent with previous studies of patch testing nickel-allergic patients with nickel sulfate. It was reported that the shortest time of reactivity was 1 hour, and that half of the individuals did not react until after 6 hours.[17] On testing for 20 minutes with nickel sulfate solution (not in pet.), 3 of 9 individuals showed a positive reaction.[18] Testing of nickel-allergic subjects for 6 hours with 7.5% nickel sulfate showed that only 4 of 8 subjects reacted, as compared with 8 of 8 tested with 5% nickel sulfate (standard patch test concentration) at 48 hours. The findings of our study are consistent with those of other studies, in which patch test reactivity to nickel required exposure in hours, and not just minutes. Given that these results were for soluble nickel salts, for which corrosion was not required for release, and the nickel ions were readily available, it is logical that additional time would be required for corrosion and solubilization of the same amount of nickel ions. However, the current study did not test longer than 2 hours, so the actual time needed for a significant number of nickel-sensitized people to develop an allergic reaction to nickel released from metal in this test situation is not known, but would be likely to be longer than 2 hours for a single exposure. Again, this study was unable to assess real-life factors such as friction resulting from handling of metal.

4.4 | Sensitivity of patch test location

The sensitivity of different parts of the body of nickel-allergic individuals has been examined with patch testing. It was found that the back was the most sensitive in all individuals.[19] However, another study showed that different areas of the back were not significantly different in reactivity.[20] Therefore, the results of the study presented here are expected to be representative of reactions in other parts of the body of the same individual.

4.5 | Short contact with nickel metal

Most nickel-sensitized individuals did not react after short contact times. The EN1811 test and the associated nickel release limit were designed for comparison of 48-hour patch test reactivity and 1-week nickel release test results, based on a study by Menné et al.[9] The 1-week time point for nickel release results correlated the best with clinical reactions at the times tested in that study. Therefore, it is not surprising that shortening of the patch testing times for nickel metal and nickel-plated discs would show a lack of predictive capacity.

This type of study has been performed by several authors to better understand potential skin exposures associated with short and frequent contact.[10,21–32] The methods used in these studies vary from nickel release tests on articles or materials, to wipe testing of surfaces or skin. Many of these studies have shown nickel release rates and/or skin exposures greater than the EU nickel restriction release limit of 0.5 μg/cm^2/wk. However, none of these studies was designed to correlate the exposures with clinical reactions. As explained above, the release limit was derived specifically for certain time periods and only for nickel release, which may not be representative of dermal exposure. Theoretically, the appropriate nickel release limit for items associated with short exposure may differ significantly from the current EU nickel restriction limit. This would need further investigation and validation with clinical reactivity, as was performed for the current nickel release rate limit for longer time periods.[9]

The study by Julander et al[30] showed high nickel release during a relatively short time period. The results for the nickel-plated coins tested in this study should be comparable to those for the nickel-plated brass discs in the present study. However, the threshold for clinical reactivity with this early higher release (and therefore higher exposure) has not been determined, so it is not known how much nickel is needed in a shorter time period to elicit a nickel dermatitis reaction. Measurement of the higher doses on the skin is relevant, but must be compared with a threshold for the equivalent duration of exposure associated with the shorter time period. Bruze[16] found that, with a shorter time of exposure (5 hours), a much higher dose is needed to elicit nickel allergic reactions in most nickel-allergic people than with a longer time (48 hours). Therefore, it is likely that the threshold for much shorter contact is even higher than the 5-hour time point measured in that study. These high doses may be rarely exceeded by metallic nickel items that are not completely soluble. Of course, thresholds in hypersensitized individuals are lower and might be exceeded, accounting for the observed (but not frequent) nickel allergic reactions after short time periods to items such as coins.

A study by Jensen et al[27] measured dermal exposure to nickel 2 hours into a work shift for individuals experiencing hand eczema, to assess what exposure amounts might correlate with elicitation of nickel dermatitis. Although these measurements provided a 2-hour estimate of exposure, the amount measured did not correlate with the clinical reaction over the normal time frame of exposure that caused nickel dermatitis reactions in these individuals. Occupational exposure estimates for risk assessment require measurement after either a full

work shift or a shorter time, with that value being adjusted for the total number of hours worked in a shift (e.g. 2-hour measured value × 4 to obtain the 8-hour work shift estimated value). A controlled study of exposure amount and time correlated with clinical reactions is needed to better estimate the measured dermal amount that would cause a reaction for a specific time.

Bridging of the existing data in the studies above and the data in the present study could include dermal measurement of nickel concentration, in association with the clinical reactivity, for the various time periods.

4.6 | Slow absorption of nickel

The need for time periods in hours (and even days) rather than minutes for an NiACD reaction is, in part, attributable to the slow and low absorption of nickel ions through the skin. Dermal absorption of nickel ions from nickel metal powder was measured by tape stripping of volunteers' skin after exposure to nickel metal powder for multiple time points from 5 minutes up to 96 hours.[33] Dermal absorption ranged from 0.07% at 5 minutes to 0.2% at 96 hours, showing both the significant amount of time needed for absorption, and the low amount of the dose absorbed from nickel metal. Similarly, an in vitro study using human skin showed that the metal is indeed oxidized and ionized before being absorbed by the skin, and that measurable absorption takes place only after at least 14 hours.[34]

4.7 | Definition of prolonged contact in the EU nickel restriction

The European REACH nickel restriction is aimed at protecting all people from becoming nickel-allergic, and most nickel-allergic people from developing NiACD.[5] This is the first study that has investigated testing for the time periods included in the ECHA guidance definition. Under the occluded conditions tested, using nickel metal and nickel-plated brass discs that significantly exceed the nickel release rate limit, we have found that the current definition protected all individuals in our sample (N = 45).

5 | CONCLUSIONS

The majority of nickel-allergic subjects did not react to to standardized nickel metal or nickel-plated brass discs after 2 hours, and did not react after repetitive exposure to nickel of 10 minutes on 3 occasions over a period of 2 weeks. This suggests that the length of time needed to cause NiACD under the conditions and with the materials tested, for most nickel-allergic individuals, is greater than the times tested in this study, and greater than that in the ECHA guidance definition. These findings are consistent with other studies in which patch test reactivity to soluble nickel required exposure in hours, and not just minutes. Although the test conditions did not adequately assess real-life factors associated with handling nickel or nickel-plated items, such as friction for some types of use, the corrosion and dose concentration in the test process would have been expected to increase nickel delivery to the skin and represent other types of use known to cause NiACD. Additional research measuring dose and time of exposure associated with clinical reactivity of NiACD could address the data gap regarding time and amount of exposure from soluble as compared with nickel metal materials.

Conflict of interest

This study was funded by NiPERA, a division of the Nickel Institute, which is the global association of leading primary nickel producers.

ORCID

Claire L. Higgins https://orcid.org/0000-0001-7364-0250
Danit Maor https://orcid.org/0000-0003-0652-8159

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