

A strategy to assess short and frequent skin contacts with nickel

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Popular Science Summary

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Nickel is a metallic element that is widely used in many types of materials and alloys used for many applications within our society, for example in metallic consumer products. Skin contact with nickel-containing materials and items in our daily life may cause contact allergy to nickel. Once allergic, any further skin contact with nickel can give eczema, often on the hands.

In order to protect the European general public from nickel allergy, certain items intended for direct and prolonged contact with the skin are not allowed to release nickel above a limit value. However, nickel has remained the most frequent cause of contact allergy.

Why is the Ni allergy problem not solved nearly 15 years after introduction of the EU nickel restriction?

It might be due to the many daily short, but frequently occurring skin contacts with nickel-releasing items such as toys, tools and coins that are not covered by the EU regulation. Many drops make up a river!



Photo: J Midander

This project presents a research strategy for assessing short and frequent skin contacts with nickel-containing consumer products. Screening of three different home environments for nickel-releasing items showed that 28% of metallic consumer products released nickel above the limit value. Furthermore nickel was detected on the skin after touching several different nickel-containing materials only once!

Abstract

Background. In order to protect the public health, the EU nickel regulation sets a limit for certain consumer products not to release more nickel than $0.5 \mu\text{g}/\text{cm}^2/\text{week}$. However, nickel remains the most frequent cause of skin allergy.

Objectives. To design and develop an experimental strategy for assessment of short and frequent skin exposure to nickel containing consumer products in daily life.

Methods. The research strategy includes: screening for nickel exposure in daily life using the dimethylglyoxime (DMG) spot test for direct qualitative detection of nickel, and assessment of nickel skin dose from relevant nickel-containing materials in two contact test models using modified skin sampling methods. Future tests for measurement of nickel release in artificial sweat were planned for in the strategy but not performed within this master thesis project.

Results. In 3 different home environments, 28% of different kinds of metallic consumer products were DMG test positive. The chemical compositions of DMG positive items were mostly detected as combinations of Ni/Cu/Zn, Ni/Fe/Cu or Ni/Fe. The lowest nickel skin dose detected as DMG test positive was $0.05 \mu\text{g}/\text{cm}^2$. The presence of nickel on the skin was identified qualitatively after single contact with all the selected nickel-containing materials except for stainless steel.

Conclusion. The presented strategy will provide new and important knowledge for better understanding the allergy risk from short and repetitive skin contacts with nickel-releasing materials. Such data can be used for safety assessment and more protective restriction of nickel release from items intended also for short and frequent contact with the skin.

List of abbreviations

ACD	allergic contact dermatitis
Cr	chromium
Cu	copper
DMG	dimethylglyoxime
Fe	iron
ICP-MS	inductively coupled plasma mass spectrometry
Ni	nickel
REACH	registration evaluation authorisation of chemicals, the EU Chemicals regulation
XRF	x-ray fluorescence
Zn	zinc

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Introduction

Nickel is a silvery white metallic element with magnetic properties, which is resistant to both oxidation and corrosion. It is employed in combination with other metallic elements to make alloys such as stainless steel (iron/nickel/chromium), nickel silver (nickel/copper/zinc) and copper-nickel. Nickel is widely used in a broad variety of industrial applications like production of metallic consumer products (coins, tools, keys, garment details, and stationery).

Unavoidable frequent skin contact with nickel-containing materials/ surfaces in daily life may cause sensitization and contact allergy. The reason is the interaction between the nickel material and various components of human sweat (salts, amino acids and proteins) and sebum (skin surface lipids), leading to release and deposition of the allergenic divalent nickel ions onto the skin ¹.

The amount of nickel being released and deposited in this manner is mainly correlated to the amount of available nickel on the surface of the material, which is to some extent dependent on the nickel content in the bulk material. Other factors such as corrosion/friction, pH, temperature, salts/proteins and the duration of the contact also play a major role in this context ¹.

The allergenic divalent nickel ions have a potential to cause skin sensitization. To become sensitized, skin contact with nickel-releasing material is required. Nickel ions can then bind to a protein and trigger the immune system causing skin sensitization (contact allergy), which is a lifelong allergy. Further repeated contact to nickel may lead to onset (elicitation) of allergic contact dermatitis (ACD) ².

Skin absorption and accumulation of nickel in the skin is essential for development of contact allergy and ACD, since a certain threshold level is needed to trigger the sensitization and contact allergy, while elicitation of ACD require a lower threshold level ³. Patch test is the medical diagnostic tool for detection of contact allergy ⁴.

In the early nineteenth century, nickel contact allergy was identified amongst men working in the plating industry ⁵. Later on, the prevalence of nickel allergy in women increased as a result of exposure to direct and prolonged contact with nickel-releasing products such as stocking suspenders, jeans' zippers and buttons, ear piercings and jewellery ⁶.

European nickel regulation

In order to decrease the frequency of nickel allergy and protect the European general public, the EU Nickel Directive was adopted in 1994 and it entered into full force in 2001. The EU Nickel Directive covered the products and items intended for prolonged use in direct contact with the skin, and limited release of nickel to not exceed a limit threshold value⁷. This limitation value is $0.5 \mu\text{g}/\text{cm}^2/\text{week}$ and it was introduced by Menné et al., based on reactivity to nickel alloys after performance of patch testing in nickel sensitive individuals⁸.

Since 2009, the former EU Nickel Directive is part of REACH (Registration, Evaluation, Authorisation and Restriction of the Chemicals) and is now referred to as the restriction of nickel release in REACH⁷.

Before the EU Nickel Directive, Denmark was the first European country to restrict the amount of nickel release, to not exceed more than $0.5 \mu\text{g}/\text{cm}^2/\text{week}$, from consumer products that are in prolonged contact with the skin such as watches, bracelets, necklaces, ear rings and jean buttons (1989)⁹. Thereafter use of piercings or rings containing more than 0.05% nickel for ear piercing were banned in Sweden (1990). In Germany, consumer products that contained nickel required to be labelled “contains nickel and may cause an allergic reaction” already in the 1991¹⁰.

Prevalence of nickel allergy

According to a study summarising patch test data from four European regions in 2005/2006, nickel has remained the most frequent allergen with high prevalence in both central Europe (19.7%) and southern Europe (24.4%)¹¹.

The prevalence of nickel allergy after adopting the EU nickel regulation, was further investigated by Garg et al., who performed a study on national patch test data from 18390 patients in Denmark, Germany, Italy, and the UK from 1985 until 2010¹². In their study, a much higher prevalence of nickel allergy in women compared with men was confirmed, as is well-known since decades.

Furthermore they demonstrated that the prevalence of nickel allergy decreased significantly in young females (younger than 30 years) and increased significantly in middle aged (between 30-60 years) and old women (older than 60 years). Finally it was suggested that, “nickel allergy and dermatitis remain very frequent”¹².

Prevalence of nickel allergy in the general population is difficult to study. In fact the literature generally is limited to patient data from patch testing dermatitis patients referred to dermatologists. There are relatively few population-based studies in which patch testing has been used, and very few where young people have been tested. Especially the prevalence of nickel allergy in the younger population is of concern, as an indication of the current exposure and risk.

A recent follow-up study on a cohort of 1501 unselected 8th grade schoolchildren in Denmark (Odense) was conducted by Mortz et al. in order to follow the course of contact allergy over 15 years, from adolescence to adulthood ¹³. They revealed that among commonly tested allergens, nickel was the most frequent cause of allergy (11.8%) and the prevalence of nickel allergy increased in women and declined among men. Also they could identify a significant raise of cases with new nickel sensitization over time. Therefore they suggested that the high incidence and prevalence of nickel allergy twenty years after the Danish nickel regulation showed the limited efficacy of the regulation ¹³.

Why is the Ni allergy problem not solved nearly 15 years after introduction of the EU nickel restriction?

Compliance with the limitation of nickel release in REACH

Studies have demonstrated that since the full enforcement of the EU Nickel Directive the percentage of nickel-releasing items that are covered by the EU nickel regulation has declined significantly on the Swedish market ¹⁴. In Denmark 10% of inexpensive jewellery and hair clasps released more nickel than the limit value in the regulation ¹⁵ despite the fact that Denmark was pioneer in nickel regulation ⁹.

According to a recent review article, the limitation of nickel release in REACH needs to be optimized and the European markets should be monitored for compliance with EU nickel regulation ¹⁶.

Aim of the study

The existing EU limitation of nickel release from products intended for use in prolonged and direct contact with the skin is not protecting the population from unnecessary skin exposure to nickel. We believe that many short but frequent contacts with nickel-releasing items in our daily life contribute largely to the total load of nickel on the skin, causing nickel allergy and dermatitis. But to what extent, needs to be better understood.

The aim of this study is to design and develop an experimental approach to assess the skin exposure to nickel from short and frequent contacts with nickel-releasing items/surfaces.

Materials and methods

Within this project, we have designed and developed a research strategy for investigating nickel skin deposition and accumulation from short and frequent contacts with the nickel-containing materials, both qualitatively and quantitatively. The strategy consists of three interlinked parts that combine established methods for nickel screening, skin dose and metal release measurements, see overview in figure 1.

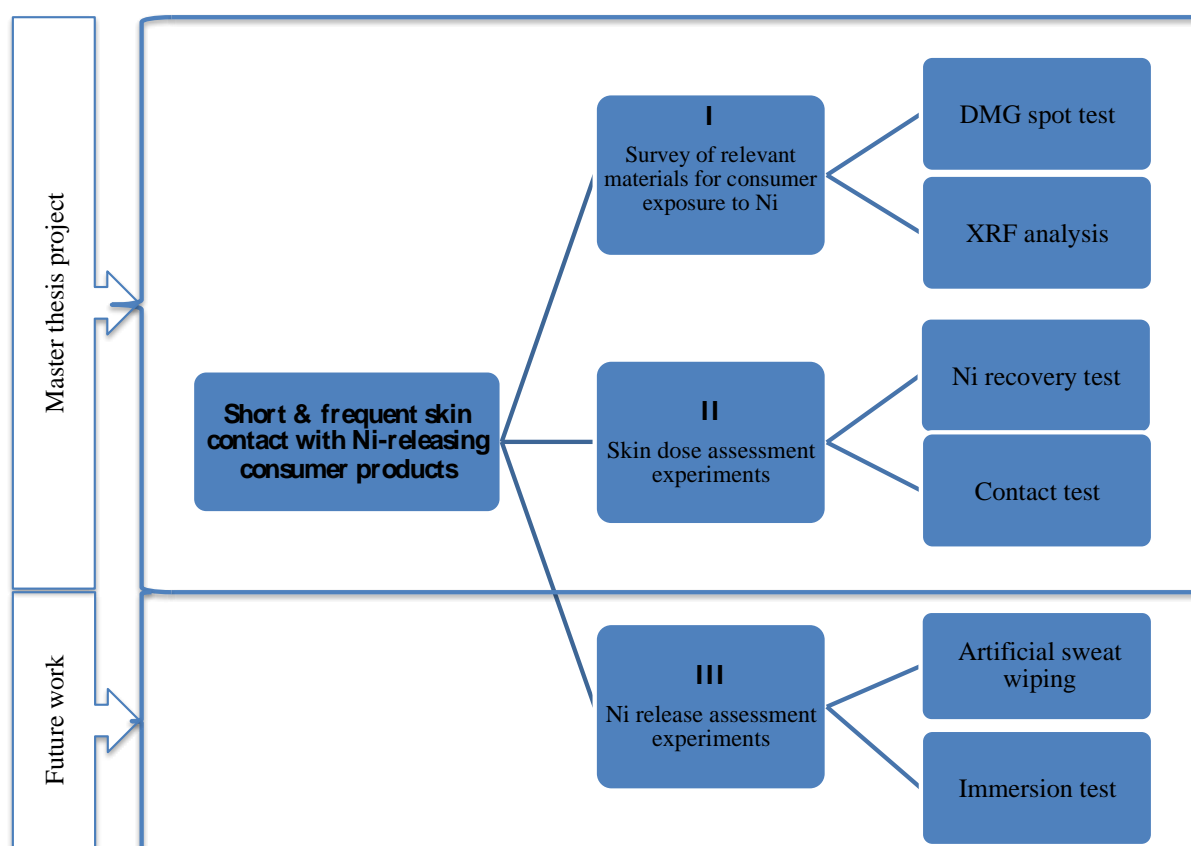


Fig. 1. Overview of the strategy consisting of three parts: I) Survey of nickel-releasing materials in 3 home environments for selection of materials that are representative of daily nickel exposure. II) Assessment of deposited skin doses of nickel as a result of short and frequent exposure using the selected materials. III) Parallel measurement of nickel release from the selected materials by artificial sweat wiping and immersion of materials in artificial sweat for short time periods.

In order to enable assessment of short contact events, the experimental methodology had to be carefully considered and sometimes optimised. Only describing the short and somehow frequent skin contact in a reproducible way is challenging. A screening of nickel-releasing

items that come into contact with skin on a daily basis was performed and resulted in a selection of representative materials for further studies (part I). Experiments to test the efficiency of a modified skin sampling method were conducted as well as two different model tests for short and frequent skin contact (part II). The experimental design of the contact tests was tried for the first time in the pilot tests of this master thesis project. Corresponding pilot experiments for assessment of nickel release from the study materials has been planned for (part III) but was not performed within the frame of this master thesis project. Also, it has not been possible to manage chemical analysis for several reasons. However, pilot experiments have been performed using a direct qualitative method for detection of nickel in parallel with samplings for quantitative analysis.

I. Survey of relevant materials for consumer exposure to nickel

The aim of this part of the study was to screen for nickel in metallic consumer products used for short period of time and to identify the chemical compositions of such materials. Based on this information, a set of representative materials to study further in project II and III was selected.

Dimethylglyoxime (DMG) spot tests

Spot tests were performed in three different home environments to identify nickel-releasing items/ products in daily life.

Chemicals. DMG test reagent solution (Chemo Nickel Test™, commercially available from Chemotechnique Diagnostics®, Vellinge, Sweden) contains DMG (1%), ammonia (9.9%) and ethanol. It was used for colorimetric detection of nickel on surface of the items. The reagent solution is originally transparent but changes to pink colour after reaction with nickel ions; hence the colour change indicates the presence of nickel ions available on the surface.

Equipment. White cotton-wool-tipped sticks (cotton swabs, Critical Swab™ double cotton tip, paper handle, 3" -76.2 mm, VWR North America) were used for DMG spot testing on surface of the items. Eppendorf® tubes (2 mL) were used for storing an appropriate volume of DMG solution (50 µL) for each spot test.

Experimental procedure. DMG spot tests were performed by moistening a white cotton swab in the Eppendorf® tube containing the 50 µL of DMG solution, which then was rubbed against the surface of the item for 30 seconds. The result of the spot tests was observed and recorded one minute after performing the experiment. A pink colour on the white cotton swab was scored as a positive reaction and no colour change was scored as a negative reaction. A

discolouration, such as brown or black colour, on the cotton swab was assumed as a doubtful reaction (?), since it may hide the pink colour of a positive test.

X-ray fluorescence spectrometry (XRF)

The chemical composition of test items that exhibited positive reaction in the DMG screening test was identified individually by a handheld X-ray fluorescence analyser (Innov-X, Alpha 4000) in the lab at IMM. The different atoms in the material become ionized by high energy X-rays (up to 35 keV and 5 μ A) and emits fluorescent radiation of a certain energy characteristic for each element, when going back to a stable state. This secondary radiation is detected and its intensity is directly related to the amount of each element in the material.

Each item was subjected in the XRF for 300 seconds and the chemical composition was analysed, using the analytical mode and including light elements in the presentation of chemical compositions (%). Due to practical reasons, the chemical composition of all of the positive items was not analysed.

II. Skin dose assessment experiments

The aim of this part of the study was to further investigate amounts of nickel that can be deposited on the skin from short contacts with representative nickel-containing materials (selected based on results from part I). To assess the nickel exposure, both qualitative and quantitative methods including DMG spot test and the acid wipe sampling technique were applied.

Recovery test of the acid wipe sampling method for measurements of low nickel skin doses

A nickel recovery test was performed to measure the efficiency of the acid wipe sampling method for quantification of low doses of nickel on the skin¹⁷. In order to measure the recovery of lower doses of nickel, the original experimental procedure was modified by using 10 mL of 1% HNO₃ instead of 25 mL as well as shaking the sample solution for 45 minutes instead of 30 minutes for extraction of nickel from the samples.

Study participants. Five healthy volunteers without any history of nickel allergy or on-going dermatitis participated in the nickel recovery test. The study was approved by the ethical committee (KI Nord at Karolinska Hospital, Stockholm). The defined skin doses of nickel were applied onto fingers, see figure 2. The right hand was used for acid wipe sampling (with 1% HNO₃, not harmful for the skin) and the left hand for DMG spot test in each participant (which is not harmful for the skin). One of the volunteers participated in an additional test

using cotton swabs moistened with 1% HNO₃ for skin sampling.

Chemicals. Nitric acid (65%) was diluted with deionized water to 1%. A mixture of 1% HNO₃ and 99.5% ethanol 1:1 were used as a vehicle for dilution of nickel stock solution and control. 1% HNO₃ was used for extraction of nickel from the acid wipe samples. A dilution series with standard concentrations of 50, 5, and 0.5 mg Ni/L was prepared from a nickel stock solution 1000 mg/L \pm 2 mg/L Tracecert[®]. The commercially available DMG test reagent solution was used for colorimetric detection of nickel on the skin.

Equipment. Containers (60 mL, PP plastic; Thermo Fisher Scientific, Nalgene[®] Labware, Waltham, MA, USA) and test tubes (25 mL, PP plastic; Sarstedt, Nümbrecht, Germany) were used for the nickel recovery test. Cellulose wipes (injection wipes; Paper-Pak Sweden AB, Sundbyberg, Sweden) were used for acid wipe sampling. White cotton swabs (Critical Swab[™] double cotton tip, paper handle, 3" -76.2 mm, VWR North America) were used for DMG spot test. Flexible plastic templates (metal free) with an aperture of 2 cm² were used for indicating the corners of the sampling areas. A marker pen with red ink (nickel free) was used for marking the sampling area.

Preparation of the equipment. All the test tubes, containers, templates, pipette tips, and other laboratory utensils were acid washed by immersing them in 10% HNO₃ for 24 hours and then they were rinsed 4 times by deionized water and dried in the ambient air in the laboratory.

Experimental procedure. Before application of defined nickel doses, the participants washed their hands with soap and water, and dried them with a paper towel. Thereafter the operator rinsed the participant's hands with 1% HNO₃ and deionized water, and dried them with a paper towel. Afterwards, the sampling areas (the thumb, index, middle, and ring fingers) were marked on the skin (only right hand, used for acid wipe sampling) by indicating the corners of a 2 cm² template.

Applied nickel doses. Twenty microliters (20 μ L) of each concentration of the nickel standard solutions (0.5, 5, and 50 mg Ni/L corresponding to nickel doses of 0.01, 0.1, 1 μ g applied on 2 cm² on the skin), as well as 20 μ L of vehicle solution) were applied on volar fingertips of the thumb, index, middle, and ring fingers of both left and right (marked area) hands in each participant as shown in the figure 2. Thereafter, the skin was allowed to dry for about 1-5 minutes.

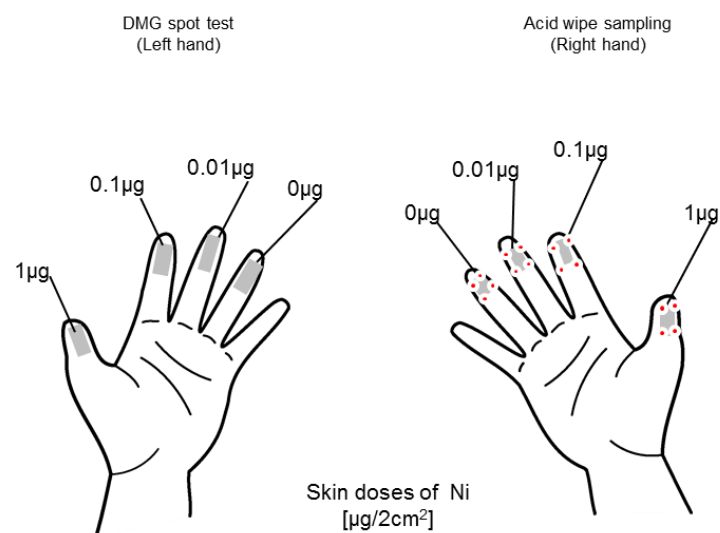


Fig. 2. Position of applied nickel doses. Nickel solutions, 20 μL of each concentration (0.5, 5, and 50 mg Ni/L), corresponding to 0.01, 0.1 and 1 μg doses of nickel, as well as 20 μL of vehicle as a control (1% HNO_3 + 99.5 ethanol) were applied by pipette and spread over the 2 cm^2 of marked areas of the volar fingertips of 4 fingers on right hand in each participant for acid wipe sampling and on the left hand (without markings) for DMG spot test.

After application of nickel doses. The operator wiped each marked area on the right hand of the participants with three consecutive cellulose wipes that were each moistened with 0.5 mL of 1% HNO_3 . Then three wipes from each finger were pooled in the same container (60 mL).

In parallel, the DMG spot test was used directly on the skin of the volar fingertips (the thumb, index, middle, and ring fingers) of the left hand in 5 participants. In this case a pink colour in white cotton swab was scored as a positive reaction, light pink colour as a weak positive reaction, and no colour changed was assigned as a negative reaction.

In order to enhance the precision, cotton swabs (instead of wipes) were used for skin sampling in one of the volunteers. This sampling procedure might also prevent any contact between operators' glove and the moistened wipe containing nickel, as well as unintended contact with the sampling areas on the skin. The operator rubbed the marked area on the right hand with 3 consecutive cotton swabs that were moistened with 0.1 mL of 1% HNO_3 . Then the 3 cotton swabs from each area were pooled in the same container (60 mL).

Dosed wipes and blank samples. Each concentration of nickel standard solutions (0.5, 5, and 50 mg Ni/L) and vehicle was applied by pipette on 3 wipes to compare the nickel recovery rate of dosed wipes with the recovery from doses applied on skin. The 3 wipes from each dose were put in the same container (60 mL). Furthermore, blank samples were prepared by filling

the containers either without any wipes (triplicate) or with 3 blank wipes (triplicate) or with 3 blank cotton swabs (duplicate).

In order to extract nickel from the samples, 8.5 mL of 1% HNO₃ solution were added to each container containing samples (wipes, cotton swabs and blanks, making up 10 mL of solution in total) and then all the containers were shaken for 45 minutes with an orbital shaker.

Subsequently, the nickel extract solutions from each container were poured into a new test tube (25 mL). To collect all of the extract solution the wipes were squeezed with the tip of the pipette before transferring the nickel extracts solution to the test tubes. The solution samples were stored in refrigerator (+8°C) until chemical analysis.

Contact tests

To assess deposited doses of nickel on skin after several short skin contacts with the selected nickel-containing materials, contact test was conducted according to two models. Model I was designed to investigate the contribution of nickel on the skin from short and frequent skin contact by touching a “fresh” surface each time versus touching the same surface of identical materials. Furthermore, model II was designed to identify the skin dose of nickel after different numbers of short contact events with each of the selected study materials.

Study participants. Healthy volunteers without any history of nickel allergy or on-going dermatitis participated in this study. Three subjects participated in model I, and five subjects in model II of contact tests. The study was approved by the ethical committee (KI Nord at Karolinska Hospital, Stockholm).

Materials of the study and preparation of the materials. The materials used for this study were selected based on the results from part I and included five different nickel-containing materials: 316L stainless steel (Fe/Cr18/Ni10/Mo3), pure nickel (90%), nickel-silver (Cu62/Ni18/Zn20), Monel alloy 400 (Ni65/Cu33/Fe2) and copper-nickel (Cu70/Ni30). Stainless steel was assigned as being a negative control in experiments. The materials were purchased from Goodfellow Cambridge Limited, see further table 2. Three samples of each material were available and both sides, denoted *a* and *b* respectively, were used in the contact tests, see table 1.

Prior to use in the different experiments (also in between each participant) all the material surfaces were grinded with wet emery paper P800 and P1200 (Mirka, Finland) on both sides. Each side was grinded by moving the sample over the emery papers back and forward 10

times in perpendicular directions. Furthermore, the materials were rinsed with distilled water, then with ethanol and again with distilled water. The material samples were gently dabbed with laboratory paper tissues and left to dry on paper tissues overnight to stabilize the surface oxide in a reproducible way. The preparation of material samples was performed using nitrile gloves.

Table 1. The material samples used in contact tests.

Material	Sample 1		Sample 2		Sample 3	
316L stainless steel	1a	1b	2a	2b	3a	3b
Nickel	1a	1b	2a	2b	3a	3b
Copper/Nickel	1a	1b	2a	2b	3a	3b
Monel	1a	1b	2a	2b	3a	3b
Nickel silver	1a	1b	2a	2b	3a	3b

Chemicals, equipment and preparation of the equipment. 1% HNO₃ was used for acid wipe sampling and for extraction of nickel from samples. The commercially available DMG test reagent solution was used to detect nickel on the skin. Acid washed material was used as previously described. A specially built sample holder, to control the force applied when touching the material surfaces, was used in the contact tests.

Performance. Prior to experiment, the hands of participants were washed as described previously and the participants were instructed how to carry out the contact tests. In model I, each person could perform the contact procedure on two different materials (each contact consisted of three strokes over the material surface) by volar fingertips of the index and middle fingers of both the right and the left hands in each participant. The same sample surface was contacted by the fingers of the right hand and different surfaces on the same material samples were contacted by the left hand, as in the example for stainless steel and nickel shown in figure 3. The contact events in model I were performed with a pace of 1 contact/minute.

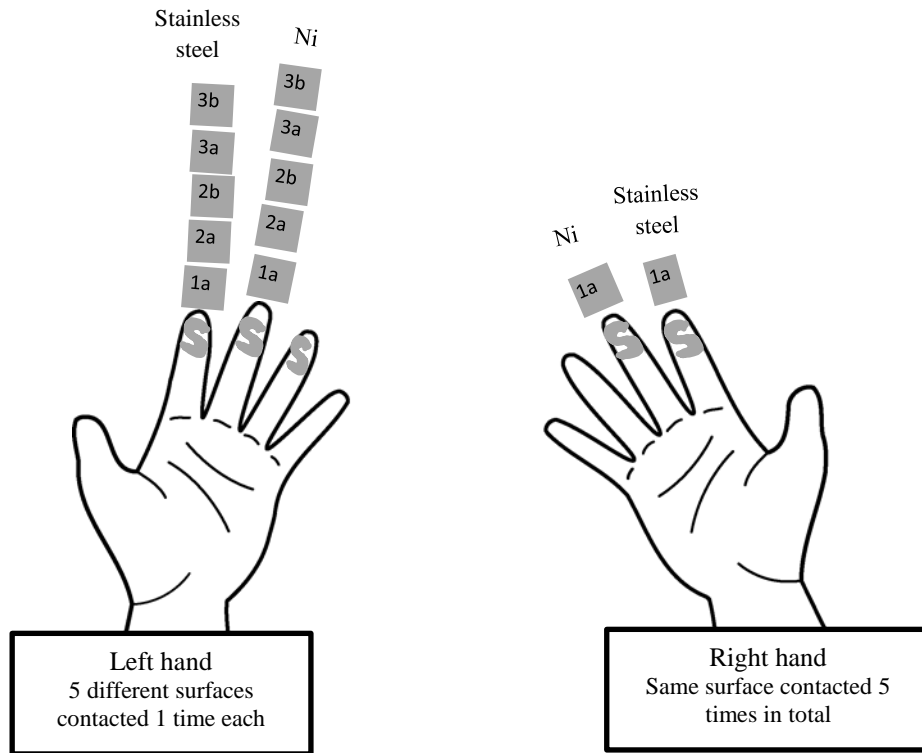


Fig. 3. Contact test model I. The same surface of the material sample was contacted 5 times in total by volar fingertips of either middle or index finger of each participant's right hand. Five different surfaces of the material samples were contacted once by the left hand in each participant (same fingers in each hand contacted the same material).

In model II, the same sample surface of the different materials was contacted 1, 5 or 10 times by volar fingertips of the ring, middle, or index fingers of both right and left hands in each participant as shown in figure 4. The contact events in model II were performed with a pace of 2 contacts/minute (one event every 30 second).

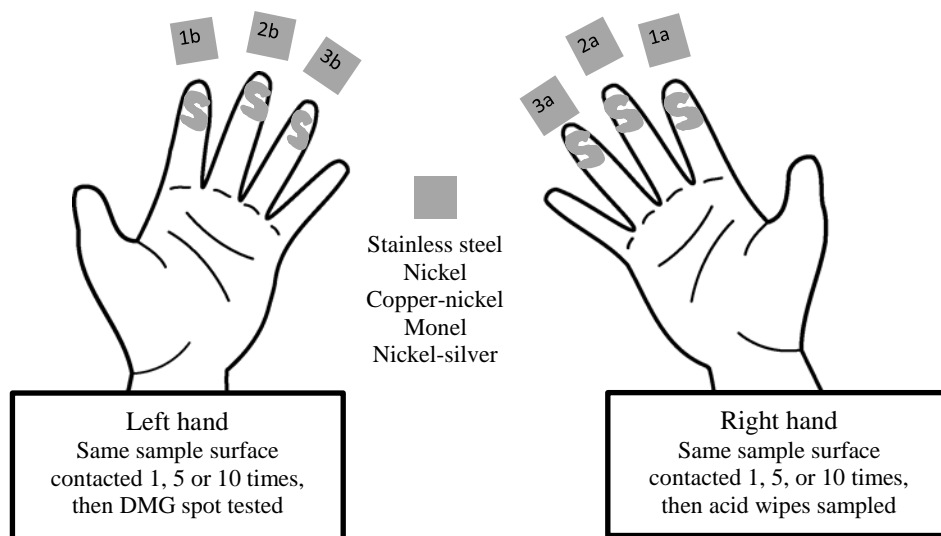


Fig. 4. Contact test model II. The same sample surfaces were contacted 1, 5 or 10 times by volar fingertips of the ring, middle, or index finger of both the right and the left hands in each participant. After contacting the materials, the acid wipe sampling and DMG spot tests were performed on the right and left hand respectively.

After contacting the study material, the operator marked the 2 cm² areas on the index and middle fingers of the right and left hands for model I and the index, middle and ring fingers of the right hand for model II, by using the template. Thereafter, acid wipe sampling was performed on the marked areas of the right and left hands. In model II, the DMG spot test was performed in parallel on the left hand in each participant.

Results and discussion

I. Survey of relevant materials for consumer exposure to nickel

Dimethylglyoxime (DMG) spot tests

Screening for nickel-releasing materials among items that may come in contact with skin for a short time period was performed by using the DMG spot test. Among 239 items that were spot tested, 68 (28%) items were positive and 175 items were negative or doubtful. The items tested were grouped into different product categories with varying numbers of DMG positive items. Relatively many DMG positive items were found among sewing materials, accessories, toys, keys and key chains, door handles and knobs (figure 5).

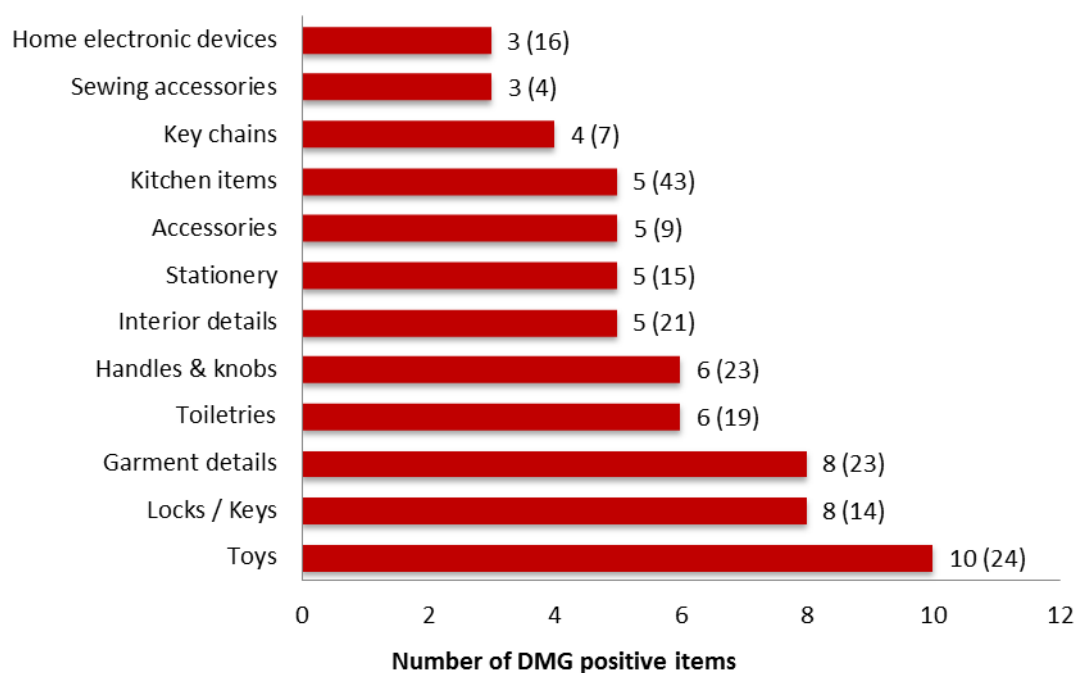


Fig.5. The overview of categories of nickel-positive items in 3 different home environments (total number of tested items in each category is represented in brackets)

The DMG test is often used by nickel allergic individuals to detect nickel-releasing items to avoid any harmful contact. It is easy to use, inexpensive, and gives a direct qualitative result.

A positive reaction in the DMG test is an indication of nickel release more than $0.5 \mu\text{g}/\text{cm}^2/\text{week}$ ¹⁸, which is the limit value in the EU nickel regulation for products intended for prolonged contact with the skin^{19 20}. The DMG spot test has previously been used for screening of nickel-releasing items on the market such as tools, consumer products and to study compliance with the EU nickel regulation^{14 15 21}. To the best of our knowledge, no such survey of nickel-releasing items among consumer products that come into short and frequent contact with skin exists in the literature. Our survey then provides new information on the daily exposure to nickel-releasing materials in products not covered by the limitation of nickel release in REACH.

Our results show that 28% of the metallic consumer products tested in three home environments released nickel more than the limit value. It was previously demonstrated that also products that may release more nickel than the limit value ($0.5 \mu\text{g}/\text{cm}^2/\text{week}$) but only come into short contact with skin, can deposit nickel skin doses in amounts that has a potential to cause sensitization and contact dermatitis²²⁻²⁵. It is obvious that the potential allergy risk of skin exposure through short and frequent contacts with nickel-releasing items needs to be better understood when such a large proportion of the general population still becomes sensitized. The aim of our survey was primarily to identify representative materials to further assess the deposited skin doses of nickel after several short contacts with nickel-releasing consumer products as well as measuring the amount of nickel release from these products. Even though the study was limited to only three home environments and the DMG test may not be sensitive enough to detect all nickel-releasing materials and also has the limitation of developing false positive or negative result, we now have a better understanding regarding daily exposure to different kinds of consumer products that release nickel at short contact with the skin. Based on this new knowledge, a more structured and comprehensive survey can be performed in the future.

X-ray fluorescence spectrometry (XRF)

Selected DMG positive items were analysed using XRF to identify the chemical composition of the tested materials. In total 41 items that were practically feasible to transfer from the home environments to the laboratory were analysed. Data on 5 of the DMG positive items is not presented, since no nickel was detected hence it is suspected that the DMG test results in these cases were erroneously scored positive but might have been doubtful or negative reactions. The results from XRF analysis of the remaining 36 DMG test positive items show

compositions mostly detected as combinations of Ni/Cu/Zn, Ni/Fe/Cu or Ni/Fe (table 2) with different proportion of nickel (figure 6).

Table 2. The results from analysis of chemical composition in 36 DMG positive items and number of items in each combination alloys.

Alloys	Ni/Cu/Zn	Ni/Fe/Cu	Ni/Fe	Ni/Fe/Cu/Cr	Ni/Fe/Cu/Zn	Ni/Fe/Zn	Ni/Cu
Number of items	12	11	9	1	1	1	1

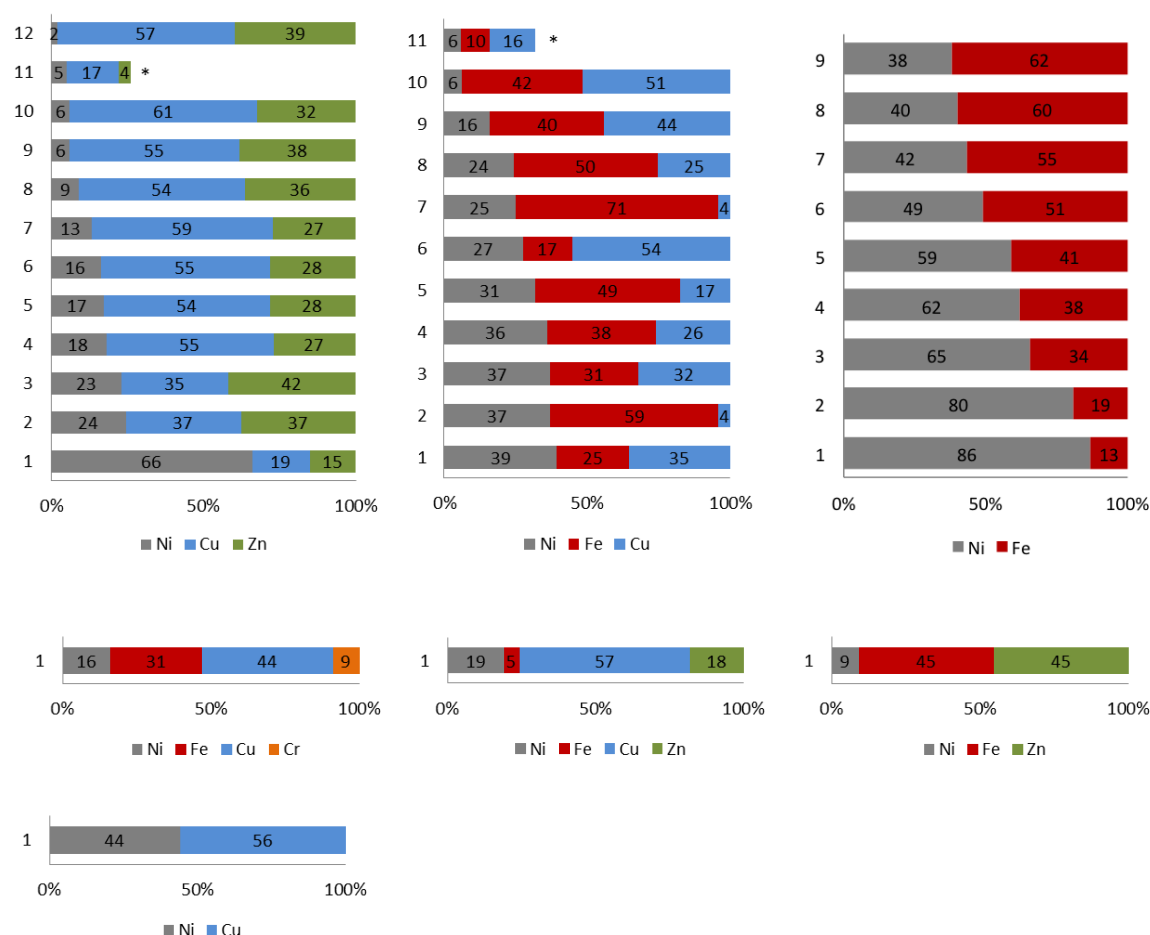


Fig. 6. The results from XRF analysis of nickel content and the main composition of alloys (%) in 36 DMG positive items. Number on the y-axis corresponds to the number of items in each group of chemical composition. The asterisk (*) indicate analysis of items with a leather background which affects the result.

The chemical compositions of the DMG positive items obtained from the XRF analysis provided important information for selection of relevant nickel-containing materials for further study despite the limitation of not including all DMG positive items from the preceding survey. Moreover, no coins (in Sweden either copper-nickel, or a nickel-free alloy) were included in the survey or XRF analysis and no stainless steels were represented however

skin contact with these types of nickel-containing materials is also very relevant. Taking this into account and also considering what materials were available for purchase, we selected 5 different nickel-containing materials for further study: 316L stainless steel, pure nickel, copper-nickel (Cu70/Ni30), Monel alloy 400 (Ni65/Cu33/Fe2), and nickel silver (Cu62/Ni18/Zn20). The stainless steel, grade 316L, is a commonly used material for various applications in consumer products and included as a negative control material. Pure nickel is included as a positive control. Copper-nickel is representative for coin materials, which also come into short and frequent contact with skin. Monel alloy 400 and nickel silver are common nickel-containing alloys that are much used in different components and details in consumer products. A detailed description of the selected study materials, purchased from Goodfellow Cambridge Ltd, is shown in table 3.

Table 3. Detailed description of the selected study materials. The materials were purchased from Goodfellow Cambridge Ltd and for further studies of the nickel skin dose and nickel release.

Material	Stainless steel ^b	Nickel	Copper/Nickel	Monel alloy 400	Nickel silver
Composition	Fe/Cr18/Ni10/Mo3	99% Ni	Cu70/Ni30	Ni65/Cu33/Fe2	Cu62/Ni18/Zn20
Thickness	2 mm	2 mm	1.6 mm	2 mm	0.6 mm
Size ^a	25*25 mm	25*25 mm	25*25 mm	25*25 mm	25*25 mm
Quantity	3	3	3	3	3
Net weight	29.8 g	34.6 g	25.6 g	33.1 g	9.81 g

^a Material samples were in form of squares.

^b Stainless steel were used as a negative control.

II. Skin dose assessment experiments

Recovery test of the acid wipe sampling method for measurements of low nickel skin doses

The result of the DMG spot test on the skin after application of defined nickel skin doses in 5 participants are shown in table 4. The largest dose of nickel ($0.5 \mu\text{g}/\text{cm}^2$) resulted in a clearly visible pink colour in all participants. Also a pink colouration of the skin was seen on the thumb in one participant as shown in figure 6. The second dose of nickel ($0.05 \mu\text{g}/\text{cm}^2$) gave rise to a weak pink colour in 4 participants and the DMG test result was negative for the smallest dose of nickel ($0.005 \mu\text{g}/\text{cm}^2$) and vehicle for all participants, see figure 6. The staining the skin with DMG test reagent in one participant and the second nickel dose of $0.05 \mu\text{g}/\text{cm}^2$ not detected in another, reflects the normal variations in skin status between individuals.

Our results show that the DMG spot test can detect nickel doses on the skin at a level of $0.05 \mu\text{g}/\text{cm}^2$. This is consistent with the findings in a previous study by Julander et al. where they showed that the lowest nickel skin dose in their study ($0.063 \mu\text{g}/\text{cm}^2$) was detectable in 3 of 5 participants and the second lowest dose of nickel ($0.13 \mu\text{g}/\text{cm}^2$) was detectable in all the participants by DMG spot test ²⁰.

Table 4. Results from the DMG spot test for investigating the identified low doses of nickel on the skin; four doses of nickel and vehicle (control) ^a were applied on left hand ^b in 5 participants.

Participants	Position of the Ni dose (Left hand)	Ni total dose (μg) ^c	Ni dose ($\mu\text{g}/\text{cm}^2$)	DMG spot test result
A	Ring finger	0	0	Negative
A	Middle finger	0.001	0.005	Negative
A	Index finger	0.01	0.05	Weak positive
A	Thumb	0.1	0.5	Positive
B	Ring finger	0	0.00	Negative
B	Middle finger	0.001	0.005	Negative
B	Index finger	0.01	0.05	Weak positive
B	Thumb	0.1	0.5	Positive
C	Ring finger	0	0	Negative
C	Middle finger	0.001	0.005	Negative
C	Index finger	0.01	0.05	Negative
C	Thumb	0.1	0.5	Positive
D	Ring finger	0	0	Negative
D	Middle finger	0.001	0.005	Negative
D	Index finger	0.01	0.05	Weak positive
D	Thumb	0.1	0.5	Positive
E	Ring finger	0	0	Negative
E	Middle finger	0.001	0.005	Negative
E	Index finger	0.01	0.05	Weak positive
E	Thumb	0.1	0.5	Positive

^a Vehicle, containing 1% HNO_3 and 99.5% ethanol 1:1 was used as a control.

^b Nickel doses and vehicle were applied on volar fingertips of 4 fingers (thumb, index, middle, and ring fingers) of the left hand.

^c the total dose of Ni was applied over 2cm^2



Fig. 6. DMG spot test for investigating the identified low doses of nickel on the skin of the volar fingertips (thumb, index, middle, and ring finger) on the right hand in participant B. The two highest nickel doses (0.5 and 0.05 $\mu\text{g}/\text{cm}^2$) developed a pink colour on the cotton wool swabs and the highest nickel dose (0.5 $\mu\text{g}/\text{cm}^2$) even caused pink colouration on the thumb in one participant.

The aim of the recovery test was to measure the efficiency of the acid wipe sampling method for quantification of low doses of nickel on the skin. The largest dose in our study (0.5 $\mu\text{g}/\text{cm}^2$) is similar to the lowest dose in the first description of the acid wipe sampling technique by Lidén et al., and the second dose in our study (0.05 $\mu\text{g}/\text{cm}^2$) corresponds to a lowest dose used in a similar recovery test for cobalt^{17 26}. The recovery rate was then 93% from a nickel skin dose of 0.5 $\mu\text{g}/\text{cm}^2$ and the recovery rate for a cobalt skin dose of 0.6 $\mu\text{g}/\text{cm}^2$ was $50\% \pm 20$, using the original acid wipe sampling method^{17 26}.

Skin exposure to such low doses of nickel will not cause larger risk than our daily skin exposure to nickel-releasing consumer products. Regarding possible side effects from using the 1% HNO_3 (pH1.5) for sampling, it has been shown not to be harmful to the skin²⁷. The method of using this acid was first developed by Berglund et al. (1994) here at IMM with the purpose of cleaning the skin of small children before blood sampling of lead and cadmium²⁷. Lidén et al. (2006) expanded the concept of cleaning the skin into a sampling method for metals on skin, since 1% HNO_3 had been proven to be both efficient regarding removal of metals from skin and also not harmful for the skin¹⁷. After the development the researchers have used the method for several studies of metals on skin^{20 22 26 28}.

In order to increase the method recovery for sampling of low nickel skin doses, the previously used procedure was modified: instead of 25 mL of 1% HNO_3 for extraction of nickel from wipes, only 10 mL was used. This will result in a more concentrated solution sample for metal analysis which likely will improve the recovery rate. However, the 10 mL of HNO_3 was

found to be no more than just enough to cover the wipes during the extraction step. To ensure as good extraction as possible, the time for shaking the samples was slightly prolonged in our case (45 minutes instead of 30 minutes). Because the three wipes absorbed significant amounts of the 10 mL volume for extraction, they were squeezed to draw out as much solution as possible. To control for the extraction step separately and to compare how much nickel is lost in the skin sampling procedure; samples with dosed wipes were added. Blank samples containing three wipes + 1% HNO₃ and HNO₃ only, were also included to check for eventual background contribution.

During the performance of acid wipe sampling for the recovery test, we realized that the skin sampling perhaps could be performed with improved precision using cotton wool swabs rather than wipes. Maybe some of the wiped nickel from skin could end up on the operator's glove as a result of contact with the moistened wipe containing nickel or unintended contact with the skin surface where the nickel skin dose was applied. If such “contamination” occurs, it will affect the recovery of the acid wipe sampling. Therefore, we performed an additional skin sampling in one participant using cotton swabs instead of wipes. The recovery of this sampling technique is not yet evaluated but we experienced that the procedure using three consecutive swabs moistened with 1% HNO₃ was rather convenient. Also the swabs were practical to handle in the extraction step; it was easier to remove the cotton swabs from the extraction solution without having significant amounts of acid absorbed in the swab.

The acid wipe and swab samples were performed in order to quantitatively measure the efficiency of our modified skin sampling procedure. The metal analysis has not been performed within the frame of this master thesis project, but will be made with a new ICP-MS (inductively coupled plasma mass spectrometry) instrument to be installed at IMM in late May/beginning of June. The different nickel skin doses (0.5, 0.05, 0.005 µg/cm²) in this test of sampling recovery, were chosen based on results from previous measurements of nickel skin doses in volunteers handling coins for one hour^{22 28}. The lowest dose however, was based on what is possible to detect with this new instrument. In theory we will be able to detect and analyse all the three applied doses of nickel, because the typical limit of detection for ICP-MS is (less than) 0.1 µg Ni/L, whereas the smallest dose in this study corresponds to a concentration of 1 µg Ni/L. This means that we will be able to measure not only the two larger Ni skin doses as was detected on skin by the DMG test (which is not a very sensitive method for detection of nickel), but also the low dose will be quantified in this analysis.

Contact tests

The contact tests included in the research strategy, describes two models for assessment of short and frequent skin contact with nickel-containing materials. The contact tests allow the quantification of the amounts of nickel that might transfer to the hands after having contact with the selected study materials by using the acid wipe sampling method, which was modified for detection of low nickel doses. The DMG spot test was used on the skin for direct qualitative detection of nickel in one model.

Model I. This contact model was designed to investigate the difference in deposited nickel skin doses from repeated contacts with the same sample surface versus repeated contacts with different sample surfaces for each contact event. The metal analysis of acid wipe samples was not performed within the frame of this master thesis project. No DMG spot test was used for detection of nickel in this model, since it is not a sensitive method for identifying the differences between the two different contact patterns. However our hypothesis is that we will measure higher doses of nickel deposited on skin from contacts with different (fresh) surfaces. Several studies have measured relatively high initial release rates of nickel from materials in contact with artificial sweat^{22 28 29}. Metal release occurs via different processes depending on contact duration, friction and wear and other surrounding conditions such as pH, presence of salts, proteins etc.¹. All metallic surfaces are naturally oxidized and this surface oxide is present also on the metallic consumer products that we come in contact with on a daily basis. The state of the surface oxide is dynamic and it can be changed over time and use as well as with skin contact²⁸. A newly formed surface oxide can be more reactive than an aged oxide that has been allowed to grow and stabilize, as was shown in relation to skin contact in a study of newly introduced UK coins by Julander et al.²⁸. Also the initial nickel release rates from new coins (directly from production) of copper nickel alloy were higher than from used (old) coins²⁸. The initial release process is assigned to dissolution of the surface oxide whereas corrosion will occur in prolonged contact with artificial sweat^{1 28}. We expect to measure slightly higher nickel skin doses from the contacts with different, “fresh” sample surfaces compared to repeated contacts with the same sample surface for identical materials, hence the dose variation will not be in the same range among different nickel-containing materials.

The results that will be obtained from this contact test will enable us to understand more about the variation between different nickel-containing materials as well as the importance of the surface condition of the material regarding nickel release and skin deposition. A limitation in

the performance of this pilot experiment so far is that few participants were in contact with each kind of materials due to limited time.

Model II. This contact model was designed to study the deposited nickel doses from one and several (5 and 10) contacts with the same sample surface and evaluate after how many contacts we were able to detect a nickel dose on the skin. The ultimate aim was to quantify the nickel skin dose from “one touch”. Metal analysis of the acid wipe samples was not performed but results from DMG spot tests on the skin of the left hand in five participants are shown in table 5.

We expect to be able to measure the nickel dose from single contact with the nickel-containing materials by the modified acid wipe sampling method. However, it was somehow surprising to detect nickel on the skin with the DMG test for a single contact for all study materials except the stainless steel, see figure 7. The reason for having several contacts was in case of not being able to detect the nickel dose from a single contact; it might be possible to extrapolate the dose from five or ten contacts. This is still a relevant question for further study of materials that deposit very little nickel on the skin upon contact. It is important for making a correct extrapolation to know the results from model I.

Table 5. Results from the nickel spot test on the skin for assessing the accumulated doses of nickel after contacting the materials; each participant^a contacted the same surface^b of the same kind of materials.

Participants	Materials	Position of touch	Number of touches	DMG spot test result
A	Stainless steel ^c	Ring finger	1 time	Doubtful (black)
A	Stainless steel	Middle finger	5 times	Doubtful (black)
A	Stainless steel	Index finger	10 times	Doubtful(black)
B	Nickel	Ring finger	1 time	Positive
B	Nickel	Middle finger	5 times	Positive
B	Nickel	Index finger	10 times	Positive
C	Copper nickel	Ring finger	1 time	Positive
C	Copper nickel	Middle finger	5 times	Positive
C	Copper nickel	Index finger	10 times	Positive
D	Monel	Ring finger	1 time	Positive
D	Monel	Middle finger	5 times	Positive
D	Monel	Index finger	10 times	Positive
E	Nickel silver	Ring finger	1 time	Positive
E	Nickel silver	Middle finger	5 times	Positive
E	Nickel silver	Index finger	10 times	Positive

^a Five participants were participated.

^b The same surface of the same material was touched 1, 5 or 10 times by the volar fingertips of the ring, middle or index finger of the left hand.

^c Stainless steel were used as a negative control.

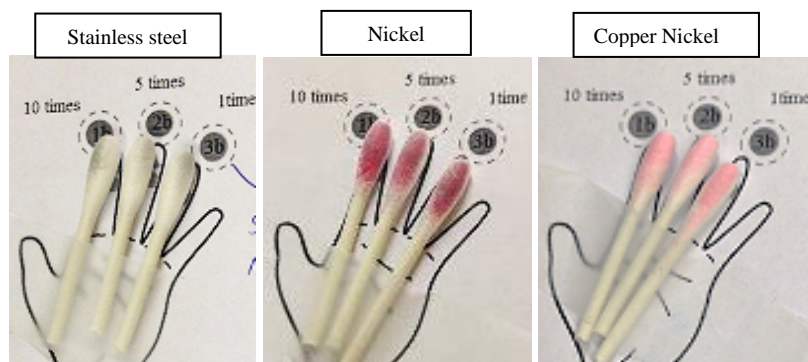


Fig. 7. DMG spot test was performed on the skin to investigate the accumulated doses of nickel after contacting nickel-containing study materials. The volar fingertips (index, middle, and ring finger) on the left hand in participants A, B and C were spot tested and doubtful reactions (with a discolouration) were observed for skin dose from stainless steel, while DMG positive results were seen for spot tests on skin that contacted pure nickel and copper nickel. The photographs of participant D and E are not shown, furthermore in the laboratory protocols circles were used to indicate the material samples, even though the tested materials were in the shape of squares.

Nickel was identified qualitatively after one, five, ten contacts with DMG spot test with the exception for stainless steel (our negative control), which developed doubtful results by appearance of black discolouration on the cotton swab. DMG spot test reagent is known to react also with other metals such as copper (brown colour) and iron (red); hence interference with other metal might be a reason for hiding the pink colour from reaction with nickel or reading a false positive reaction³⁰.

Another limitation of this contact test is that so far only five individuals participated and we were not able to identify any individual variations affecting the results. Only one person contacted one of the nickel-containing materials. To complete with five participants for all study materials would improve the results.

The pace in which the five repeated contact events were performed was different in model I and model II, 5 contacts/5 min vs. 5 contacts/2.5 min respectively). This will allow us to evaluate if there is any time-dependent difference in the deposited skin doses for the nickel-containing study materials.

Conclusions

Within this master project, we have developed and introduced a research strategy for assessing short and frequent skin contacts with nickel-containing materials both qualitatively and quantitatively. The strategy consists of three parts including nickel screening, nickel skin dose assessment and nickel release assessment.

The nickel screening provided us with useful information for the selection of study materials, representative of consumer exposure to nickel. We showed that a considerable proportion (28%) of different kinds of metallic consumer products that are in short contact with the skin repeatedly, such as toys, door handles, locks, keys, various garment and interior details, were positive with the DMG spot test. This is indicative for a release of nickel more than the restricted value for items intended for direct and prolonged skin contact within the EU nickel regulation.

Skin dose assessment helped us to better understand the total load of nickel on skin as a result of short and frequent exposure to selected nickel-containing materials. We were able to qualitatively detect nickel on the skin after only one contact with the selected material samples with the exception for the 316L stainless steel. Our ultimate aim, to quantify the nickel skin dose from “one contact”, will be achieved when the collected samples from skin dose assessment experiments are analysed in near future.

For assessment of nickel release, we have included a new type of experiment; wiping the nickel-containing material surfaces with artificial sweat corresponding similar to the contacts in assessment of skin dose. This method has the potential to allow quantification of the nickel release from material samples, in a way that simulate realistic skin contact. Future experiments will verify this.

Nickel allergy is still very frequent and better protection is needed both for consumers and workers. The research strategy that we have introduced here will provide useful knowledge regarding short and repeated skin contacts with nickel-containing consumer products that is necessary in assessment of the allergy risk from such short exposure to nickel. Moreover, this new information is needed for authorities to be able to extend the EU nickel regulation to also

cover products that may deposit considerable amounts of nickel on the skin when used only for short periods of time but repeatedly.

Future work

The research strategy for assessment of short and frequent skin contacts with nickel consists of three parts combining different methods to screen for nickel exposure in daily life, measure nickel skin doses and nickel release from representative materials. The main focus of this master thesis project has been to develop the strategy and suggest modifications of existing experimental methods and sampling techniques. Moreover, pilot experiments have been performed within the first two parts of the project. The DMG test has been used as a tool for direct qualitative detection of nickel in these experiments but quantitative results will be generated when IMM's new instrument for metal analysis is installed and running. The analysis of the acid wipe samples from recovery and contact test is future work. Future work will also be to perform pilot experiments for assessment of nickel release, planned in part III of the research strategy.

III. Nickel release assessment experiments

The aim of this part of the study will be to assess nickel release quantitatively, identifying the amounts of nickel release from selected nickel-containing materials by using a new experiment based on wiping with artificial sweat and immersion tests.

Artificial sweat wiping

In order to identify the level of nickel release from nickel-containing materials at short and frequent skin contacts, material samples will be wiped with the same procedure of acid wipe sampling on the skin. By wiping the material surfaces directly with wipes moistened with artificial sweat (similar to the way they were contacted by the finger in contact tests) a measure of nickel release at skin contact can be obtained.

Artificial sweat wiping will be performed in experiments using the same approach as in contact tests of two different models. Hence, it will be possible to investigate how much nickel will be released at contacts with the same surface versus contact with fresh surfaces as well as quantifying the amount of nickel released after one, five and ten wipes (each wipe consists of three strokes).

Wiping the material samples, stimulate the friction inherent of skin contacts, which is perhaps the most most predominant key factor regarding transferring the metal to the skin ³¹. Hence, this experimental technique has a potential to assess the correlation between nickel release from wiping and nickel skin doses deposited from skin contact with the study materials. Furthermore it may be applied in skin exposure assessments of various materials without recruiting any research subjects, which would be ethically as well as more time efficient.

Immersion tests

In parallel with artificial sweat wiping experiments, immersion tests for short time durations will be performed. These experiments are based on EN1811:2011, which is the reference method for investigating compliance with the EU nickel regulation ¹⁹. In this reference method, items/materials shall be immersed in artificial sweat solution for one week and then the amount of nickel released into the sweat will be analysed. Immersion of material for one week in artificial sweat is neither representative of human exposure nor suitable for mimicking short and frequent skin exposure. It has been shown by Julander et al. that the initial nickel release rate from the coins was highest within the first two minutes of an immersion period (seven days) in artificial sweat, which was a time dependent process. It was also concluded that immersing the material in artificial sweat for one week is not an appropriate end point for items such as coins, which are in contact with skin frequently but for a short period of time ²⁸. Therefore the nickel-containing material samples in this study will be immersed in artificial sweat for very short periods of time similar to contact tests and artificial sweat wiping, hence the immersion experiments planned in this part of the study will be more a dipping in artificial sweat procedure.

Sustainable development

Nickel is a part of the modern society through its many uses as an alloying element in a broad variety of materials. Due to its sensitizing properties, frequently causing nickel allergy in the general population, an increased awareness of the allergy risk from unnecessary exposure would lead to sustainable development with reduced costs for the society if more people were protected. At present, the European Chemicals Agency has to define the concept of “prolonged contact with skin” in relation to the existing nickel restriction³². In this context, the importance of short and frequent skin exposure to nickel-releasing items and the contribution to the total load of nickel on the skin needs to be better understood. The research

strategy presented within this master thesis project could provide new and important information on the amounts of nickel that can deposit on the skin from short and frequent contacts with different nickel-containing materials. Quantified nickel skin doses from “one touch” would be useful for risk assessment and exposure could be modelled based on such data. This research strategy presents a new type of experiment, artificial sweat wiping that could be used to assess nickel release at skin contact and deposited nickel skin dose without using research subjects. This could also be seen as a contribution to a more sustainable development on an experimental level.

Contribution

Since the topic of this master thesis project is a part of Klara Midander’s research plan, the work has partly been performed in close collaboration. Hereby my contribution to the project is specified:

- I have carefully read suggested articles and searched related scientific literature independently. I worked with the literature in a structured way and documented my thoughts and conclusions before drafting the introduction part in this report. I have discussed the literature findings with my supervisor and the colleagues at the Unit of Occupational and Environmental dermatology.
- The ideas for building this research strategy was based on previous work within the Unit of Environmental and Occupational Dermatology however, the details, not least the practical ones, are a product from many discussions in which I have made an acknowledged contribution.
- I have performed all different parts of the pilot tests within this master thesis project together with my supervisor and independently. After supervision I have prepared most of the material for the experiments, made the protocols and I have managed the documentation in the electronic laboratory notebook (ELN).
- I have drafted this report and together with my supervisor and other colleagues at the Unit of Occupational and Environmental Dermatology improved the presentation of its content and the text in several stages.

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