

Nickel deposited on the skin – visualization by DMG test

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Summary

Background. Nickel is the most common cause of contact allergy and an important risk factor for hand eczema. Visualization techniques may be powerful in showing exposures. The dimethylglyoxime (DMG) test might be used to establish skin exposure to nickel.

Objectives. To develop and evaluate methods for visualization of nickel on the skin by the DMG test and hand imprints.

Methods. Nickel solutions at different concentrations were applied in duplicate on the hands in healthy subjects ($n = 5$). The DMG test and acid wipe sampling for quantification were then performed. Hand imprints were taken after manipulation of nickel-releasing tools ($n = 1$), and in workers performing their normal tasks ($n = 7$). The imprints were developed by the DMG test.

Results. The DMG test on hands gave positive results in all subjects. The lowest concentration giving rise to a colour change was set to $0.13 \mu\text{g}/\text{cm}^2$ for DMG testing on skin. DMG test-developed imprints worked well except when hands were heavily contaminated by other particles/dust.

Conclusions. The DMG test may be used as a simple and powerful tool for visualization of nickel on skin. DMG test-developed hand imprints may, in the future, be used for semi-quantitative or quantitative exposure assessment.

Key words: acid wipe sampling; DMG test; imprint; nickel; risk assessment; skin exposure; visualization.

Nickel is the most common cause of contact allergy, and nickel is an important risk factor for hand eczema, although this association weakened after the early nickel regulation limiting nickel exposure in Denmark (1, 2). Nearly all epidemiological studies on nickel allergy lack data on how much nickel the skin has been exposed to. Only a few studies have assessed the amounts of nickel on the skin (3–10).

Interest in the assessment of skin exposure to hazardous chemicals, skin sensitizers and irritants has increased during the last decade. Most attention has been given to

pesticides and to some other substances with systemic toxicity. Methods for assessment of skin exposure to a few skin sensitizers, such as metals, hair dyes, and acrylates, have been developed and applied in workplace studies (4–7, 9, 11, 12). The methods include skin wiping, rinsing, and tape stripping, and all of these methods require advanced laboratory equipment.

Imaging techniques are widely used in radiology, thermography, and chemical imaging. The fluorescent tracer technique, by which invisible contaminants on the skin are made visible by a fluorescent tracer and ultraviolet light, has been used in education, prevention and exposure assessment concerning pesticide contamination (13, 14). Dimethylglyoxime (DMG; CAS 95-45-4) is used as a simple spot test for nickel release from surfaces, and it is widely used in chemistry for gravimetric analysis of nickel. Applications in clinical dermatology and research have focused on metal items, particularly items covered

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by the EU Nickel Directive, but also on handheld tools (1, 15–19). Dithio-oxamide, producing a blue complex with nickel, has been used to stain nickel in skin biopsies from nickel patch test reactions (20).

On the basis of our experience with the DMG test for nickel release, the fluorescent tracer technique for visualization of skin exposure to pesticides (13), and knowledge about the quantification of sweating by imprints (21, 22), it is hypothesized that visualization of skin exposure to nickel, based on the DMG test, would be useful in skin exposure assessment and prevention of nickel dermatitis. The aims of the present study were to investigate the usefulness of the DMG test for detection of nickel deposited on the skin, by establishing at which concentration level a colour change would be visible, and by testing a novel technique for visualization of nickel exposure by hand imprints.

Materials and Methods

The present study consisted of two different approaches, the first to establish the lowest concentration giving rise to a colour change with the DMG test applied on the skin; and the second to visualize the distribution of nickel deposited on the hands by DMG test-developed hand imprints.

Subjects

Five healthy subjects with no history indicating nickel allergy, and no ongoing dermatitis or skin lesions on their hands, participated in DMG testing on the skin. For the hand imprint study, 1 healthy subject participated in the taking of hand imprints in the laboratory, and 7 workers performing their normal tasks (1 cashier, 1 locksmith, 1 secretary, and 4 metal workers) participated in the taking of hand imprints in their workplace. The study was approved by the Regional Ethical Research Board in Stockholm. All participants gave their informed consent.

Chemicals

A standard nickel stock solution of 1000 ± 3 mg/ml in 2% nitric acid (High Purity Standards, Charleston, SC, USA) was used to prepare nickel solutions for the two approaches. For DMG testing on the skin, concentrations of 100, 50, 25, 12.5 and 0 µg/ml, respectively, were prepared in a vehicle containing 1:1 nitric acid (1%) and ethanol (99.5%). The concentrations were chosen after pretests to cover strong positives to negatives, and considering exposure levels detected in our previous studies (6, 7). Solutions with 100, 50, 25, 10, 5 and 0 µg/ml nickel in 1% nitric acid were prepared for a calibration curve on blotting paper.

Nitric acid 0.5% and 1% were prepared from nitric acid 65% (pro analysis, Merck KGaA, Darmstadt, Germany) diluted with deionized water. The 0.5% solution was used for taking hand imprints, and the 1% solution was used for cleaning skin and surfaces before dosing nickel onto the skin for acid wipe sampling (5) from the skin surface, with subsequent analysis of nickel by inductively coupled plasma mass spectrometry (ICP-MS). Wiping of the skin with nitric acid 1% is well tolerated, and is used for cleaning skin before taking blood samples for metal analysis and for sampling from the skin surface (4–7).

The DMG test (Chemo Nickel Test™; Chemotechnique Diagnostics, Vellinge, Sweden) containing DMG (1%), ethanol, and ammonia (9.9%), mixed in one bottle, was used for DMG testing on skin. For development of hand imprints, we prepared two solutions: one containing DMG (1%; Merck) in ethanol, and the other ammonium solution (10%).

Equipment

All glassware and plastic (polypropylene) test tubes and containers (Sarstedts, Landskrona, Sweden) were acid-washed before use. For the acid wipe sampling, cellulose wipes (injection wipes; Paper-Pak Sweden AB, Sundbyberg, Sweden) were used. An acid-washed and metal-free flexible plastic template with an aperture of 2 cm² and a marking pen with red ink (Lumocolor® permanent universal pen F 318-2; Mars GmbH & Co. KG, Nürnberg, Germany) were used for marking test areas. For taking hand imprints, a transit Lumbar support mattress (30 × 26 × 6 cm; Tempur Sverige AB, Stockholm, Sweden) was used as support for a white sheet of blotting paper, laminated with an impermeable layer of polyethylene (Benchkote™ 46 × 57 mm, Whatman™; Schleicher & Schnell, London, UK).

An ICP-MS instrument (Agilent 7500CE; Agilent Technologies, Waldbronn, Germany) with a collision/reaction cell system operating in helium mode (5.0 ml/min) was used to determine the levels of nickel in the acid wipe samples, as previously described (4). Nickel was monitored at m/z 60. The limit of detection (LOD, calculated as three standard deviations in blank samples) for nickel was 0.015 µg/l, corresponding to 0.00018 µg/cm². The analytical work was performed in clean room facilities at the Institute of Environmental Medicine, Karolinska Institutet.

Experimental Design

DMG testing on skin and acid wipe sampling

Prior to dosing of the nickel solutions on skin, the subjects washed their hands with soap and water. The operator

then carefully cleaned their hands with wipes soaked with nitric acid (1%), rinsed them with deionized water and dried them with paper towels to remove nickel deposited on the skin surface prior to the experiment. Areas on the volar fingertips (except for the thumbs) and the centre of the palm in each hand were then marked with the marking pen, indicating the corners of the template.

The four solutions with different concentrations of nickel and a nickel-free vehicle control were used. Ten millilitres of each solution was applied by pipette and spread over the 2-cm² demarcated areas on the fingers and palm of each hand, respectively. Thus, 1.0, 0.50, 0.26, 0.13 or 0.00 µg of nickel was dosed onto each area, corresponding to 0.50, 0.25, 0.13, 0.063 and 0.00 µg/cm². The solutions were left to dry. The drying time was shortened by exposing the areas to heat from a spotlight for 3–4 min the temperature close to the skin was not above 30°C. After drying, and within 5 min after application of the solutions, the areas were subjected to either DMG testing or acid wipe sampling.

The DMG test was performed on the skin of the left hand in each subject, in the same order in which the nickel solutions had been applied. Two to three drops of the DMG test solution were applied on a cotton-wool-tipped stick, which was then rubbed gently for 30 seconds over the entire surface of the demarked area. The test was performed with a fresh stick for each area. For identification, the sticks had been marked by numbers 1–25 in advance. The numbers were concealed before reading of the result; that is, the reading was blind. When all areas in all subjects had been tested, and the acid wipe sampling had been completed, the sticks were mixed, and the results were assessed in random order, against a white background, independently by the operator and by a second person. A pink colour on the cotton indicated that nickel ions were present. The DMG test results were categorized as pink, weak pink (so faint that it might easily be missed), or negative.

Recovery by acid wipe sampling (5) was assessed in parallel with the DMG test on the skin. The five areas (2 cm² each) on the right hand in each subject were wiped for assessment of nickel, and for calculation of recovery. Sampling was performed with wipes, moistened with 0.5 ml of nitric acid (1%). Each demarcated area was wiped, with application of gentle pressure, using three consecutive wipes per area. The wipes from each area were pooled in the same container, and 23.5 ml of nitric acid (1%) was added for extraction of nickel. The containers were shaken for 30 min to extract nickel from the wipes, and the extracts were then poured into test tubes for ICP-MS analysis (4) to determine the nickel concentration. Immediately after all samples had been taken, the subjects washed their hands with soap and water.

Pilot study—DMG test-developed hand imprints

A calibration curve was made in duplicate by applying 30 µl of six concentrations of nickel (100, 50, 25, 10, 5 and, 0 µg/ml, corresponding to 3, 1.5, 0.75, 0.3, 0.15 and 0 µg of nickel) on a blotting paper, and leaving until dry. The paper was then sprayed in a ventilated hood, first with ammonia (10%) until moist, and immediately after this with DMG (1%) in ethanol.

Different approaches had been tested in the development of the technique for hand imprints applied in the present pilot study, first in the laboratory and then in workplaces. The hands were cleaned with soap, water, and nitric acid (1%), as above. Then, the subject handled a set of tools for 30 min in the laboratory. The tools had previously been shown, with the DMG test, to release nickel. A blotting paper was soaked with nitric acid (0.5%) with a Pasteur pipette. Then, the paper was placed on top of a transit Lumbar support mattress on a table. The subject pressed the hands against the blotting paper, while the operator added some additional pressure to the hands and fingers to increase contact with the blotting paper. The pressure was applied for approximately 20 seconds. Before the subject removed the hands from the paper, the operator indicated the borders of the imprints by drawing the contour of each hand. Hands were washed with soap and water immediately after the imprints were taken. The moist sheet of blotting paper was placed with the polyethylene side down on a tray in a rack, and dried at room temperature.

To develop the hand imprints, the blotting paper was suspended vertically by plastic holders in a tray. The paper was then first sprayed with ammonia (10%) until moist, and immediately after this with DMG (1%) in ethanol, and left to dry; all of these procedures were performed in a ventilated hood. The resulting image was then photographed, and the original imprint was stored in darkness at room temperature.

The technique was also tested in 7 workers at their workplaces. Their hands were cleaned with soap and water, followed by nitric acid (1%), and deionized water, as above. Then, they performed their normal work tasks for approximately 2 hr. After this, hand imprints were made. The same procedure as described above was used, except that the imprints were developed a few days later.

Results

DMG testing on skin

The outcomes of the DMG test on the skin in 5 subjects, where known amounts of nickel solution had been applied, are shown in detail in Table 1. A photograph

Table 1. Results from assessment of nickel on the skin by acid wipe sampling and analysis by inductively coupled plasma mass spectrometry^a, and by the dimethylglyoxime (DMG) test; four concentrations of nickel and a vehicle control^b were applied onto demarked areas on both hands in five subjects (A–E)

Subject and location number	Area on right and left hand ^c	Nickel dose ($\mu\text{g}/\text{cm}^2$)	Nickel measured on right hand ($\mu\text{g}/\text{cm}^2$)	DMG test on left hand
A1	Palm	0.00	0.00	Negative
A2	Index finger	0.063	0.058	Negative
A3	Middle finger	0.13	0.11	Weak pink
A4	Ring finger	0.25	0.25	Weak pink
A5	Little finger	0.50	0.46	Pink
B1	Palm	0.00	0.00	Negative
B2	Index finger	0.063	0.059	Weak pink
B3	Middle finger	0.13	0.12	Weak pink
B4	Ring finger	0.25	0.24	Weak pink
B5	Little finger	0.50	0.46	Pink
C1	Palm	0.00	0.00	Negative
C2	Index finger	0.063	0.034	Negative
C3	Middle finger	0.13	0.10	Weak pink
C4	Ring finger	0.25	0.23	Weak pink
C5	Little finger	0.50	0.45	Pink
D1	Palm	0.00	0.00	Negative
D2	Index finger	0.063	0.052	Weak pink
D3	Middle finger	0.13	0.12	Weak pink
D4	Ring finger	0.25	0.24	Weak pink
D5	Little finger	0.50	0.65	Pink
E1	Palm	0.00	0.00	Negative
E2	Index finger	0.063	0.062	Weak pink
E3	Middle finger	0.13	0.13	Weak pink
E4	Ring finger	0.25	0.24	Weak pink
E5	Little finger	0.50	0.46	Pink

^aLimit of detection (LOD) was set at $0.00018 \mu\text{g}/\text{cm}^2$.

^bVehicle: nitric acid (1% HNO_3) and 99.5% ethanol (1:1).

^c2 cm^2 on volar fingertip or centre of the palm.

of the test in subject B is shown in Fig. 1. A total of 20 areas with applied nickel doses and five vehicle control areas were tested. Positive DMG test reactions, shown by a pink colour of the cotton wool, occurred in all subjects at the concentrations 0.50, 0.25 and $0.13 \mu\text{g}/\text{cm}^2$. In 3 of 5 subjects, the lowest concentration ($0.063 \mu\text{g}/\text{cm}^2$) applied to the skin gave rise to a weak pink colour, although it was very weak and could easily have been missed. No false-positive DMG test reactions were found. The assessment of all DMG test reactions was identical between the two independent assessors. It was also noted that DMG testing of the highest concentrations of nickel gave rise to pink coloration of the skin that lasted for approximately 24 hr.

The results of the recovery test performed by acid wipe sampling in the same 5 subjects are presented in Table 1. A total of 25 samples were analysed by ICP-MS. The average recovery of the applied amounts of nickel as assessed with the acid wipe sampling technique was 93%.

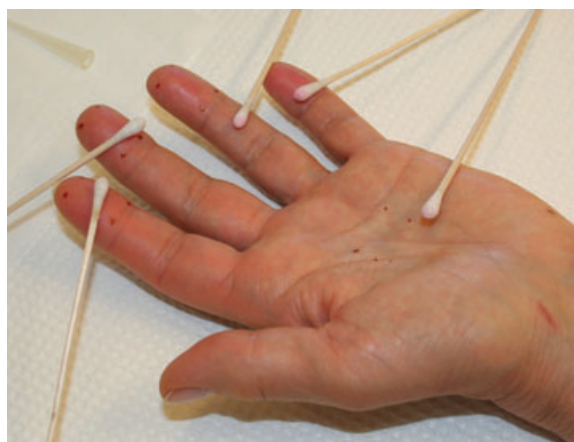


Fig. 1. Dimethylglyoxime (DMG) test on the skin in subject B. The cotton-tipped sticks with DMG test solution, after rubbing of areas where a concentration curve of nickel (from 0.063 to $0.50 \mu\text{g}/\text{cm}^2$ and vehicle control) had been applied. Pink colour is seen at the two highest nickel concentrations (digits 4 and 5); no pink colour is seen with the vehicle control (palm) or at the two lowest concentrations (digits 2 and 3). The skin had been carefully cleaned with soap and water and wiped with nitric acid (1%) prior to application of the nickel solutions.

Nickel hand imprints developed by the DMG test

The novel method for visualization of nickel on the skin, developed in the laboratory by a calibration curve (Fig. 2) and taking imprints after manipulation of nickel-releasing tools (Figs. 3 and 4), was shown to work. The calibration curve showed that nickel at levels as low as $0.3 \mu\text{g}$ gave rise to a weak pink DMG test reaction on the blotting paper. At 0.75 , 1.5 and $3.0 \mu\text{g}$, an obvious pink colour change appeared on the blotting paper (Fig. 2). When 7 workers performing their normal work were tested, the results were mixed. The test was clearly impaired if the worker had a work task that generated a lot of other particles/dirt on the hands. This made it difficult to assess any pink colour by the DMG test in the imprints of the locksmith and 2 of the metal workers.

Discussion

The aim of this study was to assess the usefulness of the DMG test for visualization of nickel contamination of the skin. We also wanted to establish the lowest concentration giving rise to a colour change for nickel deposited onto the skin. The first of our tests, DMG testing on skin under laboratory conditions, proved to be useful. When a cotton-tipped stick was moistened with two to three drops of DMG test solution and rubbed over a skin area dosed with nickel, a positive DMG test reaction, with a pink colour, was achieved. We were able to establish the lowest



Fig. 2. Test of nickel concentration curve on blotting paper, to visualize the lowest concentrations on paper that can be detected by the dimethylglyoxime test. Thirty millilitres of the stock solution (100, 50, 25, 10, 5 and, 0 µg/ml) was added to the blotting paper, corresponding to 3, 1.5, 0.75, 0.3, 0.15 and 0 µg of nickel, distributed over 3 cm² each. No pink coloration is seen with 0 and 0.15 µg of nickel. Weak pink colour is visible at 0.3 µg, which is considered to easily be missed. Obvious pink colour is present at 0.75 up to 3.0 µg.

concentration giving a colour change as 0.13 µg/cm² for DMG testing on skin. This concentration gave rise to a weak pink colour in all five test subjects. Concentrations above 0.13 µg/cm² resulted in clearly pink DMG test reactions on the sticks. The method is very quick and has low costs, although the person assessing the outcome must have normal colour vision and should have some training, as low concentrations of nickel might otherwise be missed. In comparison with other methods, such as the acid wipe sampling technique, the DMG test on skin may, after further experience and validation, be preferred when only qualitative assessment of nickel exposure is required. With the acid wipe sampling technique, it is



Fig. 3. Manipulation of nickel-releasing tools for 30 min prior to taking hand imprints for visualization of nickel by the dimethylglyoxime test (Fig. 4).



Fig. 4. Hand imprint, developed by the dimethylglyoxime (DMG) test, indicating skin areas contaminated by nickel after 30 min of manipulating nickel-releasing tools (Fig. 3). The imprint was taken on blotting paper moistened with nitric acid (1%), and the contour of the hand was marked. The imprint was developed with DMG solution and ammonia after drying. The pink colour on the paper indicates nickel transferred from the skin that had been contaminated by the tools.

possible to detect very low doses and to quantify nickel, as well as other metals deposited on the skin to be used in risk assessments (4–7, 10). We suggest that DMG testing on the skin may be used primarily as a screening test for visualization of nickel contamination in different work tasks, in contact dermatitis patients, and as a tool in follow-up of interventions for exposure reduction.

The colour change at 0.13 µg/cm², the lowest concentration to give a colour change in this study, would have detected the amounts of nickel deposited on the skin by 1 hr of work in cashiers, carpenters, locksmiths and industry workers previously studied by us (4, 6). This LOD value is also considered to be highly relevant in comparison with the elicitation threshold presented by Fischer et al. (23), obtained with the repeated open application test. They reported a response frequency of 50% in nickel-sensitive individuals when nickel was applied twice daily for 1 week at a concentration of 0.35 µg/cm². In a recent pilot study of 6 dermatitis patients with nickel allergy, work-related exposure to nickel was assessed by acid wipe sampling after 2 hr of

work (10). The amount of nickel detected on their hands varied from 0.05 to 0.3 $\mu\text{g}/\text{cm}^2$. We consider it likely that DMG testing on the skin would have shown positive results in at least 3 of these cases, and thus, by simple means, establish occupational skin exposure to nickel.

DMG testing on the skin and the taking of hand imprints do have some limitations. DMG testing on the skin may cause temporary pink coloration of the tested skin surface, if the concentration of nickel is higher than 0.5 $\mu\text{g}/\text{cm}^2$. For the DMG test-developed hand imprints and, to some extent, possibly also DMG testing on the skin, the pink colour produced by nickel may be masked by staining from other particles/dirt on the hands. It is known that metals other than nickel may produce colours other than the typical pink colour produced by nickel, for example reddish-brown for iron and black or blue for cobalt (15, 16). Such discoloration was noted in imprints from the locksmith and 2 of the metal workers who had handled pieces of metal with dust from laser cutting on the surface. Our previous observation, that nickel deposited on the skin as a result of handling items with high nickel release can be visualized by the DMG test (5), has now been confirmed.

The current, simple DMG test may be applied on hands as a qualitative screening test with which to detect and visualize contamination of skin with nickel. The lowest level of colour change, obtained with 0.13 $\mu\text{g}/\text{cm}^2$, refers to the experimental conditions of the study, when nickel solutions were deposited onto the skin. DMG testing in parallel with acid wipe sampling under realistic exposure conditions will give important information on the detection limit of DMG testing on the skin. Further guidelines may support clinical application and detailed exposure assessment in patients, workers, and consumers. The hand imprint DMG test is more complicated to perform.

To optimize the technique, it should be further developed, refined and standardized with regard to materials, pressure and duration of hand contact, and evaluation. Computer-aided image analysis of the size and colour intensity of imprints may be used for quantitative exposure assessment.

There is a need to further develop and apply methods for the assessment of skin exposure to skin sensitizers and other hazardous substances. For example, how the recently described cobalt spot test (24) may be used for assessment of cobalt on the skin will be explored. Such methods will help us to increase our understanding of skin contamination, dose-response relationships, and possibilities for prevention. The results may be used in risk assessment, in setting occupational dermal exposure limits, and in follow-up after intervention for exposure reduction. Skin exposure assessment may also be applied in the evaluation of patients with contact dermatitis. As nickel is the most frequent skin sensitizer, assessment of skin exposure to nickel should be given high priority. Visualization of skin contaminants by the fluorescent tracer technique has proven extremely efficient in exposure assessment, and education and training for prevention. The DMG test can be used as a powerful tool for the detection of nickel contaminants on the skin. DMG test-developed hand imprints may be used for semi-quantitative, and in the future possibly also quantitative, assessment of nickel contamination.

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References

- Lidén C, Bruze M, Menné T. Metals. In: *Contact Dermatitis*, 4th edition, Chapter 32, Frosch P J, Menné T, Lepoittevin J-P (eds): Berlin, Heidelberg, Springer, 2006: pp. 537–568.
- Thyssen J P, Linneberg A, Menné T, Nielsen N H, Johansen J D. The association between hand eczema and nickel allergy has weakened among young women in the general population following the Danish nickel regulation: results from two cross-sectional studies. *Contact Dermatitis* 2009; **61**: 342–348.
- Fournier P G, Govers T R. Contamination by nickel, copper and zinc during the handling of euro coins. *Contact Dermatitis* 2003; **48**: 181–188.
- Julander A, Skare L, Mulder M, Grandér M, Vahter M, Lidén C. Skin deposition of nickel, cobalt and chromium in production of gas turbines and space propulsion components. *Ann Occup Hyg* 2010; **54**: 340–350.
- Lidén C, Skare L, Lind B, Nise G, Vahter M. Assessment of skin exposure to nickel, chromium and cobalt by acid wipe sampling and ICP-MS. *Contact Dermatitis* 2006; **54**: 233–238.
- Lidén C, Skare L, Nise G, Vahter M. Deposition of nickel, chromium and cobalt on the skin in some occupations – assessment by acid wipe sampling. *Contact Dermatitis* 2008; **58**: 347–354.
- Lidén C, Skare L, Vahter M. Release of nickel from coins and deposition onto skin from coin handling – comparing euro coins and SEK. *Contact Dermatitis* 2008; **59**: 31–37.
- Pedersen N B, Fregert S, Brodelius P, Gruvberger B. Release of nickel from silver coins. *Acta Derm Venereol* 1974; **54**: 231–234.
- Staton I, Ma R, Evans N, Hutchinson R W, McLeod C W, Gawkrödger D J. Dermal nickel exposure associated with coin handling and in various occupational settings: assessment using a newly

- developed finger immersion method. *Br J Dermatol* 2006; **154**: 658–664.
- 10 Jensen P, Thyssen J P, Johansen J D, Skare L, Menné T, Lidén C. Occupational hand eczema caused by nickel and evaluated by quantitative exposure assessment. *Contact Dermatitis* 2011; **64**: 32–36.
 - 11 Lind M-L, Boman A, Surakka J, Sollenberg J, Meding B. A method for assessing occupational dermal exposure to permanent hair dyes. *Ann Occup Hyg* 2004; **48**: 533–539.
 - 12 Surakka J, Lindh T, Rosén G, Fischer T. Workers' dermal exposure to UV-curable acrylates in the furniture and parquet industry. *Ann Occup Hyg* 2000; **44**: 635–644.
 - 13 Aragón A, Blanco L E, Funez A, Ruepert C, Lidén C, Nise G, Wesseling C. Assessment of dermal pesticide exposure with fluorescent tracer: a modification of a visual scoring system for developing countries. *Ann Occup Hyg* 2006; **50**: 75–83.
 - 14 Fenske R A, Wong S M, Leffingwell J T, Spear R C. A video imaging technique for assessing dermal exposure. II. Fluorescent tracer testing. *Am Ind Hyg Assoc J* 1986; **47**: 771–775.
 - 15 Biesterbos J, Yazar K, Lidén C. Nickel on the Swedish market: follow-up ten years after entry into force of the EU Nickel Directive. *Contact Dermatitis* 2010; **63**: 333–339.
 - 16 Lidén C, Norberg K. Nickel on the Swedish market. Follow-up after implementation of the nickel directive. *Contact Dermatitis* 2005; **52**: 29–35.
 - 17 Lidén C, Røndell E, Skare L, Nalbanti A. Nickel release from tools on the Swedish market. *Contact Dermatitis* 1998; **39**: 127–131.
 - 18 Thyssen J P, Maibach H I. Nickel release from earrings purchased in the United States: the San Francisco earring study. *J Am Acad Dermatol* 2008; **58**: 1000–1005.
 - 19 Thyssen J P, Skare L, Lundgren L, Menné T, Johansen J D, Maibach H I, Lidén C. Sensitivity and specificity of the nickel spot (dimethylglyoxime) test. *Contact Dermatitis* 2010; **62**: 279–288.
 - 20 Wells G C. Effects of nickel on the skin. *Br J Dermatol* 1956; **68**: 237–242.
 - 21 Ferreira A J, Winter W D. The palmar sweat print: a methodological study. *Psychosom Med* 1963; **25**: 377–384.
 - 22 Rouse C, Siegfried E, Breer W, Nahass G. Hair and sweat glands in families with hypohidrotic ectodermal dysplasia. Further characterization. *Arch Dermatol* 2004; **140**: 850–855.
 - 23 Fischer L A, Johansen J D, Menné T. Nickel allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2007; **157**: 723–729.
 - 24 Thyssen J P, Menné T, Johansen J D, Lidén C, Julander A, Møller P, Jellesen M S. A spot test for detection of cobalt release – early experience and findings. *Contact Dermatitis* 2010; **63**: 63–69.