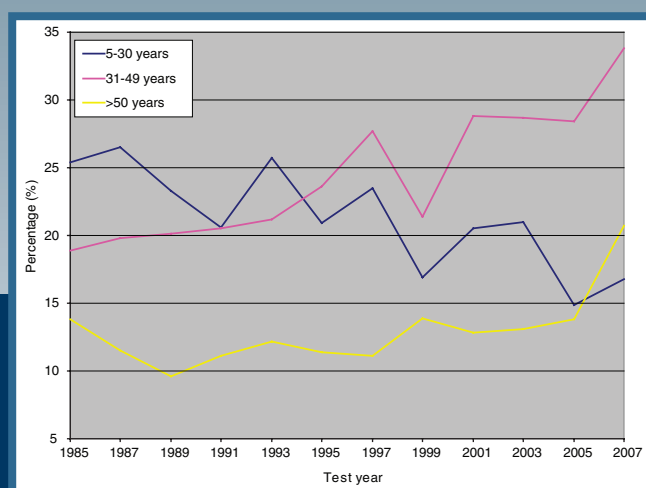


CONTACT DERMATITIS

CUTANEOUS ALLERGY

ENVIRONMENTAL AND OCCUPATIONAL DERMATITIS

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**Nickel and Cobalt Allergy Before and After Nickel
Regulation – Evaluation of a Public Health Intervention**

Jacob Pontoppidan Thyssen

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Nickel and cobalt allergy before and after nickel regulation – evaluation of a public health intervention

Doctoral thesis

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- I Thyssen JP, Johansen JD, Menné T, Nielsen NH, Linneberg A. Nickel allergy in Danish women before and after nickel regulation. *N Engl J Med* 2009; **360**: 2259–2260.
- II Thyssen JP, Linneberg A, Menné T, Nielsen NH, Johansen JD. 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.
- III Thyssen JP, Johansen JD, Linneberg A, Menné T, Nielsen NH, Melgaard M, Szecsi PB, Stender S, Carlsen BC. The association between null mutations in the *Filaggrin* gene and contact sensitization to nickel and other chemicals in the general population. *Br J Dermatol* 2010; **162**: 1278–1285.
- IV Thyssen JP, Johansen JD, Carlsen BC, Menné T. Prevalence of nickel and cobalt allergy among female dermatitis patients before and after Danish government regulation: a 23-year retrospective study. *J Am Acad Dermatol* 2009; **61**: 799–805.
- V Thyssen JP, Skare L, Lundgren L, Menné T, Johansen JD, Maibach HI, Lidén C. Sensitivity and specificity of the nickel spot (dimethylglyoxime) test. *Contact Dermatitis* 2010; **62**: 279–288.
- VI Thyssen JP, Menné T, Johansen JD. Nickel release from inexpensive jewellery and hair clasps purchased in an EU country –are consumers sufficiently protected from nickel exposure? *Sci Total Environ* 2009; **407**: 5315–5318.
- VII Thyssen JP, Menné T, Lidén C, White IR, White J, Spiewak R, Johansen JD. Excessive nickel release from earrings purchased from independent shops and street markets—a field study from Warsaw and London. *J EADV*. DOI: 10/1111/j.1468-3083.2010.0309.x.
- VIII Thyssen JP, Menné T, Johansen JD, Lidén C, Julander A, Møller P, Jellesen MS. A spot test for detection of cobalt release—early experience and findings. *Contact Dermatitis* 2010; **63**: 63–69.
- IX Thyssen JP, Jellesen MS, Menné T, Lidén C, Julander A, Møller P, Johansen JD. Cobalt release from inexpensive jewellery—has the use of cobalt replaced nickel following regulatory intervention? *Contact Dermatitis* 2010; **63**: 70–76.

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Abbreviations

CEN	European Committee for Standardization
CI	confidence interval
DMG	dimethylglyoxime
DZ	dizygotic
EDS	energy dispersive spectroscopy
EDTA	ethylenediaminetetraacetic acid
HLA	human leukocyte antigen
ICDRG	International Contact Dermatitis Research Group
IFN	interferon
IL	interleukin
IQR	interquartile range
LCs	Langerhans cells
MHC	major histocompatibility complex
MZ	monozygotic
nitroso-R salt	disodium-1-nitroso-2-naphthol-3,6-disulfonate
NK	natural killer
OR	odds ratio
ROAT	repeated open application test
SEM	scanning electron microscopy
SLS	sodium lauryl sulfate
TAP	transporter associated with antigen processing
TCR	T-cell receptor
Th	T-helper
TNF	tumour necrosis factor
TRUE test	thin-layer rapid use epicutaneous test

Nickel and cobalt allergy before and after nickel regulation – evaluation of a public health intervention

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Summary (English)

Over the 20th century, the frequent use of nickel in consumer products resulted in an increasing prevalence of nickel allergy. Risk items included suspenders in the 1950s–1960s; buttons, zippers and rivets in the 1970s; and ear-piercing jewellery in the 1980s. When subjects allergic to nickel were exposed to nickel in high concentrations, it often resulted in allergic nickel contact dermatitis and hand eczema.

In 1990, the Danish government began to regulate consumer nickel exposure as a response to the increasing nickel allergy problem. In 1994, the EU Nickel Directive was passed, a regulation that was based on the Danish and Swedish nickel regulations. These major public health interventions were expected to change the epidemiology of nickel allergy and dermatitis in Europe. Furthermore, it was debated whether nickel would be replaced by cobalt in inexpensive jewellery and result in higher prevalence of cobalt allergy. An evaluation of the possible effects of the European nickel regulations is of importance to ensure protection of consumers and dermatitis patients.

This doctoral thesis aimed to evaluate the effects of regulatory interventions on nickel exposure by investigating the development of nickel allergy and dermatitis before and after nickel regulation. Furthermore, a change in the association between nickel allergy and hand eczema was evaluated. The nickel spot test was validated to determine its value when used for screening purposes. Possible explanations for the persistence of nickel allergy were explored including genetic predisposition and consumer nickel exposure from jewellery and accessories. A cobalt spot test was developed and validated. Finally, it was evaluated whether a cobalt allergy epidemic had replaced the nickel allergy epidemic after nickel regulation in terms of increasing cobalt sensitization and cobalt exposure.

The thesis showed that the prevalence of nickel allergy decreased significantly after nickel regulation in young Danish women from the general population (18–35 years) and in young Danish female dermatitis patients from a university patch test clinic (0–30 years). Stratification by ear-piercing status revealed that women ear pierced before 1990 had a significantly higher prevalence of nickel allergy and dermatitis than women ear pierced after 1990. Furthermore, the association between hand eczema and nickel allergy decreased in young women aged 18–35 years when a comparison was made between women who were patch tested and questioned in, respectively, 1990 and 2006. Despite the decreasing prevalence of nickel allergy, this condition remains prevalent in young Danish women, as about 10% are nickel allergic. Genetic predisposition to nickel allergy was evaluated by investigating the possible association between filaggrin null mutation status and nickel allergy. A positive association was found but it was also concluded that environmental nickel exposure is of much greater importance. Thus, of 354 consumer items purchased from 36 different stores in Copenhagen, 22% released nickel in concentrations that may result in nickel dermatitis in sensitized subjects. Therefore excessive consumer nickel exposure remains common in Denmark and may be an important explanation for the persistence of nickel allergy. Excessive nickel release

was also frequent when samples of earrings purchased in Warsaw and London were examined with the nickel spot test, in particular earrings purchased from street markets and shops with independent ownership. Only very weak indications of an emerging cobalt allergy epidemic were found, as the prevalence of cobalt allergy has not yet increased in young people and only four of 354 consumer items purchased in Copenhagen released cobalt in concentrations that may result in cobalt dermatitis in sensitized individuals. The specificity of the nickel spot test was 98% and the sensitivity 59%. Also, a cobalt spot test was developed and validated and seemed to be a useful diagnostic tool.

In conclusion, the Danish nickel regulation and the EU Nickel Directive have changed the epidemiology of nickel allergy in Denmark. However, the Nickel Directive and its reference methods need to be revised to better protect consumers and dermatitis patients.

Resumé (Danish)

I løbet af det 20. århundrede blev nikkel i tiltagende grad anvendt i billige forbrugerprodukter såsom bæltespænder, smykker, ure, knapper og lynlåse da metallet var billigt, gav en pæn og skinnende overflade og kun i ringe grad rustede. Ved længerevarende hudkontakt frigav mange af disse metalgenstande nikkellioner i koncentrationer der medførte nikkellallergi og eksem. Industrialisering gjorde således vestlige forbrugere rigere, men samtidig med de ændrede forbrugsvaner, førte den til en stigende forekomst af nikkellallergi. Foruden nikkeleksem på det eksponerede hudområde øger nikkellallergi risikoen for håndeksem.

Den danske regering valgte fra 1990 at regulere nikkelludsættelsen fra udvalgte forbrugergerstande. I 1994, indførte EU et nikkel direktiv, bl.a. baseret på den danske nikkellovgivning. Lovgivere og hudlæger forventede at de Europæiske nikkellreguleringer ville medføre et fald i forekomsten af nikkellallergi og eksem. Desuden diskuterede man om forekomsten af koboltallergi kunne stige såfremt industrien valgte at anvende kobolt frem for nikkel i forbrugerprodukter. Det er vigtigt at evaluere effekten af de Europæiske nikkellovgivninger for at sikre at Europæiske forbrugere og eksempatienter er tilstrækkeligt beskyttet mod nikkellallergi og eksem.

Denne doktorafhandling havde som formål at sammenligne forekomsten af nikkel- og koboltallergi før og efter den danske nikkellforordning trådte i kraft for hermed at kunne evaluere effekten af lovgivningen. Forekomsten af nikkellallergi og nikkeleksem samt sammenhængen mellem nikkellallergi og håndeksem blev bestemt og sammenlignet for årene 1990 og 2006 ved hjælp af data fra 2 uafhængige befolkningsundersøgelser samt for årene 1985–2007 ved hjælp af den kliniske database fra Gentofte Hospital. Årsagen til den fortsat høje forekomst af nikkellallergi i befolkningen blev undersøgt ved at 354 smykker og hårspænder fra 36 Københavnske butikker blev undersøgt for nikkellfrigivelse. Nikkeltesten blev valideret for at bestemme dens anvendelighed. Betydningen af genetisk disposition til nikkellallergi blev undersøgt ved hjælp af filaggrin genmutationsanalyse i den danske befolkning. En kobolttest blev udviklet og valideret, hvorefter 354 smykker og hårspænder blev undersøgt for koboltfrigivelse. Endelig blev øreringe købt i Warszawa og London undersøgt for nikkellfrigivelse.

Afhandlingen viste at forekomsten af nikkellallergi faldt signifikant blandt yngre kvindelige eksempatienter (0–30 år) der blev lappetestet i perioden 1985–2007 samt blandt 18–35 årige kvinder fra baggrundsbefolkningen der blev testet i 1990 og 2006. Derudover faldt forekomsten af selv-rapporteret nikkeleksem signifikant mens sammenhængen mellem nikkellallergi og håndeksem blev reduceret betydeligt. Forekomsten af nikkellallergi og nikkeleksem var lavere blandt kvinder der fik lavet huller i ørerne efter den danske nikkellforordning trådte i kraft set i forhold til kvinder der fik lavet huller i ørerne før. Nikkelallergi var associeret med filaggrin genmutationer, hvormed det blev vist at en defekt hudbarriere øger risikoen for nikkellallergi. Betydning var dog minimal set i forhold til den miljømæssige nikkeleksposering. Nikkel blev

frigivet fra 22% af 354 indkøbte smykker og hårspænder fra Københavnske butikker. Nikkeltesten viste sig at være meget specifik (98%) men kun moderat sensitiv (59%). Omkring 15% af øreringe købt i Warszawa og London frigav for meget nikkel. Det var muligt at definere markeder og butikker der ikke tilhører en kæde som lokaliteter hvor risikoen for nikkeludsættelse er betydelig. Forekomsten af koboltallergi syntes ikke at være tiltagende blandt eksempelpatienter og ligeledes frigav kun 4 af 354 smykker og hårspænder kobolt da de blev undersøgt med den nyudviklede kobolttest.

Det konkluderes at den danske nikkelforordning samt EU's nikkel direktiv har været succesrige i det omfang at de har reduceret forekomsten af nikkelallergi. Trods dette, er der behov for yderligere tiltag for at beskytte Europæiske forbrugere og eksempelpatienter, heriblandt flere kontrolinspektioner samt en stramning af reference metoderne til vurdering af om metalgenstande overholder lovgivningen.

Background

Nickel is the fifth most common element on Earth although it only constitutes about 0.008% of the Earth's crust (1). All nickel was formed billions of years ago in supernova explosions, the only place where the temperature and pressure conditions were sufficient for the nickel atom to form. In nature, nickel usually occurs together with cobalt, ranging from a trace of cobalt to as much as 1 part cobalt to 10 parts nickel (1). Nickel may also occur together with copper, zinc, iron, or arsenic whereas cobalt may occur together with copper (2). The nickel sulfides and oxides found in nature are not allergenic. Only free nickel ions may act as haptens. In Germany, cobalt was used to denote a goblin and nickel to denote a scamp, as these were believed to haunt the mines and harm the miners (2). Since around 2000 BC cobalt has been used as a blue colouring agent for pottery, jewellery, and glass (3). From the 17th century, traders from China supplied Europe with German silver. It consisted of 25% nickel, 25% zinc, and 50% copper (2). Nickel was described in 1751 by the Swedish chemist and mineralogist Baron Axel Fredrik Cronstedt as he attempted to extract copper from niccolite (kupfernickel) (2) whereas cobalt was first isolated in 1735 by Swedish chemist George Brandt (3).

Over the 19th century, nickel was used in coins, in the steel industry, and in military-related industries (4). This resulted in allergic nickel dermatitis on the hands and forearms of workers in the plating industry (5). In 1889, Blascho referred to nickel dermatitis as 'das Galvaniseur-Ekzem' (galvanization eczema) (5). In 1925 in Kiel, nickel was proven, by patch testing, to be the aetiological factor for the development of dermatitis in workers from the electroplating industry (6). Further reports on occupational nickel dermatitis were released through the 1920s and 1930s (7, 8). Cobalt was used from the 1920s in industrial applications such as cutting tools

and high-speed drills as cobalt bound tungsten carbide into a hard substance (referred to as hard metal) (3). In the 1930s, it was discovered that, when cobalt was alloyed with other metals, very strong magnets were created (3). The use of cobalt increased rapidly following the Second World War (3). In the 1930s, the first case reports on consumer nickel dermatitis to spectacles, watches and suspenders were published (9–11). In 1939, Poul Bonnevie noted that 88% of 58 female dermatitis patients with nickel allergy had causative exposure to stocking suspenders (12). From that time, the burden of occupational nickel allergy began to level off and at the same time the burden of consumer-related nickel allergy accelerated (13). The following passages offer background information that will put into perspective the complexity of the nickel and cobalt allergy problem.

Metals and alloys

Nickel and cobalt are a constituent of many alloys and platings (14, 15); for example, nickel plating of non-precious metals and alloys such as iron, brass, and stainless steel (0.5–30% nickel) but also white gold (10–15% nickel), German silver (also referred to as nickel silver) (10–15% nickel), solder, hard gold plating and sterling silver (4). German silver, sterling silver and white gold were often used in inexpensive jewellery during the 1970s and 1980s and frequently led to nickel allergy and dermatitis (16–18). White gold was previously made of platinum and gold but from the 1970s it mostly consisted of gold, nickel, palladium, zinc, and sometimes cadmium (18). Fischer et al. patch tested 18 nickel allergic female patients with 16 white gold discs (18). The discs had varying nickel content (2.5–15%) and eight were plated with rhodium. Nickel release from unplated discs was 0.09–0.82 µg after 1 week in synthetic sweat and 0.04–0.54 µg/week from the rhodium-plated

discs (18). All discs gave negative dimethylglyoxime (DMG) test outcomes even though all patients reacted to the discs upon patch testing. Finally, bright nickel plate contains sulfur which makes it more active and likely to release nickel ions (19). In line with this, black nickel has a high sulfur content and releases high amounts of nickel.

To give jewellery a golden appearance, nickel may be plated with uniform layers of gold as thin as 0.3 µm (15). Items are typically covered with a transparent varnish based on acrylic resins to increase resistance against wear (15). Many other gold and nickel alloys have also been patented and used for inexpensive jewellery (15). However, gold-plated items may release nickel as the layer of gold is thin and porous and easily cracks (14). This was underscored by a Japanese investigation that performed the DMG test on 127 gold or gold-plated products (20). Watches, wristbands, spectacle frames, jewellery and lighters were investigated and 57% gave positive DMG test reactions. Another Japanese study investigated the distribution of nickel on the surface of four sets of used gold earrings that elicited nickel dermatitis and three sets of unused gold earrings (21). Gold plating defects were found in both used and unused earrings and nickel was detected in scratches and pits. Nickel was found in crusts and blood from the pierced region of the earlobe in one of three nickel allergic patients (22). Finally, Lidén et al. patch tested 100 nickel allergic patients with 21 different nickel-containing alloys and coatings (17). Nickel interliners under gold, silver or chromium plating as well as nickel silver and some white gold alloys gave positive patch test reactions (17). Patch testing was performed on the earlobe of a subset of patients with materials that had not resulted in reactions in 40 nickel allergic subjects. However, only two positive reactions were observed (to brass and white gold) (17).

Cavelier et al. investigated whether seven different metallic samples with nickel as the underlying metal, surface plated with chrome (different structures and thickness), would elicit nickel dermatitis upon patch testing in, respectively, guinea pigs and 20 nickel allergic subjects (23). They found that all discs, independent of the thickness of the chromium deposits, resulted in dermatitis in humans and animals. The best tolerated discs also contained copper (23). In further studies, the same group investigated whether nickel and cobalt allergic individuals and guinea pigs would react to metallic discs containing either nickel or cobalt as the underlying metal surface and a deposit of chromium (+/- copper) (24). All guinea pigs sensitized to either cobalt or nickel reacted to all discs. However, in human test subjects, those allergic to nickel only tolerated cobalt discs but not nickel discs (24).

In the 1980s–1990s, the CuNiStan alloy (consisting of copper, nickel, and tin) was increasingly used for imitation jewellery as it was highly corrosion resistant and had a white silver to pink silver appearance (15). Furthermore, pewter made from lead, copper, and tin with an exterior layer of nickel that offered shine and hardness was frequently used (15). Vilaplana et al. concluded in 1991 that nickel was present in imitation jewellery and that it was set to be included into the indefinite future (15). Nevertheless, the authors emphasized that attempts were made by manufacturers to substitute nickel with other metals such as palladium and bronze (25). Seven years later, Vilaplana and Romaguera discussed recent advances in the development of materials used in jewellery and stated that alloys for jewellery and watches rather contained iron, aluminium, titanium, copper and magnesium than nickel and cobalt (26). Cobalt is believed to be used rarely in inexpensive jewellery but can be alloyed with, for example, tin and gold. However, a recent American study evaluated the allergenicity of a novel metal alloy intended for use in jewellery made of cobalt and platinum (27). Bocca et al. recently showed that earrings and earring components had complex structures (28). Components typically had cores of brass or iron, which were plated with gold or silver. In some cases, nickel was used as an interliner (28). Finally, a recent study of 26 inexpensive earrings purchased in Thailand and China showed that copper, zinc and nickel were the most predominant metals (29).

Corrosion and passivation

Corrosion is the disintegration of an alloy into its constituent atoms due to chemical reactions with its surroundings. Corrosion resistance is important for items that come into direct contact with the skin as metal ions may be released and result in contact sensitization. Two metals in electrical contact share the same electrons and will therefore tend to compete for free electrons. Metals can be ordered in a hierarchy, the galvanic series, depending on their individual ability to take electrons. Noble metals (e.g. gold, platinum, and silver) are rarely attacked by corrosive solutions as these are electropositive (30) whereas electronegative metals (e.g. zinc, iron, and aluminium) are more easily attacked (30). Nickel and cobalt have weak electronegative dissolution potentials and therefore have good resistance to corrosion. Cobalt is less noble than nickel. Previously, it was believed that plating with a thin layer of a precious (noble) metal could prevent nickel release from the core. However, since the deposit of precious metal was rarely impervious, the more noble metal would actually work as a galvanic

couple and favour nickel corrosion (30). Plating with gold and silver should therefore be thicker than 0.5 μg to prevent nickel release (30). One may also choose to plate nickel with a more electronegative metal, e.g. chromium. This is generally a very efficient way to prevent nickel release (30).

Corrosion can be concentrated locally to form a pit or crack, or it can extend across a wide area more or less uniformly corroding the surface. Because corrosion is a diffusion-controlled process, it occurs only on exposed surfaces. Methods to reduce the activity of the exposed surface, such as passivation or conversion coating, can increase a material's corrosion resistance [passivation is the spontaneous formation of a hard non-reactive surface film (oxide) that inhibits further corrosion]. Although passivation is useful in alleviating corrosion damage, corrosion may still happen due to a change in pH or temperature. Nickel is difficult to passivate and passivation never occurs spontaneously (30). However, chromium and iron easily passivate and this effect is used in stainless steel, which is generally a corrosion resistant material. Accordingly, it contains 12–20% chromium (30). There are many different grades of stainless steel with varying degrees of nickel (31). Stainless steel also contains molybdenum as this metal improves corrosion resistance in chloride-containing media (31). Sulfur is known to decrease corrosion resistance but enhance machinability (31). Haudrechy et al. showed that stainless steel with a high sulfur content elicited nickel dermatitis in some nickel allergic patients whereas stainless steel with a middle to low sulfur content did not elicit nickel dermatitis (31, 32). An HNO_3 test was more useful for detection of nickel release than the DMG test, since the latter test resulted in false positive reactions due to complexation between DMG and iron. Case series have confirmed that some grades of stainless steel may elicit nickel dermatitis (17, 33, 34).

The composition of human sweat varies between individuals and also fluctuates as a function of time of secretion, skin temperature, relative humidity, supply of water and salt, diet, and other factors (19, 35). It typically consists of chlorides, sodium, potassium, urea, ammonia, amino acids, and lactic acid. The pH usually varies between 3.8 and 6.5 but may even range from 2.1 to 6.9 (19, 35). Although sweat increases metal release, both nickel and cobalt only react slowly to form the divalent ions (19). Menné and Solgaard showed that nickel release from a Danish crown (DKK) in synthetic sweat increased when the temperature was raised from 20°C to 35°C (36). Hemingway and Molokhia showed that the quantities of dissolved nickel ions increased with contact time, acidity and temperature (37). When pH

was lowered from 5.5 to 4.5, nickel release increased by a factor of 10 (37). The authors showed that nickel leaching was inhibited when oxygen was excluded from the test system (37). Their findings were in line with those of Emmett et al. who showed that pH 5.3 was more effective at releasing nickel than pH 6.6 when identical earrings were exposed to synthetic sweat. A 'ruster' is an individual who produces a corrosive effect by fingering a freshly polished metallic surface. When 'rusters' touch such surfaces, rusty fingerprints may appear after a few days. Abnormally high concentrations of sodium and chloride in the sweat was suspected to be responsible for this effect (38) but a Danish study showed that it was caused instead by hyperhidrosis (39, 40).

In experimental corrosion studies, Randin showed that nickel had a small region of thermodynamic stability at $\text{pH} > 7$, that it corrodes readily in acid solutions, and that it only passivates in pH 9–12 (35). He divided nickel alloys into two groups based on potentiostatic measurements: (i) alloys that are in an active state and dissolve readily (e.g. white gold, Ni, NiAl, NiP) and (ii) alloys that are in a passive state and only pit under extreme conditions (e.g. stainless steel, SnNi, PdNi) (35). Randin concluded that, except for Stellite 20, cobalt and its alloys had poor corrosion resistance. Pedersen et al. showed that nickel was more readily released from coins in synthetic sweat than in distilled water (41). Emmett et al. showed that plasma was the most effective solution for removing nickel from earrings compared with synthetic sweat and normal saline solution (42). Finally, it is important to emphasize that when one attempts to assess the allergenicity of an alloy the dose is defined, not by the metal concentration in the alloy, but by the rate of formation of allergenic metal ions (19). If the exposure time is long and/or the contact area is large, alloys with low concentrations of allergenic metal may result in allergic contact dermatitis. Thus, it is not possible to define a lower limit for the content of allergenic metal in an alloy below which elicitation is unlikely (19).

Dimethylglyoxime test

The DMG test was first described by Fritz Feigl in 1958 (43). 1% DMG in alcoholic solution gives a bright pink or cerise coloured insoluble salt when it complexes with nickel in a 10% ammonium hydroxide solution. The detection limit of the DMG test is 10 ppm, corresponding to 0.5 μg nickel/ cm^2 /week (16, 44). When the DMG test is used to detect nickel release from stainless steel, false positive reactions may occur due to complexation between DMG and iron (31, 32). The addition of hydrogen peroxide may decolour the complexes of iron but not those of

nickel. False negative reactions may occur if there is a high concentration of cobalt salts (43). Palladium may give yellow coloration and copper salts may give violet coloration (43). The European Committee for Standardisation (CEN) developed the CR12471 screening test (45). To increase sensitivity of the DMG test, CEN recommended that items under investigation should be pre-treated with artificial sweat and heating to induce corrosion (45). In practice, pre-treatment results in more positive test reactions (46).

Skin penetration

Nickel and cobalt allergy may develop following repeated or prolonged skin contact with metallic items. It is a delayed type hypersensitivity reaction (type IV) characterized by development of memory CD4+ T helper (Th) cells. When a sensitized subject is re-exposed to the relevant metal ion in concentrations that exceed the individual threshold, allergic contact dermatitis is elicited. Thus, two distinct phases are recognized, an induction phase referred to as contact sensitization and an effector phase referred to as allergic contact dermatitis (47).

Before metal ions can elicit a cutaneous immune response, they must gain access to the viable epidermis. Exogenous (e.g. dose, size, counter-ions, polarity, valence, and pH) and endogenous (e.g. age of the skin, anatomical site, oxidation, and reduction) factors are important and should be accounted for when investigating metal absorption (48–50). Little is known about how aging affects penetration aside from an incomplete barrier function in infants and young children which tends to increase penetration of chemicals (51). However, no studies have been made using metals. Permeability depends on anatomical location (scrotum > forehead > postauricular > abdomen > forearm > leg > back) (51). Differences are explained by the thickness of the stratum corneum but also by intercellular lipid composition and shunt density (51).

Nickel chloride is more rapidly absorbed than nickel sulfate when experiments are done with occlusion (52–54). Without occlusion, permeation of nickel chloride is reduced by 90% and permeation of nickel sulfate is hardly detectable (51, 54). The counter-ion (sulfate or chloride) plays a role in the passive diffusion of nickel through the stratum corneum (51, 54). Nickel diffuses slowly (time lag of 50 hr) through the stratum corneum and passage is believed to be mainly intercellular (51). Once nickel is through the stratum corneum, diffusion rates accelerate (51). Nickel is mostly accumulated in the stratum corneum where 90% is bound and only minor amounts are found in the lower layers, independent of the counter-ion (52, 55, 56).

Little is known about cobalt absorption and penetration. Volunteers who were cutaneously exposed to cobalt had higher concentrations of urinary cobalt than controls (57, 58). Linnainmaa and Kiilunen found an increase in urinary cobalt concentration 4–6 hr after skin exposure to cobalt powders in five subjects (58). One *in vitro* study confirmed cobalt penetration and showed that cobalt remained in the skin after penetration (59). Also, the permeation of both nickel and cobalt ions may be closely associated with the oxidizing capacity of sweat (48, 60). It was recently demonstrated that cobalt and nickel powders penetrate damaged skin more easily than intact skin (61–65).

Metal binding and sensitization

The epidermis contains an interdigitating network of Langerhans cells (LCs). These cells form part of a wider family of dendritic cells, the main function of which is to present antigen to the immune system. Haptens are bound, internalized, processed, and transported by LCs or macrophages to the regional skin-draining lymph node where they are presented to specific naïve T-cells that become activated and clonally expand to memory T-cells (66). Re-exposure to the relevant allergen initiates the efferent phase and clinical expression of allergic contact dermatitis (67). Kaplan et al. recently performed experimental studies using genetically modified mice to observe LC function. They identified a novel population of dermal dendritic cells that appeared to capture, process, and present antigens rather than traditional LCs (68).

Traditionally, the antigen-specific immune response is initiated by CD4+ Th cells that are activated by the antigen bound to a relevant major histocompatibility complex (MHC) class II protein on the surface of antigen presenting cells (69). Contact allergens are either inherently protein reactive or can be metabolized in the skin to protein-reactive species (70). However, it has been shown that nickel may activate T-cells in other ways (71). From a chemical point of view, metal ions like nickel and cobalt are unlikely to covalently bind to proteins (72). The dominating theory is that nickel (and cobalt) form geometrically highly defined coordination complexes with electron-rich ligands such as nitrogen or oxygen (71, 73), or that antigenic structures can be formed by a free radical mediated mechanism (72, 74). There is evidence to suggest that the trivalent nickel ion is the actual immunogenic form rather than the bivalent nickel ion. Thus, *de novo* sensitization has proved difficult in animal studies (75, 76), and Artik et al. showed that Ni(III) and Ni(IV) but not Ni(II) alone could sensitize naïve T-cells (75). Bio-oxidation

may occur through endogenous reactive oxygen in the form of hydrogen peroxide or hypochlorite in inflamed skin. Recently, a German study showed that the human Toll-like receptor 4 binds nickel resulting in immune activation (77). Furthermore, the mouse Toll-like receptor 4 did not bind nickel, perhaps explaining the difficulty of sensitizing mice (77).

The coordination complexes are reversible and allow the exchange of allergenic metal ions between different acceptor sites (71). It has been debated whether metal ions can be considered real haptens as T-cell recognition normally requires covalent hapten attachment to MHC-associated peptides (71). However, nickel may act much as a classic hapten by binding to amino acid residues of MHC and the associated peptide (78). It was even hypothesized that nickel may induce alterations of protein processing and that nickel-free cryptic self-peptides may activate T-cells (79). On the other hand, experiments have shown that in 50–60% of nickel-reactive T-cell clones from nickel allergic subjects, antigen processing is not required (80). Gamerding et al. showed that nickel activation of T-cells (clone SE9) was independent of the kind of peptide associated with the restricting human leukocyte antigen (HLA)-DR molecule (81). Pre-formation of a low-affinity T-cell receptor (TCR)–MHC complex which is then stabilized by the insertion of nickel is reminiscent of T-cell activation by superantigens as these bridge the TCR with MHC (71, 81). The authors proposed that this peptide-independent activation of T-cells could explain the high prevalence of nickel allergy (81). However, nickel recognition is limited to an extremely small selection of receptors (they require a contact site within the N-region-determined third hypervariable region of the TCR α -chain) whereas this is not the case for superantigens (71). Finally, nickel, unlike chemically reactive haptens, may use particular carrier proteins to ensure its transport through the epidermis and dermis (71).

When LCs and macrophages bind nickel or cobalt ions, they produce large amounts of interleukin (IL) 12 which promotes mainly Th1 cell differentiation in the tissue through the suppression of IL-4 and concurrent promotion of interferon (IFN) γ secretion (82). A positive feedback is generated since IFN- γ released by Th1 cells promotes further release of IL-12 from LCs and macrophages. CD4+ T-cells activate keratinocytes through the secretion of IFN- γ , tumour necrosis factor (TNF) α and IL-17 which in turn contributes to the amplification of the inflammatory response (83). A recent study showed that memory T-cells from the peripheral blood of individuals with nickel allergy, but not controls, contained Th1 cells but also Th17 cells (84). Furthermore, inflamed skin of nickel allergic subjects contained cells

expressing IL-17, IL-22, CCR6 and IL-22R (84). IL-22, a cytokine secreted by Th17 cells, was significantly higher in serum levels from patients with allergic nickel dermatitis than in controls (85). Furthermore, *in vitro* studies have shown that cobalt and nickel allergy may result in a mixed Th1 and Th2 type cytokine profile (86–88). Minang et al. showed that IL-10 may counteract the allergic reaction mediated by Th1 type cytokines such as IFN- γ (89). Allergic nickel and cobalt dermatitis is generally characterized by infiltration of numerous CD4+ Th1 cells that secrete pro-inflammatory cytokines as well as CD8+ cytotoxic T-cells, the latter considered the major effector population in allergic contact dermatitis as they induce keratinocyte apoptosis (90–92). A Finnish group showed that CD4+ Th cells act more vividly to nickel than CD8+ T-cells and that they obtain more adhesion molecules (93, 94). Natural killer (NK) cells are innate immune cells that lack rearranged TCR for antigen recognition as observed for adaptive T-cells. An early and rapid innate immune response to nickel mediated by NK cells can be observed in allergic nickel dermatitis (95). Full expression of allergic contact dermatitis requires a coordinated interaction between the adaptive and innate immune system (92). Thus, Th1 and Th17 cells activate NK cells that become effector cells in the inflammatory process of allergic contact dermatitis by secreting cytokines and inducing apoptosis of keratinocytes (92). Finally, nickel and cobalt, but not zinc, chromium, copper, and magnesium, induce gene transcription of adhesion molecules in endothelial cells (96, 97). This results in recruitment of neutrophil cells, monocytes, and memory T-cells (96) and might to some degree explain the sensitizing effect of nickel and the high frequency of nickel allergy.

Genetic disposition

Several studies have investigated whether nickel allergy is associated with genetic factors (98). Forsbeck et al. showed that genetic predisposition to allergic contact dermatitis (including nickel allergy) could be of significance when performing family studies (99, 100). Fleming et al. identified increased familial disposition (risk ratio 2.83) to develop nickel allergy among first-degree relatives of 39 probands with nickel allergy (101). They concluded that the risk ratio was low compared with for example insulin-dependent diabetes (101).

Twin studies have also investigated the heritability of nickel allergy. No difference in the prevalence of nickel allergy was found when data from 101 pairs of Swedish monozygotic (MZ) and dizygotic (DZ) twins were analysed (102). A Danish study including 1546

pairs of twins performed genetic statistical analyses on the outcome of nickel patch testing as well as personal interviews about nickel dermatitis (103). Menné and Holm found that for nickel patch test reactivity the pairwise concordance rate for MZ twins was 0.29 compared with 0.08 for DZ twins ($p < 0.05$), whereas for self-reported nickel dermatitis the pairwise concordance rate for MZ twins was 0.32 and 0.14 for DZ twins ($p < 0.05$). The heritability of nickel allergy was 60% (103). Surprisingly, a subsequent study including 630 Danish female twins showed that nickel allergy was mainly explained by environmental factors and only to a lesser degree by genetic factors (104). A weak, non-significant increase in the odds ratio (OR) for MZ twins was found compared with the OR for DZ twins (104).

Ishii et al. sensitized and challenged congenic and recombinant strains of H-2 (the mouse MHC antigen) mice with nickel. The I-A subregion of mouse H-2 was important for nickel allergy (105). The possible association between HLA genes and nickel allergy has also been investigated. HLA molecules are polymorphic membrane glycoproteins found on the cell surface. They are encoded on the short arm of chromosome 6 and are divided into classes I (HLA-A, HLA-B, and HLA-C), II (HLA-DR, HLA-DQ, and HLA-DP), and III (TNF and heat shock proteins) (98). Emtestam et al. reviewed the literature and found that only two of 14 studies showed significant associations between nickel and cobalt allergy and HLA typing after correction for the number of HLA alleles that were investigated (106). In their second genomic analysis on subjects with nickel, cobalt and chromium allergy, the authors concluded that it was unlikely that metal allergy was associated with alleles of the HLA II region (106). A Finnish study found that genes encoding for transporter associated with antigen processing (TAP) 2B proteins were associated with nickel allergy as the allele frequency of TAP2B was 40% in nickel allergic patients and 26% in controls (107). Finally, a Finnish study investigated whether nickel-induced CD4+ and CD8+ T-cells exhibited restricted TCR variable β -gene use (108). The authors found an individual pattern in nickel allergic patients.

Filaggrin is a highly phosphorylated, histidine-rich polypeptide (109). Loss of filaggrin expression may lead to deficiencies in the terminal formation of the stratum corneum and disrupt skin-barrier function. Recently, Novak et al. found that null mutations in the filaggrin gene were positively associated with nickel allergy and significantly associated with nickel allergy combined with self-reported intolerance to costume jewellery (110). A Danish twin study found no association between filaggrin null mutations and contact allergy, perhaps because

of low study power (111). Since histidine and histidine-rich polypeptides are strong nickel chelating agents, the accumulation of nickel in the upper part of the stratum corneum may be partly explained by nickel binding to histidine side-chains of the filaggrin protein (112). Thus, nickel may possibly permeate freely or more rapidly through filaggrin-deficient skin in comparison with normal skin. If so, different routes of nickel exposure may explain the contradictory results found in past twin studies. The majority of twins in the study by Menné and Holm were cutaneously exposed to nickel from stocking suspenders and buttons (103) whereas most twins in the study by Bryld et al. were exposed to nickel ions from ear piercing (104). In the latter case, the immune system was directly exposed to nickel ions as the skin and its compartments (including nickel chelating filaggrin) were bypassed by perforation. This may explain why no genetic predisposition could be found in the second twin study (112). The histidine bypass theory was recently supported since a reduced level of histidine was found in the stratum corneum of patients with null mutations in the filaggrin gene (113).

Exposure

Occupational–nickel. Nickel is used to create stainless steel, nickel alloys, and nickel cast iron that compromise coins, electrical equipment, tools, appliances, and household utensils (4). Also, occupational exposure includes mining, extraction and refining, plating, casting, grinding, and polishing (1, 4). Occupational nickel dermatitis was observed in workers in the plating industry in the late 19th century and early 20th century (5, 6). Bulmer and Mackenzie concluded that insufficient cooling power in the workers' environment was the single most important risk factor for nickel dermatitis (8). Furthermore, they found that increased skin temperature together with sweat would increase the risk of irritation and sensitization (8).

Marcussen noted that although the nickel allergy problem had been greatly reduced in major industries between 1930 and 1960, it remained present in smaller occupations such as hairdressers, sewers, tailors, cashiers, and office workers. The prevalence of nickel allergy increased significantly in Swedish female occupational dermatitis patients from 9% in 1960–1964 to 19% in 1965–1969 (114). The highest proportion was found in cleaners (114). Relevance of nickel allergy was found in 65% of women and 53% of men (114). Clemmensen et al. investigated nickel exposure in hospital cleaners and found that the nickel content of water gradually increased during the cleaning process due to nickel contamination from the cleaning procedure (115). The nickel content of water after cleaning reached $1.5 \mu\text{M}$

nickel/1(115). Olumide reported occupational nickel dermatitis on the hands in security personnel due to handling of steel gates (116). Emmett et al. reported nickel dermatitis in doctors and nurses following skin contact with stethoscopes and metal backs of medical charts (42). Samitz and Katz found no nickel release from procedures in which grinding fluids were used in factories and plants (117). Wall showed that the inside lining of protective rubber gloves from workers in an electroforming factory was DMG test positive (118) and that this was likely to be explained by nickel penetration through rubber gloves (119).

Katz and Samitz investigated nickel release from various stainless steel commodities such as flashlight cases, knives, spigot handles, and coins (120). They placed 100 items in different leaching media (e.g. sweat, soap, physiological salt solution) and measured nickel release (given as micrograms) (120). The authors concluded that the increasing use of stainless steel kitchenware in combination with their ability to release nickel could result in nickel dermatitis (120). Accordingly, nickel allergy was most prevalent in cooks, where nickel allergy was evaluated in 72 caterers (121). Menné and Bachmann showed that, for the period 1970–1976, 564 Danes applied for permanent disability due to skin disease (122). The most common cause was dermatitis ($n = 298$) of which 33% were due to nickel allergy. Of 91 nickel allergic women, only 25% were sensitized to nickel alone. Nickel allergic subjects worked in the metal industry, as cashiers, kitchen workers, seamstresses, restaurant workers, and hairdressers. Primary hand eczema was common, as about half did not report metal dermatitis from other skin sites prior to hand eczema.

In the early 1990s, up to 12% of British patients diagnosed with occupational contact dermatitis had relevant nickel allergy (123). The highest frequencies were found in hairdressers, cleaners, and cooks (123). An Australian study showed that nickel was the most prevalent occupational allergen among female patients with dermatitis whereas nickel only ranked 10 in men (124). Kanerva et al. investigated the incidence of occupational nickel dermatitis in Finland over a 7-year period (1991–1997) (125). Among 2543 occupational cases, nickel caused 6.9%. It was mainly observed in footwear workers, machine and metal product assemblers, electrical and tele-technical equipment assemblers, precision instrument mechanics, postal officials, industrial tailors and seamstresses, hairdressers and beauticians (125). Of 332 Polish patients with occupational contact dermatitis 30.7% had clinically relevant nickel allergy (126). In the late 1990s, Shah et al. found occupational relevance of nickel allergy in 22.8% of 368 nickel allergic dermatitis

patients (127). The most common occupations were retail clerks, hairdressers, domestic cleaners, metal workers, and caterers (127). Dickel et al. found that nickel was the most frequent sensitizer among patients with occupational skin disease but also that occupational relevance was only present in 11% (128).

Nickel concentrations were significantly higher in fingernails than in toenails in 71 nickel allergic patients and 20 controls (129). Nickel allergic women had significantly higher levels of nickel in toenails, hair and plasma than controls, whereas no significant difference was found in fingernails between the two groups (129). Another study showed that the nickel concentration was significantly elevated in fingernails from subjects that were heavily (employees in electronic nickel plating industry) or moderately (assemblers of electrical switches, relays, and locks) exposed to occupational nickel compared with controls (130). The authors suggested that nail nickel analysis could be performed in subjects who were suspected of occupational nickel exposure and defined occupational nickel exposure by a cut-off value of 8 µg nickel/g (130). Staton et al. developed and piloted a method to measure nickel on the skin by direct immersion of the fingers into tubes of extractant with evaluation of the nickel content of the water using inductively coupled plasma optical emission spectrometry (131). Nickel contamination of fingers was in direct proportion to exposure time of rubbing coins but variation existed between different individuals even though strict criteria were followed. Total nickel concentrations differed between various occupations; thus, nickel refinery workers (7.9–1310 ng/cm²) and nickel platers (20–7159 ng/cm²) had by far the highest nickel concentrations on the fingers whereas cashiers, hairdressers, bar staff, and department store workers had markedly lower nickel concentrations. The levels of nickel on the skin of cashiers, shop assistants, bar staff, and hairdressers would only result in nickel dermatitis in a minority (131). Lidén et al. recently found that nickel deposition on the hands of locksmiths, cashiers, and carpenters following work was capable of eliciting nickel dermatitis and possibly causing or contributing to sensitization (132). A recent Swedish study found high nickel skin deposition in workers from the thermal application department in a plant producing gas turbines and space propulsion components (up to 15 µg nickel/cm²/hr) (133).

Nickel dermatitis may occur following skin contact with cold-impregnated aluminium (134). A case report showed that the DMG test was positive when applied to 30 cold-impregnated aluminium products in three different plating firms (134). Furthermore, Lidén studied occupational nickel exposure by performing patch testing

in workers and visiting factories (135). Different tools as well as the inner side of protective gloves gave positive DMG test reactions (135). The prevalence of nickel allergy was 30% in female patients and 8% in male patients. In further studies, Lidén et al. investigated 565 handheld tools with metal parts that came into contact with the skin and found that 27% gave positive DMG test reactions (136). No relevant information was displayed about nickel release or nickel content on the tools or in catalogues. Also, different DMG test results were observed on the same products that had been produced in different countries (136).

A recent Danish report described two female hairdressers with occupational nickel hand eczema following contact with DMG-test-positive scissors and crochet hooks (137). Of 200 scissors investigated with the DMG test one was positive, whereas seven of 13 crochet hooks were DMG test positive when random hairdresser salons in Copenhagen were visited (137). These findings were in line with those of Wahlberg who showed that hairdressers were often exposed to nickel from the use of work tools (scissors and clips) (138) and that 40% of 35 hairdressers had nickel allergy (139). Ammonium thioglycolate in permanent wave liquids accelerated nickel release from clips compared with synthetic sweat (140).

In a pioneering study, Pedersen et al. showed that nickel was found in a film-like coating on used, but not new, Swedish coins and notes, and that nickel was released from silver coins in synthetic sweat (41). Nickel was found in various concentrations (5–122 µg/person) on the hands of persons who had been in contact with coins in their profession or from counting coins (>5 min) (41). They concluded that coin handling could result in nickel dermatitis in subjects with a disrupted skin barrier and maintain existing hand eczema (41). Marcussen found that the prevalence of hand eczema was much higher in nickel allergic cashiers than in nickel allergic subjects with other occupations (13). van Ketel found hand eczema in four of seven cashiers with nickel allergy and concluded that absence of hand eczema was mainly explained by the fact that cashiers were not exposed to water and irritants (141). A Swedish study supported the very limited risk of nickel dermatitis following repeated handling of coins (142) whereas a Finnish provocation study showed worsening of nickel hand eczema in two of three nickel allergic cashiers (143). Lidén and Carter determined the soluble surface nickel content (and its nature) of used coinage from the UK, France, and Sweden (144). They found that all coins dissolved nickel into water and artificial sweat, respectively, following a 2 min washing procedure (144). Nickel was mainly present as nickel chloride rather than

nickel citrate or sulfate. Most coins released more than 0.5 µg nickel/cm²/week when analysed with the 1-week sweat test (EN 1811). The authors predicted that the 1 and 2 euro coins would also release nickel as they were cupro-nickel coins (144). On 1 January 2002, selected EU member states replaced their national currencies with the euro, and eight coins were introduced (1, 2, 5, 10, 20, and 50 cent coins as well as the 1 and 2 euro coins). Composition differed as the bicoloured 1 and 2 euro coins contained nickel in the white and yellow parts (25% and 5%, respectively) (144). In accordance, Aberer and Kränke found positive patch test reactions to the 1 and 2 euro coins and positive DMG test results (145). Foti et al. found low reactivity on the thenar region and thumb of nickel allergic patients who were provoked with 1 euro coins for 48 hr (146). Nestlé et al. found positive patch test reactions to the 1 and 2 euro coins and high nickel release rates (>100 µg nickel/cm²/week) when the coins were analysed with the EN 1811 sweat test (147). They measured the galvanic potential between the yellow and the white part of the coins and found that galvanic coupling increased nickel release markedly from the yellow part (147). Jellesen et al. highlighted the complexity of the coin problem by emphasizing that contact with coins is an intermittent frictional contact and that the nickel coin problem should be studied with tribocorrosion (148). They showed that nickel release decreased after rubbing and that oxygen, copper, and zinc rather than nickel was found on the surface of used coins (148). Their findings were in line with results from a cross-over exposure study where 18 subjects (10 nickel allergic and eight non-nickel allergic) handled 10 coins (either nickel-containing or non-nickel-containing) for 5 min followed by 5 min rest, 8 hr a day over a 12-day period (149). No differences were found between the two groups and no signs of dermatitis were observed in nickel allergic subjects (149). Recently, Lidén et al. showed that nickel was released from 1 and 2 euro coins in similar amounts and that nickel was deposited on the fingers and hands after 1 hr of handling (0.09–4.1 µg/cm²) (150). The authors concluded that coin handling could play a role in nickel dermatitis (150) as it is known that a few patients may react to low nickel concentration on the hands (151). Although the euro coin debate has recently calmed down, several studies and letters have addressed this issue over the years (152, 153).

Studies have found high prevalences of nickel allergy in certain occupations (e.g. hairdressers, restaurant workers, and cleaning personnel) but few of these have taken into account socio-economic variables. At present little research has been conducted on the possible association between contact allergy and social status. However,

van den Burg et al. showed that the prevalence of nickel allergy was, respectively, 17% and 27% in male and female hairdresser apprentices and 0% and 13% in male and female nursing apprentices (154). The difference was due to greater nickel exposure prior to occupational exposure as hairdresser apprentices got their ears pierced at a younger age (154). Data from Malmö, Sweden, suggested that the prevalence of nickel allergy was significantly higher among immigrants, the unemployed, and patients on social security than among patients from higher socio-economic groups (155). Furthermore, a German study showed that the prevalence of nickel allergy was higher among nurses (24.9%) and receptionists (29.3%) than among physicians (12.1%), indicating that nickel allergy may be more prevalent in low income groups (156). Thus, occupational study results may have been confounded to a certain degree.

Occupational-cobalt. Since the 1930s, there has been a large increase in cobalt production, which reached 53 700 tons in 2007 (157). Cobalt allergy and dermatitis may develop following exposure to cobalt in the work environment. Cobalt is used to make different alloys: (i) high temperature corrosion resistant superalloys that can be used in the production of jet engines and gas turbine engines; (ii) magnetic alloys; (iii) electro-deposited alloys; and (iv) high strength steels (3). Cobalt is also used as a pigment and drier in paint, for catalysts, and as an additive in fertilizer and animal feed (3). Historically, mainly metal workers, bricklayers and pottery workers have suffered from cobalt dermatitis. Occupational allergic contact dermatitis caused by metallic cobalt dust in a factory producing tungsten carbide alloys was reported for the first time in 1945 (158). In a study on 853 hard metal workers, grinding and etching appeared to involve the greatest risk of developing cobalt allergy (159).

Weak cobalt blue stains neutralize the faint yellow colour produced by iron oxide in clay in pottery factories (160). In addition, cobalt compounds may be used for colouring by dipping, spraying, or decorating the items (160). Pirilä concluded that cobalt compounds used for glaze and decoration played a smaller role in the development of cobalt dermatitis than cobalt exposure from clay in a Finnish factory (160). Fregert and Gruvberger analysed cobalt release from blue and black pottery (161). They found that 21 objects released less than $0.1 \mu\text{g cobalt}/\text{cm}^2/\text{week}$ and that seven objects released $2\text{--}13 \mu\text{g cobalt}/\text{cm}^2/\text{week}$. They concluded that blue and black pottery (especially with a damaged surface) in an acid environment can release cobalt (161). In 1988, two Danish pottery factories changed the production of

porcelain plates by using a special technique developed to withstand the high temperature of porcelain firing. From that point painters were exposed to cobalt dyes containing cobalt–zinc silicate or cobalt aluminate but with only very low traces of, for example, nickel. Over a 4-year period, the level of cobalt in the urine decreased 10-fold and the airborne cobalt exposure level decreased markedly (162).

The significance of exposure to cobalt in cement was underscored in a study by Salinas and Subiza who observed cobalt allergy in 59% of 37 bricklayers and by Zina and Bony who found cobalt allergy in 23% of 172 bricklayers [referred to in (163)]. Cobalt allergy was most often observed together with chromium allergy in clinical reports from that time and later (163–165). Wahlberg et al. investigated the cobalt content in eight brands of cement, 19 detergents, 28 unused mould oils and 28 unused cutting fluids (166). All eight brands of cement contained cobalt ($5\text{--}16 \mu\text{g}/\text{g}$), and three of eight detergents contained cobalt ($2\text{--}3.9 \mu\text{g}/\text{g}$); only very low concentrations of cobalt were found in mould oils and cutting fluids (166). Payne showed that cobalt in cement was soluble in cysteine, glutathione, and synthetic sweat and that cobalt was 500 times more soluble in plasma than in physiological saline (167). A study found that 78% of the 30 most popular domestic detergents and cleaners sold in Spain in 1985 contained cobalt in the range $0.1\text{--}1.4 \text{ mg}/\text{l}$ (168).

An 11-year British survey showed that cobalt allergy accounted for 4% of 22 184 cases of occupational contact dermatitis (169). No decreasing trend was observed over the study period (169). The sex ratio was 1:1 and the prevalence of cobalt allergy seemed to increase with increasing age (169). Occupations associated with cobalt allergy were hairdressers, builders, rail cash/checkout operators, machine operatives, and domestic cleaners (169). Kanerva et al. investigated the incidence of occupational cobalt dermatitis in Finland over a 7-year period (1991–1997) (125). Among 2543 cases, cobalt caused 1.6% and was mainly observed in printers, turners, mechanists, and toolmakers (125). Lidén et al. used the acid wipe sampling technique to evaluate cobalt deposition on the fingers and hands of locksmiths, carpenters, cashiers, and secretaries but found very low levels (mean $0.0002\text{--}0.004 \mu\text{g cobalt}/\text{cm}^2/\text{hr}$) (132). Recently, a broad selection of cobalt-containing hard metal and dental alloys was stored in synthetic sweat for 1 week. The release of cobalt varied significantly from different materials. The highest amount released from the hard metal alloys was $290 \mu\text{g cobalt}/\text{cm}^2/\text{week}$ (170). Furthermore, Julander et al. studied cobalt skin deposition in 24 workers from a plant producing gas turbines and

space propulsion components (133). Cobalt was detected in relatively high amounts in workers sharpening hard metal tools (up to $4.5 \mu\text{g}/\text{cm}^2/\text{hr}$). Cobalt deposition was explained by direct skin contact rather than by airborne dust exposure. Finally, a Finnish study investigated cobalt exposure in 16 different workplaces manufacturing or maintaining blades (58). Workers' exposure to cobalt could be reduced by improving skin protection and personal hygiene in workplaces (58).

Consumer–nickel and cobalt. In 1951, Rostenberg and Perkins made a list of consumer items that could result in nickel sensitization and dermatitis: jewellery, cutlery, kitchen equipment, hardware, sporting goods, wire screens, electrical equipment and many others (171). The authors gave no clinical evidence to support their assertion that these objects could result in nickel allergy and dermatitis, or to what degree. Furthermore, the authors listed possible causes of consumer exposure to cobalt: cutting tools, pigments, tattoo pigments, vitamin B12, and the adhesive of flypaper (171). However, they emphasized that the most important cause of cobalt allergy was exposure to nickel-plated items (171). Between the 1930s and 1960s, dermatologists observed that mainly stocking suspenders (but also jewellery, watches and spectacle frames) caused nickel allergy and dermatitis (10, 12, 30, 172). In 1956, Calnan found that 95% of 400 female nickel allergic patients had stocking suspender dermatitis as their first manifestation of nickel allergy (172). Despite the replacement of stocking suspenders with pantyhose, new sources of nickel exposure appeared and maintained a high prevalence of nickel allergy (173). Gaul made interesting notes on the changing consumer habits in the 1950s: 'people began wearing wristwatches instead of using pocket watches, teenagers began wearing more jewellery, e.g. wearing rings in their necklaces, and babies kept their diapers on with metallic pins' (174). In the late 1960s, Gaul stated: 'wearing earrings is the latest vogue among adolescent girls. The most fashionable earrings require the earlobes to be pierced. A physician may do this, a friend, or a relative; it is possible to buy an instrument to do it yourself ... it is common to insert the jewellery device at once so that the channel stays open' (175). Gaul predicted that the prevalence of nickel allergy and dermatitis would increase sharply if the present fashion of ear piercing remained popular (175).

In 1969, Malten and Spruit reviewed the relative importance of various sources of environmental exposure to nickel (176). They concluded that the most important causative exposures in women were jewellery, nickel-plated garment appliances, and American style kitchenware (176). Also, they suggested that nickel-containing

detergents could increase the prevalence of nickel allergy (176) as Wells found that the nickel content of detergents in England was up to 10 ppm (56) and Malten et al. found that detergents in Holland contained 2–9 ppm nickel (177). From 1965, ethylenediaminetetraacetic acid (EDTA) was added to detergent powders in the Netherlands to reduce bioavailable nickel (177). In England, detergent powders contained no free nickel ions capable of inducing or eliciting dermatitis (56). Malten et al. applied a nickel–EDTA complex on the volar aspect of the arm in three nickel allergic subjects and saw no dermatitis reactions (177). However, since the incidence of nickel allergy did not decrease following the use of EDTA in washing powders, the authors concluded that other nickel exposures were of greater importance (177). In a later study, Allenby and Goodwin showed that the minimum level of nickel required to elicit a positive patch test reaction was higher than the concentration found in fabric washing powders (61). Furthermore, fabric washing powder did not significantly alter the patch test reaction to nickel or provoke reactions in nickel allergic subjects (61).

In 1976–1977, Brandrup and Larsen showed that 63% of 79 nickel allergic patients had or had had dermatitis at the site of skin contact with metallic buttons in blue jeans (178). Most patients were aged 10–30 years and about 21–25% had nickel button dermatitis as their first manifestation of nickel allergy (178). Nickel release was determined in seven of 10 buttons by using the DMG test (178). Thirty-seven metallic clothing objects which gave dermatitis in nickel allergic patients were investigated by energy dispersive X-ray analysis and electron microscopy and by the DMG test (30). In 1982, Boss and Menné showed that ear piercing was an important risk factor for the development of nickel allergy (179); 75% of 16–22-year-old girls from a school for hairdressers had pierced ears done by a jeweller whereas 25% had ear piercings done by friends or colleagues (179). Healing time ranged from 2 days to 8 months and 66.4% of 107 girls reported dermatitis or oozing following the use of jewellery (179). A 1979 general population study from San Francisco showed that ear piercing was a strong risk factor for nickel allergy (180). In 1989, Santucci et al. reported that 98% of patients with earlobe dermatitis were also nickel allergic (181). Furthermore, they noted that Italian girls often had more than one ear piercing (181). Analyses showed that 33 earrings contained and released nickel (30). Santucci et al. showed that nine clasps and clips commonly used in earrings released between 49 and $103 \mu\text{g}$ nickel/12 hr when stored in synthetic sweat (181). Kalimo et al. showed that jean buttons, belt buckles, clasps, wristwatches and

jewellery were frequently reported as items nickel allergic patients first reacted to (182). Menné et al. purchased 54 random metallic consumer items in 1987 and found that 66.6% gave positive DMG test reactions (44). Ingber et al. showed that 36.8% of 1037 metallic consumer items (e.g. watches, rings, earrings, spectacle frames) collected from 500 random patients gave positive DMG test reactions (183). The authors noted that 90% of patients with known nickel allergy were aware of their sensitivity but continued to wear at least one piece of nickel-releasing jewellery (183). Walsh and Mitchell investigated nickel release from 373 new and 50 used spectacle frames by using the DMG test (184); 96% of used frames tested positive and 24.5% of new ones tested positive. In further studies, 6.5% of 395 new spectacle frames gave positive DMG test outcomes (185).

Today, nickel may be used as a constituent in a variety of products including zippers, rivets, safety pins, metal toys, magnets, doorknobs, keys, scissors, handles, and thimbles (4). Reports have investigated nickel release from various consumer items in recent years. Heim and McKean found that 6% of 173 children's clothing fasteners purchased in the USA released nickel when examined by the DMG test (186). The authors reflected that the globalized nature of industry means that fasteners regardless of brand, country of manufacture, or intended market were likely to come from a few large common sources (186). Byer and Morrell found that 10% of 90 pairs of blue jeans and 53% of 47 belts purchased in Chapel Hill, North Carolina, gave positive DMG test reactions (187). A Finnish study investigated pairs of piercing earrings (30 piercing earrings and 36 back pieces) with the 1-week sweat test and found that 17% of the 66 components released more than $0.5 \mu\text{g nickel}/\text{cm}^2/\text{week}$ (46). The only three consecutive DMG test studies have been performed in Stockholm, Sweden. Lidén and Johnsson made a baseline study in 1999, before the EU Nickel Directive came into force, when they assessed nickel release from various consumer products on the Swedish market (188). Head buyers, quality control staff, and shops and chains selling jewellery, watches, spectacles, underwear, and shoes were invited to participate (188). The authors selected 725 different consumer items (jewellery, accessories, watches, spectacle frames, clothing, and shoes) and used the DMG test to assess nickel release. Furthermore, nickel content was investigated in 15 piercing posts by using flame atomic absorption spectroscopy. They found that 25% of the items gave positive DMG test reactions (shoes 59%, hair slides 45%, jewellery and accessories 23%, watches 17%, clothes 16%, and spectacle frames 11%) (188). The nickel content was above 0.05% in 60% of 15 piercing posts. In their follow-up study in 2002–2003, they tried

to make a nearly identical study but made an extension to also include markets (189). They showed that 8% of 786 items were DMG positive (57% of hair slides and more than 20% of belts, bracelets, and necklaces). The largest decrease was observed for shoes (59% in 1999 and 3% in 2002–2003); however, very different types of shoes were tested in the two studies. Also, 17% of 18 piercing posts contained more than 0.05% nickel, a significant decrease when data were compared with the baseline study (189). Finally, in 2010 the same authors showed that 9% of 659 items gave positive DMG test results emphasizing that the decrease had been maintained (190). Occasional reports have emphasized the high frequency of significant nickel release from hair clasps and head bands (191–193). A recent Danish report described a woman with mild scalp dermatitis who accidentally purchased both expensive and inexpensive hair clasps that gave positive DMG test reactions (193). Also, a Danish report from 2002 showed that two (10%) (a finger ring and a toe ring) of 20 jewellery items purchased in 12 different stores in Copenhagen released nickel when examined by the EN 1811 reference test method (194).

In 2007, Bocca et al. showed that three of 10 earring components purchased in Italy released more than $0.5 \mu\text{g nickel}/\text{cm}^2/\text{week}$, but also that less nickel was released from earrings when the results were compared with Santucci's data from 1989 (28, 181). Of note, three pieces also released cobalt (0.013 – $0.18 \mu\text{g}/\text{cm}^2/\text{week}$). In a Korean study, nine pairs of earrings, two belt buckles, two metal buttons and one hook were analysed for nickel release. The belt buckles and the hook released more than $0.5 \mu\text{g nickel}/\text{cm}^2/\text{week}$ (195). A study from 2007 found that 30.7% of 277 inexpensive earrings purchased in San Francisco gave positive DMG test reactions (196). Earrings from stores targeting young girls and women often sold earrings that released more than $0.5 \mu\text{g}/\text{cm}^2/\text{week}$ (196). A recent study performed in major cities in Thailand and China found positive DMG test results in around 30% of inexpensive earrings (29). Due to the growing popularity of body piercing, youngsters and young adults are increasingly exposed to metals (197). Erlich et al. showed that the prevalence of nickel allergy in men increased with the number of piercings (198). Thus, the prevalence of nickel allergy was 4% in non-pierced, 11.1% in one site pierced, and 14.6% in multiply pierced men. Recently, a case of cobalt and nickel dermatitis was observed on the feet of a nurse who used green plastic shoes with a high nickel and cobalt content (199).

The law in EU member states does not permit the use of nickel and cobalt in make-up products whereas nickel as an 'impurity' is permitted. A possible association between

nickel allergy and eyelid dermatitis has previously been suggested following exposure to, for example, eyelash curlers (200), eye pencils (201), mascara (202) and eye shadow (203, 204). In theory, all pigmented make-up products may contain metal allergens including nickel and cobalt (205). Basketter et al. investigated safe levels of nickel in consumer products (mainly household products) and found that they should not contain more than 5 ppm of nickel and that the ultimate target level should be 1 ppm (206, 207). A large Finnish study showed that, among 49 eye shadows, the nickel content was up to 44 ppm and that no product contained less than 1 ppm nickel (205). In addition, the Information Centre for Environment and Health which is funded by the Danish Ministry of the Environment performed an investigation on the content of nickel in mascaras (<http://www.miljoeogsundhed.dk/DEFAULT.aspx?node=5193>). This was done as the Centre repeatedly received complaints from nickel allergic consumers who reported eyelid dermatitis from mascara use. The study found that 19 mascaras on the Danish market contained 16–41 ppm nickel. A recent Danish general population study found no association between nickel allergy and self-reported cosmetic dermatitis from eye shadow or mascara (208). Bocca et al. investigated the content of nickel in moisturizing creams on the Italian market labelled as 'Ni-tested' (i.e. nickel content <100 ng/g). Nickel was present in all the products with levels between 17.5 and 153 ng/g; three skin creams were slightly above the concentration reported on the labels (209). Recently another Italian study showed that 27% of 52 toy make-up products released more than 5 ppm nickel (210).

Exposure to mobile phones with metallic buttons and cases may result in nickel dermatitis (211–213). A Canadian study investigated with the DMG test 22 random mobile phones from eight different manufacturers and found that 10 of these gave positive test reactions (214). Danish investigators visited two international mobile phone manufacturers in Denmark and performed the DMG test at site on 41 mobile phones (215). Eight phones gave positive DMG test reactions and one DMG-test-positive phone had been produced in more than 100 million copies worldwide (215).

A recent Swedish report described allergic cobalt dermatitis following exposure to a necklace that contained and released cobalt (216). Metal release was estimated after 5 min in artificial sweat and revealed that 411 µg cobalt and 6 µg nickel were released. An earlier Danish study warned against the use of metallic cobalt in jewellery as 11 of 19 cobalt allergic patients reacted to discs with 100% cobalt (217). Finally, cobalt exposure may derive from beauty products (218).

Epidemiology of nickel and cobalt allergy

Age and gender. Differential nickel exposure heavily influences age- and gender-specific prevalences of nickel allergy. Thus, the prevalence of nickel allergy was higher in male Kuwaiti dermatitis patients, mainly due to nickel in wristwatches and press studs in underpants (219). Bonnevie, Calnan and Gaul found that nickel allergy was much more common in female dermatitis patients due to nickel in suspenders and jewellery (12, 172, 174). Even today, a strong female predominance is observed worldwide. A recent German study using patch test data from 74 940 patients revealed a strong association between female gender and nickel allergy (prevalence ratio = 3.74; 95% confidence interval (CI) = 3.51–3.98) (220). A review of studies performed in the general population showed that the median prevalence of nickel allergy was higher among women than men (17.1%, range 3.9–38.8% versus 3%, range 0.7–6.8%) (221). The higher prevalence of nickel allergy in women was explained by a higher median prevalence of pierced ears in women than in men (81.5%, range 38.0–91.6% versus 12%, range 4.9–29.2%) (221). Two recent studies have suggested that tobacco smoking might be associated with nickel allergy (222, 223). However, since nickel allergy is uncommon in men and they tend to smoke more than women, the effect of smoking is likely to be very limited.

Calnan found that the majority of female patients with nickel allergy were aged 10–50 years (172). In Lodz, Poland, the prevalence of nickel allergy was significantly higher in female patients under 30 years of age compared with older women (224). Christensen and Möller as well as Menné found that the average age of onset of metal dermatitis was 25 years (225, 226). Kalimo et al. found that the mean age at onset of nickel dermatitis was 29 years in women and 34 years in men (182). Gawkrödger et al. found that the mean age at onset of nickel dermatitis in female dermatitis patients was 20 years and it was much higher in male patients (227). Uter et al. showed that nickel allergy was significantly more prevalent in German patients aged less than 30 years than in patients older than 68 years (prevalence ratio = 3.23; 95% CI = 3.03–3.45) (220). Mørtz et al. patch tested and questioned Danish schoolchildren aged 12–16 years and found that 8.6% of 1146 children were nickel allergic and that 69% of positive reactions were relevant (228). Taken together, the collection of studies supports that individuals are mainly sensitized in their younger years.

Atopic dermatitis. Several authors have been interested in the possible association between nickel allergy and atopic

dermatitis. Steiner was probably the first to call attention to this area as nine of 16 patients with atopic dermatitis had nickel allergy (229). Watt and Baumann found a strong correlation as 15 of 17 girls with earlobe dermatitis were atopic (230). Wahlberg and Skog did not find higher frequencies of atopy in nickel allergic subjects (231). The number of patch test studies that have evaluated the association since then seems endless. A rejection of a possible association between atopic dermatitis and nickel allergy was made by two large German studies including children and adults (220, 232). Furthermore, no general population studies have supported a positive association. Thus, Fedler and Strömer found no higher risk of nickel allergy in subjects with atopic dermatitis than in healthy individuals (233). Nielsen et al. showed that nickel allergy was equally prevalent among those with and without a positive prick test (234). A Polish general population study with 135 participants also rejected an association between atopy and contact allergy (235). Perhaps the introduction of filaggrin genotyping will throw light on this difficult area of allergic contact dermatitis.

Temporal trends over the 20th century. Since the consumer nickel allergy epidemic took off, epidemiological reports have demonstrated increasing prevalence of nickel allergy. In general, the development of cobalt allergy has paralleled the development of nickel allergy. However, patch test centres with large groups of occupational dermatitis patients traditionally report higher prevalences of cobalt allergy than patch test centres with few occupational patients. Marcussen presented patch test data from 36 000 patients seen at the Finsen Institute in Copenhagen between 1938 and 1955 (236). The prevalence of nickel allergy decreased from approximately 1.7% in 1938 to 0.8% in 1945 and then increased to 3.2% in 1955 (236). He concluded that the prevalence of nickel allergy followed the pattern of nickel import in Denmark as both decreased simultaneously with the Second World War and then increased (236, 237). No data on cobalt allergy were given. Baer et al. showed that the prevalence of nickel allergy remained stable in New York at 12% for the period 1937–1962 (238). Modée and Skog showed that the prevalence of nickel allergy in female dermatitis patients seen at the Karolinska Institutet, Stockholm, was 9.4% in 1948–1951 and 10.5% in 1958–1961 (239). However, in male dermatitis patients the prevalence decreased from 7.9% to 1.5% over the same 10-year period, illustrating the decreasing burden of occupational nickel exposure (239).

In 1968, patch test data from 5558 patients seen in six Scandinavian patch test clinics showed that the overall prevalence of nickel allergy was 2% in male patients

and 9% in female patients (240). Cobalt allergy was observed in 5% of male and 5% of female dermatitis patients (240). In Copenhagen, the prevalence of nickel allergy was 1% in male patients and 6% in female patients whereas the prevalence of cobalt allergy was 3% in male and 3% in female patients (240). Similar prevalence data were reported in a European multicentre study from 1967 to 1968 (241). This study showed that nickel and cobalt allergy frequently were clinically relevant (62–84% of all cases) (241). Between 1969 and 1979, the prevalence of nickel and cobalt allergy increased significantly in Swedish female dermatitis patients from, respectively, 10.5% to 18% and 7% to 9% (242). In 1977, Menné questioned 241 hospitalized female patients from different departments and found that 8.3% had a history compatible with nickel allergy (226).

In Lodz, Poland, the prevalence of nickel allergy increased in female and male patients from 7.1% to 43.7% and from 4.3% to 8.5%, respectively, over the period from 1977–1980 to 1985–1988 (224). In Budapest, Hungary, the prevalence of nickel allergy increased from 6.9% in 1978 to 10.7% in 1982 (243). In Spain the prevalence of cobalt allergy increased from 4.5% to 10.0% between 1977 and 1982 and further to 16% in 1985 (168). A high prevalence of isolated cobalt reactions (28%) was found in 183 cobalt allergic patients due to exposure to cobalt in household products (168). The prevalence of nickel allergy in Spanish dermatitis patients increased from 13.8% to 26.1% over a 10-year period (25). The increase was mainly explained by consumer exposure to nickel as 78% of all cases were consumer related (25). In Rome, Italy, the incidence of nickel allergy increased 27% between 1984 and 1988 (181). The authors noted that the prevalence (not given) was much higher than the 5.2% recorded in 1962–1965 (181). Another Italian report showed that the prevalence of nickel allergy increased from 3.9% in 1967 to 31.9% in 1998 among female dermatitis patients [referred in (34)]. Few general population studies have addressed the development of nickel allergy over time. The prevalence of nickel allergy increased in Swedish women from 12.1% in 1964–1965 to 21.9% in 1982 (244). Furthermore, the prevalence of nickel dermatitis in Finnish schoolgirls was 1% in 1975 and 16% in 1987–1988 [referred in (34)]. Nielsen et al. showed that the prevalence of nickel allergy remained stable between 1990 and 1998 in Denmark (245). However, the prevalence of nickel allergy decreased from 16.7% to 11.8% in 15–22-year-old Danes (245). Finally, a review gathered patch test studies performed in the general population between 1966 and 2007 and showed that the proportion of nickel allergy out

of the overall prevalence of contact allergy increased significantly from approximately 5% in 1966 to 60% in 2007 ($p < 0.003$) (221). Taken together, the second half of the 20th century presented consistent increasing prevalences of nickel allergy in Eastern and Western European dermatitis patients.

Recent trends. The prevalence of nickel allergy observed in Asian and North American dermatitis patients has increased significantly in recent decades (246) (personal communication, Professor Li, Beijing University, June 2010). Rietschel et al. showed that young men were 2.33 times more likely to be nickel allergic than old men (95% CI, 1.80–3.01; $p < 0.0001$) and that young women were 1.51 times more likely to be nickel allergic than old women (95% CI, 1.34–1.72; $p < 0.0001$) (247). Between 2001 and 2004, the prevalence of nickel and cobalt allergy in 319 American children with dermatitis was 28.3% and 17.9%, respectively (248). Nickel allergy was relevant in 26% and cobalt allergy in 12% (248). In Asia, the nickel problem is also increasing, e.g. in Korea (195). In Taiwan, the prevalence of nickel allergy increased from 14.3% in 1978–1990 to 23.0% in 1991–2003 (249). In China, the prevalence of nickel allergy increased from 16.1% in 1999–2003 to 23.1% in recent years among dermatitis patients (personal communication, Professor Li, Beijing University, June 2010). In Singapore, the prevalence of nickel allergy in children aged 0–18 years was 40%, markedly higher than the prevalence of nickel allergy in adult patients (250). Finally, in Ethiopia, the prevalence of nickel allergy is also high, reaching nearly 40% (251).

The increasing trends of nickel allergy contrast with the decreasing trend observed in parts of Europe. Johansen et al. showed that the prevalence of nickel allergy decreased significantly from 24.8% in 1985–1986 to 9.2% in 1997–1998 in Danish children aged 0–18 years ($p = 0.0008$) (252). Jensen et al. showed that the prevalence of nickel allergy was 3.9% in Danish schoolgirls who were ear pierced after 1992 and 17.1% in schoolgirls who were ear pierced before 1992 (253). Veien et al. found a significantly decreasing trend of nickel allergy in female patients under 20 years of age in a retrospective longitudinal study performed in a private practice in Denmark (254). The prevalence in young women decreased from 22.1% in 1986–1989 to 16.7% in 1996–1999 ($p < 0.05$) whereas no decrease was observed for older women or men. A recent Danish multicentre study from private practice revealed a lower prevalence of nickel allergy among women from the youngest age group (14.6% in 17–22 year olds) compared with older age groups (31.8% in 23–34 year olds and 31.9% in 35–46 year olds) ($p = 0.03$) (255).

A significantly lower prevalence of nickel allergy was also observed in young Danish female hairdressers compared with middle-aged and older hairdressers (137). In Germany, a significantly decreasing prevalence of nickel allergy was observed following the EU Nickel Directive. Thus, the prevalence of nickel allergy in female dermatitis patients under 30 years of age decreased from 36.7% in 1992 to 25.8% in 2001 (256) and at the same time an increase from 27.9% to 33.2% was observed in 31–44-year-old women. No clear pattern was observed in male patients although the prevalence of nickel allergy seemed to decrease in men younger than 30 years (257). In Sweden, the prevalence of nickel allergy decreased significantly in young women from 33.8% in 1992 to 29.4% in 2000 (258). In Poland, the prevalence of nickel allergy decreased from 15.9% in 1995 to 10.0% in 2004 in female dermatitis patients aged under 20 years (259). Conversely, the prevalence increased in older female age groups. The authors speculated that the changing epidemiology could be explained by increased awareness among consumers or could be a result of the close economic ties to the EU. Bocca et al. recently investigated the development of nickel allergy in Rome, Italy (28). The prevalence of nickel allergy was 54.3% in 931 patients tested in 1994 and 53.5% in 867 patients tested in 2005 (28). Although the authors did not present gender- and age-specific strata, they concluded that the EU Nickel Directive had so far been insufficient (28). Of interest, a very recent Italian study showed that nickel allergy was significantly higher in female patients aged 26–35 years compared with female dermatitis patients aged 15–25 years (260). Although studies have indicated a change in the epidemiology of nickel allergy in Europe, research is still warranted to estimate the effect of nickel regulations.

Clinical expression

Allergic contact dermatitis. Nickel and cobalt contact dermatitis may involve all body parts but is most frequently located on the hands and face. The clinical picture varies depending on its chronicity. Acute dermatitis is characterized by erythema, oedema, papules, vesicles, and weeping, whereas chronic dermatitis is scaly, dry, and fissured. In general, risk factors of allergic contact dermatitis include the inherent sensitizing potential of an allergen (261), elevated allergen concentration (dose per unit area) (262), high frequency of exposure (263), occlusion (264), long exposure time (265), the presence of penetration-enhancing factors and an altered skin-barrier function (266).

In 1956, Calnan suggested that nickel dermatitis could be divided into primary eruptions at the site of metal

contact and secondary eruptions at areas of spread. The secondary sites were elbow flexures (79.3%), eyelids (26.4%), sides of neck and face (25.4%), inner aspects of the thighs (2.1%), and the entire body (generalized) (4.7%) (172). Calnan suggested that only suspender dermatitis resulted in secondary eruptions; however, this belief was later rejected by Marcussen (267). In 622 nickel allergic patients, he found that secondary eruptions occurred in 79.7% but that they were different in men and women as a result of different exposures (267). He therefore suggested that secondary eruptions to a certain degree reflected direct contamination from skin contact with nickel-plated items (267). He also found that spread to elbow flexures was independent of the primary site of eruption but positively associated with the strength of the positive nickel patch test reactions and to the duration of the primary eruption prior to spread (267). Marcussen also patch tested 300 dermatitis patients and noted that 10 of 12 cobalt allergic patients had the same clinical pattern and distribution as patients with nickel allergy (268). Christensen and Möller found secondary spread in 51% of 66 female nickel allergic patients with hand eczema (225). Wahlberg and Skog showed that the hands were the first location of nickel dermatitis in a large proportion of nickel allergic patients, suggesting a change of epidemiology (231). Pirilä found erythematous papulovesicular type dermatitis on the hands and forearms of pottery workers in Finland (160). Vilaplana et al. found no specific features of cobalt allergy but observed that 72% of cobalt allergic patients had hand eczema (168). In 1971, Cronin noted a change in the clinical pattern of nickel dermatitis (173). Thus, suspender dermatitis was rarely seen whereas earring dermatitis was often observed. Today, nickel dermatitis at the site of metallic buttons from blue jeans is referred to as prominent pruritic periumbilical papules as it is a relatively frequent clinical sign in American pediatric dermatitis patients (269).

Hand eczema. Calnan was one of the first to note that the frequency of hand eczema was elevated in subjects with nickel allergy (172). Of 400 female nickel allergic patients 20% suffered from hand eczema although direct metal contact on the hands was only observed in very few patients (172). Ingber et al. found that 60% of nickel allergic patients had hand eczema; however, the authors had no control group (183). Van Joost and Van Everdingen found that combined nickel and cobalt allergy was frequently associated with hand eczema as 48% of cobalt and nickel allergic patients had hand eczema (270). General population studies have also addressed the possible association between nickel allergy and hand

eczema. Agrup found positive patch test reactions to nickel in 56 (12.1%) of 462 females with a history of allergic hand eczema (271). In 49, nickel was found to be the main cause or a contributory cause of hand eczema (271). Peltonen showed that hand eczema was more common in nickel allergic subjects (20.5%) than in subjects without hand eczema (percentage not given) (272). Menné et al. studied the association between nickel allergy and hand eczema in a sample of female twins and found that nickel allergic women had an increased risk of developing hand eczema but also that women with hand eczema had an increased risk of developing nickel allergy (273). Mørtz et al. found that nickel allergy was associated with hand eczema in 12–16-year-old Danes ($OR = 2.36$; 95% $CI = 1.39–4.01$) as the prevalence of nickel allergy was 22.4% among those with hand eczema and only 9.4% among those without (228). Bryld et al. found that nickel allergy was a risk factor for the development of hand eczema in a sample of female twins (274). This association was stronger among subjects with strong patch test reactions (3+) than for those with weaker test reactions (274). Josefson et al. made a follow-up study of 735 schoolgirls who had been nickel patch tested 20 years earlier (275). They found no difference in the frequency of self-reported hand eczema after 15 years of age in nickel allergic and non nickel allergic women (275). The authors confirmed their initial finding when they performed clinical examinations as well as nickel patch testing in 369 of these women (276). However, they found an increased risk of hand eczema in women with nickel allergy but without a history of childhood eczema (276).

Hand eczema (externally provoked). Recent experimental studies have convincingly supported an association between hand eczema and external metal exposure. Banfield et al. found that one nickel allergic patient reacted to a lower nickel concentration on the hand than on the back (151). Nielsen et al. investigated whether it could be assumed that nickel or cobalt exposure was a contributing factor of hand eczema (63, 64). In a double-blind placebo controlled exposure study, a statistically significant aggravation of hand eczema was observed when patients with nickel allergy and mild to moderate hand eczema were exposed to 10–100 ppm nickel for 10 min per day over a 2-week period (63). In a similar experimental study with three cobalt allergic patients, exposure to 10–50 ppm cobalt in the first week and 100–200 ppm cobalt in the second week did not result in the formation of vesicles (64).

Hand eczema (internally provoked). It is generally agreed that oral ingestion of metals can result in vesicular hand

eczema (277–280). Furthermore, several reports have described the development or aggravation of vesicular hand eczema following insertion of metallic implants such as orthodontic wires, dental plates, infusion needles, clips, pacemakers, and orthopaedic plates and screws (280). Christensen and Möller made a clinical study on 66 nickel allergic female patients with hand eczema (225). This constituted a subgroup of female hand eczema patients of whom 42% were nickel allergic (225). Of these, 77% had pompholyx type hand eczema and the rest allergic, irritant, atopic or nummular hand eczema. On average, hand eczema started at age 32 years and was mostly preceded by metal allergy for 6 years. Exacerbation following skin contact with metallic items seemed to be a minor problem as it was only reported by 24% (225). Subsequently, Christensen and Möller performed a provocation study in which 12 female nickel allergic patients with hand eczema of pompholyx type were exposed externally and internally to nickel (281). External exposures included dipping of hands in a bowl of water with DMG-test-positive coins, clips, and keys, whereas internal exposure was achieved by ingestion of either 5.6 mg nickel or placebo in a double-blind manner (281). Before and after exposure, vesicles were counted under a magnifier. External exposure did not increase the number of vesicles whereas internal exposure on average increased the number of vesicles by eight times in nine of 12 patients (281). Placebo exposure did not increase the number of vesicles. The authors stated that normal daily nickel intake was around 0.3–0.76 mg, well below the 5 mg used in the study (281). In further studies, Christensen and Möller investigated whether nickel could be released from cooking utensils and exacerbate nickel dermatitis (282). Among 12 new and 15 used saucepans of different materials (stainless steel, aluminium, or Teflon/enamel coating), nickel was only released from stainless steel pans when pH was low (282). They concluded that nickel allergic subjects should avoid stainless steel cooking utensils, as different foods were acidic (apples, lingonberries, and other). Flint and Packirisamy showed that new stainless steel pans released more nickel than used pans but also that the contribution made by stainless steel cooking utensils to nickel in the diet was very small and within the day-to-day variation of nickel intake (283). They concluded that there was no reason for nickel allergic subjects to avoid the use of stainless steel utensils, even for the cooking of acid fruits (283).

Kaaber et al. carried out an open prospective study and found an effect of a 6-week low-nickel diet in nine of 17 nickel allergic patients who had previously reacted to ingestion of 2.5 mg nickel (284). Menné and Thorboe showed that the nickel concentration in urine was elevated in four nickel allergic female patients during

outbreak of vesicular eruptions (285); and de Jongh et al. found that the concentration of nickel in the urine and plasma of one nickel allergic female patient fluctuated to some degree with the clinical course of dermatitis (286). A recent study on systemic nickel allergy found a dose–response relationship between nickel ingestion and dermatitis flare-up (287). Thus, 0.3, 1 and 4 mg nickel sulfate hexahydrate resulted in dermatitis in 40%, 40% and 70% of nickel allergic subjects (287). From this study, it can be concluded that a single dose of 4 mg nickel will result in widespread dermatitis in most nickel allergic patients (287). Cobalt allergic patients may also experience worsening of their vesicular hand eczema upon oral exposure to 1 mg cobalt (288, 289). It is currently unknown why vesicular hand eczema can be observed in both metal sensitized and non-sensitized individuals (288, 290). Possible explanations include false negative patch test reactions although this is speculative (280), a ‘placebo’ effect, or direct activation of immunological reactions caused by metal ingestion (291). Recently, the degree to which systemic reactivation contributes to the pathogenesis of vesicular hand eczema was questioned by Guillet et al. (292). The authors showed that only 6.7% of 120 patients had an internal reactivation of their hand eczema from food, drugs, or metals (292). The strength of the association between metal intake and vesicular hand eczema may have decreased in recent decades, perhaps as a result of decreased external nickel exposure.

Implant or device failure. Metals in contact with body fluids corrode and release metal ions that may bind to proteins and activate T-cells. This can result in allergic contact dermatitis in the skin overlying the implant or in device failure. The majority of intracoronary stents are made from stainless steel. Coronary in-stent restenosis has been associated with nickel allergy in some studies (293–295) whereas others have rejected an association (296–298). Total hip arthroplasties are in some instances performed using second generation metal-on-metal prostheses which typically consist of a forged, high-carbon, cobalt–chromium–molybdenum material (299). They tend to produce a high load of metal particles, nanometer sized, which accumulate to a high specific surface area (300). Recent studies have shown that patients with metal-on-metal prostheses may have elevated serum and urine concentrations of cobalt and chromium (301). Accumulated reports on metal allergy in total hip arthroplasty patients showed that the prevalence of metal allergy was 25% in patients with well-functioning hip arthroplasties and 60% in patients with a failed or a poorly functioning implant (301).

Although these prevalences are much higher than general population estimates, it remains unknown whether metal sensitivity causes device failure or whether device failure causes metal sensitivity. Thomas et al. recently showed that among 16 patients with revised metal-on-metal arthroplasty and peri-implant lymphocytic inflammation, 81% had metal allergy based on patch testing and/or lymphocyte transformation testing (302). Allergic reactions were mainly observed against cobalt, chromium, nickel, and molybdenum, and the authors concluded that complications reflected allergic hyper-reactivity (302). A recent Danish case-control study found no increased risk of implant failure among dermatitis patients with metal sensitivity to nickel, cobalt, or chromium (303). This area is in need of immediate attention as western populations are aging (304).

Tolerance. Although nickel is used in many different consumer items, only a subset of nickel-exposed individuals become allergic. This may possibly be explained by uneven environmental nickel exposure, genetic predisposition, or tolerance induction. Retrospective epidemiological studies have demonstrated tolerance to nickel following oral exposure (154, 228, 305, 306). Tolerance is an important research area in immunology but the clinical implications remain to be elucidated.

Prognosis. Various authors have assessed the prognosis of nickel allergy and dermatitis. Marcussen estimated the chronicity of nickel allergy and found that most patients had primary as well as secondary eruptions for a period of 2–3 years (267). One explanation for the long duration was given by Gaul as many of his nickel allergic patients continued to wear their jewellery even though it resulted in nickel dermatitis (174). The patients would typically shift the jewellery to another skin area and still deny a cause-effect relationship. He noted that they would declare the problem to be bad nerves (174). Similar findings were reported from Israel and Italy as nickel allergic women continued to wear jewellery despite dermatitis (181, 183). A Belgian follow-up study including female patients with nickel allergy showed that 75% still suffered from nickel dermatitis when they did not avoid skin contact with metallic items and that 53% still had hand eczema (307). Schubert et al. questioned and re-patch tested 100 patients with nickel allergy 3 years after their first visit and found that nearly all patients reported intolerance to metallic consumer items such as jewellery, watches and buckles (308). A total of 68 patients were free of dermatitis whereas 13 suffered from chronic hand eczema; 42 had changed their job, mostly to white collar occupations (308). Kalimo

et al. questioned 91 patients who had been found to be nickel allergic at least 10 years earlier (182). They found that 73 had suffered from dermatitis (61 with hand eczema) and that 18 had been symptom free. They showed that current dermatitis was significantly associated with current exposure to metallic items such as buttons, belt buckles, watches, and jewellery and suggested that metal avoidance gave a better prognosis (182). The prognosis was significantly poorer in atopic dermatitis patients with nickel allergy and mild skin problems in their teens compared with atopic dermatitis patients without nickel allergy (309). Christensen made a 6-year follow-up study of nickel allergic hand eczema patients and showed that 30% of 63 female patients with nickel allergy were free from dermatitis (310). A poor prognosis was found in patients with hand eczema of pompholyx type (310).

Diagnosis of nickel allergy

Contact allergy to cobalt and nickel may be diagnosed by performing *in vitro* analyses, intradermal testing, and patch testing.

In vitro testing. *In vitro* tests such as the macrophage migration test, the lymphocyte blastic transformation test, and the lymphocyte transformation test have mostly been used in research projects (143, 311, 312). None of these has become a routine analysis in the clinical setting, even when two or three methods have been combined.

Intradermal testing. The use of intradermal testing has been evaluated on several occasions and it has been suggested that it may be useful in patients with low grade allergy (313–316). In this test, nickel sulfate or cobalt chloride is typically injected intradermally on the volar aspect of the forearm and readings are performed 1–3 days later.

Patch testing. In their pioneering patch test study, Schittenhelm and Stockinger used open application of nickel sulfate taken directly from the nickel sulfate bath (6). A compress with a 10-fold dilution of the nickel solution used during the work process was applied for 4 hr on the forearm (at the site of previous dermatitis) (6). Although nickel chloride is the relevant salt in relation to daily nickel exposure, nickel sulfate is generally used for patch testing. The background for this may be that Bonnevie used nickel sulfate in his baseline test series (12). Nickel chloride is more irritant than nickel sulfate which could increase the allergenic potential of nickel (317). Sulfate may inhibit nickel penetration through the skin compartments (54)

and the bioavailability of nickel sulfate is therefore lower than for nickel chloride. Gawkrödger et al. patch tested 111 patients with 5% nickel chloride in water as well as 5% nickel sulfate in petrolatum and found that nickel sulfate was more sensitive (318). They found that 71% reacted to both but that 22% reacted to nickel sulfate alone and 8% to nickel chloride alone. The authors concluded that nickel sulfate was more likely to detect nickel allergy than nickel chloride even though this belief opposed previous findings (118, 318, 319). Nethercott and Holness summarized 12 patch test studies where nickel patch testing was performed with either 2.5% or 5% nickel sulfate (320). They found that 12% reacted to 2.5% and only 10% to 5% nickel sulfate and concluded that the difference was due to variations in interpretation of patch test reactions as well as differences in the populations studied (320). The association between clinical relevance and patch test response to nickel was evaluated in 447 patients and it was found that more than 90% of positive responses could be related to a past or present problem with contact dermatitis (320).

In an experimental model, Hindsén and Bruze demonstrated that nickel allergic patients had higher patch test reactivity at sites with previous allergic nickel dermatitis reactions and also that they had lower test reactivity at sites of previous sodium lauryl sulfate (SLS) application (321). In further studies, Hindsén et al. induced nickel dermatitis on the lower back on three different occasions (and sites) in 25 nickel allergic patients (322). This was done 8, 4 and 1 month before final nickel challenge patch testing. At that point, four nickel dilution test series were applied at the three different sites of previous nickel dermatitis and at one control site (322). Significantly higher test reactivity was found at the sites of previous nickel dermatitis and there was a stronger reaction at sites with most recent dermatitis (322). Hindsén et al. also found significantly more flare-up reactions of the most recent positive nickel patch test reactions (1 month versus 8 months) in nickel allergic patients challenged orally with nickel (323).

Wells showed that nickel was found in high concentrations in the orifices of the sweat ducts in positive patch test reactions (56). Follicular reactions may be divided into pustular reactions (one or more follicular pustules) or poral reactions (punctuate erythema, sometimes slightly papular and/or haemorrhagic around the hair follicle openings, presenting as small dots) (324). Nickel and cobalt may produce non-allergic 'poral' reactions, presumably because of the toxic effects on the acrosyringium. As Menné and Calvin found inflammatory reactions around the orifices of both the sweat ducts and the hair follicles in a biopsy of a follicular

reaction (317), they concluded that follicular reactions could be reactions in the sweat ducts and the hair follicles that were allergic rather than irritant. They found several follicular reactions at concentrations that frequently gave allergic reactions but also at concentrations that did not elicit allergic reactions (317). Fischer and Rystedt found that follicular reactions were more common when testing was performed with petrolatum than with water and also that they were reproducible for nickel in 18% of cases and for cobalt in 48% of cases (324). History and clinical examination did not suggest that poral reactions were suggestive of allergic sensitization (324). Storrs and White showed that 32% of 222 patients had irritant reactions to cobalt and that these reactions were poral and seemed to reflect a toxic effect of cobalt on the acrosyringium (325). These data prove that it may be difficult to interpret follicular reactions and make clinical judgments from them. Recently, it was shown that it is possible to make a distinction between early (6–8 hr after exposure) allergic and irritant patch test reactions as follicular spongiosis may be observed in allergic reactions (326).

Cobalt is a difficult substance to investigate. Thus, Veien and Svejgaard found negative lymphocyte transformation in six patients with weak test reactions but positive lymphocyte transformation in eight patients with definite positive reactions (327). Fischer and Rystedt retested cobalt allergic subjects over a 6-month period and found that seven (100%) of seven patients with +++ reactions were retrieved, that 22 (73.3%) of 30 ++ reactions were retrieved and finally that three (17.6%) of 17 + reactions were retrieved (159). Based on history, retesting, and serial dilution testing, they found that only 39 (62.9%) reactions of the initial 62 positive reactions were truly allergic (159).

Longitudinal studies have shown that nickel patch test reactivity may fluctuate over time. When Marcussen re-patch tested 27 nickel allergic patients after 1–20 years, only one patient had lost nickel sensitivity (328). Keczkes et al. showed that 14 of 14 nickel allergic patients maintained their nickel allergy over a 10-year period (329). A Swedish follow-up study found that 96% maintained their nickel allergy over a 6-year period (310). However, a more recent Danish study found that 19 (33%) of 56 positive reactions to nickel sulfate were lost over a 12-year period (330). For cobalt chloride, five of 14 positive patch test reactions were lost (330). Katsarou et al. investigated persistence of cobalt allergy in 180 cement workers over a period of 2–6 years (331). At baseline patch testing, 98 workers had a positive test reaction to cobalt (either ++ or +++). On follow-up patch testing, 39 (40%) had lost their reactivity (331). The authors found that in subjects

with persistent reactivity circulating CD45RO+ T-cells could be isolated, whereas in subjects with lost reactivity CD45RA+ could be isolated (331). Two Danish and one Swedish prospective patch test studies performed in the general population supported a high reproducibility rate for nickel allergy (276, 332, 333). Thus nickel allergy persisted in 79% and 83% of Danish adults and twins, respectively, over an 8-year period and in 74% of Swedish children growing older. In Danish twins, the persistence rate for cobalt allergy was 75% (332). It is worth considering that the level of nickel exposure may have decreased and that some of the lost reactions are because of allergen avoidance. Finally, studies have also compared the reactivity to identical allergens by using different patch test systems. Lazarov et al. compared the TRUE® (thin-layer rapid use epicutaneous) test with IQ chambers and found that the concordance rate was 79% for nickel but only 27% for cobalt (334). Jensen and Andersen concluded that our knowledge is still inadequate and that it is unknown whether lost reactions are explained by allergen avoidance or other factors (330).

The outcome of patch testing may vary depending on the time of patch test reading. Geier et al. showed that when nickel sulfate patches were read beyond day 3, more positive reactions were found (day 4, 10.5%; day 5, 19.4%; day 6, 17.5%) (335). Jonker and Bruynzeel showed that the incidence of positive nickel patch test reactions was 10.5% when readings were done on day 6 or 7 and not only on day 3 (336). Uter et al. showed that 26.6% of negative patch test reactions to nickel sulfate read on day 2 became positive on day 3 (337). A Danish general population study including young girls showed that additional readings performed on day 4 (and not only on day 2) resulted in 18–29% more positive reactions to nickel sulfate (338). A Swedish general population study nickel patch tested adult women and found that 15 had doubtful reactions on day 3 (276). These were further evaluated with a nickel dilution test series and seven had negative test reactions and eight had positive test reactions.

One case letter suggested that the outcome of patch testing could fluctuate with the menstruation cycle (339). However, other patch test studies have not confirmed this in series of nickel allergic patients (340–343). One recent Italian study by Bonamonte et al. showed that the ovulatory phase of the menstruation cycle inhibited allergic responses in 30 nickel allergic subjects (344). A recent Swedish study showed that the variability of nickel patch test reactivity over time was to some degree associated with fluctuations in systemic T-cell reactivity but the authors concluded that other factors were also of importance (345). A German study showed

that the outcome of patch testing may be influenced by local weather conditions (346). The odds of irritant and doubtful reactions to cobalt and nickel increased during cold or arid conditions. Strong allergic reactions were essentially independent of weather conditions (346). Taken together, it may sometimes be advisable to repeat testing at a later occasion or to perform serial dilution testing and repeated open application testing (ROAT) in subjects where contact allergy is strongly suspected.

Sensitization

Cobalt (isolated). Isolated cobalt allergy is generally uncommon although a previous Spanish study showed that 28% of 183 cobalt allergic patients had isolated cobalt allergy due to cobalt exposure from household products (168). Traditionally, isolated patch test reactions to cobalt have been associated with occupational exposure as they were often seen in pottery and metal workers (158, 160). Only 1.2% of 4034 Swedish dermatitis patients had isolated cobalt allergy (i.e. no simultaneous reaction to nickel or chromium) whereas 5.8% had cobalt allergy in combination with other contact allergies (347). Patients with isolated cobalt allergy tended to react to other contact allergens such as rubber (347). It was therefore hypothesized that cobalt allergy was a secondary phenomenon indicating general skin vulnerability (347). Patients with isolated cobalt allergy were of both genders and all age groups (347). Fischer and Rystedt found that in 39 metal workers with true allergy to cobalt, 24 (61.5%) had isolated cobalt allergy (159). A recent Spanish study showed that 10.8% of 10 092 patients had isolated cobalt allergy (348). Hegewald et al. showed that isolated cobalt allergy was significantly associated with young age. A modest positive association was observed between female gender and cobalt allergy (OR = 1.36; 95% CI = 1.16–1.59) (349). Finally, the odds of being allergic to other contact allergies were elevated in patients with cobalt allergy (349).

Cobalt and nickel (concomitant). From the 1930s onwards, it was believed that concomitant nickel and cobalt allergy was a result of cross-sensitivity rather than dual sensitization (350–352). It was postulated that cross-sensitivity acted on the atomic level and that it was likely to depend on the valence state of the metals (351). At the time, cobalt testing was complicated by nickel contamination in the cobalt salts (347). Today, this is not likely to be a problem although it may affect the outcome of patch testing in very sensitive subjects (353). Haxthausen used chemically pure nickel and cobalt to sensitize 12 subjects (eight with nickel and four with cobalt), and found that four of

seven sensitized subjects reacted to both allergens upon re-exposure [referred in (354)]. However, there were several possible errors in the study (354). Epstein reviewed the literature in 1955 and found that, among 102 patients tested, 65% had concomitant positive patch test reactions to cobalt and nickel (351). Some authors even chose to discard the use of cobalt chloride for routine patch testing as positive cobalt test reactions were mostly found in nickel allergic patients (355). Rostenberg and Perkins reflected on the curious finding that metals with similar reactivity such as chromium, copper, and platinum did not cross-react with nickel and stated that there were serious defects in current knowledge (171). Pirilä and Förström showed that the pattern of concomitant cobalt and nickel allergy differed between various groups and that it relied on different quantities of exposure (354). Thus, nickel platers mainly suffered from nickel allergy alone whereas pottery workers suffered from cobalt allergy, even though some subjects in both groups were also sensitized to cobalt and nickel, respectively (354). The authors predicted that the high prevalence of concomitant nickel and cobalt allergy would decrease, and even disappear, as the use of cobalt as a brightener was gradually replaced by organic brighteners (354). A suspender factory that used large amounts of cobalt to produce 8 million pairs of suspenders between 1949 and 1955 abandoned cobalt as it was expensive and less corrosion resistant than imidazole for example (354).

Vandenberg and Epstein showed that experimental cobalt exposure did not result in dermatitis in eight nickel allergic subjects (356). Cavellier et al. made an exposure study in guinea pigs and showed that cobalt allergic animals reacted to nickel and vice versa (24). Subsequently, Lidén and Wahlberg studied cross-reactivity by exposing 15 cobalt sensitized guinea pigs to nickel sulfate (357). Nickel and cobalt did not cross-react and the authors concluded that cross-reactivity among metals was not restricted to periods in the periodic table (357). This finding was supported in a later study (358). Moulon et al. isolated nickel-specific T-cell clones from the peripheral blood of metal sensitized human donors and tested them for cross-reactivity to other sensitizing metals (80). They found only one nickel-specific clone that was able to weakly cross-react with cobalt (80). They concluded that no cross-reactivity exists between cobalt and nickel as they have different coordination geometries in their protein bound state (80). Van den Broeke et al. found that the extent of nickel and cobalt mediated free radical generation depended on metal-ion complexes and the biological oxidants present (72). This could possibly support findings from previous animal and cellular studies that have found absence of cross-sensitivity between nickel and cobalt.

Taken together, most studies show that there is no cross-reactivity between nickel and cobalt. However, Hindsén et al. recently performed an oral nickel challenge in dermatitis patients and found that previous cobalt patch test reactions flared up in some patients (359). This finding may point towards a degree of cross-sensitivity between nickel and cobalt and deserves further attention (359).

Menné found that 36 (30.3%) of 119 female dermatitis patients had simultaneous nickel and cobalt allergy (360). Furthermore, nearly all (34 of 36) patients with hand eczema had concomitant nickel and cobalt allergy whereas no trend was observed for those with isolated cobalt allergy. The same pattern was observed in 71 female twins from the general population although the prevalence was much lower (360). Thus, combined cobalt and nickel allergy was mainly seen in patients with hand eczema. Menné hypothesized that cobalt allergy was a secondary phenomenon to nickel allergy in patients with hand eczema. This finding was reconfirmed in Swedish metal workers where hand eczema was more severe in subjects with combined nickel and cobalt allergy (361). A 6-year follow-up study also found a poor prognosis for dermatitis patients with combined cobalt and nickel allergy (310). Lammintausta and Kalimo evaluated whether nickel allergic patients had an increased risk of developing other allergic reactions but only found an increased number of positive reactions to cobalt which was likely to be explained by simultaneous exposure (362). In an animal study, Lammintausta et al. showed that animals sensitized to both nickel and cobalt reacted more readily than animals that were only sensitized to one of the two metals (363). In 11 516 patients, Kränke showed that 23.4% of nickel allergic patients were also sensitized to cobalt and that 78.9% of cobalt allergic patients were sensitized to nickel (364). Lisi et al. performed extended testing in 60 cobalt allergic patients and showed that 93% had other metal allergies (365). Prevalent allergens included nickel, palladium, and chromium (365). Concomitant allergy to cobalt and nickel was strongly associated with female gender and young age in German dermatitis patients (OR = 6.80; 95% CI = 5.65–8.19) (349). Uter et al. performed serial dilution patch testing with nickel and included 332 nickel allergic patients in their data analyses (366). Most patients were tested with cobalt, chromium, and palladium and 103 were tested with nickel and palladium metal plates. Subjects who reacted to low concentrations of nickel sulfate (0.1% or less) also tended to react to nickel metal plates, palladium, and cobalt (366). Finally, Brasch and Geier showed that the frequency of concomitant cobalt and nickel allergy was related to the strength of nickel allergy (232).

Thus, individuals with strong nickel reactions had higher prevalences of concomitant cobalt allergy (232).

Determination of threshold

Cobalt. Cobalt is a strong sensitizer (grade 5) (367). Wahlberg investigated the elicitation threshold of dermatitis to cobalt and nickel by performing serial dilution testing in patients with isolated and simultaneous allergy to cobalt and nickel (368). He was not able to establish a lower threshold level for cobalt allergic patients but found that the mean threshold value was higher for patients with simultaneous nickel and cobalt allergy than the threshold level for patients with isolated cobalt allergy (368). Fischer and Rystedt performed serial dilution testing in 32 cobalt allergic hard metal workers and found that some reacted to even very low concentrations (19 ppm) of cobalt (159). Allenby and Basketter also investigated the minimum eliciting patch test concentration of cobalt in nine cobalt allergic patients (369). They performed serial dilution patch testing with aqueous cobalt chloride on normal skin and on skin pretreated for 24 hr with sodium dodecylsulfate (369). Of note, five of the patients who had previously reacted to 1% cobalt in petrolatum did not react to this substance and concentration in the new study. On normal skin, the minimum eliciting concentration of cobalt chloride was 10 000 ppm. When skin was pretreated with surfactant, five of six subjects reacted to 100 ppm and three subjects reacted to 10 ppm (369). In guinea pigs, 10–100 ppm cobalt chloride gave positive test reactions (369). A Polish study with 72 cobalt allergic patients identified an elicitation concentration at 50 ppm (370). Julander et al. recently showed that four of 19 cobalt allergic dermatitis patients reacted to 320 ppm cobalt upon patch testing, the lowest concentration used in their serial dilution which was not used to set the elicitation threshold of cobalt (170).

Nickel. Nickel is a moderate sensitizer (grade 3) (371). The threshold level of nickel allergy has never been accurately established. Vandenberg and Epstein showed that exposure to 25% nickel chloride dissolved in water with 0.1% SLS resulted in nickel allergy in 9% of 178 healthy male volunteers (356). Kligman showed that 12 of 25 subjects became nickel allergic when they were exposed to 10% nickel sulfate following skin irritation (261). Räsänen et al. observed nine women, with no prior symptoms suggestive of nickel dermatitis, following ear piercing (372). Six developed itching, swelling, or discharge on the earlobes, which was sufficient to terminate the use of earrings. They all displayed positive

nickel patch test reactions and had their earrings analysed for nickel release (372). Higher nickel concentrations were found in plasma (in $\mu\text{g}/\text{cm}^2/\text{week}$: 0.15, 0.17, 2.03, 10.06, 22.44, and 104.59) than in distilled water (in $\mu\text{g}/\text{cm}^2/\text{week}$: range 0.02–0.79) (372). These data suggested that there is wide variation in susceptibility to nickel sensitization. Finally, Larsen and Brandrup found that nickel release was 100–1000 times higher from buttons suspected of inducing primary nickel allergy compared with buttons eliciting nickel dermatitis in patients already nickel allergic (373). This emphasizes that the elicitation threshold is generally the same or lower than the sensitization threshold (207).

By using a cup method (6 hr exposure to the allergen on the forearm), Malten and Spruit estimated that 1 μM nickel/l was a safe environmental concentration for nickel allergic individuals (176). However, a corrosion study suggested that this limit would be reached within seconds if different nickel-containing items were placed in a sweat solution (35). In a Swedish nickel release study investigating ear-piercing kits and earrings, Fischer et al. found that nickel allergic patients would react to earrings releasing nickel down to 0.05 μg (33). Unfortunately, no area measurements were given. A Korean woman had facial dermatitis following repeated exposure to underground water that contained 53 μg nickel/l (374). Emmett et al. investigated the provocation threshold by patch testing 12 nickel allergic subjects with nickel dilution series and defined 1.5 μg nickel (0.0316%) as the concentration that was significantly different from zero according to Duncan's multiple range test (42). However, the authors found a 250-fold variation in the provocation threshold in nickel allergic patients and defined 0.15 μg nickel as a safe limit (42). A 250-fold variation has also been found by other investigators (341). When Emmett et al. compared the nickel patch test provocation threshold with the concentration of nickel release from earrings after 7 days in the sweat test, they found that nickel release exceeded the threshold of nickel allergic subjects by a factor of 1.4 to 93 (42). Recently, Fischer et al. found that the elicitation of nickel dermatitis was influenced by the size of the area and therefore the total amount of applied nickel (at low concentrations but not high concentrations) (375).

Wall and Calnan found allergic nickel dermatitis on the hands in seven workers from the electroforming industry who had been exposed to 42.5 ppm nickel (118). Menné and Calvin established the minimum eliciting threshold concentration of nickel dermatitis in 51 nickel allergic subjects after open application of nickel on the forearm skin with and without surfactant (317). They found no positive reactions to 100 ppm nickel or less

(with or without the addition of SLS) and underscored that this level was much higher than that found in water, detergents, and household products (317). Allenby and Goodwin used occluded patch testing on the back and found that 14 of 25 nickel allergic subjects reacted to 112 ppm and that one subject reacted to 1 ppm on healthy skin (61). In further studies, Allenby and Basketter showed that three of 20 nickel allergic subjects reacted to 10 ppm on the back and that the dorsal aspect of the arm was more reactive than the back with three of 20 patients reacting to 5 ppm (62). Despite a threefold increase of nickel in fingernails following 23 days of daily treatments with surfactant and nickel in concentrations up to 1 ppm on the hands, no allergic dermatitis reactions were observed (62). Based on exposure studies, two repeated reviews of the literature recommended that consumer products should not contain more than 5 ppm of nickel and cobalt and that the ultimate target level should be 1 ppm (206, 207). Fischer et al. recently reviewed the literature of studies using occluded nickel exposure and concluded that studies were often difficult to compare as materials, substances, methods, and details of the study conditions varied (377). Ten patch test studies fulfilled the inclusion criteria and were used to make a dose–response curve (377). The effective dose for 1% of the population (ED1%) was 0.07 $\mu\text{g nickel}/\text{cm}^2$ (range 0.08–0.38), the ED5% was 0.44 $\mu\text{g nickel}/\text{cm}^2$ (range not given), and the ED10% was 1.04 $\mu\text{g nickel}/\text{cm}^2$ (range 0.24–3.05) (377). The authors concluded that open exposure studies gave remarkably similar results but that the elicitation threshold for single open exposure was six times higher than for single occluded exposure (377). A recent experimental study aimed at extrapolating patch test exposure data into real-life exposure conditions found that the dose–response relationship for the ROAT and the patch test was very similar but that test subjects reacted to lower doses of nickel per unit area in the ROAT than in the patch test (378). The authors suggested that the accumulation of nickel in the skin determined elicitation rather than dose per application (378).

Menné and colleagues aimed to identify a general threshold limit below which nickel allergic subjects could safely wear metallic items that contained nickel (16). In total, 267 nickel allergic patients were patch tested with 15 metal alloys of known composition (nickel content ranged from 0–100%) (16). The discs were also analysed in synthetic sweat for 1 week and with energy dispersive X-ray analysis to confirm their composition. Discs that released more than 1 $\mu\text{g nickel}/\text{cm}^2/\text{week}$ gave strong patch test reactions whereas discs that released less than 0.5 $\mu\text{g nickel}/\text{cm}^2/\text{week}$ showed weak reactivity (16). However, one disc, Inconel 600 (77% nickel, 8% iron,

15% chromium), gave dermatitis reactions in a large proportion of patients but released less than 0.5 $\mu\text{g nickel}/\text{cm}^2/\text{week}$ when analysed in synthetic sweat. This finding could be explained by the formation of chromium oxides on the surface during corrosion testing (16). The authors concluded that the nickel allergy problem could be minimized by the use of alloys that released less than 0.5 $\mu\text{g nickel}/\text{cm}^2/\text{week}$. When the DMG test was applied on the discs used for patch testing, it mostly gave positive reactions in alloys that released more than 0.5 $\mu\text{g nickel}/\text{cm}^2/\text{week}$ (44). Based on this finding, the DMG test became a rapid and inexpensive screening method to discriminate between safe and unsafe nickel alloys.

Regulatory interventions

In the late 1980s and early 1990s, authorities in northern European countries began to introduce regulations aimed at the prevention of nickel exposure and allergy (14). At that time, neither industry nor authorities took responsibility for preventing the most common and important contact allergy in industrialized countries (17). In Denmark, a statutory order (no. 472, 27 June 1989) based on work by Menné and colleagues was implemented to reduce nickel allergy and dermatitis (Table 1) (16, 44, 379). The order restricted nickel release from certain consumer items but did not cover hair clasps. Companies should instead display 'contains nickel' in case hair clasps released more than 0.5 $\mu\text{g nickel}/\text{cm}^2/\text{week}$ (193). Test methods included the DMG test but also chemical analyses. Menné and Rasmussen predicted that the regulation would result in decreasing prevalence of nickel allergy but also felt that it did not cover all individuals as some subjects reacted to nickel concentrations below the 0.5 $\mu\text{g nickel}/\text{cm}^2/\text{week}$ limit (379). In 1990 in Sweden, ear piercing with nickel-containing piercers or rings was banned if the alloy contained more than 0.05% nickel (14). From 1991 in Germany, nickel-containing objects had to be labelled 'contains nickel and may cause an allergic reaction' (14). In July 1991, the CEN began to work on a new standardization project (CEN/TC 283) referred to as 'Precious Metals–Applications in Jewellery and Associated Products' (14). It was foreseen that the CEN/TC 283 could be an initial step towards a European regulation on nickel exposure (14). The EEC, Directorate-general III, Internal Market and Industry (DG III), prepared a regulation aimed at the prevention of nickel allergy (17).

In 1994, the EU Nickel Directive was adopted by the member states (Table 2) (380). The delay between the adoption of the EU Nickel Directive in 1994 and its coming into full force in July 2001 was mainly explained by intensive lobbying by certain industries (188). Lidén

Table 1. Contents of the 1989–1990 Danish nickel regulation

<i>Scope of the order</i>	
<i>Section 1</i>	This order applies to the following metal objects made from nickel-containing surface coating, provided the release of nickel exceeds 0.5 µg/cm ² /week (I) ear ornaments and ear stickers (II) necklaces, bracelets and chains, anklets, finger rings, nail clips (III) back of wristwatch cases, watch straps and tighteners (IV) spectacle frames (V) garments equipped with buttons, tighteners, rivets, zippers, and metal marks which will by normal use come into close contact with the skin
<i>Regulation of use</i>	
<i>Section 2</i>	The objects specified in section 1 of this Order must not be sold by manufacturers or importers
<i>Subsection 2</i>	The release of nickel will be considered as less than 0.5 µg/cm ² /week if the object treated by the analysis method described in the Annex to this order does not give the red colour described in the annex
<i>Supervision and control</i>	
<i>Section 3</i>	The national Agency of Environmental Protection will supervise the observance of the rules laid in this Order
<i>Section 4</i>	The national Agency of Environmental Protection may in special cases allow deviations from the rules laid down in this Order
<i>Penalty and entry into force</i>	
<i>Section 5</i>	Offenders of the provision of section 2 of this Order will be liable to detention or imprisonment for up to 1 year
<i>Subsection 2</i>	For violations committed by limited liability companies, cooperative societies or the like, the company or society as such may be held liable to pay the fine
<i>Section 6</i>	This Order enters into force on 10 July 1989
<i>Subsection 2</i>	Irrespective of the provisions of section 2 of this Order, metal objects covered by section 1 which were imported or manufactured before 10 July 1989 may be sold until 10 January 1991
<i>Annex</i>	
<i>Analysis method</i>	Analysis method to determine the release of nickel from metal objects and surface coatings The following reagents must be used: – a 1% solution of dimethylglyoxime in alcohol – a 10% solution of ammonium hydroxide in water Place 2 drops of each solution on a cotton swab, and rub it evenly against the test item for 30 seconds. The appearance of a red colour, from light pink to strong cerise, indicates that the release of nickel exceeds 0.5 µg/cm²/week. As regards analysis of painted or varnished surfaces, the test must be made before as well as after removal of surface

Table 2. The EU Nickel Directive and reference methods

		CEN standard
Part 1	<i>Original requirement</i> (before 2005): Nickel was prohibited in post assemblies which were inserted into pierced ears and other pierced parts of the human body during epithelialization of the wound, unless they were homogeneous and the nickel concentration was below 0.05%	EN 1810 (Flame Atomic Absorption Spectrometry. Nickel content is expressed as mass of nickel to total mass)
	<i>New requirement</i> (from 2005): Nickel release from all items inserted into pierced parts of the body (not only during epithelialization after piercing) should be less than 0.2 µg/cm ² /week.	EN 1811 (Items under investigation are placed in artificial sweat for 1 week and the concentration of dissolved nickel in the solution is determined by atomic absorption spectrometry (or other methodology) and expressed in µg/cm ² /week)
Part 2	Nickel may not be used in products intended to come into direct and prolonged contact with the skin such as earrings, necklaces, bracelets, chains, anklets, finger rings, spectacle frames, wristwatch cases, watch straps, zippers, buttons, and mobile phones if nickel release from the parts coming into direct and prolonged contact with the skin is greater than 0.5 µg/cm ² /week	EN 1811
Part 3	Nickel is prohibited in products such as those listed under point 2 if they have a coating and if they do not fulfil the requirement under point 2 for a period of at least 2 years of normal use of the product	EN 12472 (Method for simulation of wear and corrosion for the detection of nickel release from coated items. The item under investigation is exposed to a corrosive atmosphere and then placed in a container together with abrasive chips, water, and a wetting agent. The container is rotated to smooth the surface and abrade the coating. Finally, the item is subjected to EN 1811)

et al. mentioned that companies producing hair slides, spectacle frames, watches, and piercing posts claimed that their products did not cause clinical problems and that they should not be covered by the EU Nickel Directive (188). Other parts of the industry, particularly those participating in the development of the CEN test methods, were more constructive. It was decided that all EU member states should adopt the EU Nickel Directive by January 2000, that from July 2000 importers and manufacturers could not place products on the market which did not comply with the Nickel Directive, and finally that, from July 2001, no such products could be sold or made available to customers within the EU market (188).

In 2004, an amendment was made to the EU Nickel Directive (381). Based on risk assessment performed at the time, it was decided that a migration limit ($0.2 \mu\text{g nickel/cm}^2/\text{week}$) for piercing-post assemblies was more appropriate than the 0.05% nickel content limit. The scientific background for this change is not clear, however. Prior to the amendment, Ingber et al. performed a small study on AISI316L stainless steel ear-piercing-post assemblies with a nickel content between 10% and 14% (382). Ear Piercing Manufacturers of Europe Ltd, Spalding, UK, supported the study financially. Nickel release from piercing-post assemblies was between 0.11 and $0.21 \mu\text{g nickel/cm}^2/\text{week}$ for 10 unused posts (382). Ingber et al. argued that a nickel release limit would be a more appropriate protective regulation than a nickel content limit (382).

In Denmark, a mobile phone DMG test study received much attention from national media (215, 383). The mobile phone story was taken up by national television (the Danish Broadcasting Corporation). An evening programme about mobile phone dermatitis was broadcast. The TV station performed their own investigation for this programme and found that 26% of 50 mobile phones released more than $0.5 \mu\text{g nickel/cm}^2/\text{week}$ (383).

In January 2009, the Danish Ministry of the Environment officially suggested that the EU Nickel Directive should cover mobile phones. The European Commission agreed to this shortly after (383).

Study Aims

The aim of this thesis was to evaluate the effect of regulatory interventions on nickel exposure. The prevalence of nickel allergy and nickel dermatitis as well as the association between hand eczema and nickel allergy was studied before and after Danish nickel regulation. Causes that could explain the persistence of nickel allergy in Denmark, namely consumer exposures and genetic factors, were evaluated and discussed. Finally, it was investigated whether the use of nickel has been replaced by the use of cobalt in consumer products and whether a change in the epidemiology of cobalt allergy has occurred following nickel regulation. In addition, earrings randomly purchased in the capital of a new and an old EU country were examined for nickel release by using the DMG test to estimate the magnitude of nickel exposure in some EU capitals. The aims were thus as follows.

Materials and Methods

Part 1: Cross-sectional general population studies

Patch test and questionnaire data from two consecutive cross-sectional studies performed in the general population of Copenhagen in 1990 and 2006 were analysed. Danish adults (with Danish citizenship and born in Denmark) were randomly drawn from the Danish Civil Registration System and invited to participate in a general health examination including patch testing. The Ethical Committee of Copenhagen County approved all studies (KA-20060011).

Study I	To estimate the prevalence of nickel allergy and dermatitis in adult Danish women from the general population before and after nickel regulation.
Study II	To estimate the association between nickel allergy and hand eczema in Danish adults from the general population before and after nickel regulation.
Study III	To investigate the association between filaggrin null mutation status and nickel allergy in Danish adults.
Study IV	To estimate the effect of nickel regulation on temporal trends of nickel and cobalt allergy in Danish female dermatitis patients from a tertiary referral patch test centre.
Study V	To investigate the sensitivity and specificity of the DMG spot test.
Study VI	To estimate the proportion of consumer products purchased in Copenhagen, Denmark, that release nickel in amounts that can elicit nickel dermatitis (by using the DMG spot test).
Study VII	To determine the proportion of inexpensive earrings randomly purchased from stores and street vendors in Warsaw and London that release nickel in amounts that can elicit nickel dermatitis and to investigate whether the degree of nickel release from inexpensive earrings is related to shop category.
Study VIII	To develop and validate a new spot test for the detection of cobalt ions.
Study IX	To estimate the proportion of consumer products purchased in Copenhagen that release cobalt in concentrations that may elicit cobalt dermatitis in cobalt allergic individuals by using the cobalt spot test.

1990 study. A screening questionnaire on respiratory symptoms was mailed to a random sample of 8000 15–69 year olds (384). A total of 6998 (87.5%) subjects responded and 793 were randomly selected for the patch test study. Between February 1990 and January 1991, 567 (71.5%) participants were patch tested with nickel sulfate. To compare the results with those from the 2006 study, participants aged 15–17 years were excluded. Thus, 543 participants aged 18–69 years old were patch tested with nickel by using the TRUE test. On the test day, all participants filled out another questionnaire with a variety of health questions including questions on lifestyle factors, socio-economic factors, ear piercing and dermatitis. Cross-sectional nickel patch test data from the random and overlap groups in 1990 were used for comparison with nickel patch test data from the 2006 study (Study I + II).

2006 study. Between June 2006 and May 2008, 3471 (43.7%) of 7931 invited subjects aged 18–69 years showed up for a general health examination. Of these, 3460 participants were patch tested with panels 1 and 2 from the TRUE test but only 3338 participants were tested with nickel sulfate. For practical reasons, persons below the age of 18 years were not invited for the 2006 study since an informed, written consent from parents is now mandatory in Denmark. Data from this study were used to estimate the prevalence of nickel allergy, nickel dermatitis and hand eczema in 2006 (Study I + II). Furthermore, filaggrin genotyping was performed on blood samples from participants (Study III).

Patch testing. Patch testing was performed by using panels 1 and 2 of the TRUE test (suppliers were ALK-Abelló A/S, Hørsholm, Denmark, in 1990 and Mekos Laboratories, Hillerød, Denmark, in 2006). Directions to apply the patch tests panels to the upper back 2 days before examination were mailed together with the patch tests. They were read and photographed 1–1.5 hr after removal by Nielsen in 1990 and by trained healthcare personnel in 2006 (supervised by Thyssen and Linneberg) at the Research Centre for Prevention and Health, Glostrup Hospital. Subsequently, photographs were reviewed in a blinded manner by Nielsen and Menné in 1990 and by Nielsen, Menné, Linneberg, and Thyssen in 2006. The group had no information about gender, age, or previous medical history of participants when reviewing the photographs. The set-up should ensure that the International Contact Dermatitis Research Group (ICDRG) criteria were used consistently over time (385). Prior to study start, the healthcare staff ($n = 6$) who performed patch test readings in the 2006 study underwent 1 week of training at

the patch test clinic at Gentofte Hospital. A combination of theoretical education provided by Thyssen and hands-on training provided by experienced nurses at Gentofte Hospital was given to ensure a high validity of patch test reading. A laboratory technician with 10 years of patch test reading experience from the Department of Dermatology at Bispebjerg Hospital had the daily overall patch test reading responsibility when Thyssen was not present at Glostrup Hospital. Nickel allergy was defined as a positive patch test (1+, 2+, or 3+ according to ICDRG recommendations) (385). If the patch had no skin contact upon patch test reading, or if the subject had removed it prior to testing as a result of known nickel allergy, it was regarded as missing data.

Filaggrin genotyping. Filaggrin genotyping was successfully performed on blood samples from 3335 participants in the 2006 study. Regions covering the mutations R501X and 2282del4 of the filaggrin gene were amplified from genomic DNA by PCR, and DNA fragments obtained were hybridized to both mutation-specific and wild-type probes attached to paramagnetic beads (Luminex, Austin, TX, USA) and analysed on a BioPlex 200 (Biorad, Hercules, CA, USA).

Questionnaires. Participating researchers prepared questionnaires with mostly identical questions prior to the 1990 and 2006 studies. Questionnaires were mailed to participants prior to the health examination in 2006 but not in 1990. At the day of the health examination, the staff made sure that all questions had been answered to ensure high response rates. The questionnaires included a description of eczema: 'Eczema is an itching skin disorder showing redness, dryness, and possibly vesicles and exudation. Eczema is present at the same area for some time.' Among a broad variety of questions on general health and living, participants were asked 'Have you ever had eczema under the tightener of your watch strap, under the button of your jeans or from wearing ear pins or earrings?' (an affirmative answer was used to define allergic nickel dermatitis in Study I whereas an affirmative answer together with a positive patch test reaction to nickel sulfate was used to define allergic nickel dermatitis in Studies II and III) and 'Have you ever had your ears pierced?' Participants who gave an affirmative answer to this question were further asked 'What year were your ears pierced the first time?' A history of self-reported hand eczema was defined as an affirmative answer to the question 'Have you ever had hand eczema?' Finally, atopic dermatitis was defined by an affirmative answer to the question 'Has your doctor ever told you that you suffered from atopic dermatitis?' in Study II and by the UK

Working Party's diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria in Study III (386). The major criterion was an itchy skin condition and the minor criteria were (i) a history of involvement of the skin creases, (ii) a personal history of asthma or hay fever, (iii) a history of general dry skin in the last year, and (iv) onset under the age of 2 years.

Part 2: Longitudinal patch test study

Female dermatitis patients who were patch tested at Gentofte University Hospital between 1 January 1985 and 31 December 2007 were included in Study IV. This population included newly referred patients as well as hospitalized and ambulatory patients. Patch testing was performed with the European baseline series using 8-mm Finn chambers® (Epitest Ltd, Oy, Finland) on Scanpor tape (Norgesplaster A/S, Alpharma, Vennessla, Norway). Nickel sulfate and cobalt chloride were tested in petrolatum in concentrations of 5% and 1%, respectively. The patch tests were applied to the upper aspect of the back and were occluded for 48 hr. Readings were done on days 2, 3 or 4, and 7 during the test period according to the recommendation from the ICDRG (385). A 1+, 2+, or 3+ reading was interpreted as a positive response. An irritant response, a doubtful reading (+?), or a negative reading was interpreted as a negative response.

Statistical methods. Comparisons were generally made by using the χ^2 -test. Fisher's exact test was used when the expected values were less than 5. In Study IV, a Mann–Whitney non-parametric test was performed to compare the median age of patients with nickel and cobalt allergy as age was not normally distributed when analysed with the Kolmogorov–Smirnov test ($p < 0.001$). Hence, continuous data were summarized by median and interquartile (IQR) ranges. In Study III, deviation from the Hardy–Weinberg equilibrium was tested using the free online calculator at the Online Encyclopedia for Genetic Epidemiology (OEGE) (<http://www.oege.org/software/hwe-mr-calc.shtml>) for both filaggrin null mutations. Logistic regression models were performed to adjust for known confounders when investigating associations between contact allergy and selected variables (Studies II–IV). Associations were expressed as ORs with 95% CIs. Data analyses were performed using the Statistical Products and Service Solutions package (SPSS Inc., Chicago, IL, USA) for Windows (release 15.0).

Personal data and questions were used to define the following independent variables that were used in the logistic regression analyses:

- Gender: men, women (Studies I, II, III, IV)
- Age group: 18–35, 36–55, 56–69 (Studies I, II, III)
- Age group: 5–30, 31–49, > 50 (Study IV)
- Study year: 1990, 2006 (Studies I and II)
- Filaggrin null mutation: yes, no (Study III)
- Ear-piercing status: yes, no (Studies I, II, III)
- Nickel dermatitis: yes, no (Studies I, II, III)
- Atopic dermatitis: yes, no (Studies II, III)

Part 3: Metal exposure assessment studies

Jewellery and accessories sample from Copenhagen. Between December 2008 and March 2009 inexpensive jewellery and hair clasps were purchased from stores in Copenhagen. No duplicates or items labelled 'nickel free' were bought. Each store was only visited once. When buying earrings, inexpensive metallic pieces with perforating piercing posts (hoops, studs, and drops) were given priority. Metallic finger rings, bracelets, hair clasps and necklaces were also randomly purchased. Approximately 3000 € (range 5–50 €) were spent buying 354 metallic jewellery items and hair clasps from a total of 36 places: eight stores selling clothing and accessories to infants, toddlers, and children; four accessory stores targeting young and middle-aged women; five jewellery and accessory sections in three large supermarkets and two department stores; two jewellery stores selling popular jewellery to young and middle-aged women; two street vendors; one Danish internet homepage selling jewellery; and 12 clothing stores targeting mainly young and middle-aged women. Two of the latter catered to immigrants and were located in Nørrebro, a neighbourhood with a large Middle Eastern population.

Most jewellery and accessories were intended for girls and young women but the accumulation of jewellery and hair clasps was believed to be representative of possible nickel exposure in consumers from all age groups. Adults and adolescents were defined as persons above 12 years of age and children as persons below 12 years of age. The majority of hair clasps for children were probably made for toddlers. Overall, 170 (48%) earrings, 117 (33.1%) hair clasps, 33 (9.3%) necklaces, 18 (5.1%) bracelets, and 16 (4.5%) finger rings were bought, totalling 354 items.

Earring samples from London and Warsaw. In January 2010, inexpensive earrings were purchased from stores in Warsaw and London. Prior to shopping, it was agreed that around 200 earrings should be purchased in each city, preferably 10 items from each store, and that only 30–40 earrings should be purchased from street markets. In the event that fewer than 10 earrings could be found in one store, it was acceptable to buy more than 10 earrings from stores that were visited subsequently to reach the target

number. A broad variety of stores were visited and a focus was put on earrings targeting girls and young women. No duplicates were bought. Each store was only visited once. Inexpensive metallic pieces with perforating piercing posts (hoops, studs, and drops) were given priority. A total of 1100 € (range 1–10 €) was spent buying 205 earrings in London and 700 € (range 1–12 €) was spent buying 206 earrings in Warsaw. In London, earrings from 23 stores were purchased: 11 chain clothing stores in Oxford Street; two chain accessory stores in Westfield Shopping Centre and Oxford Street, respectively; one drug store in Oxford Street; three market stalls (one in Covent Garden and two at the Saturday morning Church Street Market); one freestanding jewellery booth in the underground station at Westminster; one local artist in Covent Garden; and four accessory and clothing stores located in Soho, East London and the Paddington areas. In Warsaw, earrings from 20 stores were purchased: eight chain clothing stores, three chain accessory stores and two freestanding jewellery booths located in two different shopping centres (Złote Tarasy and Warszawa Wilenska Shopping Centre); three street market stalls in the shopping area next to the 10th Anniversary Stadium (Stadion Dziesięciolecia) in Praga; one combined clothing and accessory store in Praga; one small local shop selling jewellery and art from local artists in the Praga area; and finally two accessory stores located in the underground terrain near the central railway station.

Nickel spot testing. Testing for nickel release was performed by using the DMG test. For Study V, the Chemo Nickel Test™ from Chemotechnique Diagnostics®, Vellinge, Sweden, was used (dimethylglyoxime 1%, ethanol and ammonium (9.9%) solution; in one bottle together). For Studies VI and VII, the DMG test was prepared by a hospital pharmacy in the Capital Region of Copenhagen. The test solutions were 1% DMG in alcohol, and 10% ammonium hydroxide in water. All individual parts of the jewellery that came into direct and prolonged skin contact were examined. The two-bottle test was administered by placing two drops of each solution in succession on a white cotton-wool-tipped applicator that was rubbed for up to 20 seconds against the test object. A pink or a cerise coloration of the applicator indicated a positive reaction whereas a negative reaction was registered when no colour change was observed. Doubtful reactions were retested, and if the reaction remained doubtful it was considered negative.

Cobalt spot testing. The cobalt test reagent was prepared by mixing 0.1% (w/w) oxalic acid, 0.02% (w/w) disodium-1-nitroso-2-naphthol-3,6-disulfonate (nitroso-R salt) and

5.0% (w/w) sodium acetate in deionized water. To do the cobalt gel test, 15 ml of the cobalt test reagent was added to 100 ml agar solution (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and stirred at low rotation speed for a few minutes to produce 115 ml of the cobalt gel ready for use. (The aqueous agar solution was prepared by placing 3 g of agar powder in a flask and adding 100 ml of demineralized water. Using a stir hot plate and agitation with a stirring magnet, the agar powder was dissolved by heating to 80°C. The stirring magnet was started on low rotation and the rotation speed slowly increased to avoid vigorous stirring that might produce unwanted bubbles.)

One drop of the cobalt test solution, a clear yellow solution, was added to the tip of a white cotton stick, which was then rubbed for 20–30 seconds against the test item. A positive reaction was indicated by a red (or orange-red) coloration of the applicator whereas a negative reaction was registered when the applicator remained yellow (Fig. 1). Doubtful reactions were retested, and if the reaction remained doubtful it was considered negative. The cobalt gel test is similar to the cobalt cotton stick test except that the yellow fluid has been replaced with a yellow gel and no rubbing is involved. The sample was placed on a white plastic dish and the gel was poured over the sample to cover it (Fig. 2).

In a subsample of 40 earrings, the cobalt gel test was performed to verify the outcome of the cobalt cotton stick test (Fig. 2). This was done at the Department of Mechanical Engineering, Technical University of Denmark. The selection of test items for the gel test was based on previous DMG testing (20 were DMG test positive and 20 were DMG test negative) and on the outcome of cobalt cotton stick testing (four positive reactions were re-investigated of which one was also DMG positive). All samples were rinsed and washed in tap water prior to cobalt gel testing. The samples were placed on white plastic dishes and the gel was poured to

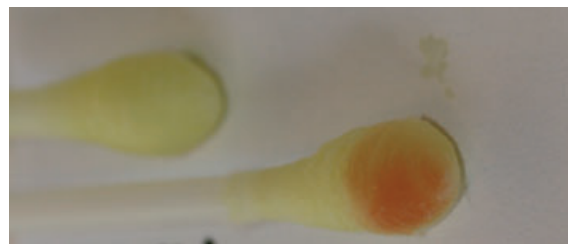


Fig. 1. The cobalt cotton stick test reveals a yellow colour (cotton stick on the left) when the tested item does not release cobalt (it was rubbed against aluminium, disc H). The cobalt cotton stick test gives a red colour when the item releases cobalt (it was rubbed against a hard metal alloy containing 6 wt% Co, disc A) (cotton stick on the right). Rubbing for 30 seconds is recommended.



Fig. 2. The cobalt gel test prepared from the cobalt cotton stick test was used to detect cobalt release from an earring purchased in Copenhagen, Denmark. Note that cobalt release is restricted to the decorative part and that cobalt is not released from the ear post hook.

cover the samples. The gel was then left for 30 min and was inspected for a colour change. A red colour indicated cobalt release whereas no change of the yellow colour indicated absence of cobalt release. A final interpretation of the gel test was made after 30 min to confirm results from the cobalt cotton stick test.

Scanning electron microscopy and energy dispersive spectroscopy analysis. Microstructural characterization and chemical analysis of four cobalt positive items with a dark metallic appearance was done using a scanning electron microscope (SEM) (Model JEOL 5900, JEOL Ltd, Tokyo, Japan) in combination with energy dispersive spectroscopy (EDS) (Oxford Link ISIS analyzer, Oxford, UK) using INCA Energy EDS software. After initial SEM/EDS characterization of the four items, they were cut with a nipper, hot moulded in a conductive embedding material, and ground with SiC grit paper size 320, 500, 1000, 2000, and 4000 before polishing to a mirror-like surface. The polished cross-sections were then analysed using SEM/EDS.

Part 4: Validation of the nickel spot test

Sample. Among 277 pairs of earrings previously purchased and DMG tested (196), 73 pairs were selected to ensure a broad variety of metallic components (rings, discs, posts, and wires). The selection was based on size, shape, colour, surface area, producer, and previous DMG test outcome (196). Each earring was carefully inspected to identify components in skin contact in normal use. Metallic parts that were not in skin contact as well as non-metallic parts (e.g. plastic, wood, glass, and stone) were removed from the earrings with a pair of DMG-test-negative tongs. For practical reasons, components with a

diameter larger than 21 mm were excluded as they did not fit in the uniform sample bottles used. As most hoops were larger, these did not fit in the bottles and were excluded. For the above reasons, a large proportion of the metallic components were hooks, posts, and post assemblies. A total of 96 sets of metallic parts were analysed (73 pairs of posts and hooks, 23 pairs of decorative attachments).

Cleaning procedure. The cleaning procedure was performed in accordance with EN 1811 at Karolinska Institutet, Stockholm. Thus, all components were gently swirled for 2 min in a degreasing solution containing 5 g dodecylbenzene sulfonate (CAS 25155-30-0, Sigma-Aldrich) in 1000 ml deionized water at room temperature (20°C–22°C) to remove extraneous grease and skin secretions as well as nickel contamination. The components were then rinsed with deionized water and left to dry. From this step, all handling was performed using protective plastic gloves and plastic forceps.

X-ray fluorescence spectrometry. Metallic sample components were individually examined with a handheld X-ray fluorescence analyser (Innov-X, Alpha 4000, Innov-X-Europe, The Netherlands) to determine the composition of the outer layers. X-ray fluorescence spectroscopy (XRF) is a non-destructive analytical technique widely used for elemental and chemical analysis. Briefly, high-energy X-rays bombard the metal and lead to emission of characteristic fluorescent X-rays that are registered. The detected X-ray intensities were evaluated with the use of the instrument's own standard module for alloy analysis.

Area measurement. Area measurement was performed using computerized image analysis, instead of the relatively rough area assessment described in EN 1811. Individual components were digitally photographed together with a ruler (Fig. 3). The pictures were then viewed and evaluated with image analysis software, IMAGEJ, a software program available for different platforms and freely downloaded from the internet (<http://rsbweb.nih.gov/ij/>). This program allows the user to measure the size after calibration with the ruler of different parts of the metallic components. By using basic measurement formulae, areas of these components were calculated. All areas expected to become exposed to artificial sweat during analysis were measured and calculated, e.g. the interior of a square-tube-shaped component was included in the total sample area of that component.

Nickel release and chemical analysis (EN 1811). To determine nickel release, the European Standard EN 1811 developed by CEN/TC 283/WG4 was followed (387). All 96 pairs of earring components were simultaneously investigated for



Fig. 3. A pair of earrings has been dismantled prior to area measurement. Both the interior and the exterior of the square tubes in the lower part of the figure were included in the total area as these will become exposed to artificial sweat during chemical analysis.

nickel release by immersion in artificial sweat consisting of deionized and aerated water containing sodium chloride 0.5%, lactic acid 0.1% and urea 0.1%. pH was adjusted to 6.5 with ammonia. The solution was used within 3 h of preparation. Furthermore, nickel-free acid prewashed plastic vessels with lid (Nalgene polypropylene) 30 and 60 ml in size were prepared according to EN 1811. Each pair of earring components was totally immersed in approximately 1 ml artificial sweat per square centimetre of sample area. The minimum volume of artificial sweat required by the analytical laboratory for this chemical analysis was 6 ml. The vessels were left untouched in an oven for nickel extraction at 30°C for 1 week. The earring components were then removed by plastic forceps which were rinsed between samples. Next 400 µl of 25% nitric acid was added to the vessels to obtain 1% nitric acid concentration. Finally, the solutions were stored at 8°C until analysed, which was done within 4 weeks by the SP Technical Research Institute of Sweden, Borås. Inductively coupled plasma optical emission spectroscopy (Perkin Elmer Optima 5300DV) was used for the analysis of nickel in the artificial sweat extracts. The detection limit of nickel was 0.01 µg/l. The final results were expressed in µg/cm²/week. The analytical values of nickel release were reported, and the values were not multiplied by the adjustment factor of 0.1 described in the current version of EN 1811.

Calculation of specificity and sensitivity. When the sensitivity and specificity of a test is assessed, one needs to correlate test results from the test that is under validation against

test results from a gold standard. The DMG test was validated against the chemical analysis described in EN 1811. Sensitivity and specificity were defined as follows and given with 95% CIs (388):

$$\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

True positive reactions were defined as positive DMG test reactions that were confirmed by nickel release above 0.5 µg/cm²/week. False positive reactions were defined as positive DMG test reactions but with a nickel release concentration below 0.5 µg/cm²/week. True negative reactions were defined as negative DMG test confirmed by a nickel release below 0.5 µg/cm²/week. Finally, false negative reactions were defined as negative DMG test reactions but where nickel release was above 0.5 µg/cm²/week.

Part 5: Validation of the cobalt spot test

Threshold determination. Cobalt nitrate standard solution for atomic absorption spectroscopy (BDH Chemicals Ltd) was used to estimate the threshold value of the cobalt test reagent. To determine the threshold level (detection level), i.e. a change in colour from yellow to red, the cobalt test reagent (50 µl) was mixed with 10 µl of a cobalt nitrate standard solution that contained different amounts of cobalt (0, 0.01, 0.05, 0.1, 0.5, and 1 µg cobalt, respectively).

Effect of temperature and storage. Cobalt spot testing was performed on a tool steel specimen containing 6% (w/w) cobalt, after storage of the cobalt test reagent in a closed vessel under different conditions (5°C for 10 months; 24 hr at -21°C or +60°C). Furthermore, threshold determination of a red colour change was reassessed after cooling of the cobalt test for 24 hr at -24°C, 5°C and storage for 24 hr at 60°C, respectively.

Testing of other metals and alloys. Samples of pure metals and alloys known not to contain cobalt were tested with the cobalt gel test. Thus, solid cylindrical samples with a diameter of 10 mm and height of 10 mm of pure metals and alloys made of massive nickel, copper, aluminium, tin, zinc, lead, titanium, iron, magnesium, silver, or iron were tested. Coatings of gold, chromium, a silver-palladium alloy (77 Pd/23 Ag), and a standard austenitic stainless steel [main alloying element is iron (>70 wt%), secondary elements are 18 wt% chromium and 18 wt% nickel]

were also tested. The silver–palladium alloy was supplied by Polyteknik A/S (Østervrå, Denmark); the rest were samples used for teaching purposes at the Technical University of Denmark, Lyngby. The samples were subjected to the cobalt gel test to assess the specificity of the test and possible reactivity to metals other than cobalt. Each sample was placed in the cobalt gel test on a white plastic dish and left for at least 1 hr at room temperature.

Testing clinical relevance of threshold level. The test materials were nine different custom-made cobalt-containing metal discs of the same batch as used by Julander et al. (170). The authors had shown that all discs released cobalt in artificial sweat and also elicited positive cobalt patch test reactions in cobalt allergic patients (170). The alloys are used as raw material in the manufacturing of wear parts for hard metal tools (Sandvik AB, Sandviken, Sweden) and as dental alloys (Dentaurum, Ispringen, Germany). A titanium carbonitride (TiCN) coated cobalt-containing bulk chip cutting tool produced for conventional use (e.g. in turning lathes), supplied by Sandvik AB, Sweden, was also included. The latter had not been used for patch testing in the study by Julander et al. and was not analysed chemically. The cobalt gel test was used to determine cobalt release from the materials and the results were compared with the outcome of the previous patch testing and chemical analysis (170).

Results

Part 1: Cross-sectional general population studies

Participation rate. Decreasing participation rates were observed in both sexes and all age groups when the 1990 study was compared with the 2006 study (Table 3). Young men in particular had a low participation rate in the 2006 study (25.0%).

Nickel allergy in 1990 and 2006. A total of 543 and 3338 persons aged 18–69 years were nickel tested in 1990 and

2006, respectively. The prevalence of nickel allergy was very similar in 1990 (overall 6.6%; men 2.2% and women 10.9%) and 2006 (overall 6.1%; men 1.0% and women 10.3%). However, the change in the prevalence of nickel allergy from 1990 to 2006 was significantly dependent on age group (the *p*-value for interaction between age group and study year was 0.008) in a logistic regression analysis with nickel allergy as the dependent variable and age group and study year as the explanatory variables. This means that the change in the prevalence of nickel allergy from 1990 to 2006 cannot be presented as one overall estimate and should be stratified by age group. Thus, among women aged 18–35 years, the prevalence of nickel allergy decreased from 19.8% in 1990 to 11.4% in 2006 (OR = 0.52; 95% CI = 0.29–0.92), whereas the prevalence increased among women aged 36–55 years from 6.5% in 1990 to 13.0% in 2006 (OR = 2.17; 95% CI = 1.03–4.56) (Table 4). In 1990, women aged 18–35 years had a higher prevalence of nickel allergy and ear piercing than older age groups ($p_{\text{trend}} < 0.001$). The prevalence of ear piercing remained at the same level among 18–35-year-old women over the study period whereas it increased significantly among 36–55 year olds (OR = 5.72; 95% CI = 3.82–8.56) and 56–69 year olds (OR = 3.33; 95% CI = 1.77–6.27) between 1990 and 2006 (Table 4). The prevalence of body piercing among women in the 2006 study was 32.4% among 18–35 year olds, 6.8% among 36–55 year olds, and 0.2% among 56–69 year olds. Figure 4 presents age-specific prevalences of nickel allergy among women in 1990 and 2006. Consistent with the above analyses, an increase was seen in older age groups (age above 35 years) and a decrease in younger age groups (age below 35 years).

Prevalences of nickel allergy and nickel dermatitis were significantly lower among women who had their ears pierced after the start of nickel regulation in 1990 (between 1990 and 2006) than among women who had their ears pierced before 1990 (nickel allergy, 6.9% versus 15.6%; $p = 0.004$; and nickel dermatitis, 30.5% versus

Table 3. The rate of participation among 753 and 7931 subjects invited in 1990 and 2006, respectively, to a cross-sectional study in Copenhagen, Denmark (Studies I, II and III)

Age (years)	Participation rate % (attendants/invited)					
	Men			Women		
	1990	2006	Crude OR with 95% CI	1990	2006	Crude OR with 95% CI
18–35	70.1 (94/134)	25.0 (265/1058)	0.14 (0.10–0.21)	78.7 (111/141)	38.4 (382/996)	0.17 (0.11–0.26)
36–55	79.5 (128/161)	43.0 (742/1724)	0.19 (0.13–0.29)	78.2 (129/165)	53.8 (938/1742)	0.33 (0.22–0.48)
56–69	81.3 (65/80)	45.9 (546/1189)	0.20 (0.11–0.35)	63.9 (46/72)	48.9 (598/1222)	0.54 (0.33–0.89)
Total	76.5 (287/375)	39.1 (1553/3971)	0.20 (0.15–0.25)	75.6 (286/378)	48.4 (1918/3960)	0.30 (0.24–0.39)

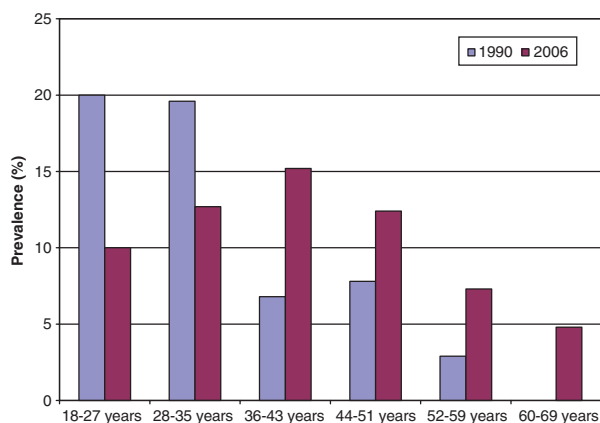
Odds ratio (OR) with 95% confidence interval (CI) comparing the age- and gender-specific rates of participation in 2006 with those in 1990.

Table 4. The prevalence of patch test sensitivity to nickel, self-reported nickel dermatitis, ear piercing and body piercing among women aged 18–35 years, 36–55 years and 56–69 years in two repeated cross-sectional studies performed in the same general population in Copenhagen, Denmark (1990 and 2006)

Age group		Year of patch testing (women)		Odds ratio (95%CI) [†]
		1990, <i>n</i> = 274	2006, <i>n</i> = 1843	
18–35 years <i>n</i> = 475	Nickel allergy (%)	19.8 (21/106) [‡]	11.4 (42/369)	0.52 (0.29–0.92)
	Have you ever had your ears pierced?	89.6 (95/106) [‡]	91.2 (332/364)	1.20 (0.58–2.47)
	Have you ever had a body piercing?	—	32.4 (121/252)	—
	Self-reported nickel dermatitis* among nickel allergic subjects	90.5 (19/21)	69.0 (29/42)	0.24 (0.05–1.16)
36–55 years <i>n</i> = 1031	Nickel allergy (%)	6.5 (8/124) [‡]	13.0 (118/907)	2.17 (1.03–4.56)
	Have you ever had your ears pierced?	54.0 (67/124) [‡]	87.0 (786/903)	5.72 (3.82–8.56)
	Have you ever had a body piercing?	—	6.8 (63/868)	—
	Self-reported nickel dermatitis* among nickel allergic subjects	100.0 (8/8)	85.5 (100/117)	—
56–69 years <i>n</i> = 611	Nickel allergy (%)	2.3 (1/44) [‡]	5.1 (29/567)	2.32 (0.31–17.43)
	Have you ever had your ears pierced?	38.6 (17/44) [‡]	67.7 (380/561)	3.33 (1.77–6.27)
	Have you ever had a body piercing?	—	0.2 (1/584)	—
	Self reported nickel dermatitis* among nickel allergic subjects	100.0 (1/1)	69.0 (20/29)	—

—, not available.

*Have you ever had eczema under the tightener of your watch strap, or under the button of your jeans, or from wearing earpins or earrings?

[†]Crude odds ratio for the comparison of individuals tested and questioned in 1990 and 2006.[‡]Chi squared (χ^2) trend test compares the variables nickel allergy and 'have you ever had your ears pierced?' within three different age groups (18–35, 36–55, 56–69 years) in 1990; $p_{\text{trend}} < 0.001$.**Fig. 4.** Age-specific changes in the prevalence of nickel allergy among women in two repeated cross-sectional population based studies (1990 and 2006).

44.1%; $p = 0.004$) (Table 5). The decrease in prevalence was most pronounced among women 18–35 years of age. The prevalence of nickel allergy among men was non-significantly higher among those with ears pierced before 1990 than those with ears pierced between 1990 and 2006 (6.5% versus 3.3%).

A logistic regression analysis including men and women was performed with nickel allergy as the dependent variable and with the following explanatory variables: sex (women versus men), age (18–35, 36–55,

56–69), study year (2006 versus 1990), year of first ear piercing (before 1990, after 1990, never ear pierced). A significantly lower prevalence of nickel allergy was identified among subjects ear pierced later than 1990 (OR = 0.45; 95% CI = 0.25–0.82) and among subjects never ear pierced (OR = 0.28; 95% CI = 0.18–0.45) compared with subjects ear pierced before 1990. These associations became stronger when the regression analysis was performed with women only. A similar logistic regression analysis was performed with nickel dermatitis as the dependent variable and, in addition to the above variables, nickel allergy as explanatory variable (Table 6). It showed that the prevalence of nickel dermatitis was lower among subjects ear pierced later than 1990 (OR = 0.69; 95% CI = 0.49–0.97) and among subjects never ear pierced (OR = 0.53; 95% CI = 0.43–0.67) than among subjects ear pierced before 1990. A similar logistic regression analysis performed for women only showed a borderline significantly lower prevalence of nickel dermatitis among women ear pierced later than 1990 (OR = 0.71; 95% CI = 0.50–1.01) and a significantly lower prevalence among subjects never ear pierced (OR = 0.43; 95% CI = 0.33–0.56).

Nickel allergy and hand eczema. The overall prevalence of a self-reported history of hand eczema (i.e. an affirmative answer to the question 'Have you ever had hand eczema') and atopic dermatitis (i.e. an affirmative answer to

Table 5. The prevalence of nickel allergy and self-reported nickel dermatitis[†] among women aged 18–35 years, 36–55 years and 56–69 years in two independent cross-sectional patch test studies performed in the same general population in Copenhagen, Denmark (1990 and 2006)

Age group		Year of patch testing (women)				
		1990, n = 274		2006, n = 1843		
		Ears pierced before 1990 % (n)	Never ear pierced % (n)	Ears pierced before 1990 % (n)	Ears pierced between 1990 and 2006 % (n)	Never ear pierced % (n)
18–35 years, n = 475	Nickel allergy	22.1 (21/95)	0 (0/11)	16.1 (28/174)	8.3 (12/145), <i>p</i> = 0.002*	3.1 (1/32)
	Nickel dermatitis [†]	52.6 (50/95)	9.1 (1/11)	42.2 (73/173)	31.9 (46/144), <i>p</i> = 0.002*	21.9 (7/32)
36–55 years, n = 1031	Nickel allergy	9.0 (6/67)	3.5 (2/57)	14.8 (109/736)	7.7 (3/39), <i>p</i> = 0.82*	4.3 (5/117)
	Nickel dermatitis [†]	35.8 (25/67)	16.7 (10/60)	47.8 (351/734)	35.9 (14/39), <i>p</i> = 1.00*	21.4 (25/117)
56–69 years, n = 611	Nickel allergy	5.9 (1/17)	0 (0/27)	7.2 (22/307)	3.2 (2/62), <i>p</i> = 0.62*	2.2 (4/181)
	Nickel dermatitis [†]	29.4 (4/17)	18.5 (5/27)	29.2 (89/305)	23.3 (14/60), <i>p</i> = 0.75*	17.2 (31/180)
All ages, n = 2117	Nickel allergy	15.6 (28/179)	2.1 (2/95)	13.1 (159/1217)	6.9 (17/245), <i>p</i> = 0.004*	3.0 (10/330)
	Nickel dermatitis [†]	44.1 (79/179)	16.3 (16/98)	42.3 (513/1212)	30.5 (74/243), <i>p</i> = 0.004*	19.1 (63/329)

**p*-value for comparison of the prevalence of nickel allergy and nickel dermatitis among participants ear pierced between 1990 and 2006 (2006 study) and the prevalence of nickel allergy and nickel dermatitis among participants ear pierced before 1990 (1990 study) (χ^2 test). Fisher's exact test was used when the expected frequency in one of the cells was lower than 5.

[†]'Have you ever had eczema under the fastener of your watch strap, or under the button of your jeans, or from wearing earpins or earrings?'

Table 6. The risk of nickel dermatitis* associated with different potential risk factors in 3881 subjects tested with nickel in 1990 and 2006

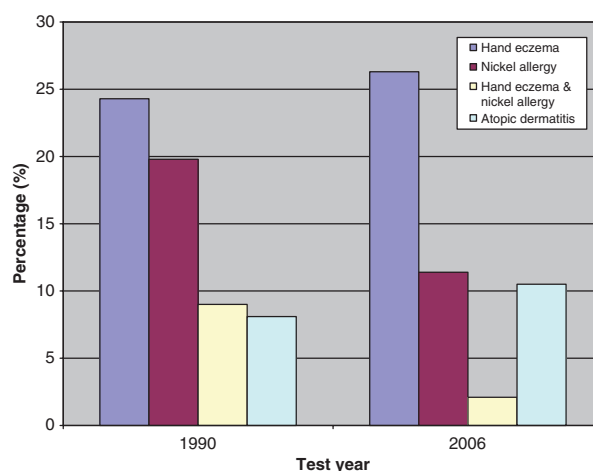
Explanatory variables	Adjusted odds ratio [†] (95% CI)	
Nickel allergy		
No	1	(reference)
Yes	8.28	(5.95–11.53)
Gender		
Male	1	(reference)
Female	3.68	(2.91–4.65)
First ear piercing		
Before 1990	1	(reference)
Later than 1990	0.69	(0.49–0.97)
Never pierced	0.53	(0.43–0.67)

*Nickel dermatitis is defined as a positive answer to the question 'Have you ever had eczema under the tightener of your watch strap, or under the button of your jeans, or from wearing earpins or earrings?'

[†]Odds ratios have been obtained by using a logistic regression analysis mutually adjusted for variables shown in the table as well as age group, study year (2006 versus 1990), and an interaction term between study year and age group.

the question 'Has your doctor ever told you that you suffered from atopic dermatitis?') was 21.4% (men, 16.7%; women, 25.8%) and 4.9% (men, 3.3%; women, 6.5%), respectively, when data from the 1990 and 2006 studies were analysed together. The overall prevalence of hand eczema increased slightly from 20.6% in 1990 to 21.8% in 2006 whereas the overall prevalence of atopic dermatitis increased from 2.8% in 1990 to 5.5% in 2006.

Table 7 shows gender-specific characteristics of the participants. Hand eczema tended to increase in all age groups during the two study periods except among

**Fig. 5.** Changes in the prevalence of hand eczema (i.e. a history of hand eczema), nickel allergy, and atopic dermatitis among female participants aged 18–35 years examined in 1990 and 2006.

56–69-year-old men and 36–55-year-old women where slight decreases were observed. The prevalence of atopic dermatitis increased in all age groups between 1990 and 2006 and was generally higher among women than men. A decrease in the prevalence of nickel allergy was observed among male age groups and among 18–35-year-old women whereas increasing prevalences were observed among women older than 35 years (Tables 4 and 7). The prevalence of combined nickel allergy and hand eczema (i.e. participants who reported hand eczema and also had a positive patch test reaction to nickel) decreased from 9.4% (10/106) in 1990 to 2.2% (8/363) in 2006 among women in the youngest age group (18–35

Table 7. Characteristics of male and female participants tested in 1990 and 2006

Age group (years)		Test year					
		Men			Women		
		1990 % (n/n _{total})	2006 % (n/n _{total})	p-value [†]	1990 % (n/n _{total})	2006 % (n/n _{total})	p-value [†]
18–35 n = 852	Nickel allergy	3.6 (3/84)	1.2 (3/253)	0.17	19.8 (21/106)	11.4 (42/369)	0.02
	History of hand eczema	10.6 (10/94)	14.2 (37/260)	0.38	24.3 (27/111)	26.3 (99/376)	0.67
	Atopic dermatitis*	0 (0/94)	5.5 (14/253)	0.01	8.1 (9/111)	10.5 (39/371)	0.46
	Nickel allergy and a history of hand eczema	0 (0/84)	0.8 (2/248)	1.00	9.4 (10/106)	2.2 (8/363)	0.002
36–55 n = 1937	Nickel allergy	1.7 (2/121)	1.4 (10/718)	0.68	6.5 (8/124)	13.0 (118/907)	0.03
	History of hand eczema	14.8 (19/128)	18.3 (134/732)	0.35	32.6 (42/129)	27.9 (259/928)	0.27
	Atopic dermatitis*	1.6 (2/128)	5.0 (36/721)	0.08	3.9 (5/129)	7.5 (69/916)	0.13
	Nickel allergy and a history of hand eczema	0.4 (1/121)	0.3 (2/708)	0.37	1.6 (2/124)	4.2 (38/897)	0.22
56–69 n = 1255	Nickel allergy	1.6 (1/64)	0.4 (2/524)	0.29	2.3 (1/44)	5.1 (29/567)	0.4
	History of hand eczema	21.5 (14/65)	16.6 (90/543)	0.32	13.0 (6/46)	21.9 (129/590)	0.16
	Atopic dermatitis*	0 (0/65)	1.5 (8/537)	1.00	0 (0/46)	3.1 (18/576)	0.39
	Nickel allergy and a history of hand eczema	0 (0/64)	0 (0/521)	—	0 (0/44)	1.0 (6/559)	1.00
All ages n = 4044	Nickel allergy	2.2 (6/263)	1.0 (15/1495)	0.12	10.9 (30/274)	10.3 (189/1843)	0.72
	History of hand eczema	15.0 (43/287)	17.0 (261/1535)	0.40	26.2 (75/286)	25.7 (487/1894)	0.85
	Atopic dermatitis*	1.0 (2/287)	3.8 (58/1511)	0.007	5.3 (14/286)	6.8 (126/1863)	0.23
	Nickel allergy and a history of hand eczema	0.4 (1/269)	0.3 (4/1477)	0.57	4.4 (12/274)	2.9 (52/1819)	0.17

* Affirmative answer to the question 'Has your doctor ever told you that you suffered from atopic dermatitis?'

[†]Univariate analysis with χ^2 test and Fisher's exact test (when the expected values were less than 5) was used to test for statistical differences between study year 1990 and 2006.

Table 8. Nickel allergy stratified by a history of hand eczema among female participants examined in 1990 and 2006

Age group (years)		1990			2006		
		Nickel allergy		Crude OR	Nickel allergy		Crude OR
		Yes	No		Yes	No	
18–35	Yes	9.4 (10)	16.0 (17)	3.63 (1.33–9.96)	2.2 (8)	23.4 (85)	0.65 (0.29–1.46)
	No	10.4 (11)	64.2 (68)		9.4 (34)	65.0 (236)	
36–55	Yes	1.6 (2)	32.3 (40)	0.63 (0.12–3.28)	4.2 (38)	23.4 (210)	1.36 (0.86–1.98)
	No	4.8 (6)	61.3 (76)		8.8 (79)	63.5 (570)	
56–69	Yes	0 (0)	13.6 (6)	0.97 (0.92–1.03)	1.1 (6)	19.3 (108)	1.12 (0.44–2.85)
	No	2.3 (1)	84.1 (37)		3.8 (21)	75.8 (424)	

years) ($p < 0.002$) (Fig. 5 and Table 7) but increased from 1.6% (2/124) to 4.2% (38/897) among women aged 36–55 years ($p = 0.22$) and from 0% in 1990 to 1.0% (6/559) in 2006 among 56–69-year-old women ($p = 1.00$). Among 18–35-year-old nickel allergic women, the prevalence of hand eczema decreased from 47.6% (10/21) to 19.0% (8/42) ($p < 0.02$). In comparison, the prevalence of hand eczema increased among nickel allergic women aged 36–55 years from 25.0% (2/8) in 1990 to 32.5% (38/117) in 2006 ($p = 0.66$).

Table 8 shows unadjusted associations between nickel allergy and hand eczema among female participants. This approach was justified as the proportion of self-reported

atopic dermatitis did not differ significantly among female age groups in the two study years. Among women aged 18–35 years the association was markedly stronger in 1990 (OR = 3.63; 95% CI = 1.33–9.96) than in 2006 (OR = 0.65; 95% CI = 0.29–1.46), whereas this was not the case among women in older age groups (Table 8). Three identical logistic regression analyses with hand eczema as the dependent variable and with atopic dermatitis, nickel allergy, and study year (1990 versus 2006) as the explanatory variables were performed for women aged 18–35 years, 36–55 years, and 56–69 years, respectively. In these models, an interaction term between nickel allergy and study year was inserted to investigate

Table 9. Characteristics of patch tested participants stratified by null mutations in filaggrin – null alleles R501X and 2282del4

	All % (n/n _{total})	Filaggrin null mutation [†]		Odds ratio (95%CI)
		Yes, % (n/n _{total})	No, % (n/n _{total})	
Men	44.7 (1491/3335)	41.6 (112/269)	45.0 (1379/3066)	0.87 (0.68–1.12)
Women	55.3 (1844/3335)	58.4 (157/269)	55.0 (1687/3066)	1.15 (0.89–1.48)
Atopic dermatitis [‡]	10.4 (324/3202)	21.8 (56/257)	9.1 (268/2945)	2.78 (2.02–3.83)*
Contact sensitization to at least one allergen	9.8 (328/3335)	12.3 (33/269)	9.6 (295/3066)	1.31 (0.89–1.93)
Nickel sensitization, all respondents	6.1 (195/3213)	6.2 (16/258)	6.1 (179/2955)	1.03 (0.61–1.74)
Nickel sensitization, respondents without ear piercing	1.1 (17/1507)	1.9 (2/105)	1.1 (15/1402)	1.80 (0.41–7.96)
Allergic nickel dermatitis, [‡] all respondents	4.4 (147/3333)	4.8 (13/269)	4.4 (134/3064)	1.11 (0.62–1.99)
Allergic nickel dermatitis, [‡] respondents without ear piercing	0.6 (9/1551)	1.8 (2/111)	0.5 (7/1440)	3.76 (0.77–18.30)

[†]Genotyping was done for two common null mutations in the filaggrin gene (R501X and 2282del4).

^{‡†}Odds ratio with 95% confidence interval for the comparison of patients with and without null mutations in the filaggrin gene.

[‡]Atopic dermatitis was defined by the UK Working Party's diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria (386).

*Significant difference.

[‡]An affirmative answer to the question 'have you ever had eczema under the tightener of your watch strap, under the button of your jeans, or from wearing ear pins or earrings?' together with a positive patch test reaction to nickel sulfate.

Table 10. Null mutation status of R501X and 2282del4 among 3335 patch tested participants

		2282del4, % (n)		
		Wild-type	Heterozygote	Homozygote
R501X, % (n)	Wild-type	91.9 (3066)	4.5 (151)	0.1 (3)
	Heterozygote	3.3 (109)	0.1 (2)	0.03 (1)
	Homozygote	0.1 (3)	0	0

whether the association between nickel allergy and hand eczema was dependent on study year. This interaction term was found to be statistically significant for women aged 18–35 years ($p = 0.017$) whereas it was not significant for women aged 36–55 years ($p = 0.44$) and for women aged 56–69 years ($p = 1.00$).

Nickel allergy and filaggrin null mutations. The characteristics of participants are shown in Table 9. The genotyping success rate was 99.5% for both mutations. A total of 3335 participants (55.3% women and 44.7% men) were patch tested and tested for the R501X and 2282del4 null mutations. The observed genotype prevalences of the two polymorphisms did not deviate significantly ($p > 0.05$) from the expected prevalences under the Hardy–Weinberg equilibrium assumption. Filaggrin mutation status among participants is presented in Table 10. The combined carrier frequency was 8.1%.

Stratification by filaggrin mutation status revealed that the prevalence of self-reported atopic dermatitis was significantly higher among participants with null

mutations in the filaggrin gene than among participants without mutations (21.8% versus 9.1%) (Table 9). This positive association was found in both men and women (Table 11). A possible association between contact sensitization and filaggrin null mutation status was investigated. Nickel allergy or nickel dermatitis was not associated with filaggrin null mutations in the entire population (Table 9). Stratification by age group did not change the outcome of our crude data analyses.

The possible association between nickel allergy or allergic nickel dermatitis and filaggrin null mutations among subjects who did not report ear piercing was investigated (Tables 9 and 11). Crude data analysis revealed that, among female participants without ear piercing, filaggrin null mutation status was positively but not significantly associated with nickel allergy (8.3% versus 2.4%; OR = 3.71; 95% CI = 0.73–18.96) and positively and significantly associated with allergic nickel dermatitis (8.3% versus 1.3%; OR = 6.75; 95% CI = 1.17–38.91). Of note, the prevalence of atopic dermatitis was 13.4% in participants who were ear pierced and 6.4% in participants who were not ear pierced (for women these numbers were 14.5% and 6.6%, respectively). Thus, the association between filaggrin null mutations and nickel allergy in those without ear piercings were not explained by an over-representation of atopic dermatitis among subjects without ear piercings.

It was evaluated whether it could be assumed that the effect of filaggrin null mutation status was independent of nickel allergy. Thus, a logistic regression analysis was

Table 11. Characteristics of male and female participants stratified by filaggrin mutation status

	Filaggrin null mutation [†]					
	Men			Women		
	Yes, % (n)	No, % (n)	Crude OR with 95% CI ^{††}	Yes, % (n)	No, % (n)	Crude OR with 95% CI
Atopic dermatitis [‡]						
Contact sensitization to at least one allergen	18.1 (19/105)	5.4 (72/1323)	3.84 (2.21–6.66)*	24.3 (37/152)	12.1 (196/1622)	2.34 (1.57–3.49)*
Nickel sensitization, all respondents	3.6 (4/112)	4.7 (65/1379)	0.75 (0.27–2.10)	18.5 (29/157)	13.6 (230/1687)	1.44 (0.94–2.20)
Nickel sensitization, respondents without ear piercing	0 (0/104)	1.00 (14/1335)	—	10.4 (16/154)	10.2 (165/1620)	1.00 (0.94–1.06)
Allergic nickel dermatitis, [‡] all respondents	0 (0/81)	0.7 (8/1109)	—	8.3 (2/24)	2.4 (7/293)	3.71 (0.73–18.96)
Allergic nickel dermatitis, [‡] respondents without ear piercing	0 (0/112)	0.4 (6/1379)	—	8.3 (13/157)	7.6 (128/1685)	1.10 (0.61–1.99)
Ear piercing	21.6 (24/111)	16.6 (226/1365)	1.39 (0.86–2.23)	84.6 (132/156)	82.0 (1372/1673)	1.21 (0.77–1.90)

[†]Genotyping was done for two common null mutations in the filaggrin gene (R501X and 2282del4).

^{††}Odds ratio with 95% confidence interval for the comparison of patients with and without null mutations in the filaggrin gene.

[‡]Atopic dermatitis was defined by the UK Working Party's diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria (386), *Significant difference.

[‡]An affirmative answer to the question 'Have you ever had eczema under the tightener of your watch strap, under the button of your jeans, or from wearing ear pins or earrings?' together with a positive patch test reaction to nickel sulfate.

Table 12. Logistic regression analyses with the outcome allergic nickel dermatitis and with different explanatory variables performed in 3335 patch tested and filaggrin genotyped subjects (R501X and 2282del4)

Explanatory variables	Adjusted OR [†] with 95%CI	
	All participants	Female participants
Filaggrin null mutations		
No	1 (reference)	1 (reference)
Yes	1.03 (0.57–1.89)	1.08 (0.59–1.98)
Gender		
Male	1 (reference)	—
Female	8.91 (3.67–21.62)	—
Age		
18–35	1 (reference)	1 (reference)
36–55	1.37 (0.89–2.13)	1.44 (0.92–2.26)
56–69	0.52 (0.27–0.94)	0.53 (0.29–0.97)
Ear piercing		
No	1 (reference)	1 (reference)
Yes	4.23 (2.01–8.87)	4.27 (1.85–9.86)
Atopic dermatitis [‡]		
No	1 (reference)	1 (reference)
Yes	0.73 (0.42–1.25)	0.74 (0.43–1.28)

OR, odds ratio; CI, confidence interval.

[†]Mutually adjusted for variables shown in the table.[‡]Atopic dermatitis was defined by the UK Working Party's diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria (386).

performed with allergic nickel dermatitis as the dependent variable and with the following explanatory variables: gender, age group, atopic dermatitis, filaggrin mutation status, nickel allergy. In this model, a test for interaction between nickel allergy and filaggrin mutation status was performed by using a log-likelihood ratio test. The analysis revealed an insignificant *p*-value of the interaction term (*p* = 0.99). The result means that the possible effect of filaggrin null mutations on the prevalence of allergic nickel dermatitis did not differ significantly between those with or without nickel allergy. A similar logistic regression analysis was performed in female participants but it did not reveal any association between nickel allergy and filaggrin null mutation status (Table 12). An adjusted logistic regression analysis in female participants without ear piercing gave empty strata and is therefore not shown.

A logistic regression analysis with nickel allergy as the dependent variable and with gender, age group, ear-piercing status, and filaggrin mutation status as the independent variables showed that filaggrin null mutation status was not significantly associated with nickel allergy (Table 13). Also, the interaction term between filaggrin mutation status and ear-piercing status was *p* = 0.36. Thus, the effect of filaggrin null mutations on the prevalence of nickel allergy did not depend significantly on ear-piercing status. However, in a similar logistic regression

Table 13. Logistic regression analyses with the outcome nickel sensitization and with different explanatory variables performed in 3335 patch tested and filaggrin genotyped subjects (R501X and 2282del4)

Explanatory variables	Adjusted OR [†] with 95%CI	
	All participants	Female participants
Filaggrin null mutations		
No	1 (reference)	1 (reference)
Yes	0.90 (0.52–1.55)	0.97 (0.55–1.71)
Sex		
Male	1 (reference)	—
Female	5.34 (2.88–9.90)	—
Age		
18–35	1 (reference)	1 (reference)
36–55	1.19 (0.82–1.72)	1.15 (0.77–1.70)
56–69	0.51 (0.31–0.83)	0.48 (0.28–0.81)
Ear piercing		
No	1 (reference)	1 (reference)
Yes	3.58 (2.02–6.35)	3.77 (1.88–7.52)

OR, odds ratio; CI, confidence interval.

[†]Mutually adjusted for variables shown in the table.

analysis including only female participants, the interaction term between filaggrin mutation status and ear piercing was borderline significant (*p* = 0.08). The outcome of a logistic regression analysis performed for female participants is shown in Table 13.

Part 2: Longitudinal patch test study

Nickel allergy and dermatitis. A total of 10 335 female dermatitis patients aged 5–99 years were patch tested. The median age (IQR) was 46.0 (28.0) years among all tested women, 40.0 (22.0) years among patients with nickel allergy, 42.0 (25.25) years among patients with cobalt allergy, 37.0 (19.5) years among patients with concomitant nickel and cobalt allergy, and finally 52.5 (26.0) years among patients with isolated cobalt allergy (i.e. patch test reactivity to cobalt but not nickel). The median age was significantly higher among patients with isolated cobalt allergy than among patients with nickel allergy (*p* < 0.001).

The overall prevalence of nickel allergy was 18.6% and increased from 17.9% in the period 1985–1990 to 20.2% in the period 2002–2007 (Table 14). A logistic regression analysis with nickel allergy as the dependent variable and with test year, age group, and an interaction term between age group and test year as the explanatory variables showed that the change in prevalence of nickel allergy was significantly dependent on age group (the *p*-value for interaction was 0.001). This means that the change in the prevalence of nickel allergy from 1985 to 2007 cannot be presented as one overall estimate and should be stratified by age group (Table 14 and Fig. 6). Thus, the

Table 14. Patch test reactivity to nickel and cobalt among 10 335 female dermatitis patients tested at the Department of Dermato-Allergology, Gentofte Hospital, Denmark, between 1985 and 2007 stratified by age group (5–30 years, 31–49 years, >50 years) and test year (1985–1990, 1991–1996, 1997–2001, 2002–2007)

	Test year				$p_{\text{trend}} (\chi^2)$
	1985–1990 % (n = 2103)	1991–1996 % (n = 2649)	1997–2001 % (n = 2688)	2002–2007 % (n = 2895)	
Nickel allergy					
All age groups	17.9 (377)	17.5 (464)	18.6 (500)	20.2 (586)	0.01
5–30	25.1 (149)	22.8 (143)	20.2 (116)	18.3 (110)	0.002
31–49	19.6 (131)	21.8 (186)	25.6 (236)	29.0 (287)	0.001
>50	11.5 (97)	11.6 (135)	12.4 (148)	14.5 (189)	0.02
Concomitant nickel and cobalt allergy					
All age groups	3.2 (68)	2.2 (58)	2.9 (77)	3.5 (101)	0.21
5–30	4.7 (28)	3.2 (20)	4.2 (24)	3.8 (23)	0.64
31–49	3.9 (26)	2.7 (23)	4.0 (37)	5.6 (55)	0.02
>50	1.7 (14)	1.3 (15)	1.3 (16)	1.8 (23)	0.71
Cobalt allergy only (not nickel allergy)					
All age groups	2.3 (40)	1.3 (28)	2.4 (53)	2.3 (53)	0.42
5–30	1.8 (8)	1.0 (5)	2.2 (10)	1.6 (8)	0.66
31–49	3.2 (17)	0.7 (5)	2.0 (14)	1.6 (11)	0.12
>50	2.0 (15)	1.7 (18)	2.8 (29)	3.1 (34)	0.07
Cobalt allergy (and possibly nickel allergy)					
All age groups	5.1 (108)	3.2 (86)	4.8 (130)	5.3 (154)	0.13
5–30	6.1 (36)	4.0 (25)	5.9 (34)	5.1 (31)	0.85
31–49	6.4 (43)	3.3 (28)	5.5 (51)	6.7 (66)	0.22
>50	3.4 (29)	2.8 (33)	3.8 (45)	4.4 (57)	0.10

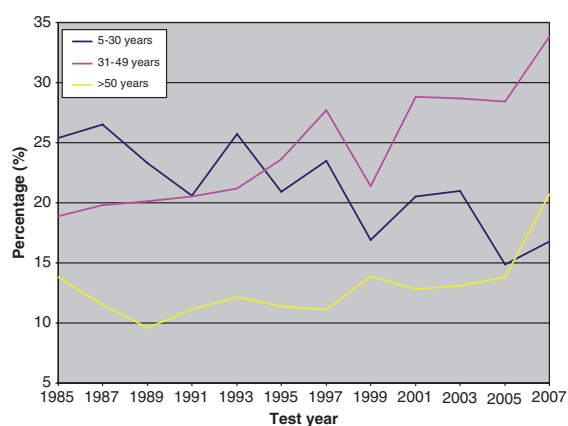


Fig. 6. The prevalence of nickel contact allergy observed between 1985 and 2007 among female dermatitis patients patch tested at the Department of Dermato-Allergology, Gentofte University Hospital, Denmark. A χ^2 trend test was performed to test for possible significant differences over time. The decline of reactions observed between 1985 and 2007 was significant among 5–30 year olds ($p_{\text{trend}} < 0.01$) whereas the increase during the same period was significant among 31–49 year olds ($p_{\text{trend}} < 0.001$) and among patients above 50 years of age ($p_{\text{trend}} < 0.01$).

prevalence of nickel allergy decreased significantly among 5–30 year olds from 27.6% in 1985 to 16.8% in 2007 ($p_{\text{trend}} < 0.002$) but increased among 31–49 year olds from 21.3% to 33.8% in the same period ($p_{\text{trend}} < 0.001$),

and from 13.2% to 20.7% among patients older than 50 years ($p_{\text{trend}} < 0.02$). The prevalence of nickel allergy was generally lower among patients older than 50 years as it was 12.6% during the study period in comparison with 21.6% and 24.1%, respectively, for patients aged 5–30 years and 31–49 years. Thus, patients older than 50 years had a significantly lower prevalence of nickel allergy than patients aged 5–30 years (OR = 0.52; 95% CI = 0.46–0.60) (Table 15). Finally, a logistic regression analysis with nickel allergy as the dependent variable and test year, cobalt allergy, and an interaction term between test year and cobalt allergy as the explanatory variables did not reveal any significant changes in the association of cobalt and nickel allergy over the study period in different age groups.

The overall prevalence of cobalt allergy, concomitant nickel and cobalt allergy, and isolated cobalt allergy was 4.6%, 2.9%, and 1.7%, respectively. A logistic regression analysis with cobalt allergy as the dependent variable and study year and age group as the explanatory variables showed that the development in the prevalence of cobalt allergy was not dependent on age group (the p -value for interaction between age group and test year was 0.77). However, it is possible that the prevalence of cobalt allergy to some degree was dependent on age group as the prevalence increased among middle-aged and older patients but appeared stable among the youngest patients (5–30

Table 15. The prevalence of nickel allergy among 10 335 female dermatitis patients stratified by age group, patch test year, and cobalt allergy

Frequency of variables	Nickel allergy % (n/total)	Crude OR with 95% CI	Adjusted OR [†] with 95% CI
Age group (years)			
5–30	21.6 (518/2397)	1.00 (ref)	1.00 (ref)
31–49	24.5 (840/3433)	1.18 (1.03–1.33)	1.17 (1.03–1.33)
>50	12.6 (569/4505)	0.52 (0.46–0.60)	0.52 (0.46–0.60)
Patch test year			
1985–1990	17.9 (377/2103)	1.00 (ref)	1.00 (ref)
1991–1996	17.5 (464/2649)	0.97 (0.83–1.13)	1.06 (0.91–1.24)
1997–2001	18.6 (500/2688)	1.05 (0.90–1.21)	1.09 (0.93–1.27)
2002–2007	20.2 (586/2895)	1.16 (1.00–1.34)	1.20 (1.03–1.40)
Cobalt allergy			
No	16.5 (1623/9857)	1.00 (ref)	1.00 (ref)
Yes	63.6 (304/478)	8.86 (7.30–10.76)	8.73 (7.17–10.64)

Risk estimates were calculated in a binary logistic regression analysis.

OR, odds ratio; CI, confidence interval.

[†]Age group, test year, and cobalt sensitization.

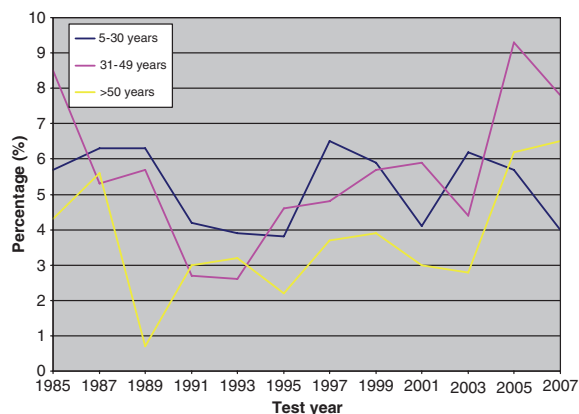


Fig. 7. The prevalence of cobalt contact allergy observed between 1985 and 2007 among female dermatitis patients patch tested at the Department of Dermato-Allergology, Gentofte University Hospital, Denmark.

years) (Fig. 7). A regression analysis was also performed with isolated cobalt allergy as the dependent variable and the same explanatory variables as mentioned above (age group and study year). It also revealed that the change in the prevalence of isolated cobalt allergy was not dependent on age group (the *p*-value for interaction between age group and test year was 0.22). However, because of the low number of isolated positive patch test reactions to cobalt and fluctuations over time, the data were difficult to interpret. Nevertheless, a general increase in the prevalence of isolated cobalt allergy was observed during the study period among patients above 50 years of age as 0.3% were sensitized in 1989 and 2.7% in 2007 (*p* < 0.008).

Part 3: Metal exposure assessment studies

Nickel release, Copenhagen. Of 354 metallic pieces of jewellery and accessories randomly purchased from 36 stores

in Copenhagen, Denmark, 22.0% gave positive DMG test reactions. Stratification by sort of item (earring, bracelet, necklace, finger ring, or hair clasp) and by age category (children or adult/adolescent) was performed (Table 16). It revealed that for items produced for adults and adolescents, hair clasps (19.3%), earrings (14.8%), and necklaces (12.9%) frequently released an excessive amount of nickel as assessed by the DMG test. Of 25 stores visited in Copenhagen where jewellery for adults was purchased, DMG-test-positive items were purchased from nine (36.0%). Furthermore, for items designed for children, DMG positivity was identified for hair clasps (79.4%) and for finger rings (20%). Only very small samples of necklaces, bracelets, and earrings were purchased for children but a positive DMG test result was identified in one necklace and in one bracelet purchased from two different expensive children stores. Four (50.0%) of eight children's clothing stores sold jewellery that released too much nickel.

Table 17 shows the outcome of DMG testing for various items stratified by category of store. The highest proportion of DMG-test-positive earrings was purchased from two street vendors (57.1%) at the busiest pedestrian street in downtown Copenhagen (Stroeget) and from one of two immigrant clothing stores (50.0%) in a different neighbourhood of the city (Nørrebro). DMG-test-positive jewellery was also purchased from downtown clothing and accessory stores of which some were national (but not international) chains. Of note, excessive nickel release was never identified in jewellery from any supermarket or department store whereas one earring purchased from a jewellery homepage on the internet was positive upon DMG testing. Although the sample size for necklaces and bracelets was very small, the study suggested that excessive nickel release was relatively frequent in

Table 16. Dimethylglyoxime (DMG) testing in a random sample of various jewellery items and hair clasps purchased in Copenhagen, Denmark, between December 2008 and March 2009

Item examined	DMG test result positive, % (n/n _{total})
Hair clasps	
total	36.8 (43/117)
for adults or adolescents	19.3 (16/83)
for children	79.4 (27/34)
Finger rings	
total	18.8 (3/16)
for adults or adolescents	0 (0/1)
for children	20.0 (3/15)
Necklaces	
total	15.2 (5/33)
for adults or adolescents	12.9 (4/31)
for children	50.0 (1/2)
Earrings	
total	14.7 (25/170)
for adults or adolescents	14.8 (25/169)
for children	0 (0/1)
Bracelets	
total	11.1 (2/18)
for adults or adolescents	5.9 (1/17)
for children	100.0 (1/1)
Accumulated	
total	22.0 (78/354)
for adults or adolescents	15.3 (46/301)
for children	60.4 (32/53)

jewellery intended for Danish children and adults. Finally, DMG testing revealed that hair clasps for adults and especially for children frequently released an excessive amount of nickel and that this finding was independent of the store that sold them. When all categories of jewellery were combined and hair clasps were excluded, 30 (13.7%) of 218 items released too much nickel. If street vendors and children's stores were excluded from a similar calculation, 16 (8.3%) of 192 items released an excessive amount of nickel.

Observations from Copenhagen. One of the street vendors guaranteed that all his jewellery was nickel free and stated that he had personally tested the items himself. Also, he said that the Danish authorities had fined him previously and that customers should return for reimbursement if they identified nickel release. In one expensive children's clothing store, the owner spontaneously said that she had personally examined the three purchased items for nickel release. However, two of three gave positive DMG test results. Finally, the internet jewellery homepage was written in Danish but clearly did not have Danish owners but belonged to an Asian company.

Table 17. The outcome of dimethylglyoxime (DMG) testing of a random sample of jewellery and hair clasps purchased in different categories of stores in Copenhagen, Denmark, between December 2008 and March 2009

Shop category	Item tested	DMG test result positive % (n/n _{total})
Clothing stores	Earrings	4.0 (2/50)
	Bracelets	20.0 (1/5)
	Necklaces	12.5 (2/16)
Immigrant clothing stores	Hair clasps	40.0 (4/10)
	Earrings	50.0 (6/12)
	Necklaces	0 (0/1)
Accessory stores	Hair clasps	0 (0/3)
	Earrings	7.7 (4/52)
	Bracelets	0 (0/4)
Street vendors	Necklaces	0 (0/2)
	Hair clasps	28.0 (7/25)
	Earrings	57.1 (12/21)
Supermarkets	Bracelets	0 (0/3)
	Necklaces	100.0 (2/2)
	Earrings	0 (0/19)
Department stores	Necklaces	0 (0/8)
	Hair clasps	15.4 (4/26)
	Finger rings	0 (0/1)
Internet jewellery homepage	Earrings	0 (0/8)
	Bracelets	0 (0/2)
	Necklaces	0 (0/2)
Children clothing stores	Hair clasps	5.3 (1/19)
	Earrings	13.3 (1/7)
	Bracelets	0 (0/3)
	Earrings	0 (0/1)
	Bracelets	100.0 (1/1)
	Necklaces	50.0 (1/2)
	Hair clasps	79.4 (27/34)
	Finger rings	20.0 (3/15)

Cobalt release, Copenhagen. For the entire study collection of jewellery and hair clasps, 1.1% (four of 354 items) gave a positive reaction with the cobalt spot test. Since none of the hair clasps released cobalt, the proportion of positive reactions in the jewellery group was 1.7% (four of 237 items). Cobalt-releasing jewellery included three earrings and one necklace which all had a dark coloured appearance (Fig. 8). Thus, cobalt release was detected from four of seven items that had a dark appearance. Among cobalt-releasing jewellery, only the piercing post of the earring that had a decorative pendant (butterfly) also gave a positive DMG test reaction (Fig. 6). Confirmatory testing with the gel test in a subsample of 40 jewellery items revealed no further positive cobalt reactions. One earring was purchased from a street vendor at the busiest pedestrian street in downtown Copenhagen (Stroeget) whereas the remaining pieces were purchased from three different accessory stores within the centre of Copenhagen.



Fig. 8. Three earrings and one necklace that released cobalt as assessed by the cobalt gel test. Red coloration of the yellow gel indicates cobalt release. All items had a dark metallic appearance.

Cross-sectional SEM/EDS analyses of cobalt-spot-test-positive jewellery revealed a thin surface layer (under $1\ \mu\text{m}$ thickness) that contained cobalt in all items except for the piercing post used for the earring that had a butterfly pendant (Table 18 and Figs. 2 and 9). One example of a cross-sectional SEM/EDS analysis is shown for the butterfly pendant (Fig. 10) but not for remaining jewellery. It revealed a bulk made mainly of lead. Similar results were obtained when cross-sections of remaining cobalt-releasing items were analysed although copper instead of lead was used as bulk material in these. The surface results seemed to be in accordance with the cobalt spot test results. Thus, all items contained cobalt in the surface and also released cobalt when investigated with the cobalt spot test. Of interest, tin–cobalt plating was found on the surface of all items, probably as a decorative coating to obtain a ‘black pearl’ colour effect (Table 18).

Nickel release, London and Warsaw. DMG testing revealed that 15.1% and 18.4%, respectively, of earrings purchased in London and Warsaw released nickel as indicated by positive test outcomes. Stratification by store category showed that DMG-test-positive jewellery was mainly purchased from street markets (36.7% in Warsaw and 38.7% in London) and from stores that seemed to have independent ownership and were not part of national

or international chains, e.g. local accessory or clothing stores, freestanding booths in underground stations or malls, drug stores, and local artists (39.7% in Warsaw and 25.0% in London). Positive DMG test results were much less frequent in earrings purchased from chain accessory or clothing stores. The proportion of piercing posts (the metallic part that perforates the ear) that gave positive DMG test reactions was 17.5% in earrings from Warsaw and 9.3% in earrings from London. The proportion of pendants (the decorative part hanging from the post or ring) that gave positive DMG tests was 5.2% from earrings purchased in Warsaw and 15.0% from earrings purchased in London.

Observations from London and Warsaw. Identical earrings were found on two occasions in different clothing chain stores in London and Warsaw (all DMG test negative). This may indicate that large earring producers cover the European market. Furthermore, salespeople and vendors in smaller individually owned stores and market booths were asked where they had purchased the earrings. Most earrings in both London and Warsaw were imported from Thailand, China, the Philippines, Indonesia, and India. According to a Turkish salesperson from the Church Street Market in London, individuals from an Asian background contacted him and other salespersons from time to time to sell jewellery they had personally imported from Asian countries. Such vendors will typically cover their travel expenses for visiting family and friends in their former home countries by selling jewellery in their new home country. A salesperson from the underground at Westminster tube station (next to the Houses of Parliament) spontaneously said that his products were nickel free and that authorities had once checked his jewellery for nickel release and found the levels acceptable. He said that authorities typically visit new stores or booths soon after they open (two DMG-test-positive jewellery items were purchased from this vendor).

Part 4: Validation of the nickel spot test

Sensitivity and specificity. Tables 19 and 20 show test results obtained by concomitantly performing the DMG spot test as well as the chemical analysis described in the EN 1811 on 96 pairs of metallic components. DMG testing revealed that 33 (34.4%) pairs of components were positive, 61 (63.5%) were negative and that two (2.1%) were doubtful. The median area of the metallic components was $2.61\ \text{cm}^2$ (range $0.63\text{--}28.34\ \text{cm}^2$) and chemical analysis showed that 55 (57.3%) pairs of components released more than $0.5\ \mu\text{g}\ \text{nickel}/\text{cm}^2/\text{week}$ and that 41 (42.3%)

Table 18. Distribution of metals after surface energy dispersive spectroscopy analysis on items that gave cobalt release (Figs. 8 and 9)

Element spectrum (wt%)	Co	Ni	Co	Sn	Fe	Zn	O
Earring, piercing post (a)	0	83.9	0	6.1	9.5	0	0
Earring, decorative part (a)	3.3	0	90.7	5.8	0	0	0
Neck of cat used as a decorative part (b)	3.0	0	85.8	11.2	0	0	0
Stud used for earring with a cat as decorative part (b)	6.0	0	77.2	15.8	0	0	0.8
Decorative anchor (c)	23.0	0	44.7	28.8	0	2.0	1.5
Setting for stone (d)	2.9	2.0	84.9	10.2	0	0	0

(a), (b), (c), and (d) refer to Fig. 9.

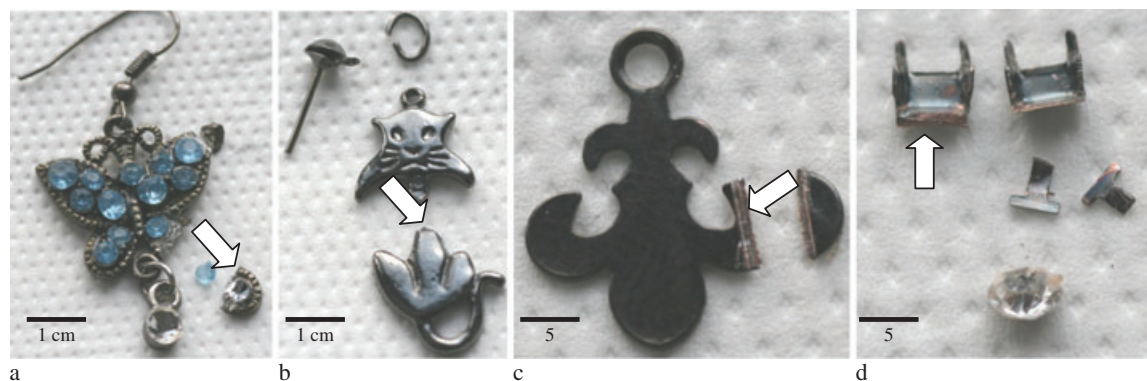


Fig. 9. Pieces of the dark appearing jewellery that gave positive cobalt spot test results were mounted in a conductive embedding material and polished to a mirror-like finish. This embedding method made it possible to investigate the cross-sections (marked with arrows) by scanning electron microscopy and energy dispersive spectroscopy analysis.

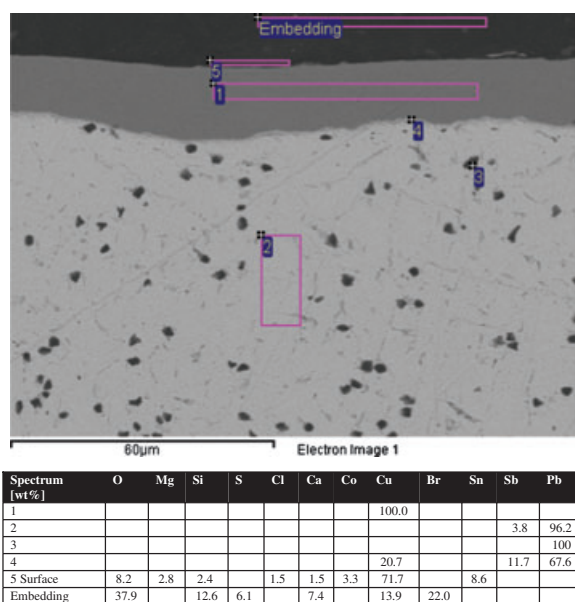


Fig. 10. Scanning electron micrograph of the cross-section of the butterfly pendant (Fig. 9a). Energy dispersive spectroscopy analysis indicates that lead (spectra 2 and 3) was coated with copper (spectrum 1) and then a thin layer (below 1 μ m) of a cobalt- and tin-containing alloy (spectrum 5).

pairs released less than 0.5 μ g nickel/cm²/week. Median nickel release after 1 week in artificial sweat was 18.9 μ g nickel/cm²/week (range 0.1–598.4) among DMG-test-positive metallic components and 0.11 μ g nickel/cm²/week (range 0.01–88.1) among DMG-test-negative metallic components (Tables 1 and 2). A calculation excluding the two doubtful DMG test results showed that the sensitivity of the DMG test was 59.3% (95% CI = 13.1–46.2%) and that the specificity was 97.5% (95% CI = 92.7–100%) (Table 21).

Nickel release after chemical analysis. When the DMG test was repeated after immersion in artificial sweat, eight (24.2%) of 33 initially DMG-test-positive components had a negative DMG test result (Table 19). Of the 63 initially DMG-test-negative or doubtful components, 28 were retested, excluding those with a negative XRF result and the lowest nickel release values. It was revealed that five (17.9%) of the initially DMG-test-negative components had a positive DMG test (Table 20). When the surface of all components was visually inspected after immersion for 1 week in artificial sweat, by comparison with photos taken for the area measurement the appearance had often changed (Tables 19 and 20). Only qualitative assessment was done with no attempt to

Table 19. Results from testing in total 96 pairs of earring components by the dimethylglyoxime (DMG) screening test for nickel release, by immersion in artificial sweat and chemical analysis according to EN 1811 for assessment of the amount of nickel release, and by X-ray fluorescence spectrometry (XRF) for nickel in the outer layers – results of the 33 initially DMG test positives

Sample (no.)	DMG test result [†]	DMG test result after artificial sweat [‡]	Chemical analysis (µg nickel/cm ² /week)	XRF* (% nickel)	Visible surface change after artificial sweat ^{‡‡}
1	+	–	0.11 [¶]	2.6	–
2	+	+	0.88	28	–
3	+	+	1.43	26	–
4	+	+	1.54	45	–
5	+	+	1.74	36	–
6	+	–	1.91	4.7	+
7	+	+	2.07	1.7	+
8	+	–	2.19	17	–
9	+	–	2.24	63	–
10	+	+	2.42	36	–
11	+	+	2.83	7.7	+
12	+	+	3.67	11	+
13	+	+	3.94	3.5	+
14	+	–	4.01	65	–
15	+	+	5.05	31	+
16	+	+	18.9	13	+
17	+	–	189	6.3	+
18	+	–	26.1	1.2	+
19	+	+	36.3	13	+
20	+	–	38.4	44	–
21	+	+	45.9	8.2	+
22	+	+	49.0	20	+
23	+	+	59.8	28	+
24	+	+	67.1	43	–
25	+	+	68.3	13	+
26	+	+	80.8	24	+
27	+	+	129	25	+
28	+	+	145	22	+
29	+	+	155	25	+
30	+	+	200	27	+
31	+	+	204	38	+
32	+	+	410	42	+
33	+	+	598	18	+
All samples (n = 33)	33 pos	8 neg 25 pos	1 < 0.5 µg/cm ² /week 32 ≥ 0.5 µg/cm ² /week	33 pos	11 no visible change 22 visible change

+, positive DMG test result or visible surface change; –, negative DMG test result or no visible surface change.

[¶]Discrepancy.

*XRF performed prior to immersion for 1 week in artificial sweat.

[†]DMG test prior to immersion for 1 week in artificial sweat.

[‡]DMG test after immersion for 1 week in artificial sweat according to EN 1811.

^{‡‡}Assessed by visual inspection after immersion in artificial sweat and comparing with photographs for area measurement before immersion.

quantify or describe the changes, which varied from small to large.

XRF analysis. Metallic components were examined for their metal content by XRF (Tables 19 and 20). All components releasing more than 0.5 µg nickel/cm²/week also contained nickel. Furthermore, few components with high nickel content did not release nickel or released nickel in amounts that were lower than 0.5 µg nickel/cm²/week.

Part 5: Validation of the cobalt spot test

Threshold determination. A weak colour change from yellow to bright red was visually detectable at 1.7 ppm cobalt and a clear red colour was visible at 8.3 ppm cobalt (Fig. 11), since 50 µl reagent plus 10 µl cobalt nitrate standard solution containing 0.1 µg cobalt equals 1.7 ppm cobalt; and 50 µl reagent plus 10 µl cobalt nitrate standard solution containing 0.5 µg cobalt equals 8.3 ppm cobalt. Under test conditions, trained as well as untrained users

Table 20. Results from testing in total 96 pairs of earring components by the dimethylglyoxime (DMG) screening test for nickel release, by immersion in artificial sweat and chemical analysis according to EN 1811 for assessment of the amount of nickel release, and by X-ray fluorescence spectrometry (XRF) for nickel in the outer layers – results of the 61 initially DMG test negatives and two DMG test doubtful results

Sample (no.)	DMG test result before artificial sweat [†]	DMG test result after artificial sweat [‡]	Chemical analysis (µg nickel/cm ² /week)	XRF (% nickel)	Visible surface change after artificial sweat ^{‡‡}
34	—	n.t.	<0.01	—	+
35	—	n.t.	<0.02	—	+
36	—	n.t.	<0.02	—	+
37	—	n.t.	<0.02	—	+
38	—	n.t.	<0.03	—	+
39	—	n.t.	<0.03	—	+
40	—	n.t.	<0.03	—	+
41	—	n.t.	<0.03	—	+
42	—	n.t.	<0.04	12	+
43	—	n.t.	<0.04	—	+
44	—	n.t.	<0.05	—	+
45	—	n.t.	<0.05	—	+
46	—	n.t.	<0.05	—	+
47	—	n.t.	<0.05	—	+
48	—	n.t.	<0.06	—	+
49	—	n.t.	<0.10	—	—
50	—	n.t.	<0.26	0.2	+
51	—	n.t.	0.01	—	+
52	—	n.t.	0.04	—	+
53	—	n.t.	0.04	—	—
54	—	n.t.	0.05	—	—
55	—	n.t.	0.05	—	+
56	—	n.t.	0.05	—	—
57	—	n.t.	0.06	—	+
58	—	n.t.	0.06	—	+
59	—	n.t.	0.06	—	+
60	—	n.t.	0.06	—	+
61	—	n.t.	0.06	—	+
62	—	n.t.	0.06	—	+
63	—	n.t.	0.10	—	+
64	—	n.t.	0.11	—	+
65	—	n.t.	0.12	—	+
66	—	n.t.	0.13	—	+
67	—	n.t.	0.14	3.1	+
68	—	—	0.16	—	+
69	—	—	0.20	—	+
70	—	—	0.20	0.2	+
71	—	—	0.20	0.06	+
72	—	—	0.33	25	+
73	—	—	0.51	—	+
74	—	—	0.55	2.9	+
75	—	—	0.71	2.3	—
76	—	—	0.75	9.3	+
77	—	—	0.79	19	—
78	—	—	0.81	43	+
79	—	—	1.08	1.8	+
80	—	—	1.63	53	+
81	—	—	2.47	7.6	+
82	—	—	2.80	6.5	+
83	—	—	3.50	4.7	+
84	—	—	3.59	0.3	+
85	—	—	3.75	5.1	+

Table 20. *Continued*

Sample (no.)	DMG test result before artificial sweat [†]	DMG test result after artificial sweat [‡]	Chemical analysis (μg nickel/ cm^2 /week)	XRF (% nickel)	Visible surface change after artificial sweat ^{†‡}
86	—	—	4.38	9.7	+
87	—	—	4.56	13	+
88	—	—	4.73	40	+
89	—	—	5.73	4.8	—
90	—	+	11.5	13	+
91	—	+	19.2	25	+
92	—	+	20.4	13	+
93	—	+	22.9	8.6	+
94	—	+	88.1	8.5	+
95	?	n.t.	0.06	—	+
96	?	—	5.95	15	—
All samples (n = 63)	61 neg, 2 doubtful	35 not tested, 23 neg, 5 pos	40 < 0.5 $\mu\text{g}/\text{cm}^2$ /week 23 $\geq$ 0.5 $\mu\text{g}/\text{cm}^2$ /week	35 neg, 28 pos	8 no visible change 55 visible change

+, positive DMG test result or visible surface change; —, negative DMG test result, or no nickel detected by XRF, or no visible surface change;

?, doubtful DMG test result; n.t., not tested by DMG test.

[†]DMG test prior to immersion for 1 week in artificial sweat.

[‡]DMG test after immersion for 1 week in artificial sweat according to EN 1811.

^{†‡}Assessed by visual inspection after immersion in artificial sweat and comparing with photographs for area measurement before immersion.

Table 21. Comparison of test results obtained by using the dimethylglyoxime (DMG) test and assessment of nickel release by immersion in artificial sweat and chemical analysis according to EN 1811 (gold standard) in 94 pairs of earring components

		Chemical analysis		Total
		+	—	
DMG test result*	+	32	1	33
	—	22	39	61
Total		54	40	94

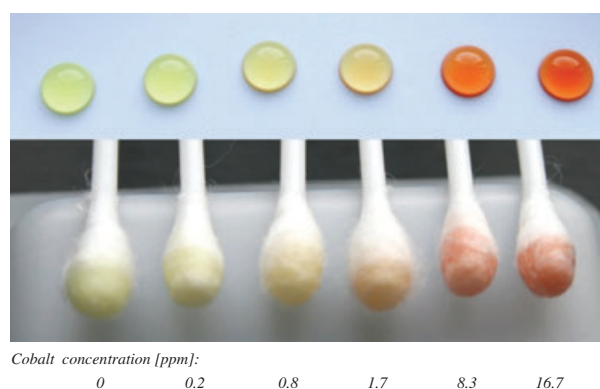
Data based on the results in Tables 19 and 20 (two samples with doubtful DMG test results in Table 20 were excluded from this table).

+, positive DMG test or nickel release $\geq 0.5 \mu\text{g}/\text{cm}^2$ /week according to chemical analysis; —, negative DMG test or nickel release $< 0.5 \mu\text{g}/\text{cm}^2$ /week according to chemical analysis.

*DMG test before immersion in artificial sweat.

of the cobalt test with normal colour vision may be able to detect a positive cobalt test when the cobalt ion concentration is above 8 ppm.

Effect of temperature and storage. The outcome of a positive test was not affected by storage of the cobalt test reagent in a closed vessel in a refrigerator at 5°C for 10 months. Also 24 hr storage at -21°C or $+60^\circ\text{C}$ did not impede the red colour formation when rubbing the cobalt test reagent with a stick on a cobalt-containing sample. Threshold determination of red colour change was reassessed after cooling of the cobalt test for 24 hr at -24°C , 5°C and storage for 24 hr at 60°C , respectively. However, cooling and warming did not affect the previously determined

**Fig. 11.** The threshold level of the cobalt test was evaluated. Thus, the threshold for a red colour change in a mixture of 10 μl cobalt nitrate standard solution and 50 μl test solution was estimated visually by the investigators. The colour change was judged to be clear and visible at a cobalt ion concentration of 8.3 ppm.

detection level at 8.3 ppm cobalt in the cobalt test reagent.

Testing of other metals and alloys. Samples of pure metals and alloys known not to contain cobalt were subjected to the cobalt gel test to assess the specificity of the test and possible reactivity to metals other than cobalt. However, no positive reactions were detected.

Testing clinical relevance of threshold level. The cobalt gel test was able to detect cobalt release from discs A, B, C, D,

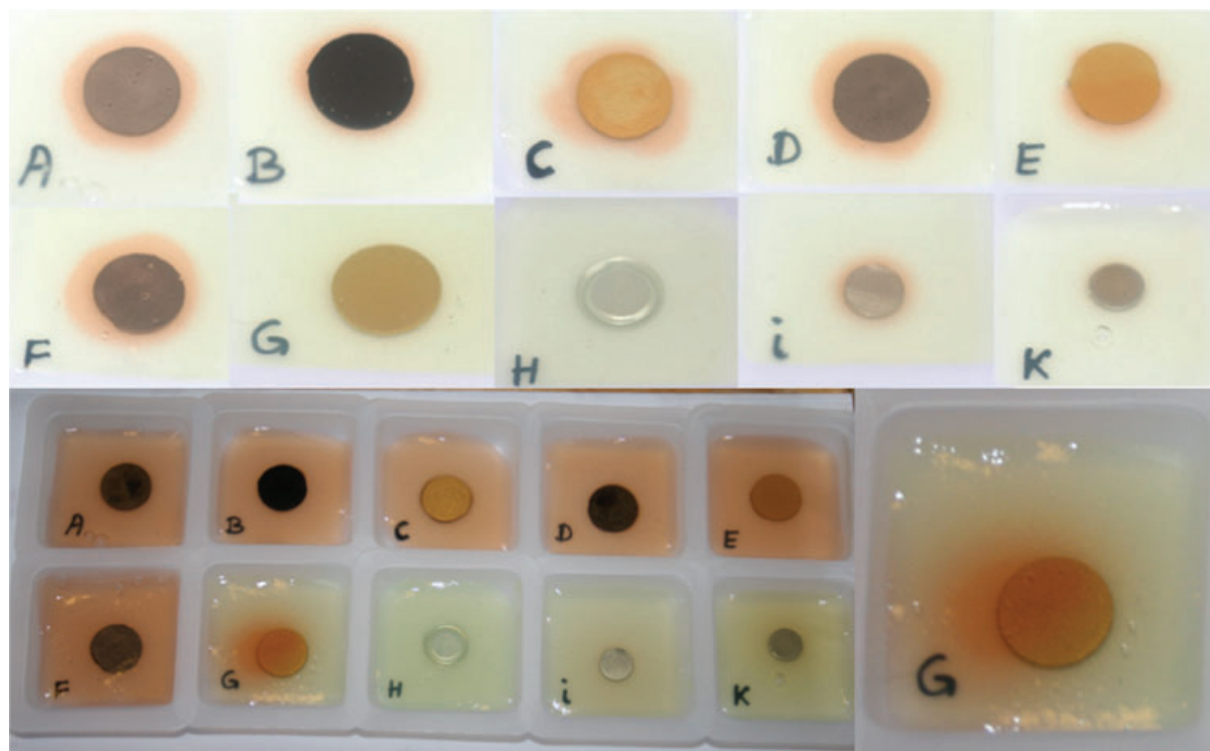


Fig. 12. The cobalt gel test prepared from the cobalt cotton stick test was used to test for cobalt release in 10 discs of which nine were made of material previously shown to release cobalt (except H) when immersed in artificial sweat (170) (Table 22). Upper lines (30 min testing): A, B, C, D, E, F, and I were judged to be positive after 30 min in the cobalt gel test (as assessed by red coloration of the gel surrounding the disc) whereas G, H, and K were negative. Lower lines (4 days of testing): A, B, C, D, E, and F coloured the entire gel red since cobalt release was continuous. Disc G, a hard metal alloy containing 15 wt% cobalt in its bulk and a coating of TiCN + Al₂O₃ + TiN, became positive after 4 days in the cobalt gel test (but was negative after 30 min). This time delay indicated slow diffusion of cobalt ions from the bulk material through the TiCN + Al₂O₃ + TiN coating pores. The gel for discs I and K was only slightly red coloured which indicates that cobalt release quickly stopped. Disc H remained negative. (TiCN, titanium carbonitride; TiN, titanium nitride; Al₂O₃, aluminium oxide.)

E, F, and I after 30 min (Fig. 12 and Table 22). These results were in accordance with those obtained following chemical analysis, whereas discordant results were found for discs G and K (170). Disc G was positive after 4 days in the gel but no positive reaction was observed for the control disk K (Fig. 12).

Discussion

The EU Nickel Directive

Over the second half of the 20th century, the nickel allergy epidemic accelerated, as European and North American consumers became increasingly nickel allergic resulting in morbidity, sick leave and increased healthcare costs. Researchers in Denmark, Sweden, the USA and elsewhere attempted to establish elicitation thresholds that could be used to prevent allergic nickel dermatitis (16, 33, 42). Menné et al. probably suggested the most operational

attempt as they patch tested nickel allergic dermatitis patients with metal discs of known composition and later investigated the discs for their nickel release by using a corrosion test method and the DMG nickel spot test (16). Based on their results, the 1990 Danish nickel regulation dictated that certain defined consumer items should not release more than 0.5 µg nickel/cm²/week and that the DMG test should be used as the analytical method to test for compliance with the regulation (Table 1) (379). Menné et al. stated that 'arbitrarily, alloys with nickel release exceeding 1.0 µg nickel/cm²/week gave strong patch test reactions, and those below 0.5 µg nickel/cm²/week gave weak reactions' and suggested 'that the nickel allergy problem could be minimized by the use of alloys with a nickel release rate of less than 0.5 µg nickel/cm²/week' (16). The original version of the EU Nickel Directive (1994) was based on the release limit from the Danish nickel regulation, although it also contained a metal content limit from the Swedish nickel regulation as

Table 22. The cobalt gel test, prepared from the cobalt cotton stick test (in 2010 or 2011 available from SmartHealth, Phoenix, Arizona), was used to verify cobalt release from 10 discs of the same batch as had been used in a previous study (170); cobalt release had been confirmed in nine of these hard metal materials (A–G) and dental alloys (I–K) by immersion in artificial sweat whereas disc H was a control made of aluminium; discs A–G and I–K had been used for patch testing in 19 cobalt allergic dermatitis patients (170)

Disc no.	Metal items examined			Test results		
	Cobalt content in base alloy %	Coating	Usage	Chemical analysis*	Patch test†	Cobalt gel test
A	6.0	None	Hard metal tools	230; 180	18	+
B	6.0	TiCN + Al ₂ O ₃	Hard metal tools	140; 180	14	+
C	6.0	TiN	Hard metal tools	220; 160	10	+
D	7.5	None	Hard metal tools	240; 290	18	+
E	7.5	TiCN + Al ₂ O ₃ + TiN	Hard metal tools	140; 160	10	+
F	15.0	None	Hard metal tools	150; 140	18	+
G	15.0	TiCN + Al ₂ O ₃ + TiN	Hard metal tools	9.4; 7.6	3	0†
H	0	None	Control disc made of aluminium	—	1	—
I	64.6	None	Dental alloy	0.69; 0.82	8	+
K	33.0	None	Dental alloy	1.4; 1.6	13	0

TiCN, titanium carbonitride; TiN, titanium nitride; Al₂O₃, aluminium oxide.

+, positive cobalt gel test; 0, negative cobalt gel test; —, not done; †, disc G became positive after 4 days in the gel (Fig. 6).

*Cobalt detected after immersion in artificial sweat for 1 week; the two different numbers indicate cobalt release in µg/cm² for two identical discs after 1 week in artificial sweat. Based on results in (170).

†Indicates the number of cobalt allergic patients who had at least a + reaction out of 19 patients that were patch tested. Based on results in (170).

well as a new 'wear and tear' limit ensuring that coatings could withstand nickel release for at least 2 years of normal use (Table 2). To evaluate whether items were in compliance with the EU Nickel Directive, three reference methods were developed, EN 1810, EN 1811 and EN 12472. The DMG test was only suggested as a screening tool (CR 12471) and not as a confirmatory test. As a result of negotiations and lobbying by the industry, the EU Nickel Directive did not come into full force until July 2001, 7 years after its introduction. In 2005, additional lobbying by the industry made the removal of the 0.05% nickel content limit in piercing posts possible and replaced it by a 0.2 µg nickel/cm²/week release limit. By this initiative, stainless steel could once again be used for piercing posts.

Obviously, a total ban on nickel in consumer products would efficiently have reduced the nickel allergy problem. However, since nickel is inexpensive and has many useful applications, this approach did not seem to be realistic or useful for all categories of products. However, a total ban in some products, e.g. piercing earrings, would probably be a meaningful way of limiting the nickel allergy problem, partly because it would eliminate inaccuracies of confirmatory test methods but also because some patients may react to very small amounts of nickel. Consumer items are often used in very different ways; e.g. a wrist watch typically has direct and occluded skin contact for 12–16 hr or more per day whereas a mobile phone is typically used for 2–30 min several times per day and with only little or limited occlusion. No model defining

a safe nickel release limit can sufficiently cover various factors such as occlusion (+/–), time of skin contact, and the number of contacts per day. The Danish nickel regulation and the EU Nickel Directive may therefore be regarded as pioneering consensus approaches aimed at reducing the nickel allergy problem, but not attempts to totally eliminate nickel allergy. In line with this, Gawkrödger predicted in 1996 that it was unlikely that nickel allergy would disappear altogether as a proportion of individuals reacted below the level defined by the EU Nickel Directive (389).

The scientific basis for the operational limit proposed by Menné et al. (16), and upon which the Danish nickel regulation and part 2 of the EU Nickel Directive are based, has several limits that separate it from real-life situations. (i) The 48 hr occlusion time used to test for allergic nickel dermatitis may not reliably predict nickel dermatitis reactions as real-life exposure time obviously may be either longer or shorter and occlusion may not be present (e.g. the pendant of an earring). Kalimo and Lammintausta showed that seven (16%) of 43 positive patch test reactions to nickel were missed if occlusion was restricted to 24 hr (319). Christensen and Wall showed that open application of nickel, although useful, resulted in a reduced number of positive test reactions in nickel allergic patients (315). (ii) Nickel penetration differs with the skin area and the threshold elicitation concentration differs depending on single or repeated applications (390). (iii) Individual threshold levels for elicitation of allergic nickel dermatitis may vary 250-fold (42) and genetic

predisposition to nickel allergy exists and may be relatively frequent (103). (iv) The production and content of sweat may vary greatly between individuals. (19, 35). Sweat production is typically higher on the hands than on the back allowing corrosion if items are handheld, e.g. phones or rings. (v) The use of the patch test method to establish the nickel elicitation threshold did not take into account the protective effect of tribocorrosion for some metal alloys. Thus, rubbing a metallic item may in some cases prevent nickel release, which is not simulated during patch testing. These observations evidently make a 'one-for-all limit' (except total prohibition of nickel) an impossible way to protect consumers from developing nickel allergy and dermatitis.

It has been claimed that when alloys are ranked according to their probability of eliciting allergic contact dermatitis, metal release is a more relevant measure than patch testing because of uncontrolled differences in the test environment and variable allergenic susceptibilities of test subjects (19). Thus, it is not reasonable to conclude from positive patch test results using soluble metal compounds that the metal itself will also sensitize the skin, since exposure time, contact area, and geometrical configuration are important and decisive factors (19). The EN 1811 reference test method used for testing compliance with the EU Nickel Directive parts 1 and 2 measures nickel release after 1 week in artificial sweat (Table 2). However, it has important limitations that should be listed. (i) It can result in false negative results as illustrated by the study by Menné et al. where Inconel 600 (77% nickel, 8% iron, and 15% chromium) gave positive patch test reactions in some patients but released less than $0.5 \mu\text{g nickel/cm}^2/\text{week}$ in the corrosion test and gave a negative DMG test reaction. (16). (ii) The rate of nickel release may not be uniform over the week (389). (iii) The amount of nickel release determined by chemical analysis may be multiplied by an adjustment factor of 0.1 before interpretation of compliance with the EU Nickel Directive. This adjustment factor was introduced to compensate for difficulties when calculating complicated area sizes (e.g. of chains) and for lack of experience using this method. However, the current adjustment factor may greatly alter the interpretation of test data. Thus, a metallic item that releases $4.9 \mu\text{g nickel/cm}^2/\text{week}$ when analysed with the EN 1811 reference method will comply with the EU Nickel Directive if it is multiplied by 0.1 (as $0.49 \mu\text{g nickel/cm}^2/\text{week}$ is below the limit).

Despite their limitations, the Danish nickel regulation and the EU Nickel Directive are pioneering attempts aimed at the protection of Danish and European consumers. An evaluation of these regulatory interventions is of great interest as they may serve as models for future

regulations of contact allergy epidemics. Also, they should be evaluated as they may not prove strict enough to efficiently prevent nickel allergy and dermatitis. In general, it may be difficult to evaluate the direct effect of regulatory interventions on nickel exposure as parts of the industry, already before their introduction, may have felt the 'wind of change' and begun using safe nickel-free alloys. However, the indirect effects of nickel regulations can certainly be measured by using epidemiological tools and by using chemical analysis of nickel release from consumer products.

Nickel allergy following nickel regulation

This thesis offers evidence to suggest that the epidemiology of nickel allergy has changed in Danish women following the 1990 Danish nickel regulation. Studies focused on women, as the prevalence of nickel allergy is much higher in women than in men, allowing for easier detection of changes. However, a similar change in the epidemiology of nickel allergy was observed for men in the Glostrup allergy study (Table 7). By using general population data and clinical data from patch tested female dermatitis patients from a tertiary referral centre, it was possible to document a significant decrease of nickel allergy in young women and a significant increase of nickel allergy in middle-aged and older women after 1990 (Tables 4, 7 and 14). The decrease observed in the youngest age groups (18–35 years and 5–30 years) is possibly explained by the Danish nickel regulation whereas the concomitant increase in middle-aged (36–55 years and 31–49 years) and older (56–69 years and >50 years) women may be explained by a cohort effect as these women were most probably sensitized before 1990 and nickel allergy is a persistent condition (333). The longitudinal patch test study used nearly the same age group intervals as previously used by Schnuch et al. when they investigated the development of nickel allergy in German female dermatitis patients following the introduction of the EU Nickel Directive (257). It was therefore reassuring that the findings from the Danish and German studies were similar, suggesting that nickel allergy has truly decreased in the youngest female age groups following regulation. Furthermore, it was convincing to see that the decrease of nickel allergy could be observed in young adults, as some of these were adolescents when the nickel regulation was introduced (and therefore had already been exposed to nickel and developed nickel allergy). Thus, the decrease of nickel allergy could possibly be even stronger if children or adolescents had also been patch tested. Finally, the results are in line with those from other studies from Denmark, Italy, Poland, and Sweden (254, 255, 258–260), where

lower prevalences of nickel allergy have been observed in young female age groups (Table 23).

The decrease in the prevalence of nickel allergy in young women (18–35 years) was not explained by a decrease in the frequency of ear piercing (Table 4). Stratification by ear-piercing status revealed that women who were ear pierced before 1990 had a significantly higher prevalence of nickel allergy than women ear pierced between 1990 and 2006 (Table 6). This finding was in line with a previous study where Danish schoolgirls were nickel patch tested (253). Jensen et al. showed that the prevalence of nickel allergy was significantly higher in girls ear pierced before 1992 compared with girls ear pierced after 1992. Furthermore, the Danish nickel regulation may have prevented additional nickel exposure caused by the increasing popularity of body piercings among young people in Denmark as one-third of 18–35-year-old women in the 2006 study were body pierced. No data on the frequency of body piercing was collected in the 1990 study but the frequency is expected to be much lower in 1990, as body piercings were not in fashion at that time.

Nickel dermatitis following nickel regulation

The Glostrup allergy study showed that the prevalence of self-reported nickel dermatitis was lower in nickel allergic women who were patch tested in 2006 compared with 1990 (Table 4). This difference was independent of age group and could be reconfirmed in a logistic regression analysis (Table 5). Stratification by ear-piercing status (Table 6) revealed that the prevalence of self-reported nickel dermatitis was significantly lower among women ear pierced later than 1990 (i.e. after the introduction of the Danish nickel regulation) than in those ear pierced before 1990. This difference was mainly caused by a difference in women from the youngest age group (18–35 years). The accumulation of data suggests that morbidity caused by nickel exposure has decreased in the general population following nickel regulation although these data have inherent weaknesses that will be discussed. Although nickel dermatitis in clinical patients was not investigated in this thesis, a recent study showed that the proportion of 3+ patch test reactions in nickel allergic patients has decreased over a 33-year period, supporting a reduction in morbidity (391).

The association between hand eczema and nickel allergy was also addressed by using general population data. The prevalence (and association) of concomitant nickel allergy and hand eczema decreased among Danish women aged 18–35 years between 1990 and 2006 (Table 8 and Fig. 5). *A priori*, a decreasing prevalence of concomitant nickel allergy and hand eczema was expected

to occur first in women belonging to the youngest age group for several reasons: (i) the prevalence of nickel allergy decreased among young women ear pierced after the 1990 nickel regulation; (ii) women in this age group were theoretically less exposed to nickel in 2006 than in 1990; (iii) the association between nickel allergy and hand eczema showed a decrease among young Danes tested in 1998 compared with those tested in 1990, although this was not statistically significant (392). Since the prevalence of nickel allergy decreased significantly among women aged 18–35 years at the same time as the prevalence of hand eczema remained stable (or slightly increased), this development would inevitably tend to dilute the proportion of concomitant nickel allergy and hand eczema in 2006 and lead to a decreasing prevalence (Fig. 5). Nevertheless, logistic regression analyses performed with hand eczema as the dependent variable revealed a significant interaction term between nickel allergy and study year among 18–35-year-old women but not among older women. Thus, the effect of nickel allergy on the prevalence of hand eczema depended significantly on study year and age group. These data suggest that nickel exposure has decreased in young Danish women over a 16–18-year period and that it has resulted in a reduction in morbidity.

Persistence of nickel allergy in Danish women

Although the prevalence of nickel allergy has decreased in young Danish women following regulatory intervention, nickel allergy remains prevalent (Fig. 4). There may be several explanations that should be considered (Table 24). Three studies included in this thesis were performed to address possible explanations for the persistence of nickel allergy. In addition, one study validated the DMG nickel spot test in order to determine the accuracy of the two DMG test studies.

First, the possible association between the filaggrin null genotype and nickel allergy was addressed. Filaggrin null mutation status was shown by a German research group to be associated with nickel allergy and nickel allergy in combination with self-reported nickel dermatitis in adults from the general population (110). However, no association could be identified between the two most prevalent null mutations in the filaggrin gene and nickel allergy when data from the Glostrup allergy study were analysed (Table 9). A sub-analysis on female participants without ear piercing was performed and a positive association was found between filaggrin null mutation status and nickel allergy and allergic nickel dermatitis, respectively (Table 11). Since ear piercing is highly prevalent among Danish women, the analyses had

Table 23. Studies that have compared the prevalence of nickel allergy before and after nickel regulation

Author	Country	Population	Number	Nickel allergy prevalence before regulation (study year, %)	Nickel allergy prevalence after regulation (study year, %)	Significance level	Conclusion
Jensen (253)	Denmark	Public school girls; mean age 12.4 years	305	< 1992, 15.8	> 1992, 4.3	0.02	A higher risk of ear piercing before but not after 1992 was found. Implementation of nickel exposure regulation has protected the female population from becoming allergic to nickel
Jensen (253)	Denmark	High school girls; mean age 18.8 years	275	< 1992, 26.8	> 1992, 12.4	0.01	
Thyssen	Denmark	Adult women (18–69 years), general population	2117	Ear pierced before regulation, 15.6	Ear pierced after regulation, 6.9	0.004	Regulatory control of nickel exposure may reduce the prevalence of nickel allergy
Thyssen	Denmark	Adult women (18–35 years), general population	852	1990, 19.8	2006, 11.4	0.02	
Johansen (252)	Denmark	Dermatitis patients (<18 years)	2499	1985–1986, 24.8	1997–1998, 9.2	0.0008	— The most likely explanation of this decrease in nickel sensitivity is reduced exposure to nickel and increased public awareness of the risk of nickel sensitization
Veien (254)	Denmark	Female dermatitis patients (<20 years)	1026	1986–1989, 22.1	1996–1999, 16.7	0.05	
Thyssen	Denmark	Female dermatitis patients (5–30 years), university clinic	2397	1985, 27.6	2007, 16.6	0.002	The prevalence of nickel allergy decreased among young female patients with dermatitis after the introduction of the Danish nickel regulation
Lindberg (258)	Sweden	Female dermatitis patients (<40 years), hospital departments	2368	1991–1993, 33.8	1999–2001, 29.4	0.05	The decrease seen in younger ages between the two test occasions may reflect decreased exposure due to the Nickel Directive
Schnuch (256)	Germany	Female dermatitis patients (<31 years), hospital departments	2252	1992, 36.7	2001, 25.8	0.0001	The observed decline indicates that measures to limit the release of nickel from costume jewellery helped reduce the prevalence of nickel allergy
Bocca (28)	Italy	Dermatitis patients, university clinic	1798	1994, 54.3	2005, 53.5	0.9	The frequency of allergy to nickel due to earrings has not decreased after the introduction of the nickel regulation

Table 24. Possible explanations for the persistence of nickel allergy and dermatitis following regulatory intervention on nickel exposure

Causes	Estimated contribution to persistence of nickel allergy and dermatitis (strong/moderate/weak)
<i>Consumer</i>	
Sensitization before nickel regulation	Strong/moderate/weak*
Violation of the EU Nickel Directive	Moderate
Lack of control and information by responsible authorities	Moderate
Exposure to items not covered by the regulation	Moderate/weak
Exposure to items personally imported from countries outside the EU	Weak
Exposure due to defect coatings on consumer items after 2 years of use	Weak
<i>Occupational</i>	
Exposure by contact with tools, keys, locks, handles, coins, other equipment, materials, metal working fluids etc.	Strong/moderate/weak†
<i>Other</i>	
'Adjustment' factor of 0.1 in EN 1811:1998, the reference test method for control of compliance with the EU Nickel Directive	Strong
Insufficiency of the contents of the EU Nickel Directive	Weak
Genetic vulnerability	Moderate
Medical devices in skin contact	Weak
Toys	Weak

*Depending on age group.

†Depending on occupation

low power. Nevertheless, the associations were strong enough to support the 'bypass theory' (i.e. histidine-rich filaggrin proteins in the epidermis chelate nickel ions and prevent their skin penetration and exposure to LCs, and the low degree of genetic predisposition to nickel sensitization found in a relatively recent Danish twin study (104) is explained by a high prevalence of ear piercing among participants resulting in 'bypassing' of the filaggrin proteins) (112). Despite the positive association between nickel allergy and filaggrin null mutation status in women without ear piercing, nickel allergy and dermatitis is mainly caused by excessive environmental nickel exposure, since the prevalence of nickel allergy is very low in men. Thus, filaggrin deficiency may only be of minor importance when one tries to determine the reason for the persistence of nickel allergy in Danish women. Nevertheless, the relative importance of filaggrin null mutations may gradually increase as the EU Nickel Directive seems to protect subjects from developing nickel allergy following ear piercing.

Second, qualitative nickel release from jewellery and accessories purchased in Copenhagen was determined by

using the DMG test. Thirty-six stores were visited and a total of 354 items were purchased. Prior to this study, only few studies had investigated nickel release from consumer items following the introduction of the EU Nickel Directive. Lidén and Norberg found a significant decrease of DMG-test-positive items in Sweden following nickel regulation and showed that about 8% of consumer products were DMG test positive in 2002–2003 (189). A Danish study showed that about 20% of 41 mobile phones from two major international companies were DMG test positive (215). The Copenhagen test study added new information about consumer nickel exposure in Denmark. It showed that a large proportion (22%) of inexpensive consumer products gave positive DMG test reactions and that the proportion reached 60% for items used with children. Hair clasps turned out to be an important source of excessive nickel exposure as 37% of 117 hair clasps gave positive DMG test reactions. In particular, hair clasps targeting children released nickel, as 27 (79%) of 34 hair clasps were DMG test positive. The study found that about a third of stores visited sold consumer items that gave positive DMG test reactions. Finally, nickel release was frequent from jewellery purchased from market stalls and shops targeting immigrants. Taken together, the Copenhagen study demonstrated that a large proportion of jewellery and hair clasps for sale in Denmark released nickel in amounts that may elicit nickel dermatitis. This could be the main reason for the persisting high level of nickel allergy in Danish women. Since the EN 1811 reference method was not performed on the purchased items, it could not be investigated whether the EU Nickel Directive was violated by stores selling DMG-test-positive items or whether the 0.1 adjustment factor made excessive nickel release possible without breaching the legislation. A recent Danish study showed that consumer items resulting in allergic nickel dermatitis in patients with nickel allergy also included mobile phones and jewellery (393).

Third, a second DMG test study was performed in Warsaw, Poland, and London, UK, to assess the proportion of inexpensive earrings that released nickel in excessive amounts. Since the study was performed outside of Denmark, it did not offer information that can be used to directly address the reason for the persistence of nickel allergy in Danish women. However, it showed that nickel release was also frequent from inexpensive earrings purchased in other EU capitals, but more importantly it was the first study ever to convincingly demonstrate that the degree of nickel exposure from jewellery is strongly related to the category of store (Table 25). Excessive nickel release, assessed by the DMG test, was frequent from earrings purchased from markets and stores that seemed to have an independent ownership and not be part of

Table 25. The proportion of earrings that gave positive dimethylglyoxime (DMG) test reactions stratified by store category and city of purchase in studies performed in different countries and at different times

		Type of store and DMG test positive earrings, % (no. pos/no.tested)				
Study year	City	Clothing (chain)	Accessories (chain)	Other*	Market	Total
<i>Present study</i>						
2010	London, UK	4.3 (4/93)	0 (0/21)	25.0 (15/60)	38.7 (12/31)	15.1 (31/205)
2010	Warsaw, Poland	3.8 (3/78)	2.5 (1/40)	39.7 (23/58)	36.7 (11/30)	18.4 (38/206)
<i>Results from previous studies performed in EU member states</i>						
1999	Stockholm, Sweden [†] (188)	0 (0/11)	0 (0/15)	11.1 (2/18)	—	4.5 (2/44)
2002–3	Stockholm, Sweden [†] (189)	0 (0/18)	0 (0/10)	0 (0/33)	—	0 (0/61)
2009	Copenhagen, Denmark	4.8 (4/84)	0 (0/36)	31.0 (9/29)	57.1 (12/21)	14.7 (25/170)
2010	Stockholm, Sweden [‡] (190)	0 (0/11)	0 (0/57)	8.6 (3/35)	25.0 (1/4)	3.7 (4/107)
<i>Results from previous studies performed outside Europe</i>						
2007	San Francisco, USA (196)	8.3 (8/97)	26.6 (25/94)	42.9 (12/28)	69.0 (40/58)	30.7 (85/277)
2009	Chengdu and Beijing, China (29)	—	—	—	—	31.5 (99/314)
2009	Bangkok, Phuket, Hatyai, Thailand (29)	—	—	—	—	29.2 (71/243)

—, not tested.

*Defined as stores that seem to have independent ownership and not be part of a chain. These could be accessories or clothing stores, freestanding booths in underground stations or shopping centres, drug stores, and local artists.

[†]Before the EU Nickel Directive came into force.[‡]After the EU Nickel Directive came into full force.

a chain. This information can be used by authorities to target their control inspection visits. The study also confirmed the findings from the Copenhagen study, i.e. either the EU Nickel Directive is not followed and more control inspection visits should be performed to better protect European consumers or the EN 1811 reference method should be revised to prevent unnecessary nickel exposure. Finally, the accumulation of DMG test studies showed that the proportion of earrings that gave positive DMG test reactions seemed to be markedly lower in EU member states than in the USA (especially regarding chain stores) and Thailand, which have no nickel regulations, and China who may not sufficiently enforce their nickel regulation.

A validation was performed to determine the specificity and sensitivity of the DMG test compared with the EN 1811 corrosion test. The DMG test had a high specificity (97.5%) and a moderate sensitivity (59.3%). For the first time, a validation study followed the EN 1811 reference method very carefully. Accurate area measurements made it possible to avoid using the 0.1 adjustment factor. Furthermore, the sample size was very large ($n = 96$) increasing the accuracy of the estimates. Thus, the moderate sensitivity (and the high specificity) make it reasonable to believe that DMG test studies offer reliable estimates of excessive nickel release (i.e. $>0.5 \mu\text{g nickel}/\text{cm}^2/\text{week}$) and that, in fact, an even higher proportion of jewellery and accessories release too much nickel. If the 0.1 adjustment factor had been used, the specificity would be 72% and the sensitivity 45%.

This illustrates the strong relevance of accurate area measurements.

No study included in this thesis investigated remaining explanations for the persistence of nickel allergy. Although occupational nickel exposure may be of relevance in selected cases, it is unlikely to affect the overall prevalence of nickel allergy in the general population. This belief is supported by the low prevalence of nickel allergy in men as they more often than women may be exposed to nickel at work from tools, for example. Personal importing of nickel-releasing consumer items purchased in countries without nickel regulation, e.g. Thailand, seems to be of very little importance as indicated by a recent Danish study investigating the origin of DMG-test-positive items from patients with positive nickel patch test reactions of current relevance (393). Finally, other sources of consumer nickel exposure not covered by the EU Nickel Directive are judged to be of little importance, as the EU Nickel Directive covers most products that are in long-lasting skin contact. In conclusion, the persisting high level of nickel allergy in young Danish women is mainly explained by sensitization before 1990, by violation of the Nickel Directive, and by insufficiency of the EN 1811 reference method which allows the industry to produce and sell risk items.

Cobalt allergy following nickel regulation

The introduction of regulatory interventions on nickel exposure made researchers speculate whether the nickel

allergy problem could be replaced by a cobalt allergy problem, as cobalt frequently occurs together with nickel in jewellery and other metallic items. Thus, to properly study the effect of the Danish nickel regulation and the EU Nickel Directive, one also needs to address the development of cobalt allergy after regulation. No general population data were used to assess the development after 1990 since cobalt allergy is generally infrequent. However, the prevalence of cobalt allergy decreased in men and women between 1990 and 2006 from, respectively, 0.7% and 1.5% to 0.1% and 0.4% (394). These data strongly suggest that the prevalence of cobalt allergy has not increased following nickel regulation but rather decreased. One should remember that the concordance rate between the TRUE test and the Finn chamber test is low for cobalt and that readings were only performed on day 2 (334, 395, 396). These data should therefore be carefully interpreted, as there may be misclassifications due to false negative reactions. No clinical data from European patch test clinics have investigated the temporal development of cobalt allergy in recent years. The longitudinal Danish patch test study showed that the prevalence of cobalt allergy in Danish female dermatitis patients followed the development of nickel allergy to a certain degree. Thus, the prevalence of cobalt allergy appeared stable between 1985 and 2007 among female patients in the youngest age group (5–30 years) whereas a slight increase was observed in older age groups (30–49 years and >50 years). Data analysis showed that the association between nickel and cobalt allergy remained unchanged during the study period. Although one cannot rule out a relative increase of cobalt allergy in young female dermatitis patients, the prevalence of cobalt allergy seems not yet to have increased following nickel regulation. Further studies are warranted to verify this observation, ideally from Germany where they have large patch test databases.

As stated, chemical analysis of consumer products is a rapid and inexpensive way to indirectly assess changes in exposure patterns. No studies have previously assessed cobalt release from consumer products by using a spot test. In 1958, Feigl showed that nitroso-R salt was a useful indicator for the detection of cobalt release (43). A cobalt spot test based on nitroso-R salt was developed and validated. It appeared that the cobalt spot test reliably detected cobalt release and that it gave positive test results when cobalt was released in concentrations that potentially may result in allergic dermatitis in sensitive cobalt allergic patients. When jewellery and accessories from the Copenhagen study ($n = 354$) were evaluated with the cobalt spot test, four items gave positive test reactions. Thus, cobalt release from the

sample in Copenhagen was infrequent but did occur. Apparently, this area should be closely followed since a recent American study evaluated the allergenicity of a new cobalt alloy intended for jewellery (27) and since cobalt-releasing items from the Copenhagen study all had a dark metallic appearance. About 5 years ago, this dark metallic style was very much in fashion in Denmark whereas very few items with this exterior can be found today. Fashion is dynamic and a dark metallic style can therefore rapidly become popular again, perhaps resulting in increased cobalt exposure in young women. Taken together, significant cobalt release from consumer items exists but seems to be a minor problem compared with the nickel allergy problem.

Methodological considerations

The studies included in this thesis had weaknesses that should be addressed.

General population studies. The 1990 and 2006 studies showed that the overall prevalence of nickel allergy in women remained stable around 10–11% which is lower than prevalence estimates from other countries (221). This finding may be due to the use of day 2 patch test readings only; to conservative patch test readings in the Glostrup allergy studies; to an overall lower prevalence of nickel allergy in Denmark in comparison with other countries; or to the use of different patch test systems. However, the concordance rate between the TRUE test and the Finn chamber test is generally high for reactions to nickel (334, 395, 396). A strength of the study set-up was that identical patch test methods using the standardized TRUE test were used in both studies allowing for comparisons over time. Also, seasonal influence was expected to be limited as sampling was performed throughout the year.

The significantly decreasing rate of participation in all age groups from 1990 to 2006 was problematic as it is possible that selection bias influenced the results. To limit the influence of bias, analyses were stratified by age group and sex, and therefore the observed trends were unlikely to be explained by differences in these variables between the studies. However, it cannot be ruled out that other unknown characteristics differed between participants in the two studies (leading to an over- or under-representation of nickel allergic subjects). However, it should be acknowledged that increasing rates of non-participation in general population studies represent an important limitation. Patch testing was only a small part of the approximately 2-hr-long general health examination that participants underwent in the 2006 study. Therefore,

selection could rather concern for example cardiovascular diseases or other large disease entities. To challenge these speculations, one could perform for example phone interviews with non-participants but this was never done. Furthermore, the representativity of the study population was unknown. Thus, the prevalence estimate cannot be directly transferred to the entire Danish population. However, it is conceivable that the overall homogeneous Danish population is fairly represented in this sample.

To define nickel dermatitis, participants were asked 'Have you ever had eczema under the tightener of your watch strap, under the button of your jeans or from wearing ear pins or earrings?' This question has never been validated and its sensitivity and specificity is therefore unknown. However, there has previously been great interest in the value of self-reported nickel dermatitis. In a combined questionnaire and patch test study performed with veterinary students, Kieffer found that one could not reliably predict nickel allergy based on medical history (397). Thus, 46% of subjects with a history of metal dermatitis had a negative nickel patch test whereas 35% of those with nickel allergy had never experienced metal dermatitis. Cronin found that 38% of 89 nickel allergic patients had no history of metal dermatitis when questioned before testing (398). Menné showed that 62 (72%) of 88 female twins with a history of nickel sensitivity had positive reactions to 5% nickel sulfate in petrolatum (399). van der Burg et al. found that two-thirds of aspiring nurses and hairdressers that reported metal dermatitis had nickel allergy (154). Romaguera et al. performed patch testing with metals and alloys used in inexpensive jewellery in 964 patients who at one point in their life had experienced metal dermatitis and in 200 controls (25). The prevalence of nickel allergy was 63% in female dermatitis patients who had experienced metal dermatitis and only 8% in female controls. McDonagh et al. found a significantly higher prevalence of self-reported metal dermatitis in nickel allergic patients compared with patients without nickel allergy. (400). Self-reported metal dermatitis was also associated with atopic dermatitis (400). Similarly, Christensen found that 57 of 63 nickel allergic patients had a history of metal dermatitis (310). Rystedt and Fischer could not identify nickel allergy in six atopic individuals despite a history of dermatitis under wristwatches and necklaces (361). In 12–16-year-old Danish schoolchildren, only a third of subjects who reported metal dermatitis were nickel allergic (228). Möller and Svensson evaluated 20 patients who reported metal dermatitis but had negative patch test reactions to nickel, cobalt, and chromium (401). Although they doubled the test concentrations and performed intradermal nickel testing, only one case of

nickel allergy was found (401). Instead they concluded that the majority of the patients had atopic dermatitis. In line with this finding, Fedler and Strömer showed that false positive metal dermatitis (i.e. self-reported metal dermatitis but absence of nickel allergy) was associated with atopic dermatitis but also with psoriasis (233).

Since a history of jewellery or metal dermatitis is obviously not a perfect indicator of nickel allergy, Gawkrödger et al. investigated the distribution of metal allergy in patients who reported metal dermatitis (318). In total, 449 jewellery reactors and 752 non-reactors were patch tested with nickel sulfate, cobalt chloride, and potassium dichromate, and furthermore a subset were tested with nickel chloride, gold, palladium, platinum, and a combination of nickel sulfate and cobalt chloride (318). The authors found that half of jewellery reactors had negative patch test outcomes and that the extended battery did not produce a greater number whose jewellery reactivity was explained by metals other than nickel. They concluded that nickel remains the most important allergen in jewellery but also that a great proportion of jewellery reactors had contact allergies to palladium (17%) and gold (10%) (318). Of particular interest, Boss and Menné performed a use test with jewellery that had resulted in dermatitis in young hairdressers who recently had, respectively, a negative ($n = 8$) and a positive ($n = 5$) nickel patch test reaction (179). Seven of eight girls with negative patch test reactions developed earlobe dermatitis at the site of the earrings and five of the earrings were DMG test positive (179). Besides methodological considerations and possible bias in the above studies, one explanation for the discrepancy between the outcome of patch testing and self-reported metal dermatitis could be that T-cells may be activated following exposure to nickel in subjects without nickel allergy (402, 403). Furthermore, nickel-specific T-cell clones have been identified in subjects without nickel allergy (404). However, CD4 T-cells from non-allergic subjects have lower IFN- γ and higher IL-10 than CD4 T-cells from allergic subjects (91). Finally, it remains unknown why some individuals have positive patch test reactions to nickel but deny past or current metal dermatitis. Perhaps activation of the innate immune system by nickel may explain this (77).

A large Swedish patch test study recently evaluated the validity of self-reported nickel dermatitis (405). The authors found that the sensitivity and specificity of their question was 52% and 87%, respectively, when held against cobalt and nickel allergy. A Scottish patient based study found that the sensitivity and specificity of self-reported nickel dermatitis was 82% and 77%, respectively (406). When clinical relevance was taken into account, the sensitivity rose to 100% (406).

A Norwegian general population study including schoolchildren found that the sensitivity was 37% and that the specificity was 85.2% (407). Finally, Josefson et al. recently evaluated the validity of self-reported nickel allergy in adult women from the general population (408). The sensitivity and specificity of the question 'Are you allergic to nickel?' was respectively 76% and 76%. The sensitivity and specificity of the question 'Do you get a rash from metal buttons, jewellery and other metal items that come into direct contact with your skin?' was 68% and 79%, respectively. Taken together, questions on self-reported nickel dermatitis have a reduced validity and the use of such questions in epidemiological studies cannot replace nickel allergy patch testing. Nevertheless, the prevalence of nickel dermatitis mirrored the development of nickel allergy and the questions did support a reduction of morbidity following nickel regulation.

In general, it seems to be difficult to accurately establish a causal relation between being nickel allergic and reporting hand eczema as the year of nickel sensitization is unknown and hand eczema could be an event that preceded nickel sensitization (e.g. atopic hand eczema in early childhood). In this thesis, hand eczema was defined as an affirmative answer to the question 'have you ever had hand eczema?', although this question has never been validated. Meding and Barregård have previously validated another question on hand eczema for car mechanics, dentists and office workers, i.e. 'have you had hand eczema on any occasion during the past 12 months?' (409). They found that the sensitivity was 53–59% and that the specificity was 96–99%. Thus, this question may tend to underestimate the prevalence of hand eczema within the past 12 months. It would obviously have been a better approach to use a validated question on hand eczema in this thesis, but since the question validated by Meding and Barregård was only used in the 2006 study and not in the 1990 study, this was impossible. Our definition of hand eczema may have a low validity since it estimates hand eczema at one point in the subject's life, allowing for recall bias. Another issue that should be addressed is the definition of atopic dermatitis. Participants were asked: 'Has your doctor ever told you that you suffered from atopic dermatitis?' This question has also never been validated and may also have a low validity. Thus, misclassification might have occurred in this study as two non-validated questions were used and nickel patch tests were only read on day 2. Furthermore, no adjustment was performed for wet work and social class, which theoretically may have changed over the two study periods. However, since the same questions and patch test systems were used in

the two cross-sectional studies and since a definition of eczema was given in the questionnaire, it can be argued that the method may be used to detect possible changes over time. The study outcome, i.e. that the association between hand eczema and nickel allergy has decreased in Danish women aged 18–35 years, somewhat conflicts with the results from a recent Swedish follow-up study. Josefson et al. re-questioned 735 women who had been nickel patch tested 20 years earlier (275). They found no difference in the frequency of self-reported hand eczema after age 15 years in nickel allergic and non nickel allergic women (275). The authors confirmed their initial finding when they performed a clinical examination as well as nickel patch testing in 369 of these women (276). A weakness of the Swedish study was that participants were only 8, 11, and 15 years old in 1985 when the first study was performed (410). A marked decrease of nickel exposure from that year was shown in a recent 33-year retrospective Danish patch test study and it is therefore likely that only a few of these young individuals have experienced heavy nickel exposure (391). A decreasing association of hand eczema and nickel allergy is to be expected if the Danish nickel regulation is effective and a decrease of nickel exposure should therefore reduce the prevalence of hand eczema in nickel allergic women. Further studies using validated questions are warranted to confirm this preliminary finding.

In the 2006 filaggrin study, the occurrence of atopic dermatitis was estimated according to the UK Working Party's Criteria. Williams et al. found that the sensitivity and specificity were 92% and 81%, respectively (386). Thus, atopic dermatitis was reliably estimated. Genotyping was only performed for the two most prevalent filaggrin null mutations, R501X and 2282del4, which cover approximately 85% of null mutations in the filaggrin gene among Caucasians. (411). It is possible that remaining null mutations in the filaggrin gene were unevenly distributed between those with and without nickel allergy. This could potentially have introduced misclassification and bias in our data analyses. The filaggrin study confirmed the bypass theory as no association between nickel allergy and filaggrin null mutation status could be found in women who were ear pierced.

Longitudinal patch test study. The interpretation of the temporal development of cobalt allergy in female dermatitis patients was difficult. Thus, no definite conclusion could be made. The changing prevalence of cobalt allergy could theoretically have been influenced by the development of chromium allergy as concomitant exposure to chromium and cobalt occurs. However, since this is mainly observed in male patients who have been occupationally exposed,

it may be of limited relevance in this study. Thus, the development of chromium allergy should ideally have been included in the data analysis. Furthermore, it can be argued that only 2+ and 3+ reactions to cobalt chloride should have been included in the analyses as 1+ reactivity can sometimes not be confirmed when patch testing is repeated. However, since the same patch test procedure was used every year, this is unlikely to affect the development over time. Finally, referral patterns may have changed over the long study period resulting in a reduction of young women with mild disease (again resulting in a decrease of nickel allergy).

DMG test studies. The main weakness of the DMG test studies was the randomization process. The surveys did not sample the entire market and are therefore not representative. They should rather be regarded as findings from random inspections of the market. Nevertheless, as many shops as possible were visited in both studies and attempts were made to buy a broad variety of items. However, comparisons between studies should always be performed with caution. DMG testing was done by the same investigator (JPT) in all studies, ensuring that the same visual detection threshold was applied. Since the DMG test reacts when $>0.5 \mu\text{g nickel/cm}^2/\text{week}$ is released, it was impossible to assess whether part 1 of the EU Nickel Directive was followed (Table 2). The 2005 change dictates that no more than $0.2 \mu\text{g nickel/cm}^2/\text{week}$ may be released from piercing posts. Since only jewellery and accessories were examined, no information on other consumer items, e.g. belts, buttons, zippers, and rivets, was generated. Finally, investigators in Stockholm performed the validation study very accurately. The sample was larger than in any previous study and for the first time ever the EN 1811 reference method was carefully followed.

Cobalt test studies. Sampling of jewellery and accessories had the same randomization weakness as discussed for the DMG test studies. Interpretation of test results after rubbing cotton sticks against the test item is not as easy as for the DMG test. However, 40 items were also tested with the gel test as a confirmatory test. Ideally, chemical analysis of consumer items should also have been performed. The cobalt spot test gave a false negative reaction for disc K. It cannot therefore be ruled out that false negative reactions also occurred in the jewellery test study.

Concluding Remarks and Future Perspectives

Despite methodological limitations, this thesis highlights evidence to suggest that the Danish nickel regulation and

the EU Nickel Directive have resulted in a decreasing prevalence of nickel allergy and dermatitis in young female Danes. Furthermore, the decreasing burden of nickel exposure has possibly resulted in fewer cases of hand eczema. The persisting high level of nickel allergy may have several causes. Genetic predisposition due to filaggrin null mutations seems to be much less important than environmental nickel exposure. High nickel exposure remains frequent since a high proportion of consumer items release nickel in amounts that may result in nickel allergy and dermatitis. Furthermore, the nickel allergy problem has not yet been replaced by a cobalt allergy problem. Taken together, the European regulatory interventions on nickel exposure have proved to be successful. This conclusion is supported by the high and increasing prevalence of nickel allergy in countries without regulation or enforcement of such, e.g. the USA and China. Also, nickel release is observed in a higher proportion of earrings purchased from cities outside the EU than in earrings purchased in EU capitals. However, the accumulation of studies also suggests that the nickel allergy problem remains present and that new initiatives are warranted to further reduce it. These include inspection control visits paid by authorities, national information campaigns to educate consumers and retailers, removal of the 0.1 adjustment factor from the EN 1811 reference method, and identification of 'safe alloys' that producers and consumers may use. Recently, it was shown that the elicitation threshold for nickel in the ROAT was lower than the elicitation threshold in the nickel patch test (375). This interesting finding may change the scientific basis of the EU Nickel Directive, part 2. Thus, the $0.5 \mu\text{g nickel/cm}^2/\text{week}$ limit may be too liberal as it does not sufficiently protect European consumers.

We live in a globalized world. Consumer products are often produced outside Europe, typically in Asian countries. People travel more than ever before, often to destinations far from their home countries. Regulators and politicians in Asian countries should increase focus on the nickel allergy problem as they produce most consumer items. The nickel allergy problem may also rapidly increase in Asian countries as their wealth is increasing. Health regulators cannot rely on companies to prevent the use of nickel in their products as recently demonstrated by the mobile phone story. Thus, the nickel allergy problem should be managed by regulatory control in the future as well. Regulators should consider including handheld items such as keys, scissors, needles, and tools in the EU Nickel Directive. At some point in the future, the nickel allergy epidemic will hopefully be replaced by a lower prevalence.

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