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Contact Allergy to Heavy Metals: Risk Factors and Pathogenesis

DOCTORAL DISSERTATION

Medical and Health Sciences
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This dissertation was written between 2017 and 2021 at Vilnius University, Faculty of Medicine, Institute of Clinical Medicine, Clinic of Chest Diseases, Immunology and Allergology.

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Kontaktinė alergija sunkiesiems metalams: rizikos veiksniai ir patogeneziniai mechanizmai

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ABBREVIATIONS

AAS	Atomic absorption spectrometry
ACD	allergic contact dermatitis
Aq.	Aqua
CD	Contact dermatitis
CEN	European Committee for Standardization
Co	Cobalt
Cr	Chrome
D	Day
DCs	Dendritic cells
ICD	Irritant contact dermatitis
IRD	Irritant contact dermatitis
ICDRG	International Contact Dermatitis Research Group
ICP-MS	Inductively coupled plasma spectrometry
ECHA	European Chemicals Agency
EECDRG	European Environmental and Contact Dermatitis Research Group
ESCD	european Society of Contact Dermatitis
EU	European Union
FIG	Figure
MHC	Major histocompatibility complex
MW	Metalworkers
NACDG	North American Contact Dermatitis Group
NK	Natural killer
Ni	Nickel
No	Number
OS	Office staff
Pet.	Petrolatum
ROAT	Repeated open application test
SC	Stratum corneum
SDS	Sodium dodecyl sulfate
WAO	World Allergy Organization

CONTENTS

1. INTRODUCTION

Occupational exposure to metals is still of great importance for the sensitization, elicitation, and maintaining of hand eczema [1, 2]. Skin diseases are serious yet often preventable diseases occurring in workplaces with dermal exposures to irritant and sensitizing agents. Metalworking has been identified as a high-risk occupation for contact dermatitis [3, 4]. Metalworkers (MW) are at high risk of developing work-related hand dermatitis. Currently, the prognosis of occupational hand dermatitis in metalworkers remains poorly controlled even after they change their occupation [5].

1.1. BACKGROUND

Metals are present in Earth's crust, usually as oxides, sulfides, and silicates, and only the precious metals are in metallic form. More than 50 metals exist, and an enormous number of naturally occurring and manufactured alloys and metal compounds. Metallic compounds occur naturally in drinking water and food, and some of them are essential nutrients for humans [6, 7]. The industrial use of many metals, their alloys is significant nowadays, as they possess valuable mechanical, electrical, and chemical properties. Iron, chromium, lead, nickel, cobalt, aluminum, and copper are often used metals. Metals are good conductors of electricity and heat. They are divided into overlapping groups, depending on their chemical, physical properties and use. Heavy metal refers to any metallic chemical element with a relatively high density and one that is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb) [8].

Human exposure to metals has risen dramatically due to an exponential increase in metal use in industrial, agricultural, domestic, and technological applications [6]. Skin problems related to metal are caused not primarily by metals in the natural environment. Still, they are related to human activity – by metallic metals and their compounds in consumer products and industrial processes. A few metals – foremost ions and compounds of Ni, Cr, and Co – belong to the most important contact allergens, causing ACD in a large proportion of the general population, as well as in large occupational groups [8].

Metallic items are generally made of alloys, which may be combined, soldered, plated, etc. Common Ni-containing alloys are stainless steel (iron/Ni/Cr), copper-Ni, and Ni-silver (Ni/copper/zinc). Examples of Ni-free alloys are brass (copper/zinc) and red gold (gold/silver/copper). Alloys are

compounds or solid solutions, powders of more than one element in metallic form, but cannot be considered as mixtures of metals [9]. The resistance to corrosion in contact with the skin varies widely between different alloys, depending on their composition. This is important for the probability of alloys to induce or to elicite ACD. Toxicologists often refer to metal compounds as soluble or insoluble. Their solubility in biological artificial or natural media, however, is relatively unexplored [9]. Therefore, the potential health issues related to human exposure to metallic particles and metal release in contact with the human body is an important to research object.

Why some metals act as potent or clinically significant contact allergens, and some others do not is not fully understood. The question of multiple metal reactivity, cross-reactivity, and multi-sensitizations also remains under discussion. However, co-sensitization rather than cross-reactivity generally explains concomitant allergy to Ni and Co [10]. Several metallic metals and their compounds present occupational health hazards, and several have been recognized by the international agency for Research on Cancer (IARC) as human carcinogens. The respiratory system is the most frequent target of metal-induced cancer in humans, and metal-powder-induced respiratory tumors have occurred only from inhalation exposure [9, 11].

Ni, Cr, and Co, their ions and compounds, belong to the most important skin sensitizers. Consumer products (e.g. jewelry, mobile phones, clothing fasteners, buttons, leather goods and buckles) and occupational skin exposure are the main sources of sensitization and elicitation [10]. Pure metals, their alloys, platings, and compounds have different abilities to cause ACD.

1.2. Nickel

Nickel (Ni) is a chemical element, ferromagnetic metal of Group 10 (VIIIb) in the periodic table, markedly resistant to oxidation and corrosion. Nickel is silvery-white and harder than iron. Ni is well known for its use in coin minting but is more important either as a pure metal or of alloys for its many domestic and industrial applications [12]. Ni was isolated by a Swedish chemist and mineralogist Baron Axel Fredrik Cronstedt in 1751 when attempting to extract copper from niccolite (nickel arsenide), (earlier the ore of this type was called Kupfernickel) [8, 12]. Since the late nineteenth century, Ni has been widely used in various alloys, particularly in stainless steel, as the metallurgy of Ni itself is complicated in its details, many of which vary widely, according to the particular ore being processed. The ore must first transform to dinickel trisulfide, which is then roasted in air to give nickel oxide, which is then reduced with carbon to obtain the metal [13].

1.2.1. The use of Ni and possible exposure

Nickel is used in over 300 000 products for consumers in the form of over 3000 alloys. It is used in construction, automobiles, petrochemicals, lubrication and welding, power and renewable energy, electronics, transportation and water [14, 15]. Major Ni-containing materials include stainless steel, superalloys, low expansion magnetic, and shape memory alloys. In addition, alloy steels, cast irons and cast alloys, copper alloys, pure nickel, and other alloys, plating and electroforming, nickel chemicals. In 2017 the stainless steel industry accounted for approximately 75% of all primary nickel usage and consumed nearly 900 000 tonnes of scrap nickel. The battery industry accounted for 3.7%, with the remainder of usage being the industries mentioned above [16].

Only a minor part of nickel is used in items designed to be in prolonged skin contact. Ni sulfides and oxides found in nature are not allergenic [8]. Only free Ni ions may act as haptens. The most important factor for the induction and elicitation of cutaneous nickel allergy is the amount of Ni per unit area present in the epidermis. Exposure to free Ni ions may occur in an occupational setting or due to skin contact to Ni-plated surfaces or Ni alloys as these are easily corroded by human sweat [8]. The Ni epidemic began in the 1950–1960s with an increasing rate of female dermatitis, who had eruptions at the sites of their stocking suspenders. In the 1970–1980s, nickel release from jeans buttons and zippers resulted in dermatitis of both genders. Later, the popularity of ear piercing and the use of Ni-plated jewelry lead to Ni allergy and dermatitis in a large number of women [10, 17, 18].

Occupational Ni exposure may still result in morbidity despite the increased work hygiene. The most common occupations with Ni allergy are retail clerks, hairdressers, domestic cleaners, metal workers, cashiers, locksmiths, construction workers, electricians, nurses, Ni platers, chefs and cooks, and car mechanics carpenters [19, 20]. The unit for the quantification of exposure to contact allergens is $\mu g/cm^2$. When it comes to Ni, the risk of Ni sensitization depends upon Ni release from metal items designed to be in direct and prolonged contact with the skin expressed as $\mu g/cm^2$ over time [8].

Ni is frequently detected as an impurity in consumer products, including washing liquids and other household products at a concentration of 1–5 ppm. However, such a concentration will only exceptionally result in clinical disease among the Ni-allergic individuals [21].

1.2.2. Ni-related safety risk

Occupational and consumer Ni contact allergies have been frequent for more than 100 years. Occupational Ni dermatitis was firstly recognized in 1889 among German workers in the plating industry. In 1931 consumer Ni dermatitis was reported following skin contact with spectacle frames [8].

Primarily Ni sensitization is often associated with prolonged and direct skin contact with Ni-releasing items, e.g., coins, keys, inexpensive jewelry, clothing fasteners, and occupational tools [8, 10, 19]. Besides contact allergy, Ni compounds can have carcinogens, pulmonary effects, and general toxicity. However, these toxic effects are not related to contact allergy, as they are caused by different Ni compounds and exposure routes [8, 9].

Occupational Ni dermatitis usually presents as chronic hand eczema, as this skin area is in most direct and prolonged contact with work-related tools or items, eventually complicated by long-lasting hand eczema [22-24].

1.2.3. Epidemiology

Nickel is the leading contact allergen in most industrialized countries worldwide. In Europe, the prevalence of Ni allergy has declined in some countries following the implementations of the EU Nickel directive [25]. The prevalence of contact allergy to Ni is approximately 8% to 19% in adults in the general population [26]. The positivity rate increases when patch testing individuals with suspected contact dermatitis and is approximately from 18.9% in Sweden [27], the Southern region (Spain and Italy) 24.5% [28], Central region (The Netherlands, Switzerland, Austria, and Germany) 19.7% [28], and from 22.4% reported by ESSCA group [29] to 25.7–29.6% reported by Lithuanian authors in Lithuania [27, 30]. Patch testing data published over the last 50 years have always presented Ni as the most common contact allergen among female dermatitis patients worldwide. As Denmark and Sweden were the first to start regulating Ni release from a consumer product, the sensitization decreased by more than 10% in 10 years; it was also noticed earlier than in other countries [31, 32].

Most nickel allergic individuals in the general population have healthy skin at the time of examination, but they report previous ear piercing, jewelry dermatitis, and/or hand eczema. The nickel problem in the general population seems to be a global phenomenon [33].

1.2.4. Patch testing with nickel

Ni sulfate was included in the first European baseline series by Bonnevie in the 1930s [8]. Patch testing with 5% of nickel sulfate (2.0 mg/cm^2) in pet. is used in the European baseline series, whereas 2.5% (1.0 mg/cm^2) is used in North America [34]. Doubtful or irritant patch test reactions to Ni are less common than to other metals [35]. Active patch test sensitization from nickel sulfate 5% in pet. has never been documented. The positive Ni patch test is reproducible, but its strength varies over individual patients [8].

1.2.5. Prevention and legislation

The reduction of exposure aimed to prevent nickel allergy in the general population is possible through regulations, education, and information campaigns to educate consumers about decontamination of the exposed skin areas, the use of protective creams, or proper protective gloves. Protective creams either prevent allergic effects of Ni or inhibit Ni penetration. Barrier creams with chelating agents (clioquinol, diethyldithiocarbamate, or EDTA) have been used with variable success [36]. The calorimetric dimethylglyoxime (DMG) test is commercially available, and every consumer can buy one in a pharmacy if there is a need to test the safety of used items. The use of DMG test is simple: a cotton stick moistened with the test solution is used to rub the surface of the item for up to 30 seconds, and if the object releases Ni more than 0.5 µg/cm^2 per week, it changes color to pink (Figure 1) [19, 37].

The plating of the surface of Ni-containing items offers only limited protection, as there can be pores left or micro-cracks that may lead to Ni release when in prolonged contact with human sweat [19]. In the original EU Ni directive, the duration of Prolonged skin contact was not described, and in 2014, the European Chemicals Agency (ECHA) defined „prolonged contact" as potentially more than 10 minutes on three or more occasions within two weeks, or 30 minutes on one or more occasions within two weeks [38].

To reduce the epidemic prevalence of Ni allergy in European citizens, the EU Nickel Directive was introduced in 1994, came into force in 2000, and into an entire course from 2001 [39]. Before these regulations appeared, three countries – Denmark (1990), Sweden (1991), and Germany (1991) – implemented national Ni regulations. These regulations at that time differed: the Danish Ni Regulation banned products intended to come into close contact with the skin with Ni release of >0.5 µg/cm^2/week; in Sweden – ear piercing with piercing post assemblies containing 0.05% Ni or Ni coating thicker than

0.01 μm was prohibited, and in Germany – Ni containing objects had to be labeled [25]. In Lithuania, the European Directive regulating Ni release from metal items was approved in 2002 [40]. The EU Nickel Directive was included in REACH, the EU Chemical Regulation, in 2009 (Table 1). Outside Europe, regulations exist in China but not in the United States, Thailand, or Australia [19].

The occupational exposure to tools or coins and other materials is not included. Whether such items need any regulation may depend upon future risk assessment.

Figure 1. A positive DMG test reaction indicated by the changed color to pink of a cotton stick rubbed the surface of a key.

Table 1. The EU Nickel Directive (94/27/EC, adopted 1994, in force 2000, part of REACH 2009) and analytical methods by European Committee for Standardization(CEN) [8].

Part	Nickel may not be used	CEN standard for control
1	*To September 2005:* In postassemblics used during epithelization, unless they are homogenous and the concentration of Ni is less than 0.05%	EN 1810 (Ni content by atomic absorption spectrometry (AAS))
1 rev.	*From September 2005*: In all postassemblies which are inserted into pierced ears and other pierced parts (not only during epithelization), unless the Ni release is less than 0.2 µg/cm^2 per week	EN 1811 (Ni release in artificial sweat)
2	In products intended to come into direct and prolonged contact with the skin. Such as earrings, necklaces, wristwatch cases, watch straps, buttons, tighteners, zips, and mobile phones, if Ni release is greater than 0.5 µg/cm^2 per week	EN 1811 (Ni release in artificial sweat) CR 12471 (screening test by dimethylglyoxime)
3	In coated products, unless the coating is sufficient to ensure that the Ni release will not exeed 0.5 µg/cm^2 per week after 2 years of normal use	EN 12472 (wear and corrosion test)

1.3. Cobalt

Cobalt (Co) is a chemical element, ferromagnetic metal of Group 9 (VIIIb) of the periodic table, used mainly for heat-resistant and magnetic alloys [41]. The metal was isolated by the Swedish chemist Georg Brandt in 1735, though it was used for centuries to give blue color to glazes and ceramics. Co has been detected in ancient Persian necklace beads, Pompeii ruins, in the blue porcelain of the Ming dynasty. The name *kobold* was first applied in the 16th century to the ores thought to contain copper but eventually found poisonous arsenic-bearing cobalt ores. In 1742 Brandt determined the blue color of those ores was due to the presence of cobalt [42].

1.3.1. Cobalt use and exposure

Co is used in various of industrial and high-end applications, especially in the production of superalloys for gas turbine aircraft engines and other items where elevated temperatures and high mechanical stress are encountered [43, 44]. Co is also a compound of pigments in glass, ceramics, varnishes, and paints, a catalyst in the petroleum and chemical industry, cutting edges of tools and drills [45, 46]. It is also used in electroplating to make hard, wear-resistant, and bright coatings of the items. Hard metals, magnets, batteries, jewelry, dental and surgical implants are the primary consumer contactable items. In low amounts, Co can be found in cement [10]. Exposure to Co and its compounds is generally thought to occur in hard-metal manufacturing, ceramics, and construction industries [47].

1.3.2. Co-related safety risk

Metalworkers and construction workers suffering from occupational dermatitis have an increased risk of Co allergy than other men with occupational dermatitis [3, 5]. In addition, there have been case reports published on dermatitis related to occupational exposure to Co-containing materials such as black ink, animal food, cement, dentures, and orthopedic prostheses [8, 48].

The occupational exposure of Co can occur through the respiratory tract. Pulmonary effects include hard-metal induced bronchial asthma or pneumoconiosis. Cardiomyopathy has been described among heavy cobalt-containing beer consumers. It is controversial whether cobalt can cause human cancer [8]. Cobalt is an essential element as it occurs in vitamin B12.

Nowadays, cobalt is mainly a byproduct of nickel and copper mining. As a result, today, 50% of the world's Co production is in Africa [8].

1.3.3. Epidemiology

The contact allergy to Co in the general population is approximately 1–3%, and in suspected contact allergy patients, 5–9% [27, 28, 30]. In Lithuania, the sensitization rate to Co remains stable throughout the years and is from 7.5 to 8.8% in patients with suspected contact allergies [27, 29, 30]. Diepgen et al., In their recent study, demonstrated that Co allergy amounted to 2.2% among the general population in multiple countries in Europe. The difference in sensitization between men and women was also noticed (1.1% vs. 3.0%) [26]. Allergy to Co is often seen with Ni or Cr, but cross-reactivity has not

been experimentally demonstrated. The high prevalence of Co allergy among female dermatitis patients is being explained by consumer exposure, as mixed or impure nickel alloys are used in jewelry. A higher prevalence was also noticed in pierced men compared with non-pierced men [49].

1.3.4. Patch testing with cobalt

The diagnostic patch testing recommendation in the European baseline series is to use 1% cobalt chloride in pet. [34]. Multiple studies were carried out to establish the minimal levels of Co chloride to elicit the positive patch test response. The minimal eliciting concentration on healthy skin was 2260 ppm, and when the skin was specially pretreated with sodium dodecyl sulfate (SDS), the elicitation level was lowered by 2.3–226 ppm of Co chloride [8]. The dilution series can also be used in case true sensitization is questioned.

1.3.5. Prevention and legislation

There is no such regulatory limitation for the usage of cobalt to prevent CD as there is for Ni and Cr. There is a risk, as Co is a potent skin sensitizer. However, it was expected that the Nickel directive could affect Co allergy's sensitization levels one way or another, as alloys used for unexpensive jewelry could not contain more Co. An indication for such a situation was noticed in the Danish retrospective study, where the prevalence of contact allergy to Co in young female dermatitis patients was stable, while the decrease was expected as the prevalence of Ni sensitization in this group age decreased [17]. The calorimetric cobalt spot test containing Nitroso-R salt for detecting Co in metallic objects is commercially available and can be bought in pharmacies. The spot test detects free Co down to a limit of 8.3 ppm, while the threshold of dermatitis elicitation of most Co allergic patients is above 10 ppm [8].

1.4. Chromium

Chromium is a chemical element of Group 6 (VIb) of the periodic table. It is a hard, steel-grey, corrosion-resistant metal that takes well to polishing and is used in alloys to increase strength. Niclolas-Louis Vauquelin, a French chemist, discovered chromium in 1797 and isolated it as a metal a year later. The name "chromium" was given from the Greek word *chroma*, meaning a color, as chromium compounds appear colorful [50]. Since then, Cr has found many industrial uses, including leather tanning, chrome plating, stainless steel, cement, alloy, and automotive industries.

Chromium is a widely distributed metal. The principal ore of Cr is chromite. It exists in every oxidation state from 0 to +6. The main and best known for ACD potential is hexavalent chromium, as its most compounds are freely water-soluble and pass through the epidermis more rapidly than trivalent chromium compounds, which are insoluble [8, 50].

1.4.1. Chromium use and exposure

Nowadays, Cr exposure with skin is mainly considered through leather gloves and shoes [51-53]. However, occupational exposure is reduced due to regulations, and sensitization to Cr is becoming a consumer problem [21, 51].

It is stated that 90% of the global leather production is tanned using chromium sulfates [53, 54]. Coated or chromated surfaces consist of both trivalent and hexavalent chromium compounds to prevent metal dulling. However, when such chromated metal items are handled, chromates can leach out and be transferred to the parts of the hand in contact [8, 20].

Forged cobalt-chromium-molybdenum material is used for metal on metal total hip arthroplasties. The problems with such prostheses are noticed among chrome-allergic individuals, as a higher prevalence of metal allergy among patients with implant failure has been noticed [55]. Chromium as a metal is present in various alloys, such as stainless steel, together with nickel and iron. On welding stainless and non-stainless steel, hexavalent Cr can be released, generated, and distributed to the facial area via the welding fume [56, 57].

High numbers of Cr were detected in detergents and bleaches and suggested a possible cause of chromate allergy in women [8]. Therefore, in 2006 Liden with colleagues developed a technique to sample chromium deposition on the hands using cellulose wipes with 1% nitric acid. The chemical analysis of samples collected by this technique was then analyzed using inductively coupled plasma mass spectrometry (ICP-MS), and the results were expressed in $\mu g/cm^2$ [56].

1.4.2. Cr-related safety risks

Historically, the most common cause of Cr sensitization was derived from skin contact with hexavalent chromium in wet cement. This is still the case in some parts of the world, but the epidemiological situation is changing in Europe following the regulations of Cr content in cement. Construction workers were the first to suffer from rashes and eczema, but the culprit was unknown at that time. In 1908 the first cases of cement eczema since the

introduction of Portland cement were ween among Paris metro builders. Later in the 1950s, the importance of the chromium content in cement as the cause of Cr-induced eczema among workers was brought to light by Jäeger and Pelloni [59].

Cr salts can induce skin irritation and ACD. Such clinical manifestations have been the basis for multiple studies, reports, and regulations [35, 60].

1.4.3. Epidemiology

The prevalence of contact dermatitis to Cr allergy in dermatitis patients has steadily decreased over the past 30 years. Several articles suggested that the decline in Cr allergy is due to improved work hygiene and decreased contact with construction materials, and the addition of iron sulfate to cement [52]. However, in countries where chromium sulfate is not changed to iron sulfate, chromate allergy may still be expected in construction workers [60].

In a study published in 2015 by Diepgen et al., where sensitization in the general population was studied, the contact allergy to potassium dichromate was found to be 0.8% [26]. In selected dermatitis patients, the sensitization rate to Cr is higher, for example, in Lithuania – from 5.3 to 6.6% [27, 29, 30], while in Sweden it is 2.8% [27], in Southern Europe (Spain, Italy) – 4.5%, and in Central Europe (The Netherlands, Switzerland, Austria, Germany) – 5.9% [28]. A contact allergy to Cr exceeding 10% is seen only among construction workers exposed to hexavalent chromium [8].

1.4.4. Patch testing with Cr

In the 1930s, Bonnevie included potassium dichromate in the first European baseline series [8]. Nowadays, in Europe, patch testing with potassium dichromate 1% in pet. is still present in the European baseline series, while in North America, the same salt is used in 0.25% concentration. Caution is needed while reading positive patch test results to potassium dichromate, as the elicited irritative reaction can morphologically resemble allergic reactions and may be incorrectly interpreted as an allergic reaction. The dilution series could be helpful to discreet allergic reactions from the irritation [35].

1.4.5. Prevention and legislation

Cr is widely used and found in our environment, and total avoidance is difficult. The EU Directive restricting the use and marketing of cement

containing >2ppm of chromium (VI) came into force in January 2005 (Table 2). The exposure of Cr from leather in the EU was firstly regulated in 2003 when chromium (VI) content in protective gloves was limited to <10 ppm (EN420:2003) and in 2009 limited to <3 ppm (EN420:2009) [60]. The EU Directive was implemented in Lithuania in 2006 [61]. Nowadays, it is noticed that regulations are non-exhaustive, and further descriptions of regulations are expected, as exposure to chromium remains an important health consideration.

Table 2. The EU Directive (2003/53/EC), EU Commision regulation (301/2014) analytical methods by the European Committee for Standardization (CEN)[58].

Part	From 17 January 2005	CEN standard for control
1	Cement and cement-containing preparations may not be used or placed on the market if they contain, when hydrated, more than 0.0002% soluble chromium (VI) of the total dry weight of the cement.	EN 196-10 A test portion of the cement is used to make a standard mortar with CEN Standard sand and water in accordance with current stantard EN 196-1. The mortar is mixed for a specified time and then filtered. An aliquot of filtrate is first treated with a diphenylcarbazide reagent, and then acidified within a narrow range of pH (2.1-2.5). In an acid solution, chromium (VI) forms a re-violet complex with reagent, and its absorption/colour is measured with a visible light spectrophotometer set at a wavelengh of 540 nm, although other instrumental/end-point procedures are permitted.
2	If reducing agents are used, the packaging of cement or cement-containing preparations should be marked with information on the period of time for which the reducing agent remains effective (i.e. packing date, storage conditions, and storage period).	
3	Part 1 and Part 2 do not apply when it is for use in totally automated and fully enclosed	

	processes, where there is no possibility of contact with the skin.	
Part	From 1 May 2015	ISO standard
1	Leather articles coming in contact with the skin shall not be placed on the market if they contain chromium (VI) in concentrations equal to or greater than 3 mg/kg (0.0003% by weight) of the total dry weight of the leather.	ISO 17075:2007 Soluble chromium (VI) is leached from the sample in pjosphate buffer at pH 7.5–8.0, and substances that influence the detection are removed by solid-phase extraction if necessary. Chromium (VI) content is determined with a diphnylcarbazide reagent at a wavelength of 504 nm with a spectrophotometer or a filterphotometer. The method described is suitable for quantifying the chromium (VI) content in leathers down to 3 mg/kg.
2	Articles containing leather parts coming into contact with the skin shall not be on the market if any of those leather parts contain chromium (VI) in concentrations equal to or greater than 3 mg/kg (0.0003% be weight) of total dry weight of that leather part.	
3	Part 1 and Part 2 shall not apply to the placing on the market of second-hand articles that were in end-use in the EU before 1 May 2015	

1.5. Contact dermatitis

Contact dermatitis is an inflammatory skin reaction to direct contact with various environmental agents [59, 60]. It must have accompanied humankind throughout history, as in ancient times dating to the first century A.D., it was noticed that some individuals cutting pine trees were suffering from a severe

itch. The first clinically oriented experimental studies that related CD to its suspected causative agent were done in the nineteenth century [59]. A group of noblemen had contributed to CD research during the time. Josef Jadassohn, a German professor of dermatology at Breslau University, is acknowledged as the father of patch testing, as he was the one offering this new tool to specialists. Later Bruno Bloch at Zurich University led to patch testing description in detail and initiated standardization worldwide. A former assistant of Bruno Bloch, Poul Bonnevie was a professor of occupational medicine in Copenhagen, Denmark. His work influenced all Scandinavian countries. Bonnevie expanded Bloch's standard series and published a textbook on environmental dermatology [59].

Nowadays, the CD aims for various contact dermatitis research groups to take action in epidemiological surveillance, conducting regulations, research, and legislation. The leading organizations are the European Society of Contact Dermatitis (ESCD), the European Environmental and Contact Dermatitis Research Group (EECDRG), the North American Contact Dermatitis Group (NACDG), and the International Contact Dermatitis Research Group (ICDRG).

1.5.1. Epidemiology

Allergic contact dermatitis maintains a persistent problem in all age groups and the occupational setting. CD affects about 27% of the general population in Europe [33, 61]. According to the World Allergy Organization (WAO), CD accounts for approximately 85–95% of all occupational skin diseases in industrialized countries [62]. In 2018, among all new work-related CD cases in Great Britain, 66% were female workers, and 34% were men. Among all work-related dermatoses, 86% were CD, 7% were other non-cancerous dermatoses, and 7% were skin cancer. In Lithuania, the prevalence of CD in patients with suspected contact allergy is 57% [30]. Contact dermatitis often occurs at a young age, particularly among female workers: 53% were women younger than 35 years compared with 35% men [63]. In a systematic review of the prevalence of contact allergy in the general population, the overall pooled prevalence of contact allergy was 20.1%. The prevalence in women, same as in dermatitis patients, was significantly higher, 27.9%, than men 13.2%. Most common allergens were nickel (11.4%), fragrance mix I (3.5%), cobalt (2.7%), myroxylon pereirae resin (1.8%), and chromium (1.8%). This meta-analysis confirmed that at least 20% of the general population are sensitized to common environmental allergens [60, 64]. Contact allergy is common among every population. Just prevalence varies,

highlighting the need for more effective preventive strategies in consumer goods and the workplace.

1.5.2. Types of contact dermatitis and etiology

Contact allergy is a form of a delayed IV type hypersensitivity reaction caused by skin contact with low molecular weight haptens (<1000 daltons) and, in some exceptions, by larger molecules. A hapten is a small molecule that can gain allergic potency and elicit an immune response only when it is attached to a carrier molecule, usually skin self-proteins [59, 61]. Therefore, in clinical practice, the term *contact allergen* is preferred to use.

Contact dermatitis comprises two main dermatitis groups, an irritant (ICD) and allergic (ACD) contact dermatitis (Table 3). Contact dermatitis can present as acute, subacute, and chronic eczema [24, 65]. ICD is mainly due to the toxicity of the contacted chemicals that damages the skin's protective outer layer, while ACD corresponds to a delayed-type hypersensitivity response. If the ICD symptoms are present, they can be made worse by heat, cold, low humidity, and friction [24, 65, 66].

Table 3. The differential diagnosis between ICD and ACD.

	ACD	ICD
Skin lesion	Not limited to the contact site	Limited to the contact site
Symptoms	Itch	Burning
Epidemiology	Affects some subjects handling the product	Affects the majority of subjects handling the product
Histology	Spongiosis, exocytosis	Epidermal necrosis
Patch tests	Positive	Negative
Skin immunology	Presence of activated T cells	No activated T cells
Blood immunology	Presence of specific T cells	No specific T cells

1.5.3. Structure of the skin and penetration pathways

The skin is the largest organ in the human body [67]. The skin's primary function is forming a protective barrier against the surrounding environment and maintaining body homeostasis [68]. The permeation of a substance is not straight through the skin. Instead, there are three main heterogeneous cell layers of the skin: epidermis, dermis, and hypodermis; Figure 2 [69, 70].

The Stratum Corneum (SC) is the outer layer of the epidermis. SC serves as 3–20 μm in thickness, composed of 15–25 layers of corneocytes, an effective barrier against transcutaneous water loss and entry of exogenous materials [71]. A theoretical model is a "brick and mortar" structure where bricks represent terminally differentiated nonviable keratinocytes, also known as corneocytes embedded in intercellular lipid membranes [72]. It mainly consists of dead cells with a water content of about 5–20%, depending on anatomical site, meaning that the circulation is lower and the cell turnover higher than the underlying skin layers. When protein bridges between corneocytes (corneodesmosomes) degrade, the lacunar spaces are created within the SC. These spaces can extend and form continuous networks, also creating a pathway for penetration across the SC [73].

The dermis is another layer of skin, which is 10 to 40 times thicker than the epidermis, depending upon the body area. It contains vascular structures, nerve endings, sensory receptors, and epidermally derived appendages [71, 72]. Many cell types reside in the dermis, including fibroblasts, macrophages, mast cells, and circulating immune cells. In addition, the dermis is responsible for skin elasticity and strength that protects against mechanical injury, retains water, and aids in thermal regulation [72, 74].

Hypodermis or subcutaneous tissue is a fastener for the skin to the underlying surface and fat storage [75]. It serves as a reserve energy supply, cushions and protects the skin, supports nerves, vessels, and lymphatics supplying the overlying skin [71].

The appendages of the skin include nails, hair, sebaceous glands, eccrine (sweat) glands, and apocrine glands [67, 72]. They have two distinct components: superficial and deeper components in the dermis, which are downgrowths of the epidermis. The sebaceous glands, apocrine glands (only in areas of secondary sexual maturation (i.e., axillae, groin, nipples), and the sweat glands are widely spread over the body [76]. Of these, only the sweat and sebaceous glands occur on the back area used for patch testing.

Skin can be considered a multilayered living membrane where extracellular lipids contribute to the barrier function and the route taken through the SC by all molecules [72]. There are three main transport routes:

transcellular (passing through skin cells), intercellular (passing in between skin cells), and appendageal (entering through hair follicles, sweat glands, and sebaceous glands) transportation (Figure 3) [70-72]. Trans- and intercellular absorption are the slowest mechanisms, where intercellular diffusion is the main route. Though being the fastest, Appendageal transportation is limited to a tiny fraction of the skin [70]. A range of biological factors can influence percutaneous penetration rate and extent, including anatomical site, age, and appendageal density [72]. Depending on the physicochemical properties of the substance, different delivery routes will be available. The skin structure has both moisture and sebum, allowing different substances to penetrate the skin using different routes. The transdermal permeability depends on substance concentration, distribution, diffusibility (moderate lipophilicity/ hydrophilicity, low melting degree, and low molecular weight), and exposure path [74, 77]. It is widely accepted that water-soluble substances are mainly transported via hydrated keratinocytes in the SC. Although the mechanism is not yet fully understood, it is hypothesized that lipophilic substances diffuse through the intercellular lipids [72, 78]. Generally, the diffusion of substances across the SC is passive, meaning it does not require energy from the cells. Nevertheless, many substances are unable to penetrate the SC due to its protective barrier function.

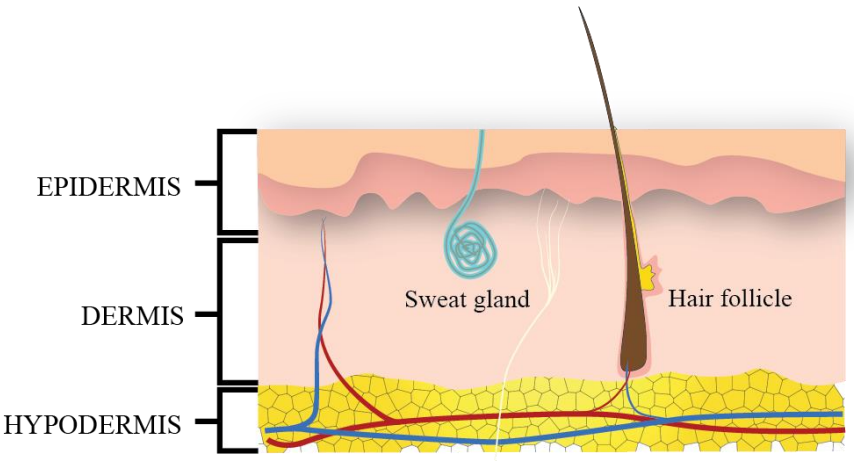


Figure 2. Structure of the human skin. Hair follicle (Drawn using Adobe After effects)

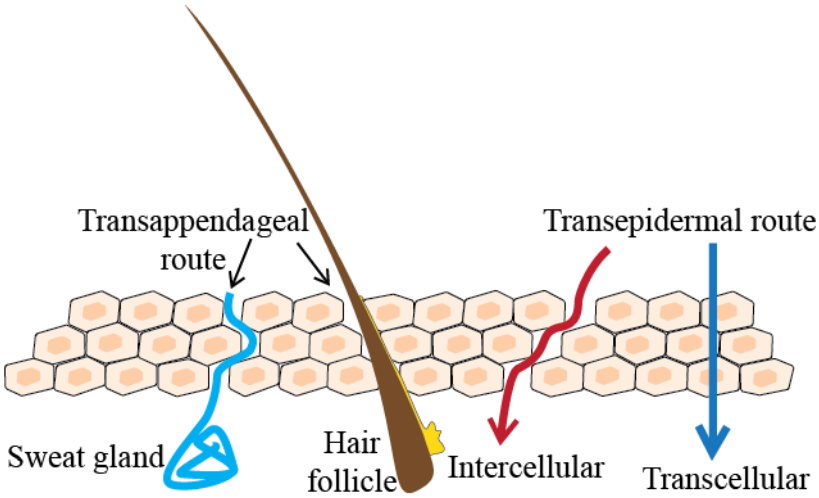


Figure 3. Transportation routes via stratum corneum. Hair follicle (Drawn using Adobe After Effects)

1.5.4. Pathophysiology and Immunology of contact dermatitis

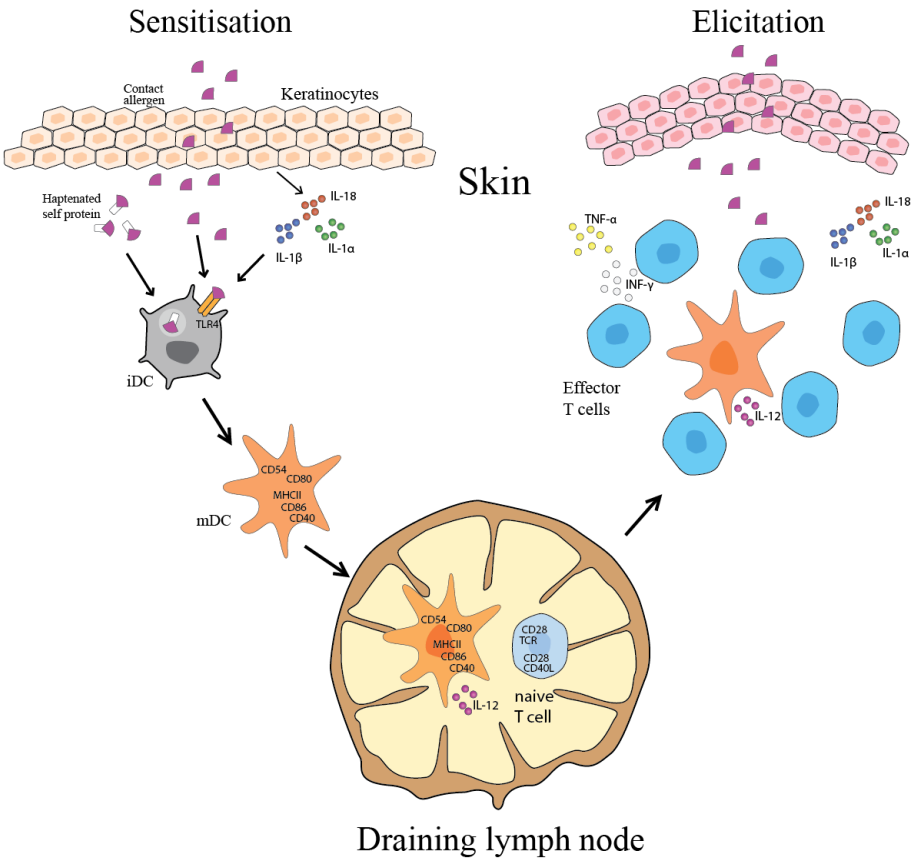
The mechanism of allergic contact dermatitis (ACD) was intensively studied during the last two decades, but the underlining immunological mechanisms are still unknown [79-82]. Keratinocytes and other skin cells produce various cytokines. They act at multiple levels of signaling molecules that affect the physiology of keratinocytes and control the skin barrier [24, 79, 83]. In most cases, irritant contact dermatitis (ICD) and ACD are clinically and histologically indistinguishable, and no markers have been identified to distinguish between the two [84]. Several in vitro and in vivo studies analyzing allergen and irritant exposures stated that the principal inflammatory pathways in ICD and ACD are essentially similar [66, 84, 85]. After the antigen has penetrated the stratum corneum, it exerts cytotoxic effects on the keratinocytes and triggers them to release alarming signals, cytokines, and chemokines. This is the way how innate immune system is triggered, and the ICD reaction is initiated. Thus, keratinocytes are the primary source of skin-derived cytokines [85, 86].

Here are 2 phases of ACD: the first is sensitization, and the second – elicitation (Figure 4). During the first phase, the chemicals in contact with the skin activate innate immunity and induce inflammation necessary for the recruitment of leukocytes and the activation of the DCs. The haptens are taken by the DCs, which migrate to the draining lymph node. In the draining lymph

nodes, the naive T cells can extravasate from capillaries. If the antigen is presented through MHC class I molecules on the surface of the dendritic cell, then CD8 + T cells do recognize it. If MHC class II molecules present the antigen, it is recognized CD4 + T cells. When the interaction and co-stimulation of the membrane-bound are sufficient, the naïve T cells develop into an antigen-experienced state. Local cytokines are generated by antigen-matured DCs and by resident stromal cells of the lymph node.

As a consequence, the generating allergen-specific T cells can either become pro-inflammatory cells of immunoregulatory T cells. The first T-cell types can be subdivided into Th1 cells, characterized by the production of IFN-γ in particular; Th2 with a predominant production of IL-4, IL-5, and IL-13, and Th17 with high IL-17 and IL-23 production. Thus, T cells have immunoregulatory properties and can either actively suppress pro-inflammatory reactions or cause antigen-specific tolerance. These cells are characterized by the release of immunosuppressive/-regulatory cytokines such as IL-10 and TGF-β. In summary, during the priming of allergen-specific T cells, functionally different subsets can develop. These subsets determine the immunological outcome and clinical appearance of the elicitation reaction in ACD [87, 88].

Elicitation phase: In case of repeated contact with the specific allergen, an elicitation reaction can occur. Small amounts of allergen manage to stimulate the immunological reaction. When the allergen is detected by allergen-specific T cells, they start to produce and secrete specific cytokines. In the ACD case, the pro-inflammatory effector T cells belonging to Th1, Th2, or Th17 subsets secrete pro-inflammatory cytokines. The massive release of inflammatory mediators causes vasodilatation, edema, spongiosis, and vesiculation [87, 89]. The development of this delayed immunological response can take several days. In later phases of acute ACD reactions, the allergen is eliminated by either metabolization or taken away by phagocytes. As a consequence of this, the inflammatory reaction starts to silence and fades away. In the case of long-lasting allergen exposures, epidermal changes can occur with acanthosis and hyperkeratosis, and desquamation of the keratinocytes. Cells get more easily activated upon repeated local allergen exposure and can already show clinical reactions within several hours.



iDC- immature dendritic cell
mDC- matured dendritic cell

Figure 4. The scheme of cellular and molecular activity during sensitization and dermatitis elicitation phases. *Sensitization phase*: the chemicals in contact with the skin activate innate immunity and induce inflammation, which is necessary for the recruitment of leukocytes and the activation of the DCs. The haptens are taken by the DCs, which migrate to the draining lymph node. Local cytokines are generated by antigen-matured DCs and by resident stromal cells of the lymph node. T cells have immunoregulatory properties and can either actively suppress pro-inflammatory reactions or cause antigen-specific tolerance. In case of repeated contact with the specific allergen, an *elicitation* reaction can occur. Small amounts of allergen manage to stimulate the immunological reaction. When the allergen is detected by allergen-specific T cells, they start to produce and secrete specific cytokines. In the ACD case, the pro-inflammatory effector T cells belonging to Th1, Th2, or Th17 subsets secrete pro-inflammatory cytokines. The massive release of inflammatory mediators causes vasodilatation, edema, spongiosis, and vesiculation. (Drawn using Adobe After effects).

1.5.5. Selected cytokines investigated in this study

In the present study, we investigated metal-induced cytokine-expression profiles representing different immune responses; Th1- (IFN-γ), IL-1 family (IL-1α and IL-1β), IL-9, Th2-type (IL-13), Th17 (IL-17A), Th22 (IL-22), and IL-12 family members (IL-23).

1.5.5.1. IFNγ

IFNγ is the key Th1-type cytokine. IFNγ is produced by irritated and damaged keratinocytes [90]. IFNγ is involved in the induction or upregulation of cell adhesion molecules mainly through effects on macrophages [91]. Studies show that IFNγ activates the inflammatory cells in ICD and ACD with higher expression levels in ICD [24, 92]. Several reports point to a critical role for IFNγ in inducing and eliciting metal-induced ACD [93-95].

1.5.5.2. IL-1α and IL-1β

L-1α and IL-1β are members of the IL-1 family of cytokines [96]. These cytokines are among the first mediators released in acute or chronic skin inflammation and are involved in a broad spectrum of skin diseases [97]. IL-1 dependent pathway of CD was investigated by Bonefeld and colleagues [98] in mice and recently in humans [99]. It is described that the IL-1 family is closely linked to the innate immune responses and is primarily associated with acute and chronic inflammation [89]. In addition, some studies describe IL-1β as a central cytokine in ACD [99, 100].

1.5.5.3. IL-9

IL-9 was first discovered in mice, where it was found to be a potent antigen-independent growth factor for T cells and mast cells [96, 101]. IL-9 expression in the skin was noticed by several authors in ACD skin lesions [24, 92]. In 2015 Liu et al. studied the IL-9 expression in Ni allergic patients' skin biopsies taken at 48 and 72 hrs and found that Ni allergic patients have a significant increase of IL-9 production in response to Ni compared to Ni non-allergic controls [92].

1.5.5.4. IL-13

IL-13 is expressed by activated Th2 cells, mast cells, basophils, eosinophils, and NK cells [96]. IL-13, directly and indirectly, regulates genes associated with skin barrier function formation and controls skin homeostasis and innate barrier function [83, 102]. It is also associated with other allergic diseases like asthma and atopic dermatitis [103].

1.5.5.5. IL-17A

IL-17A, also called IL-17 in some studies, is the founding member of this structurally distinct cytokine family. IL-17A is expressed by activated CD4+ TH17 cells, but its expression has also been detected in CD8+ T cells, NK cells, and neutrophils [96, 104]. IL-17A attracts neutrophils to mediate defenses against different pathogens [96]. Schmidt, in his study, recognized high levels of IL-17 in skin biopsies when the same site was re-exposed to the contact allergen [100].

1.5.5.6. IL-22

IL-22 is expressed by activated Th22 cells, mast cells, and NK-22 cells [96]. IL-22 induces genes that are involved in the antimicrobial defenses of keratinocytes. IL-22 is upregulated during bacterial infection, psoriasis, and atopic dermatitis [105]. In addition, IL-22 was detected in the inflamed skin of individuals with allergen-challenged skin inflammation [106].

1.5.5.7. IL-23

L23 is a member of the IL-12 family. IL-23 is mainly produced by phagocytic cells, macrophages, and activated DCs from peripheral tissues, including the skin, intestinal mucosa, and lungs [96]. IL-23 is essential in the development of Th17 cells and may be involved in the ACD pathogenesis. IL-23 was recognized to be essential for IL-22 expression and maturation and the proliferation of Th17 [107]. In addition, there are in vitro studies with human keratinocytes in which increased levels of IL-23 were observed when these cells were exposed to allergens [79, 108, 109].

1.5.6. Diagnostics

Until today the primary way of testing for ACD is patch testing. Patch testing is the method recognized for delayed-type hypersensitivity diagnostics [34, 110, 111].

During the last thirty years, much work has been done regarding standardization of the allergens, vehicles, concentrations, amounts to be placed, patch testing materials, tapes, and test reaction scoring. Due to all this work, patch testing remains accurate and reliable as multiple studies have demonstrated the reproducibility of patch testing regarding different techniques [34, 35, 112].

In Lithuania, most clinics use two types of patches: Finn chamber (Epitest, Finland), the test area is circular, and IQ chambers (Chemotechnique Diagnostics, Sweden), the test area is square. The patch test allergen series in Lithuania are mainly supplied by Chemotechnique, Sweden (Figure 5). The chemicals are placed in plastic syringes and bottles of inert material to prevent degradation or other chemical changes due to air, humidity, light, and temperature changes. There are several types of vehicles: petrolatum, which gives good occlusion, keeps allergen stable, and is inexpensive. Liquid vehicles are water and solvents (e.g., acetone, methyl ethyl ketone, ethanol) and are recommended, since they facilitate penetration of the skin, but they can evaporate [34]. All allergens in the liquid condition are recommended to be dispensed at the time of patch testing.

Figure 5. European baseline series allergens (a) (Chemotechnique Diagnostics, Sweden) and patches: IQ chambers (b).

To evaluate the significance of separate exposures – mainly occupational – many commercially produced screening series are available (e.g., cutting oils, medicaments, dental materials, plastic and glues, bakery, epoxy, photoallergens, rubber additives, metal compounds, and more others).

Before the patch testing, it is essential to evaluate if there are indications in that particular situation for patch testing. Main indications are suspected contact dermatitis, acute or chronic, including dermatitis related to occupational exposures; other types of chronic dermatitis not improving with treatment; skin and mucous membrane eruptions (including delayed-type drug eruptions in which delayed-type hypersensitivity is suspected [34, 111].

The upper back is the preferred site for patch testing. Also, the outer area of the upper arms or thighs can be used if the back is not suitable for the patch testing or is fully used already [34]. The 48-hour exposure of the patch test

allergens is recommended during patch testing. According to the best practice recommendations by ICDRG, the readings are strongly suggested to be carried out not less than two times. Best practice recommends reading after the removal of the patches and the second 2-5 days (D) later [34]. In Vilnius University Hospital Santaros Klinikos, the patch test reading by allergologists clinical immunologists is done based on best-recommended practice at D2, D3, and D7. The late reaction can be missed if not read at D5 or D7. The most valuable readings are D3 and D5/D7 [111]. The scoring is performed according to the ICDRG recommendations (Table 4) (Figure 6) [34] .

Table 4. The scoring system of the patch test reactions according to the International Contact Dermatitis Research Group (ICDRG) [34].

Symbol	Morphology	Assessment
-	No reaction	Negative reaction
?+	Faint erythema only	Doubtful reaction
+	Erythema, infiltration, possibly papules	Weak positive reaction
++	Erythema, infiltration, papules, vesicles	Strong positive reaction
+++	Intense erythema, infiltrate, coalescing vesicles	Extreme positive reaction
IR	Various morphologies, e.g. soap effect, bulla, necrosis	Irritant reaction

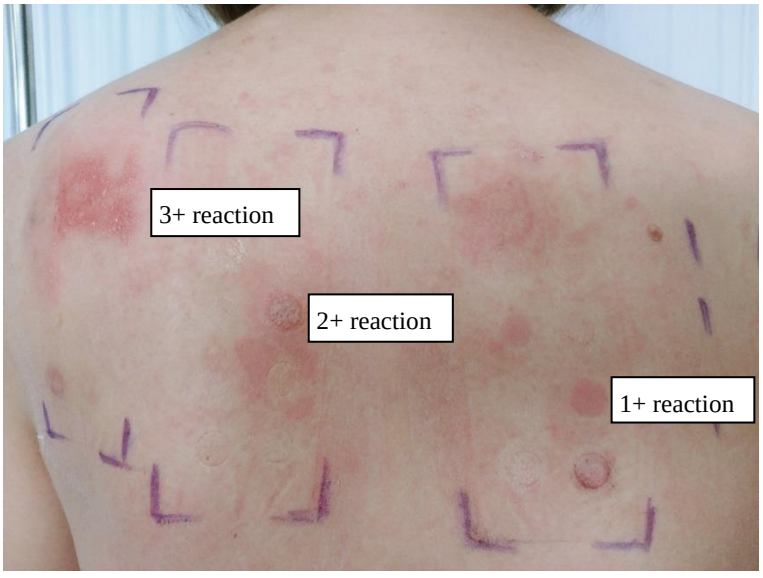


Figure 6. Positive patch test reactions.

2. AIMS

The work presented in this thesis aimed to investigate the clinical and immunological aspects of contact allergy to Ni, Co, and Cr in the occupational setting.

More specifically, the conducted studies investigated:

- Work-related skin diseases and sensitization patterns to the haptens of the European baseline series in metalworkers as a basis for possible preventive measures.
- A relation between *in vitro* release of Co, Ni, and Cr to artificial sweat from nails and wire made of different alloys and the deposition of these metals on metalworkers' fingers using the passive finger immersion method.
- The release of metals (Ni, Co, and Cr) from finished and raw material;
- The penetration of potassium dichromate 0.5% in pet. and 0.5% potassium dichromate aq. solution through pig ear skin as a model of human skin.
- Some cytokines (IFNγ and interleukins (IL-1α, IL-1β, IL-9, IL-13, IL-17A, IL-22, and IL-23) in serum and biopsied tissue, using the enzyme-linked immunosorbent assay (ELISA) in order to elucidate their role in the early phases of ACD elicitation.

3. MATERIALS AND METHODS

3.1. SUBJECTS

Metal plant workers, production workers, and office staff as the control group are the subjects of this thesis. The core activity in plant A, situated in central Lithuania, is the manufacture of nails, net, and wire (Figure 7), and in plant B – production of nails and wire.

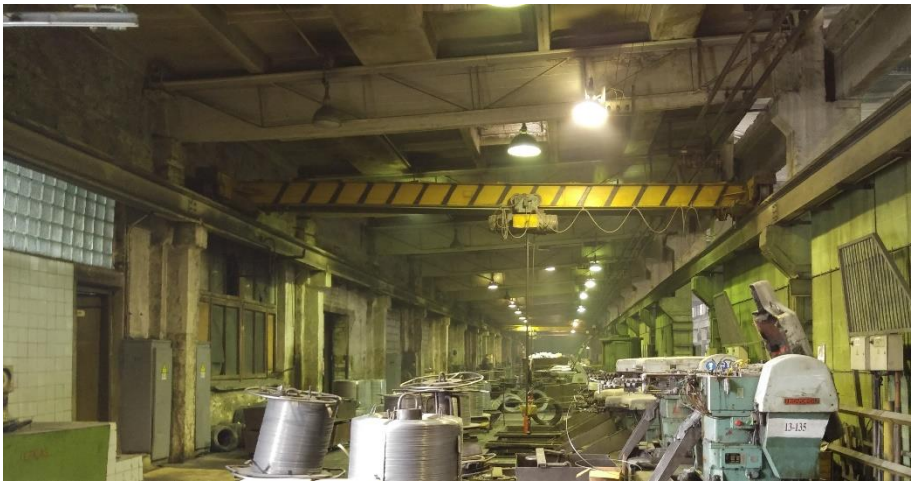


Figure 7. Wire production site in metal plant A.

The 185 workers (154 production workers and 31 office staff) filled an interviewer-administered questionnaire (Suppl. Table 1) providing information on their skin condition. The 135 workers (75 metalworkers and 60 office staff) were patch tested using the European baseline series. The characteristics of the patch-tested population are summarized in Table 5. It was 45.7% (108/236) of all the workforce from plant A and 21.9% (27/123) from plant B. There were 63 metalworkers and 45 office staff from plant A and from plant B 12 and 15, respectively. The examination of each individual's skin condition was performed by an allergologist on the same day as the patch testing but prior to it. Patch testing was offered to all personnel present at work on the day when the investigation was carried out.

Eighty-eight consecutive metal plant workers (50 metalworkers (MW) and 38 office staff (OS) from the metal plant where nails and wire were produced participated in the study. The characteristics of the participants are summarized according to the MOAHLFAP index and include characteristics

of patients such as M (male), O (occupational dermatitis (O)), A (atopic dermatitis (A)), H (hand dermatitis (H)), L (leg dermatitis (L)), F (face dermatitis (F)), A (age 40+), P (at least one positive) in Table 5.

The metals that workers most often handled were steel, stainless steel, aluminum, and iron. Mostly wire-drawing lubricants, soaps, oils, anti-rust agents, and degreasing solvents are used in the production line. The raw materials are C, Mn, Si, S, P, Cr, Ni, and Cu alloy. Both plants gained raw materials from the same suppliers. Therefore, the working conditions were considered similar in all metalworkers.

Atopy was defined according to the European Academy of Allergy and Clinical Immunology nomenclature as the presence of allergic bronchial asthma, rhinoconjunctivitis, or atopic eczema/dermatitis [113].

Table 5. MOAHLFAP index characteristics of the tested population.

	European baseline series					
			Metalworkers		Administrative personnel	
Characteristics	N	Positive reaction n, (%)	N	Positive reaction n, (%)	N	Positive reaction n, (%)
Male (M)	135	71 (52.6)	75	52 (69.3)	60	19 (31.7)
Occupational (O)	135	11135 (8.1)	75	11 14.7)	60	0 (0)
Atopic dermatitis (A)	135	2/135 (1.5)	75	0 (0)	60	2 (3.3)
Hand (H)	135	11 (8.2)	75	10 (13.3)	60	1 (1.7)
Leg (L)	135	0 (0)	75	0 (0)	60	0 (0)
Face (F)	135	16 (11.9)	75	5 (6.7)	60	11 (18.3)
Age>40 years (A)	135	81 (60.0)	75	48 (64.0)	60	33 (55.0)
Positivity (≥1 positive reaction) (P)	135	39 (28.9)	75	25 (33.3)	60	14 (23.3)

3.2. QUESTIONNAIRE

The questionnaire in the native Lithuanian language consisted of signs and symptoms of skin and respiratory conditions, suspected health

worsening factors, demographics, and time of employment. At the time of investigation, 185 workers (154 production workers and 31 office staff) employed at the factories voluntarily filled out an interviewer-administered questionnaire providing information on their skin condition. In this study, only skin will be discussed. The relationship between the symptoms, localization, contact with suspected factors, and work performed by the study members was based on occupational dermatosis criteria [4, 114]. The questionnaire in the English language is in supplement Table 1.

3.3. PATCH TESTING

One hundred and thirty-five metalworkers and office staff, as a control group, in two metal plants, gave written consent to be patch tested with the European baseline series (Suppl. Table 2). Patch testing was offered to all personnel present at work on the day when the investigation was carried out. It was 45.7% (108/236) of all the workforce from plant A and 21.9% (27/123) from plant B. There were 63 metalworkers and 45 office staff from plant A and from plant B 12 and 15, respectively. The examination of each individual's skin condition was performed by an allergologist on the same day as the patch testing but prior to it. Patch testing was performed and scored according to ESCD guidelines on-site [34]. The allergens and IQ Ultimate Chambers (9×9mm) were provided by Chemotechnique Diagnostics (Vellinge, Sweden) (Figure 8). Twenty microliters of liquid test solution were applied to the filter paper in the chamber, and 25 mg of test preparation was in pet. was applied to the test chambers [34]. The chambers were applied and left on the backs (Figure 9) for 48 hours (h), and the readings were performed on the day (D) 3/D4 and D7 by an allergologist trained to perform patch test readings. The reactions from "+" to "+++" were classified as positive and negative; irritant or doubtful reactions were classified as non-positive (Table 5). The maximum patch test reactions from either D3/D4 or D7 were considered as an outcome. All metal workers and office staff were performing their daily work during the patch testing, and the reading was done on-site.

Figure 8. The European baseline series (a) and and IQ Ultimate Chambers (9×9mm) (b), Chemotechnique Diagnostics (Vellinge, Sweden).

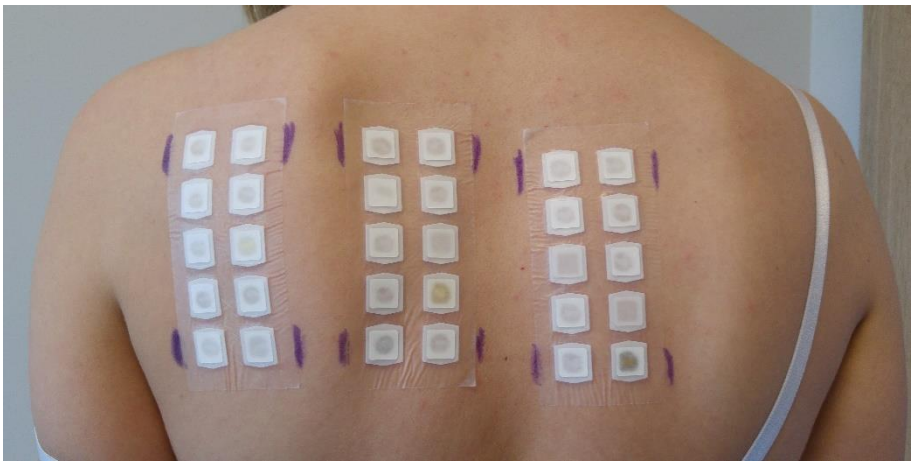


Figure 9. The European baseline series on the office staff worker‘s back.

The European baseline saries contact allergens used for the patch testing are listed in supplement table 2.

3.4. FINGER IMMERSE STUDY METHOD

All subjects (50 metalworkers (MW) and 38 office staff (OS) from the metal plant where nails and wire were produced) performed their usual work and did not wash their hands for at least 1 hour. Then they were asked to immerse their index and thumb fingers of the dominant hand in separate laboratory tubes (29 mm diameter, 50 mL, Labbox Laxware, Barcelona, Spain) filled with 35 mL of deionized water (Millipore®, Millipore, Holsheim, France) and to hold them in gently agitating for 2 minutes (Figure 10). The samples were stabilized with 35 µl of HNO3 (Merck, Darmstadt, Germany). Finger’s measurements were taken, and a corrected formula counted the affected area. The surface area in cm2 was calculated using cylinder surface area (counting one end only) $\pi r2 + 2\pi rl$ (l- length, r- radius derived from the width). All samples were stored in +4 °C temperature until the analysis (Figure 11*).*

The analysis for Cr, Co, and Ni concentrations was carried out with an inductively coupled plasma sector field mass spectrometer (ICP-SFMS, ELEMENT2 Thermo Scientific, Germany) in the Center of Physical Sciences and Technology (Department of Nuclear Research, Vilnius Lithuania). Multi-element standard solution Merck VI (Merck, Germany) was used for calibration of ICP-SFMS measurements. The chemical reporting limit was 0.001 µg/cm2.

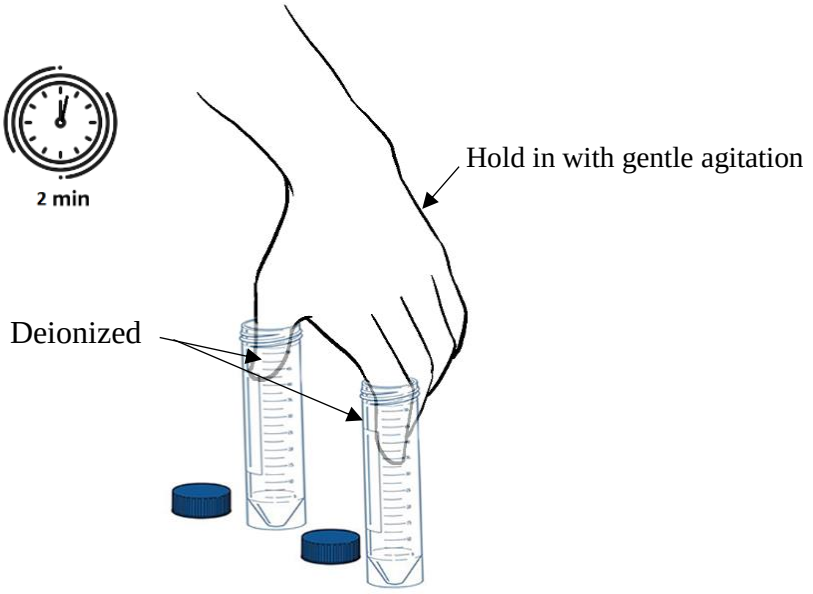


Figure 10. Passive finger immersion technique.

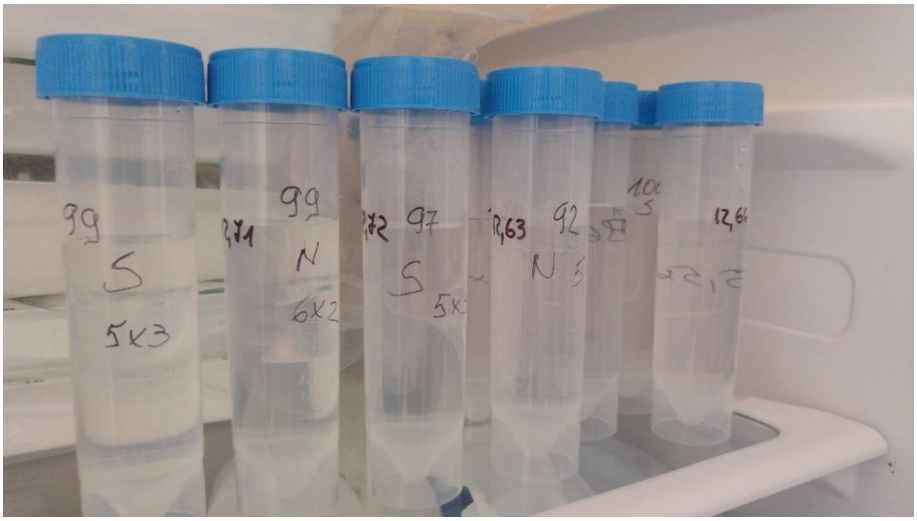


Figure 11. Finger immersion samples were stored in +4 °C temperature until the analysis.

3.5. METAL RELEASE FROM FINISHED PRODUCTS AND RAW MATERIAL

3.5.1. Metal samples

Six different samples of metal production (4 types of nails (clean and unclean) and 2 types of wire (clean and unclean) were donated by a plant producing metal items located in Kaunas, Lithuania. The cleaning of metal production involves woodchips. The steel wire rods were imported from Chelyabinsk, Russia. The chemical composition of the steel wire rod based on the supplier's information was (wt%): carbon (C) 0.08, manganese (Mn) 0.44, silicon (Si) 0.09, sulfur (S) 0.027, phosphorus (P) 0.020, chromium (Cr) 0.08, nickel (Ni) 0.07, copper (Cu) 0.03.

3.5.2. Chemicals

The artificial sweat was pre-prepared according to the reference test method EN1811:2011 (2) by mixing urea (0.1 wt%, CAS nr.57-13-6), NaCl (0.5wt%, CAS nr. 7647-14-5) and lactic acid (0.1 wt% CAS nr. 50-21-5) in deionized and aerated water (Ni, Cr and Co content below the instrumental detection limit). The final pH 6.5±0.05 was adjusted with NH3. The pH was measured before the immersion experiment started.

Ni, Co, Cr release from metal samples using modified EN1811:2011 +A1:2015

Ten milliliters (mL) of 14 mL tubes (Sarstedt, Nümbrecht, Germany) were filled with artificial sweat. A duplicate of all metal samples of the same weight was immersed in the tubes (Figure 12), the lids were sealed, and sample tubes were placed on a bilinear shaking table regulated at a maximum angle of 30^0 and an intensity of 20 cycles/min (Figure 13). This set-up gentle shaking of the test vessels ensures a good metal surface contact with the solution and prevents the eventual conglomeration of particles [115, 116]. All samples and corresponding blank were left, and 1 mL from all samples was taken after 24 hours and a week.

Chemical analysis (triplicate readings of each sample) was performed, and the total amount of Ni, Co, and Cr released from the samples was detected by flame atomic absorption spectrometry (AAS) using PerkinElmer AAnalyst™ 800 (Norwal, Shelton, USA) instrument (Figure 14*)* equipped with a graphite furnace and hollow cathode lamps. Sample analysis was

performed with Zeeman background correction. The instrumental settings and instrumental limit of detection (LOD) for Ni, Co, and Cr analysis with AAS are listed in Table 6. Samples were diluted in 0.5 M nitric acid, and 20 µL of each sample was injected. Triplicate readings of every sample were made. Standard samples (0.05 ppm of each metal) were prepared by diluting the standard solution in 0.5 M nitric acid. The released amounts of metals expressed µg/cm^2 correspond to the blank-corrected concentrations and are normalized to the solution volume and the exposed surface area. The analysis was performed at the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden.

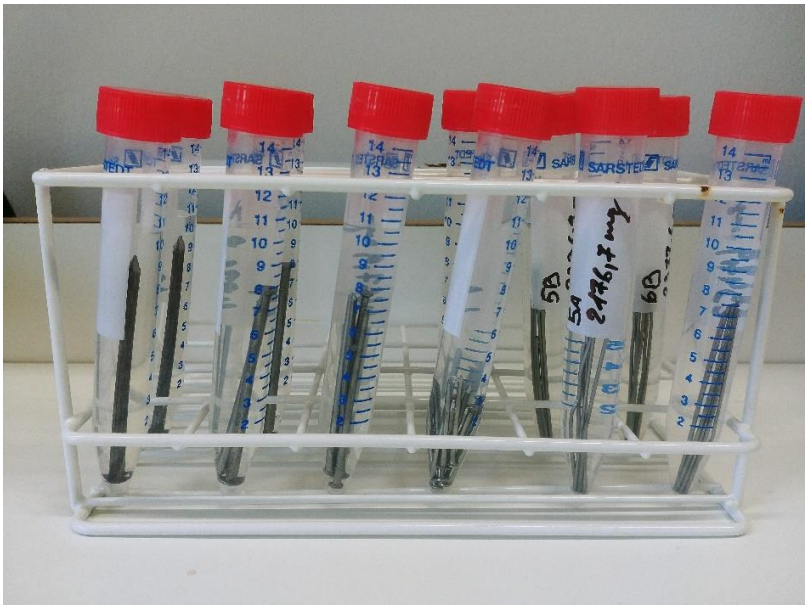


Figure 12. Duplicate of all metal samples of the same weight immersed in the tubes.

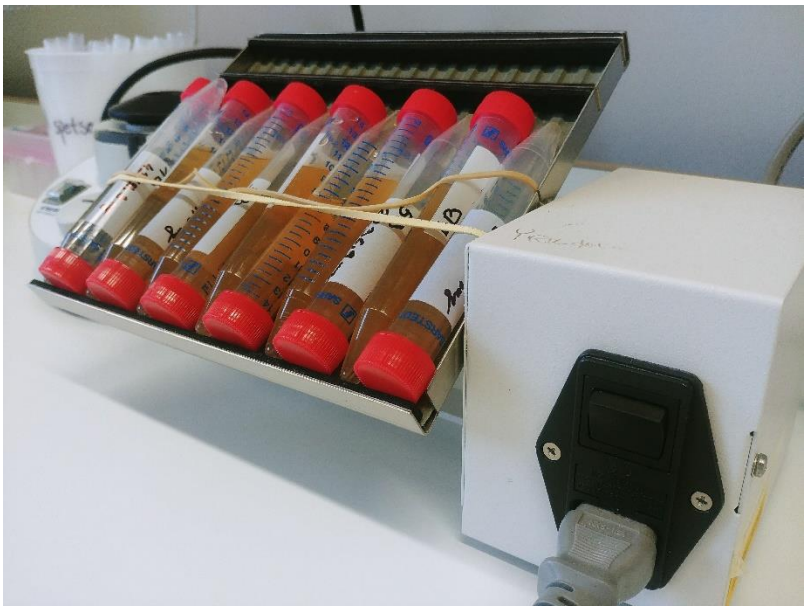


Figure 13. Sample tubes placed on a bilinear shaking table.

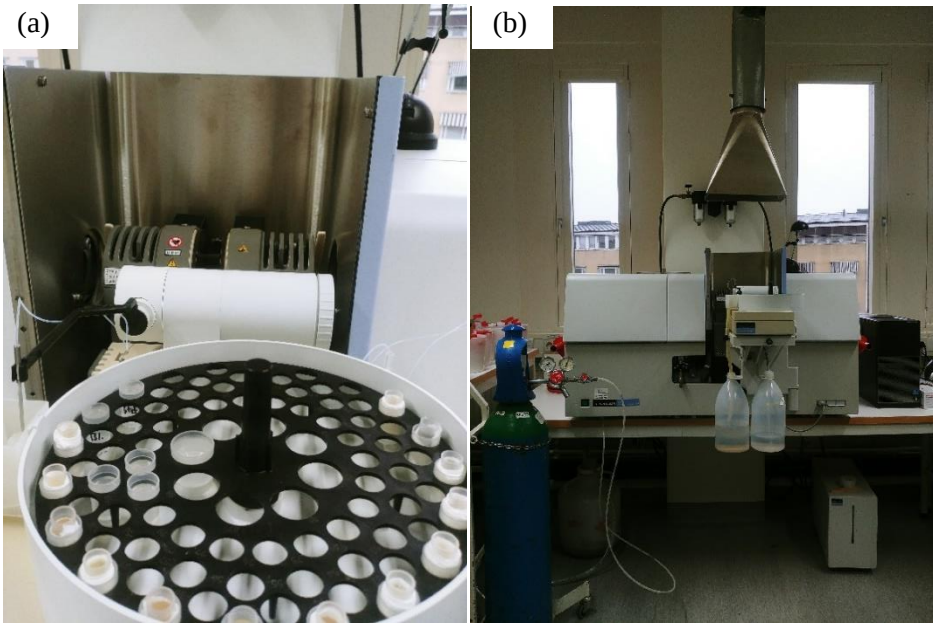


Figure 14. The chemical analysis each sample (a) was performed with flame atomic absorption spectrometry (AAS) using the PerkinElmer AAnalyst™ 800 (Shelton, USA) instrument (b).

Table 6. The instrumental settings and instrumental LOD for Ni, Co, and Cr analysis with AAS.

Metal	Wavelength (nm)	Bandwidth (nm)	Modifier	Instrumental LOD (µg/ml)
Ni	232.0	0.2	No	0.001
Cr	357.9	0.7	15 µg of $Mg(NO_3)_2$	0.001
Co	242.5	0.2	15 µg of $Mg(NO_3)_2$	0.001

3.6. PENETRATION OF CHROME USING THE FRANZ CELL DIFFUSION METHOD

3.6.1. Chemicals

Water from a Millipore purification pack system (Millipore®, Millipore, Holsheim, France) was used as the blank. One liter of pH 7.4 phosphate-buffered saline (PBS) was prepared by mixing Millipore water with 0.21g potassium hydrogen phosphate (Merck, Darmstadt, Germany), 0.908g di-Sodium hydrogen phosphate dihydrate (Merck, Darmstadt, Germany), and 7.650 sodium chloride (Merck, Darmstadt, Germany). The solution was stirred at 200 rpm for 15 minutes with a magnetic stirrer. The samples for the experiment of potassium dichromate 0.5% pet. was used from the European Baseline series (Chemotechnique Diagnostics, Vellinge, Sweden) and the standard solution of potassium dichromate 0.5% aq. (Janssen chimica, Geel, Belgium) (Figure 15). For skin digestion, extra pure HNO_3 60% (Merck, Darmstadt, Germany) was used.

3.6.2. Preparation of skin membranes

One porcine ear was stored frozen at -70^{0}C and obtained from Lund University Hospital, Sweden. The ear was allowed to thaw at room temperature before use. A machine shaver shortened the longest hairs by not disturbing the surface of the skin. Full-thickness (~1 mm) porcine skin was separated from the outer part of the ear. Due to the limited surface of one ear,

six two-centimeter diameter punches were made (Figure 16). Subcutaneous fat was carefully removed, and the thickness of all samples was measured.

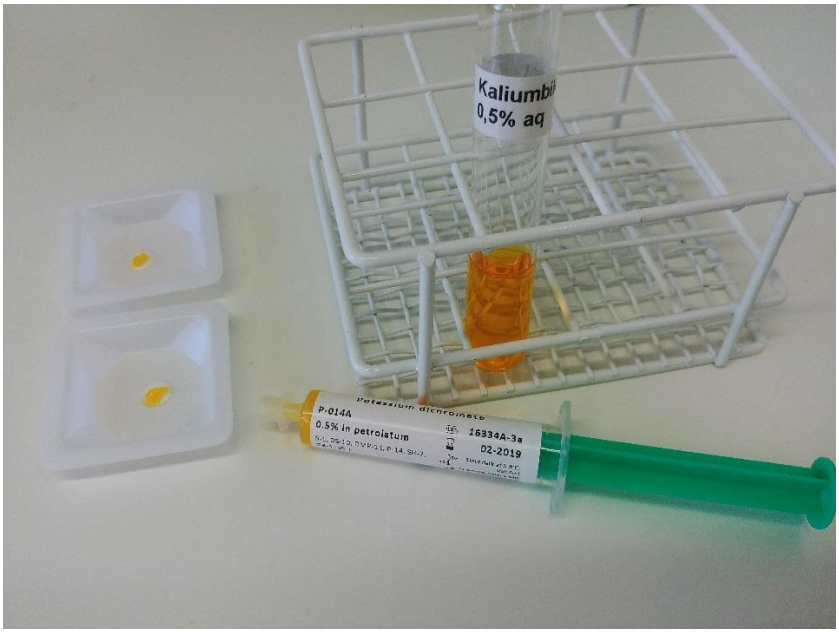


Figure 15. The samples of potassium dichromate 0.5% pet. and aq.

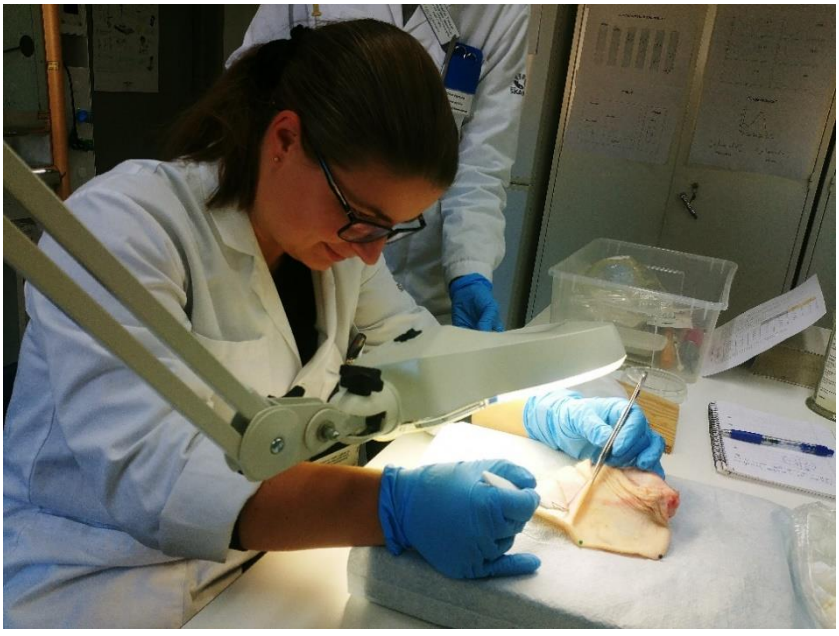


Figure 16. The separation of porcine skin.

3.6.3. In vitro diffusion system

Six PermeGear jacketed Franz cells with a 9 mm orifice diameter, flat ground joint, and 5 mL receptor volume were used (Figure 17). Full-thickness (~1 mm) porcine ear skin was mounted in Franz-type diffusion cells [117]. The donor compartment was attached using a metallic clamp. The dermal side was exposed to a recipient solution (5 mL) consisting of PBS (pH 7.4). Any bubbles were removed by tilting the cell. Magnetic stirring of 400 rpm was used for the experiment. Skin samples are left to hydrate for 2 hours under occlusion with paraffin film, thermostatically controlled bath (Grant TC120, Grant Instruments™, Cambridge, United Kingdom) was set up to 32^0C. The transepidermal water loss (TEWL) was measured after a minimal 1 h of equilibration and drying of the skin surface. The moisture on the skin surface originates from the rehydration of the frozen skin sample. TEWL was determined under closed chamber conditions. The standard limit of 10 g m^{-2} h^{-1} was used [118]; after another 2 hours, duplicate potassium dichromate preparations 20 mg of 0.5% in pet. (corresponds to 0.035mg of Cr)15µL of 0.5% in aq. (corresponds to 0.0265 mg of Cr).

Furthermore, 15µL of Millipore water as blank was applied to the donor compartments facing the epidermis for 24 hours (Figure 18). After 24 hours, the skin preparations were removed from the Franz cell system and the exposed skin in the center 10 mm punched out (Figure 19). First, the skin's surface was gently cleaned to collect the Cr left on the surface after the experiment, with a medical cloth, and this sample was named a "wipe sample". Then, the samples were frozen until cutting. The porcine skin samples were cut with a microtome (Leica CM1850, Leica Biosystems, Illinois, United States) (Figure 20). The first layer of 10 mm was named "scrapings", other slices were set to 30 mm and collected in clean, unused 1.5 mL Eppendorf safe lock microtubes (Eppendorf®, Eppendorf AG, Hamburg, Germany) without any additives (Table 7). The experiment was performed at the Department of Occupational and Environmental Dermatology in Malmö, Skåne University Hospital.

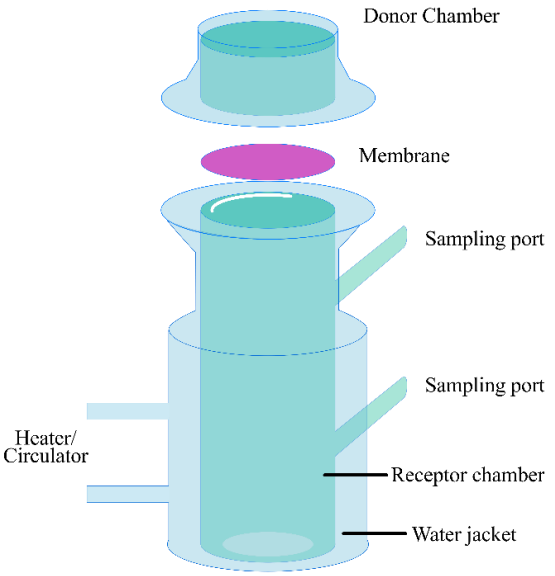


Figure 17. The Franz cell's scheme. Drawn by using Adobe After Effects.

Figure 18. Duplicate skin samples during 24-hour experiment.

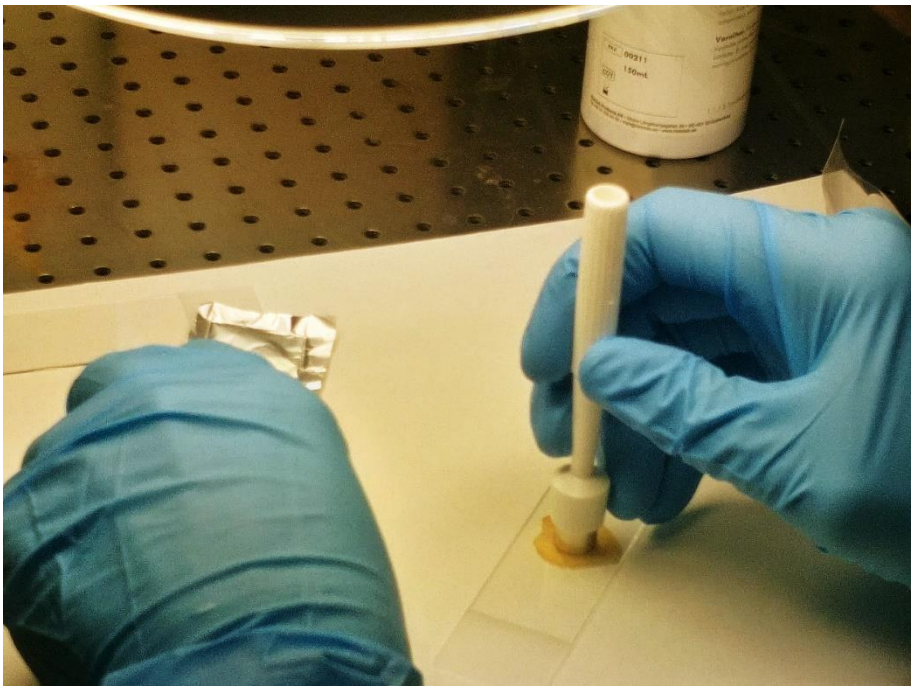


Figure 19. The puch of exposed skin sample.

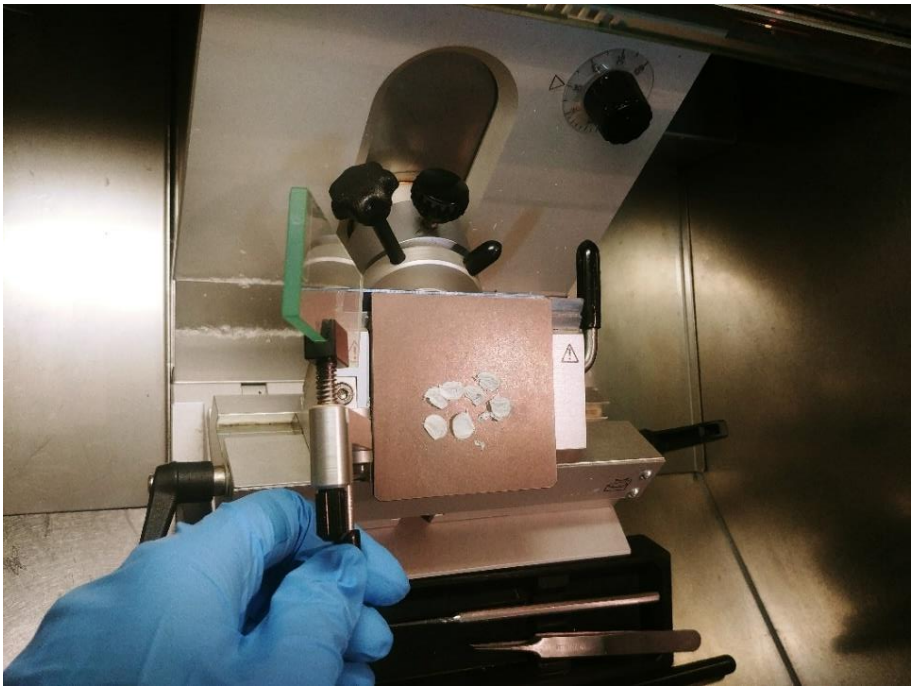


Figure 20. Cutting the frozen samples.

Table 7. The experiment plan, where A and B are blank, C and D duplicate samples in pet., E and F duplicate samples in aq.

Depth		A Blank	B Blank	C Potassium dichromate 0.5% pet.	D Potassium dichromate 0.5% pet.	E Potassium dichromate 0.5% aq.	F Potassium dichromate 0.5% aq.
	Wipe sample	A:0	B:0	C:0	D:0	E:0	F:0
	Scrapings	A:1 (10µm)	B:1 (10µm)	C:1 (10µm)	D:1 (10µm)	E:1 (10µm)	F:1 (10µm)
40µm	1x30µm	A:2 (30µm)	B:2 (3x10µm)	C:2 (3x10µm)	D:2 (3x10µm)	E:2 (3x10µm)	F:2 (3x10µm)
70µm	1x30µm	A:3 (1x30µm)	B:3 (1x30µm)	C:3 (1x30µm)	D:3 (1x30µm)	E:3 (1x30µm)	F:3 (1x30µm)
130µm	2x30µm	A:4 (2x30µm)	B:4 (2x30µm)	C:4 (2x30µm)	D:4 (2x30µm)	E:4 (2x30µm)	F:4 (2x30µm)
250µm	4x30µm	A:5 (4x30µm)	B:5 (4x30µm)	C:5 (4x30µm)	D:5 (4x30µm)	E:5 (4x30µm)	F:5 (4x30µm)
490µm	8x30µm	A:6 (8x30µm)	B:6 (8x30µm)	C:6 (8x30µm)	D:6 (8x30µm)	E:6 (8x30µm)	F:6 (8x30µm)
820µm	11x30µm	A:7 (11x30µm)	B:7 (11x30µm)	C:7 (11x30µm)	D:7 (11x30µm)	E:7 (11x30µm)	F:7 (11x30µm)
Last part	…. X 30µm	A:8 (9x30µm)	B:8 (17x30µm)	C:8 (21x30µm)	D:8 (22x30µm)	E:8 (12x30µm)	F:8 (20x30µm)
Receptor phase		A:9	B:9	C:9	D:9	E:9	F:9

A and B, duplicate control samples
C and D, duplicate Potassium dichromate 0.5% pet. samples
E and F, duplicate Potassium dichromate 0.5% aq. samples
pet., petrolatum,
aq., aqua

3.6.4. The skin digestion after the experiment

The skin samples in plastic tubes were digested using a method developed in the Department of Occupational and Environmental Dermatology, Malmö, Sweden, by adding 1 ml extra pure HNO3 60% and heated at 700 for 2 hours.

3.6.5. Quantitative analysis

All samples after the digestion were diluted to a total volume of 10 mL with Millipore water for the atomic chromium analysis using inductively coupled plasma sector field mass spectrometry (ICP-SFMS, ELEMENT2 Thermo Scientific, Germany) in the Center for Physics and Technology (Vilnius, Lithuania). Multi-element standard solution Merck VI (Merck, Germany) was used for calibration of ICP-SFMS measurements. The detection limit was 0.001 μg/cm^3.

3.7. SKIN BIOPSY METHOD FOR CYTOKINE ANALYSIS

3.7.1. Study participants

Ten participants (2 men, 8 women; mean age 42.7 (±8.6) years) were invited. Five patients had already known contact allergy to nickel (Ni), and 5 were patch test negative to Ni and served as controls. None of the participants had active dermatitis during the experiment. It was recommended that all participants do not eat 12 hrs before all visits and not consume Ni-rich food during the 48 hrs of patch testing.

3.7.2. Patch testing

Patch testing was performed and scored according to ESCD guidelines [34]. The allergens and IQ Ultimate Chambers (9×9mm) were provided by Chemotechnique Diagnostics (Vellinge, Sweden). Twenty-five milligrams (mg) of nickel sulphate 0.5% in petrolatum (pet.) were applied to the test chambers at day (D) 0. Two chambers were applied and left on the back of each participant for 24 and 48 hrs. The first test was removed after 24 hrs and the second one after 48 hrs.

3.7.3. Blood samples

Blood samples from the participants were drawn three times. The baseline sample was drawn before the patch testing at D0, others –24 hrs, and 48 hrs after the patch test application. The blood samples were left to stay in clotting (serum) (BD Vacutainer SSTTM II Advance, Plymouth, UK) laboratory vacutainers at room temperature for 30 minutes before the serum separation. After the separation serum was stored in 1.5 mL Eppendorf safe

lock microtubes (Eppendorf®, Eppendorf AG, Hamburg, Germany) without additives at -20^{0}C until further analysis.

3.7.4. Skin punch biopsy

Three skin biopsies were taken. The baseline biopsy was taken before from the nearby area approximately 7-10cm (depending on the participant) from the application of patch tests of Ni at D0, then after 24 hrs when the patch test was removed, and the third one when the second patch test was removed after 48 hrs. The area to be biopsied was cleaned with skin disinfectant and locally anesthetized with 2% lidocaine (Figure 21). A skilled allergologist performed the punch biopsies of the skin according to the best practice recommendations [119, 120] using a 4 mm dermal biopsy punch (RazormedTM , Gurgaon, India). The specimen was taken with the biopsy punch performing a rotating movement, giving a cylindrical specimen, then pulled up out of the skin, and in some cases, a scalpel was required to separate it from the base. The resulting wound was covered with a sterile dressing. The weight of the biopsied sample was measured and straight away taken to a -80^{0}C freezer until further investigation.

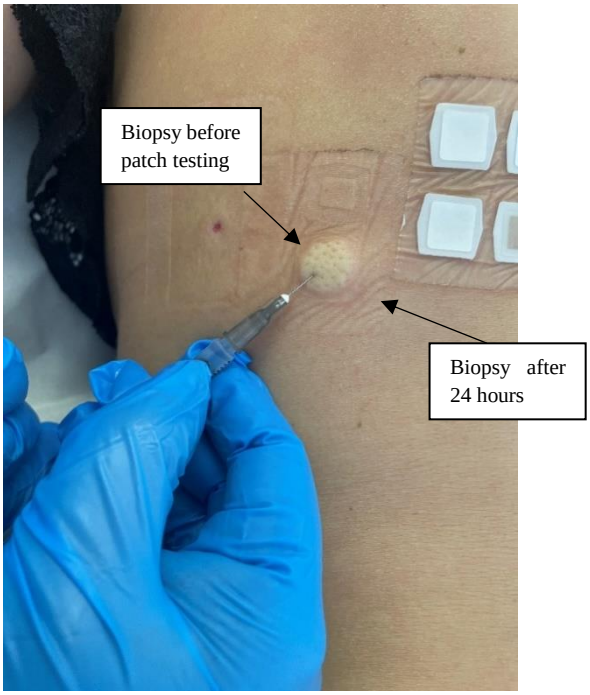


Figure 21. Local anaesthesia before the 24 hour skin punch biopsy.

3.7.5. Enzyme-linked immunosorbent assay

For quantitative detection of human IFNγ, IL-1α, IL-1β, IL-9, IL-13, IL-17A, IL-22, and IL-23, the ELISA Invitrogen (Thermo Fisher Scientific, Bender MedSystems GmgH, Vienna, Austria) kits were used. Serum samples were analyzed according to the Invitrogen procedure protocol. Biopsied tissues were lysed to become liquid before the analysis. HaltTM protease inhibitor cocktail (Thermo Scientific, Pierce Biotechnology, Rockford, Illinois, USA) was used for skin degradation and cytokine protection. The amount of protease inhibitor cocktail differed according to the sample weight. After the biopsied skin samples were degraded into liquid solution [119], further analysis due to the small number of samples was performed for IL-17A, IL-22, and IL-23. The analytical sensitivity was 4 pg/mL. The analysis was conducted in the Department of Immunology, State Research Institute, Center for Innovative Medicine, Vilnius, Lithuania.

3.8. ETHICS

The study was approved by the Regional Ethics Review Board in Vilnius, Lithuania, and conducted in accordance with ethical standards specified in the Declaration of Helsinki. All patients gave informed written consent to participate in the study.

3.9. STATISTICAL ANALYSIS

The questionnaire results and the skin patch testing were statistically analyzed using R package version 3.5-1. The $\chi 2$-test or Fisher's exact test was used where appropriate (if $n \leq 5$). All results were expressed as prevalence with 95% confidence intervals, and the threshold for statistical significance was predefined as a *P*-value of <.05.

The results of metal release from production and finger immersion sampling were statistically analyzed by R package version 3.4.3 using the Wilcoxon rank-sum test on the two independent samples. A *P* -value of <.05 was regarded as significant.

The results of cytokine analysis in biopsied skin and blood samples Statistical analysis was performed by R package version 3.5.1 using the Wilcoxon rank-sum test on the two independent samples. A *P* -value of <.05 was regarded as significant.

4. RESULTS

4.1. QUESTIONNAIRE

One hundred eighty-five metal plant workers (154 metalworkers and 31 from administrative personnel) completed the interviewer-administered questionnaire to provide information about skin symptoms (e.g., pruritus, stinging, burning or pain etc.) and signs (e.g., redness, scaling, fissures, lesions, Etc.). Metalworkers younger than 40 years statistically significantly more often complained of having skin symptoms than older ones (71.73% (33/46) vs. 49.07% (53/108), P=.009) (Figure 22). Metalworkers statistically significantly more often had complaints of face and hand dermatitis compared to administrative personnel (45.45% (70/154) vs. 16.13% (5/31) P=.0024 and 42.86% (66/154) vs. 12.9% (4/31) P=.001*)*, respectively (Table 8). Skin diseases diagnosed prior to this study by a physician were reported in 1.7% (3/185) of cases, among metalworkers 0.65% (1/154), and office staff 6.45% (2/31). Metalworkers, working less than 20 years in the factory, statistically significantly more often had skin symptoms compared with the metalworkers working longer (62.2% (70/112) vs. 38.09% (16/42), P=.007) (Figure 23*)*. Contact with chemicals at the workplace was statistically significantly more often suspected as the main factor provoking skin symptoms by metalworkers compared to office staff (20.13% (31/154) vs. 3.22% (9/31) P=.019). Office staff recognized personal hygiene products as the leading culprit agent for their skin problems 29.03% (9/31) vs. 15.58% (24/154) P=.04. The results are summarized in Table 8.

None of the metalworkers had an atopic history, while two men from the control group had mild atopic dermatitis.

4.1.1. Skin problems among metalworkers and office staff

The MOAHLFAP index summarizes the demographics of the patch-tested study members in Table 5. The average period of employment in the plants was 14.4±1.7 years (Table 9). Thirty-eight percent (29/75) of metalworkers and 25.0% (15/60) of the office staff complained of skin problems. The most common locations of dermatitis were the face and hands. On examination, prior to the patch testing, 29 of the 75 metalworkers (38.6%) and 15 of the 60 (25%) controls showed current skin signs (e.g., acne vulgaris, rosacea, psoriasis, and seborrheic eczema) (Table 10). Thus, work-related

occupational dermatitis, allergic or irritant, was suspected in 11 out of 75 metalworkers.

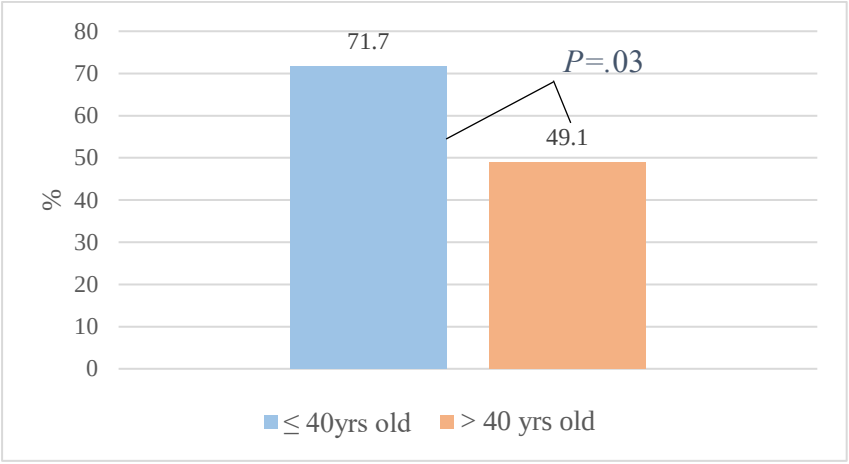


Figure 22. Skin symptom complaints according to the metalworkers' age.

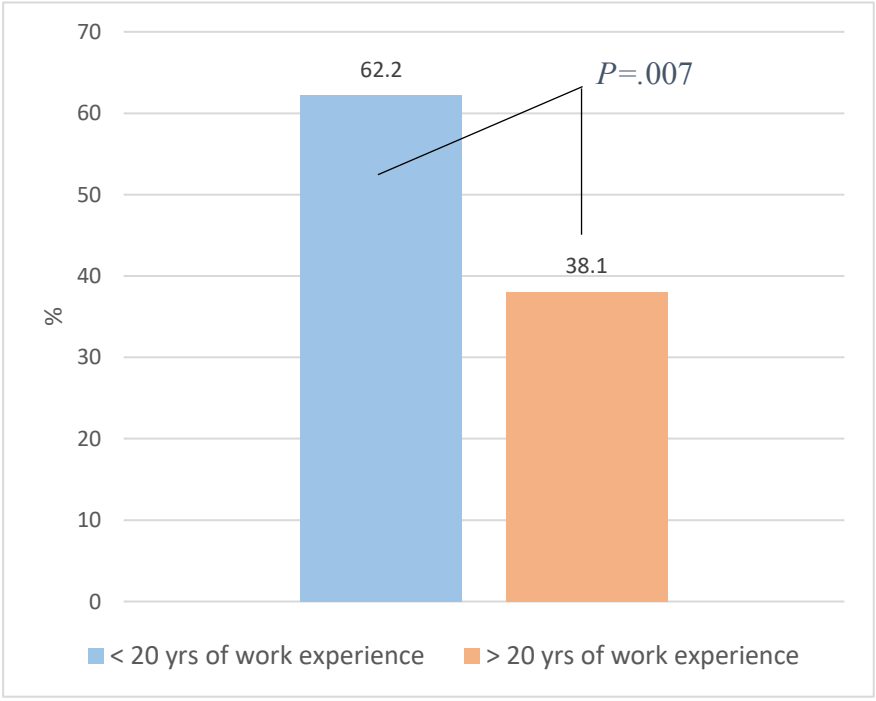


Figure 23. Skin problems and duration of work at the factory among metalworkers.

Table 8. Summarized questionnaire results according to dermatitis area and suspected offending cause.

Complaints	Metalworkers N-154		Administrative personnel N=31		Significance (p)
	n, (%)	95% CI	n, (%)	95% CI	
Face dermatitis	**70 (45.5)**	37.8-53.3	**5(16.1)**	6.6-33.1	**.0024**
Hand dermatitis	**66 (42.9)**	35.3-50.7	**4(12.9)**	4.5-29.4	**.0019**
Leg dermatitis	**2(1.3)**	0-4.9	**3(9.7)**	2.56-25.6	**.03**
Other dermatitis	0/0	-	1(3.2)	0-17.5	
Suspected offending cause					
Chemicals	**31(20.1)**	14.5-27.2	**1(3.2)**	0-17.5	**.01**
Detergents	**24(15.6)**	10.6-22.2	**9(29.0)**	15.9-46.7	**.04**
Cold	58(37.7))	29.7-44.8	8(25.8)	13.4-43.4	.2
Heat	47/(30.5)	23.7-38.2	7(22.5)	11.1-40.1	.36
Metals	13/(8.4)	4.8-14.0	0/0	-	.13
Costume jewellery	1/(0.6)	0-3.9	2(6.5)	0,7-21.7	.07
Leather	0/0	-	0/0	-	-
Rubber	2/(1.3)	0-4.9	2(6.5)	0.7-21.7	.13

N – number of participants, n – number of positive answers

CI, confidence interval.

Chi-square or Fisher's exact test (if n≤5).

Significant results (P<.05) are shown in bold

Table 9. Sex, age, and employment time of the metal workers and the office staff in the investigation during the patch testing.

Sex	Male		Female		Total	
	Metal workers	Office staff	Metal workers	Office staff	Metal workers	Office staff
Number	52	19	23	41	75	60
Mean age, years (range)	42.1 (29.1-55.1)	38.3 (26.9-49.7)	50.4 (44.5-56.3)	43.6 (30-56)	44.7 (32.8-56.6)	41.9 (29.2-54.6)
Mean time of employment, years (range)	13.7 (3.1-24.3)	12.2 (3.8-20.6)	17.6 (6.8-24.8)	14.8 (4.4-25.1)	14.8 (4.1-25.6)	13.9 (4.2-23.6)

Table 10. Relationship between skin disease, gender and workplace.

	Male		Female		Total	
	n	%	n	%	n	%
Contact dermatitis	**9**	**17.3**	**2**	**8.7**/*0*	**11**	**14.7**
Atopic dermatitis	**0**/*2*	**0**/*10.5*	0/*0*	0/*0*	*2*	*3.3*
Acne	**2**/*1*	**3.8**/*5.3*	**1**/*2*	**4.3**/*4.9*	**3**/*3*	**4**/*5*
Rosacea	**2**/*1*	**3.8**/*5.3*	**2**/*3*	**8.7**/*7.3*	**4**/*4*	**5.3**/*6.7*
Seborrheic eczema	**2**	**3.8**	**1**/*1*	**4.3**/*2.4*	**3**/*1*	**4**/*1.7*
Psoriasis	**1**/*1*	**1.9**/*5.3*	**1**/*1*	**4.3**/*2.4*	**2**/*2*	**2.7**/*3.3*
Folliculitis	**3**/*1*	**5.8**/*5.3*	**2**/*2*	**8.7**/*4.9*	**5**/*2*	**6.7**/*3.3*
Mycosis fungoides	**1**/*0*	**1.9**/*0*	0/*0*	0/*0*	**1**/*0*	**1.3**
Total	20/*6*		9/*9*		29/*15*	

Numbers in bold – metalworkers. Numbers in italics – office staff.

4.2. PATCH TESTING

Out of 135 patch-tested workers, 39 (28.9%) had at least one positive patch test reaction. Metalworkers were sensitized to cobalt chloride (6/75) significantly more often than controls (0/60) (P=.03). Women were more often sensitized to nickel sulfate than men (18.75% (12/64) and 4.22% (3/71) (P=.01)) with no difference between the workplaces. Among all tested

individuals, the top 5 contact allergens were nickel sulfate (positive in 11.11%), *Myroxylon pereirae* resin (5.93%), cobalt chloride (4.44%), fragrance mix I (3.7%), and methyl dibromo glutaronitrile (2.96%), but with no significant difference between age, gender or workplace. The frequencies of positive patch test reactions to the European baseline series are summarized in Table 11.

Table 11. Frequencies of positive patch test reactions to the European baseline series.

			Total n=135		Metalworkers (n=75)		Office staff (n=60)		Male (n= 71)		Female (n= 64)	
No.	Substance	Conc. (%)	n/% positive	95% CI	n/% positive	(95% CI)	n/% positive	(95% CI)	n/% positive	(95% CI)	n/% positive	(65% CI)
1.	Nickel (II)sulfate hexyhydrate	5.0	15/11.11	6.75-17.6	7/9.33	4.32-18.31	8/13.33	6.65-24.43	**3/4.22****	0.96-12.19	**12/18.75****	10.9-30.13
2.	*Myroxylon pereirae resin*	25.0	8/5.93	2.9-11.4	4/5.33	1.7-13.33	4/6.67	2.16-16.39	5/7.04	2.68-15.81	3/4.69	1.08-13.43
3.	Cobalt (II)chloride hexahydrate	1.0	6/4.44	1.9-9.6	**6/8***	3.4-16.7	**0/0***	-	4/5.63	1.8-14.03	2/3.13	0.2-11.33
4.	Fragrance mix I	8.0	5/3.7	1.4-8.6	3/4	0.9-11.6	2/3.33	0.25-12.03	4/5.63	1.8-14.03	1/1.56	0-9.14
5.	Methyldibromo glutaronitrile	0.5	4/2.96	0.9-7.6	2/2.67	0.2-9.77	2/3.33	0.25-12.03	3/4.23	0.96-12.19	1/1.56	0-9.14
6.	Potassium dichromate	0.5	3/2.22	0.5-6.6	3/4	0.9-11.6	0/0	-	2/2.82	0.2-10.29	1/1.56	0-9.14
7.	Formaldehyde	2.0*	3/2.22	0.5-6.6	2/2.67	0.2-9.77	1/1.67	0-9.7	2/2.82	0.2-10.29	1/1.56	0-9.14
8.	Paraben mix	16.0	2/1.48	0.07-5.6	2/2.67	0.2-9.77	0/0	-	2/2.82	0.2-10.29	0/0	-
9.	Quaternium-15	1.0	2/1.48	0.07-5.6	1/1.33	0-7.87	1/1.67	0-9.7	2/2.82	0.2-10.29	0/0	-
10.	Budesonide	0.01	2/1.48	0.07-5.6	0/0	-	2/3.33	0.25-12.03	1/1.41	0-8.29	1/1.56	0-9.14
11.	Fragrance mix II	14.0	2/1.48	0.07-5.6	2/2.67	0.2-9.77	0/0	-	1/1.41	0-8.29	1/1.56	0-9.14

12.	Methylisothiazolinone	0.2*	2/1.48	0.07-5.6	1/1.33	0-7.87	1/1.67	0-9.7	1/1.41	0-8.29	1/1.56	0-9.14
13.	Neomycin sulfate	20.0	1/0.74	0-4.5	0/0	-	1/1.67	0-9.7	0/0	-	1/1.56	0-9.14
14.	Epoxy resin	1.0	1/0.74	0-4.5	1/1.33	0-7.87	0/0	-	1/1.41	0-8.29	0/0	-
15.	4-tert-Butylphenolformaldehyde resin (PTBP)	1.0	1/0.74	0-4.5	1/1.33	0-7.87	0/0	-	1/1.41	0-8.29	0/0	-
16.	2-Mercaptobenzothiazole (MBT)	2.0	1/0.74	0-4.5	1/1.33	0-7.87	0/0	-	1/1.41	0-8.29	0/0	-
17.	Sesquiterpene lactone mix	0.1	1/0.74	0-4.5	1/1.33	0-7.87	0/0	-	1/1.41	0-8.29	0/0	-
18.	Methylisothiazolinone+Methylchloroisothiazolinone (MI+MCI)	0.02*	1/0.74	0-4.5	0/0	-	1/1.67	0-9.7	0/0	-	1/1.56	0-9.14
19.	Hydroxyisohexyl 3-cyclohexene carboxaldehyde	5.0	1/0.74	0-4.5	1/1.33	0-7.87	0/0	-	1/1.41	0-8.29	0/0	-

Cons, concentration; CI, confidence interval; n, number of patients.

Chi-square or Fisher's exact test (if n≤5).

Significant results (P<.05) are shown in bold.

*P=.03

**P=.01

Vehicle is petrolatum if not stated otherwise; *= aqua.

Negative resulted haptens are not shown in the table.

4.3. Finger immersion test

One hundred and seventy-six samples of 88 participants were analyzed. Fifty participants were MW (9 females (F) and 41 males (M)), and 38 participants were OS (21 F and 17 M). When analyzed using an inductively coupled plasma sector field mass spectrometer, Ni was detected in all samples but without statistical significance between the workplace or gender. Medians of the detected Co amount statistically significantly differed between workplaces (0.004 μg/cm^2 for MW versus 0.001 μg/cm^2 for OS (*P*=.04) (Figure 24). The median of detected Cr amount was 0.0012 μg/cm^2 in MW and 0.0011 μg/cm^2 in OS with no statistically significant difference, though there was a tendency to a difference between genders: 0.0013 μg/cm^2 in males and 0.0007 μg/cm^2 in females (*P*=.06). There was no statistically significant difference between studied fingers. Nickel amount was higher in OS, but with no significant difference compared with MW. The highest amounts of Ni in our study were detected in raw material operators (0.0174 μg/cm^2), nail heaters (0.0160 μg/cm^2), IT specialist (0.0297 μg/cm^2), and production controlee (0.0153 μg/cm^2) (Table 12). The amounts of metals on MW and OS fingers' are listed in Tables 13 and 14.

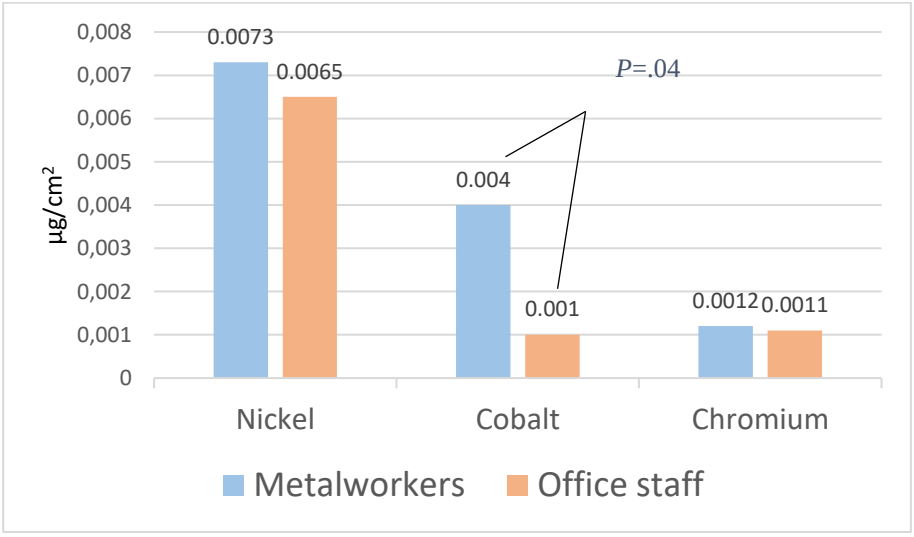


Figure 24. The medians of the total (μg/cm^2) Ni, Co and Cr on the Metalworkers (N50) and Office staff (N38) finger skin as shown by the "finger immersion" method and analysis by inductively coupled plasma mass spectrometer for comparison between occupational groups and metals. A nonparametric Wilcoxon Rank-Sum test was used.

Table 12. The median of Ni, Cr, and Co on fingers (in µg/cm^2) according to the profession (doing at least 1h their usual tasks, deposition in 2 min. finger immersion technique).

N	Occupation	Nickel µg/cm^2 (±SD)		Cobalt µg/cm^2 (±SD)		Chromium µg/cm^2 (±SD)	
		Thumb	Index finger	Thumb	Index finger	Thumb	Index finger
8	Mechanic	0.0033 (±0.0035)	0.0051 (±0.0045)	0.0001 (±0.0016)	0.0002 (±0.0016)	0.001 (±0.0024)	0.0006 (±0.0084)
4	Nail-machine operator	0.0021 (±0.0010)	0.0025 (±0.0014)	0.0002 (±0.0002)	0.0002 (±0.0002)	0.0003 (±0.0004)	0.0003 (±0.0006)
17	Wire stretchin machine operator	0.0036 (±0.0028)	0.0025 (±0.0022)	0.0002 (±0.0010)	0.0002 (±0.0007)	0.0007 (±0.0016)	0.0005 (±0.0006)
5	Nail-packer	0.0035 (±0.0017)	0.0035 (±0.0038)	0.0002 (±0.0002)	0.0002 (±0.0002)	0.0008 (±0.0005)	0.0009 (±0.0007)
5	Warehouse-worker	0.0020 (±0.0026)	0.0036 (±0.0022)	0	0	0.0002 (±0.0001)	0.0002 (±0.0002)
2	Raw material operator	0.0157 (±0.0156)	0.0174 (±0.0150)	0.0009 (±0.0004)	0.0008 (±0.0003)	0.0050 (±0.0039)	0.0056 (±0.0038)
2	Mechanic-crane operator	0.0054 (±0.0023)	0.0030 (±0.0017)	0.0005 (±0.0001)	0.0002	0.0017 (±0.0001)	0.0009
2	Locomotive driver	0.0026 (±0.0032)	0.0002	0.0004	0.0002	0.0004	0.0001
2	Weighing-machine operator	0.0016 (±0.0001)	0.0018 (±0.0014)	0.0001 (±0.0001)	0.0001 (±0.0001)	0.0003 (±0.0001)	0.0003 (±0.0001)
1	Nail heater	0.0160	0.0132	0.0007	0.0011	0.0036	0.0033
1	Security man	0.0093	0.0067	0.0002	0.0001	0.0012	0.0010
1	Electrician	0.0063	0.0065	0.0006	0.0005	0.0011	0.0011
1	IT specialist	0.0297	0.0180	0.0006	0.0005	0.0014	0.0013
1	Production controlee	0.0153	0.0123	0.0003	0.0002	0.0019	0.0014
36	Office staff	0.0034 (±0.0052)	0.0035 (±0.0040)	0.0005 (±0.0004)	0.0005 (±0.0003)	0.0006 (±0.0005)	0.0006 (±0.0004)

N - number of participants

SD - standard deviation

Table 13. The amount of Ni, Cr, and Co in sample detected (ppb) and converted to deposited sample area (μg/cm^2) in metalworkers (doing at least 1h their usual tasks, deposition in 2 min. finger immersion technique).

		Nickel				Cobalt				Chromium			
	Profession	Thumb		Index finger		Thumb		Index finger		Thumb		Index finger	
		ppb	μg/cm^2	ppb	μg/cm^2	ppb	μg/cm^2	ppb	μg/cm^2	ppb	μg/cm^2	ppb	μg/cm^2
1	Nail heater	9.62	0.0160	13.17	0.0132	0.44	0.0007	1.14	0.0011	2.18	0.0036	3.27	0.0033
2	Mechanic	1.14	0.0014	1.25	0.0012	<0.018	0	<0.018	0	0.62	0.0010	0.36	0.0003
3	Mechanic	0.92	0.0010	1.00	0.0013	<0.018	0	<0.018	0	0.54	0.0006	0.30	0.0004
4	Mechanic	2.97	0.0032	3.75	0.0055	<0.018	0	<0.018	0	6.50	0.0071	16.9	0.0247
5	Mechanic	3.31	0.0033	5.23	0.0051	0.22	0.0002	0.41	0.0004	1.04	0.0010	2.12	0.0021
6	Mechanic	2.26	0.0028	4.35	0.0036	0.14	0.0002	0.48	0.0004	0.18	0.0002	0.35	0.0003
7	Mechanic	7.73	0.0097	15.66	0.0157	0.12	0	0.25	0	2.97	0.0037	5.70	0.0057
8	Mechanic	6.21	0.0104	8.47	0.0066	2.76	0.0046	1.27	0.0010	2.13	0.0036	1.00	0.0008
9	Mechanic	4.61	0.0047	4.73	0.0051	1.73	0.0018	4.25	0.0046	0.24	0.0002	0.31	0.0003
10	Nail-machine operator	3.24	0.0028	3.49	0.0033	0.43	0.0004	0.61	0.0006	1.16	0.0010	1.55	0.0014
11	Nail-machine operator	3.12	0.0031	5.11	0.0040	0.29	0.0003	0.42	0.0003	0.41	0.0004	0.62	0.0005
12	Nail-machine operator	0.87	0.0009	1.28	0.0011	0.06	0.0001	0.09	0.0001	0.09	0.0001	0.15	0.0001
13	Nail-machine operator	1.15	0.0014	1.92	0.0016	0.05	0.0001	0.12	0.0001	0.07	0.0001	0.13	0.0001
14	Electric	6.33	0.0063	7.82	0.0065	0.56	0.0006	0.60	0.0005	1.13	0.0011	1.31	0.0001
15	Weighing-machine operator	1.29	0.0022	2.38	0.0028	0.05	0.0001	0.08	0.0001	0.09	0.0002	0.17	0.0002
16	Weighing-machine operator	0.85	0.0009	0.81	0.0008	0.04	0	0.02	0	0.27	0.0003	0.31	0.0003
17	Nail-packer	1.78	0.0026	2.34	0.0027	0.43	0.0006	0.41	0.0005	0.42	0.0006	0.75	0.0009
18	Nail-packer	0.99	0.0014	1.40	0.0012	0.12	0.0002	0.17	0.0001	0.16	0.0002	0.24	0.0002

19	Nail-packer	3.00	0.0058	9.56	0.0093	0.15	0.0003	0.16	0.0002	0.80	0.0016	1.67	0.0016
20	Nail-packer	2.79	0.0047	7.91	0.0092	0.11	0.0002	0.20	0.0002	0.50	0.0008	1.68	0.0020
21	Nail-packer	2.82	0.0035	4.24	0.0035	0.09	0.0001	0.16	0.0001	0.78	0.0010	0.89	0.0007
22	Wire stretching machine operator	1.95	0.0024	3.05	0.0025	0.08	0.0001	0.15	0.0001	0.35	0.0004	0.65	0.0005
23	Wire stretching machine operator	4.50	0.0064	4.51	0.0045	0.10	0.0001	0.07	0.0001	0.50	0.0007	0.45	0.0005
24	Wire stretching machine operator	1.57	0.0026	1.89	0.0019	0.05	0.0001	0.09	0.0001	0.32	0.0005	0.34	0.0003
25	Wire stretching machine operator	2.46	0.0041	2.45	0.0025	0.20	0.0003	0.24	0.0002	0.41	0.0007	0.57	0.0006
26	Wire stretching machine operator	3.23	0.0046	4.42	0.0044	0.19	0.0003	0.22	0.0002	1.89	0.0027	2.35	0.0024
27	Wire stretching machine operator	3.77	0.0038	4.58	0.0038	0.36	0.0004	0.52	0.0004	0.86	0.0009	1.22	0.0010
28	Wire stretching machine operator	6.23	0.0104	5.48	0.0091	0.31	0.0005	0.35	0.0006	0.31	0.0005	0.36	0.0004
29	Wire stretching machine operator	1.84	0.0036	1.88	0.0022	0.07	0.0001	0.07	0.0001	0.07	0.0001	0.10	0.0001
30	Wire stretching machine operator	1.18	0.0012	1.14	0.0013	0.14	0.0001	0.12	0.0001	0.71	0.0007	0.45	0.0005
31	Wire stretching machine operator	7.52	0.0094	2.03	0.0021	0.14	0.0002	0.09	0.0001	0.60	0.0008	1.10	0.0011
32	Wire stretching machine operator	1.89	0.0019	2.06	0.0021	0.22	0.0002	0.21	0.0002	0.54	0.0005	0.48	0.0005
33	Wire stretching machine operator	4.98	0.0048	8.33	0.0069	0.39	0.0004	0.72	0.0006	0.65	0.0006	1.70	0.0014
34	Wire stretching machine operator	1.74	0.0022	3.01	0.0029	1.49	0.0019	2.17	0.0021	0.81	0.0010	0.89	0.0009
35	Wire stretching machine operator	0.68	0.0007	0.93	0.0009	0.23	0.0002	0.18	0.0002	0.30	0.0003	0.23	0.0002
36	Wire stretching machine operator	4.34	0.0054	7.30	0.0061	0.22	0.0003	0.34	0.0003	0.46	0.0006	0.60	0.0005

37	Wire stretching machine operator	1.13	0.0008	0.93	0.0012	0.05	0.0001	0.09	0.0001	6.59	0.0071	1.65	0.0018
38	Wire stretching machine operator	2.47	0.0025	1.65	0.0024	3.90	0.0039	1.70	0.0025	0.89	0.0009	0.92	0.0013
39	Warehouse-worker	0.92	0.0013	0.74	0.0010	0.03	0	0.02	0	0.11	0.0002	0.12	0.0002
40	Warehouse-worker	2.84	0.0041	3.20	0.0045	0.05	0.0001	0.06	0.0001	0.24	0.0004	0.27	0.0004
41	Warehouse-worker	1.34	0.0020	3.38	0.0036	<0.018	0	0.03	0	0.14	0.0002	0.23	0.0002
42	Warehouse-worker	6.31	0.0074	5.28	0.0062	0.05	0.0001	0.06	0.0001	0.37	0.0004	0.39	0.0005
43	Warehouse-worker	0.95	0.0014	1.24	0.0014	<0.018	0	<0.018	0	0.08	0.0001	0.07	0.0001
44	Security man	8.01	0.0093	8.97	0.0067	0.21	0.0002	0.14	0.0001	1.00	0.0012	0.99	0.0010
45	Mechanic-crane operator	4.20	0.0070	4.20	0.0042	0.22	0.0004	0.22	0.0002	0.94	0.0016	0.94	0.0009
46	Mechanic-crane operator	1.89	0.0038	1.78	0.0018	0.27	0.0005	0.19	0.0002	0.92	0.0018	0.92	0.0009
47	Raw material operator	26.68	0.0267	33.59	0.0280	1.17	0.0012	1.24	0.0010	7.78	0.0078	9.96	0.0083
48	Raw material operator	3.77	0.0047	8.04	0.0067	0.45	0.0006	0.70	0.0006	1.85	0.0023	3.49	0.0029
49	Locomotive driver	4.89	0.0049	0.20	0.0002	0.37	0.0004	0.17	0.0001	0.68	0.0007	0.07	0.0001
50	Locomotive driver	0.29	0.0003	0.28	0.0002	0.36	0.0004	0.36	0.0003	0.03	0.0	0.03	0.0

Table 14. The amount of Ni, Cr, and Co in sample detected (ppb) and converted to deposited sample area (μg/cm^2) in office staff (doing at least 1h their usual tasks, deposition in 2 min. finger immersion technique).

	Nickel				Cobalt				Chromium			
	Thumb		Index finger		Thumb		Index finger		Thumb		Index finger	
	ppb	μg/cm^2	ppb	μg/cm^2	ppb	μg/cm^2	ppb	μg/cm^2	ppb	μg/cm^2	ppb	μg/cm^2
1	4.92	0.0066	3.29	0.0038	0.11	0.0001	0.10	0.0001	0.25	0.0003	0.30	0.0004
2	0.33	0.0006	7.57	0.0110	0.26	0.0005	0.65	0.0009	0.23	0.0004	0.51	0.0007
3	1.52	0.0030	1.68	0.0029	0.29	0.0006	0.29	0.0005	0.31	0.0006	0.31	0.0005
4	3.31	0.0064	4.42	0.0077	0.30	0.0006	0.31	0.0005	0.86	0.0017	0.97	0.0017
5	3.18	0.0062	3.58	0.0084	0.30	0.0006	0.31	0.0007	0.33	0.0006	0.50	0.0012
6	1.73	0.0034	1.94	0.0034	0.36	0.0007	0.39	0.0007	0.30	0.0006	0.32	0.0006
7	1.90	0.0032	2.47	0.0035	0.29	0.0005	0.32	0.0006	0.40	0.0007	0.50	0.0009
8	3.65	0.0046	2.91	0.0024	0.47	0.0006	0.35	0.0003	0.08	0.0001	0.07	0.0001
9	0.89	0.0021	0.77	0.0012	0.28	0.0007	0.28	0.0004	0.44	0.0010	0.32	0.0005
10	1.28	0.0027	1.64	0.0029	0.28	0.0006	0.28	0.0005	0.35	0.0007	0.34	0.0006
11	2.23	0.0040	3.85	0.0054	0.28	0.0005	0.29	0.0004	0.41	0.0007	0.64	0.0009
12	2.08	0.0040	1.57	0.0027	0.26	0.0005	0.26	0.0005	1.10	0.0021	0.74	0.0013
13	5.68	0.0102	7.72	0.0135	0.28	0.0005	0.28	0.0005	0.41	0.0007	0.47	0.0008
14	1.16	0.0027	1.42	0.0025	0.31	0.0007	0.33	0.0006	0.76	0.0018	0.74	0.0013
15	1.41	0.0025	1.17	0.0020	0.31	0.0006	0.29	0.0005	0.42	0.0008	0.38	0.0007
16	0.81	0.0016	0.64	0.0011	0.26	0.0005	0.26	0.0005	0.24	0.0005	0.23	0.0004
17	2.29	0.0033	4.00	0.0043	0.30	0.0004	0.35	0.0004	0.38	0.0005	0.51	0.0005
18	19.28	0.0297	13.86	0.0180	0.39	0.0006	0.39	0.0005	0.91	0.0014	0.97	0.0013
19	6.67	0.0095	7.50	0.0109	0.52	0.0007	0.55	0.0008	0.74	0.0011	0.70	0.0010

20	2.72	0.0049	3.82	0.0056	0.27	0.0005	0.28	0.0004	0.39	0.0007	0.67	0.0010
21	2.16	0.0036	3.40	0.0046	0.27	0.0005	0.28	0.0004	0.36	0.0006	0.43	0.0005
22	0.75	0.0013	0.55	0.0007	0.26	0.0005	0.26	0.0003	0.23	0.0004	0.23	0.0003
23	2.02	0.0036	1.46	0.0019	0.27	0.0005	0.27	0.0003	0.24	0.0004	0.27	0.0003
24	6.09	0.0089	7.12	0.0077	0.30	0.0004	0.31	0.0003	1.09	0.0016	1.09	0.0012
25	4.22	0.0082	6.56	0.0083	0.39	0.0008	0.47	0.0006	0.59	0.0011	0.84	0.0011
26	15.31	0.0153	11.28	0.0123	0.25	0.0003	0.18	0.0002	1.86	0.0019	1.27	0.0014
27	1.47	0.0018	1.42	0.0018	<0.018	0.0	<0.018	0.0	0.29	0.0004	0.28	0.0004
28	3.46	0.0047	4.45	0.0052	0.06	0.0001	0.07	0.001	0.26	0.0004	0.31	0.0004
29	0.66	0.0009	0.87	0.0010	0.02	0.0	0.02	0.0	0.13	0.0002	0.17	0.0002
30	1.51	0.0022	1.33	0.0016	0.15	0.0002	0.11	0.0001	0.32	0.0005	0.27	0.0003
31	3.58	0.0060	3.66	0.0043	1.66	0.0028	1.66	0.0019	0.50	0.0008	0.49	0.0006
32	2.53	0.0028	4.85	0.0054	0.35	0.0004	0.57	0.0006	0.22	0.0002	0.37	0.0004
33	1.56	0.0014	1.89	0.0025	1.28	0.0011	1.00	0.0013	0.55	0.0005	0.49	0.0006
34	0.26	0.0004	0.19	0.0003	<0.018	0.0	<0.018	0.0	0.05	0.0001	0.04	0.0001
35	3.16	0.0046	2.60	0.0030	0.44	0.0006	0.34	0.0004	0.28	0.0004	0.27	0.0003
36	3.05	0.0033	6.26	0.0061	0.23	0.0003	0.30	0.0003	0.64	0.0007	0.77	0.0007
37	0.81	0.0012	1.14	0.0013	0.04	0.0001	0.07	0.0001	0.15	0.0002	0.21	0.0002
38	0.86	0.0011	1.09	0.0009	0.03	0.0	0.06	0.0001	0.07	0.0001	0.10	0.0001

4.4. Metal release from raw and finished production

The atomic absorption analysis of the nails and wire detected measurable amounts of metals. The average released concentration of Ni was 0.0012 µg/cm^2, Co – 0.0007 µg/cm^2, Cr – 0.0037 µg/cm^2 after 24 hours and 0.0135 µg/cm^2, 0.0029 µg/cm^2, and 0.0042 µg/cm^2, respectively, after a week. The released concentration of Ni statistically significantly increased during a week, 0.0012 µg/cm^2 vs. 0.0135 µg/cm^2 (P=.04) (Figure 25).

Ni, Cr, and Co were detected in nearly all extracts, and the average concentration increased with the duration of extraction in contact with artificial sweat (Table 15).

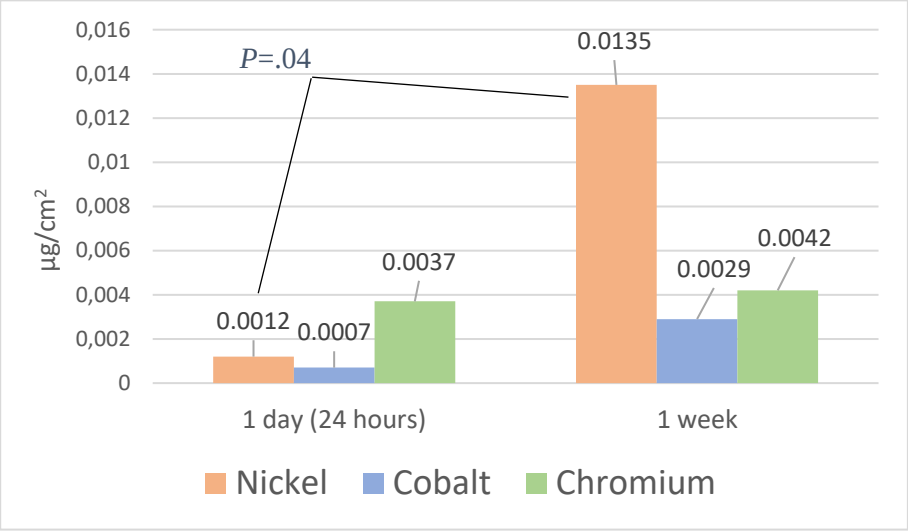


Figure 25. Average amount released Ni, Co, and Cr to artificial sweat in day 1 and a week (µg/cm^2)

Table 15. The average amount of Ni, Cr, and Co released from nails and wire to artificial sweat in 1 day and in a week (µg/cm^2).

Samples	Nickel, mean (µg/cm^2)		Cobalt, mean (µg/cm^2)		Chromium, mean (µg/cm^2)	
	1 day	1 week	1 day	1 week	1 day	1 week
Uncleaned 3 mm nails	0.0018	0.0089	0.0002	0.0040	0.0011	0.0108
Uncleaned 2.5 mm nails	0.0000	0.0207	0.0013	0.0019	0.0018	0.0016
Uncleaned 1.8 mm nails	0.0000	0.0041	0.0000	0.0002	0.0021	0.0057
Cleaned 1.5 mm nails	0.0005	0.0189	0.0005	0.0078	0.0143	0.0016
Cleaned 1 mm wire	0.0013	0.0257	0.0013	0.0026	0.0020	0.0033
Uncleaned 1 mm wire	0.0014	0.0025	0.0011	0.0011	0.0006	0.0021

4.5. THE PENETRATION OF CHROMIUM USING FRANZ CELL DIFFUSION METHOD

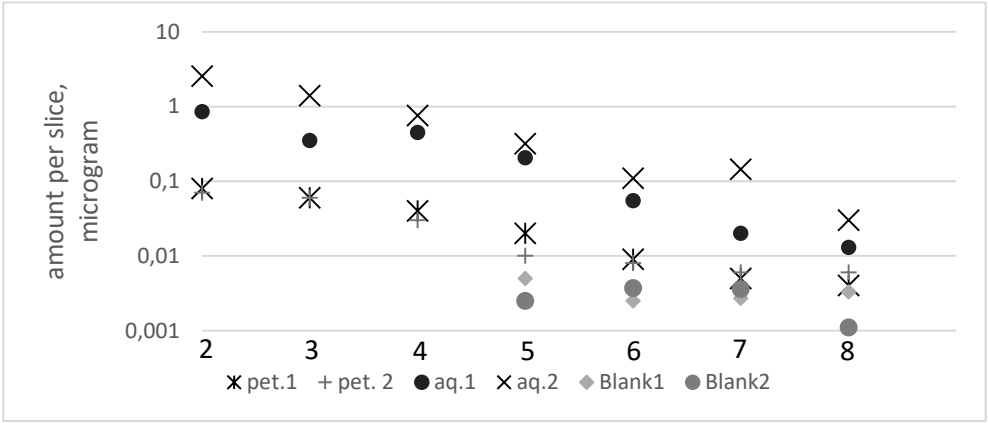
The results are presented in Table 16. Cr was detected in both recipient phases after using aq. and pet. vehicles. No Cr was detected in the wipe samples of the Potassium dichromate aq. samples. The distribution of the total Cr amount in the skin samples was very similar using pet. and aq. as vehicles in all samples illustrated per skin slice in micrograms (Figure 26) and mass balance data per slice (Figure 27).

Table 16. Cr in different skin depths, µg/%

Depth of the skin	Samples*	Tube No.	A Blank	B Blank	C 0.5% pet. Potassium dichromate, µg/%	D 0.5% pet. Potassium dichromate, µg/%	E 0.5% aqua Potassium dichromate, µg/%	F 0.5% aqua Potassium dichromate, µg/%
	Wipe samples	0	<LOD	<LOD	27.98/92.9	17.74/46.34	<LOD	<LOD
	Scapings	1	0.00	0.00	0.20/0.66	0.19/0.49	2.45/38.1	4.98/20.50
40µm	1x30µm	2	0.00	0.00	0.08/0.36	0.07/0.18	0.85/13.2	2.55/10.49
70µm	1x30µm	3	0.00	0.00	0.06/0.19	0.07/0.18	0.35/5.44	1.40/5.76
130µm	2x30µm	4	0.00	0.00	0.08/0.36	0.05/0.13	0.90/13.99	1.51/6.22
250µm	4x30µm	5	0.02	0.01	0.08/0.36	0.04/0.10	0.82/12.75	1.27/5.23
490µm	8x30µm	6	0.02	0.03	0.07/0.23	0.06/0.16	0.44/6.84	0.87/3.58
820µm	11x30µm	7	0.03	0.04	0.06/0.19	0.07/0.18	0.22/3.42	1.58/6.5
Last part	.. x 30µm	8	0.03	0.02	0.08/0.36	0.14/0.37	0.15/2.33	0.60/2.47
Receptor phase		9	<LOD	<LOD	1.41/4.68	19.85/51.85	0.25/3.88	9.53/39.23
		Sum			30.1	38.28	6.43	24.29

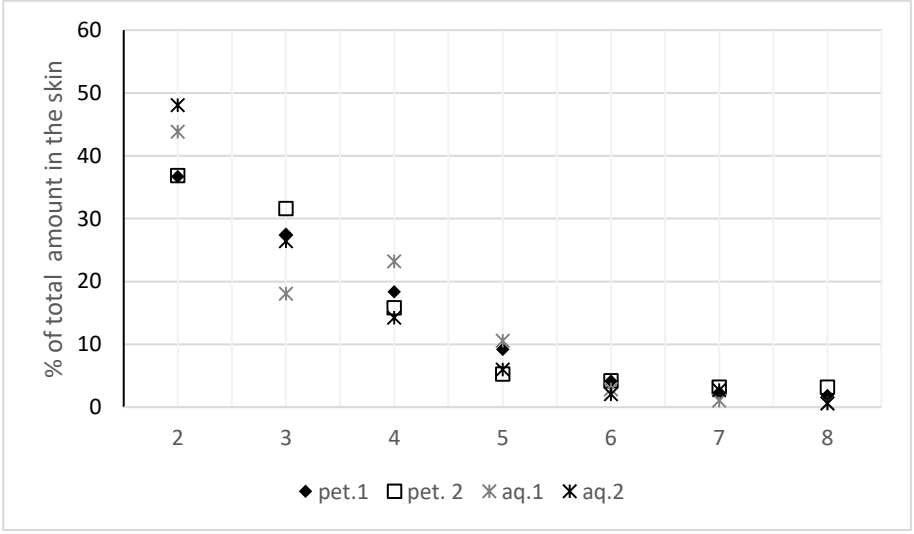
LOD – limit of detection

Samples* – in this column, the content of tube vials is listed, Tube no. 0 consists of wipe samples; tube no.6 – consists of 8 slices of 30µm.



Numbers on the X-scale represent different layers, 2 top layer and 7, 8 bottom layers.
Pet. – petrolatum.
Aq. – aqua.
Blank - water from a Millipore purification pack system.

Figure 26. The distribution of chromium in the skin per 30µm slice, µg in a logarithmic scale in different depth. Numbers on the X-scale represent different layers, 2 top layer and 7,8 bottom layers.

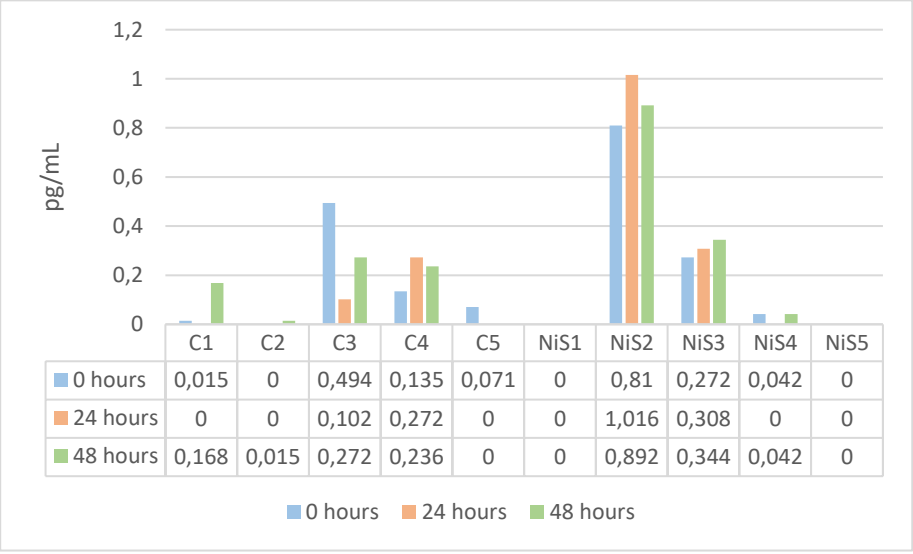


Numbers on the X-scale represent different layers, 2 top layer and 7, 8 bottom layers.
Pet. – petrolatum.
Aq. – aqua
Blank - water from a Millipore purification pack system.

Figure 27. The distribution of average chromium amount in the skin per 30µm slice, in a logarithmic scale in different skin depth, without regard to the amount left on top or in the receptor phase.

4.6. Cytokine marker analysis

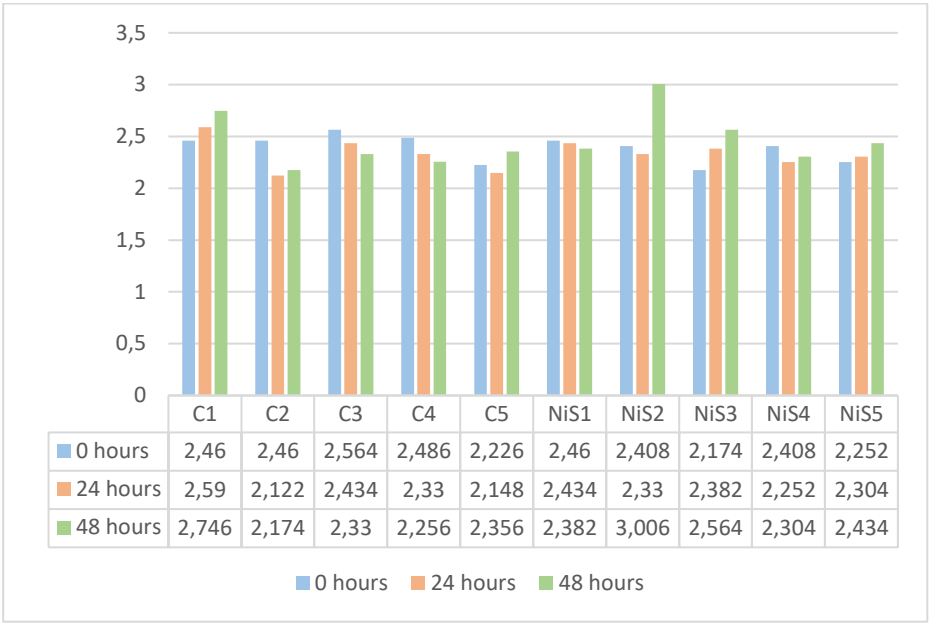
After 24 hrs, 5/5 of Ni allergic participants had a 1+ reaction to Ni. After 48 hrs 3/5 had 2+ reactions and 2/5 – 3+ positive reactions. All controls were negative to Ni at both readings. There were no IL-1β, IL-9, and IL-13 detected in the serum. IL-1α, IL-17A, and IFNγ were detectable in all serum samples, but their concentrations were less than 4 pg/mL (Figure 28;29;30;31). IL-17A concentration was found < 4 pg/mL in all biopsies irrespective of the time taken. IL-22 and IL-23 were detected in higher amounts compared to other analyzed IL, but with no statistically significant difference between the days or sensitization levels to Ni. The results are summarized in Table 17 and Figure 32, Figure 33.



	C1	C2	C3	C4	C5	NiS1	NiS2	NiS3	NiS4	NiS5
0 hours	0,015	0	0,494	0,135	0,071	0	0,81	0,272	0,042	0
24 hours	0	0	0,102	0,272	0	0	1,016	0,308	0	0
48 hours	0,168	0,015	0,272	0,236	0	0	0,892	0,344	0,042	0

C- control
NiS – Ni sensitized.

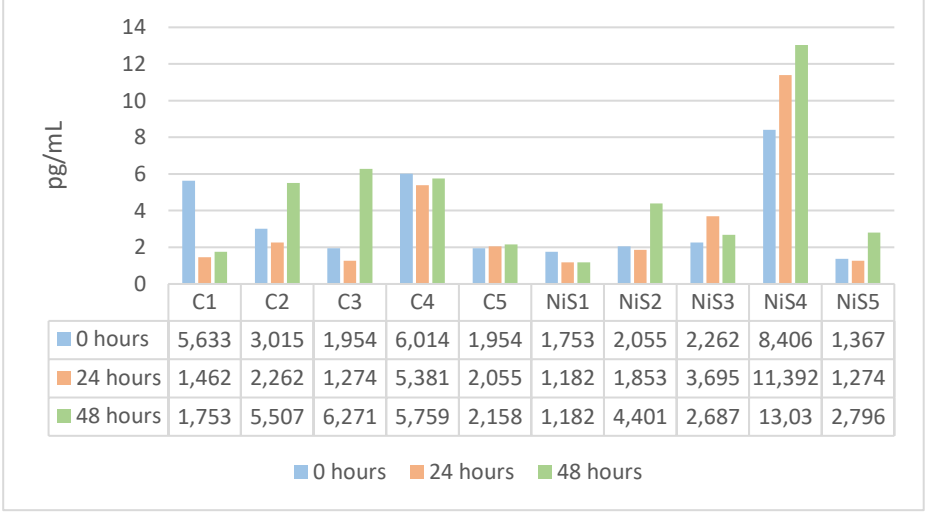
Figure 28. IL-17A in serum expressed in pg/mL in 5 controls and 5 nickel-sensitized individuals at three different time points.



	C1	C2	C3	C4	C5	NiS1	NiS2	NiS3	NiS4	NiS5
0 hours	2,46	2,46	2,564	2,486	2,226	2,46	2,408	2,174	2,408	2,252
24 hours	2,59	2,122	2,434	2,33	2,148	2,434	2,33	2,382	2,252	2,304
48 hours	2,746	2,174	2,33	2,256	2,356	2,382	3,006	2,564	2,304	2,434

C- control
NiS – Ni sensitized.

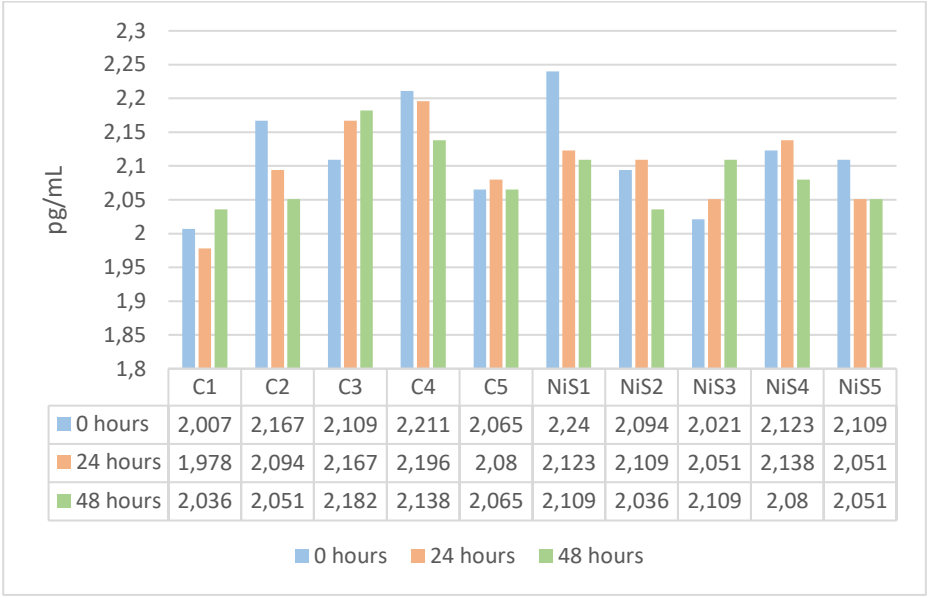
Figure 29. IL-17A concentration in skin biopsy specimens expressed in pg/mL in 5 controls and 5 nickel- sensitized individuals at three different time points.



	C1	C2	C3	C4	C5	NiS1	NiS2	NiS3	NiS4	NiS5
0 hours	5,633	3,015	1,954	6,014	1,954	1,753	2,055	2,262	8,406	1,367
24 hours	1,462	2,262	1,274	5,381	2,055	1,182	1,853	3,695	11,392	1,274
48 hours	1,753	5,507	6,271	5,759	2,158	1,182	4,401	2,687	13,03	2,796

C- control;
NiS – Ni sensitized.

Figure 30. IFNγ concentration expressed in pg/mL in serum of 5 controls and 5 nickel- sensitized individuals at three different time points.



C- control
NiS – Ni sensitized.

Figure 31. IL-1α concentration expressed in pg/mL in serum of 5 controls and 5 nickel- sensitized individuals at three different time points.

Table 17. Average amounts of IL-22 and IL-23 in serum and biopsy, in pg/mL.

IL-22	Serum, Controls, pg/mL, (SD)	Serum, Nickel allergic pg/mL, (SD)	Biopsy, Controls pg/mL, (SD)	Biopsy, Nickel allergic pg/mL, (SD)
0 hours	38.99 (±11.86)	40.36 (±10.18)	18.71 (±12.95)	25.98 (±6.35)
24 hours	40.36 (±7.78)	40.49 (±12.25)	12.41 (±7.35)	15.47 (±2.66)
48 hours	45.7 (±9.33)	46.41 (±13.62)	15.37 (±9.53)	18.43 (±4.24)
IL-23	Serum Controls pg/mL, (SD)	Serum Nickel allergic pg/mL, (SD)	Biopsy Controls pg/mL, (SD)	Biopsy Nickel allergic pg/mL, (SD)
0 hours	1.57 (±2.85)	7.41 (±17.17)	8.07 (±6.96)	8.5 (±11.81)
24 hours	7.57 (±14.18)	1.92 (±4.15)	7.37 (±11.28)	9.25 (±13.42)
48 hours	5.69 (±5.51)	3.27 (±3.05)	18.15 (±15.8)	21.18 (±15.7)

SD – standard deviation.

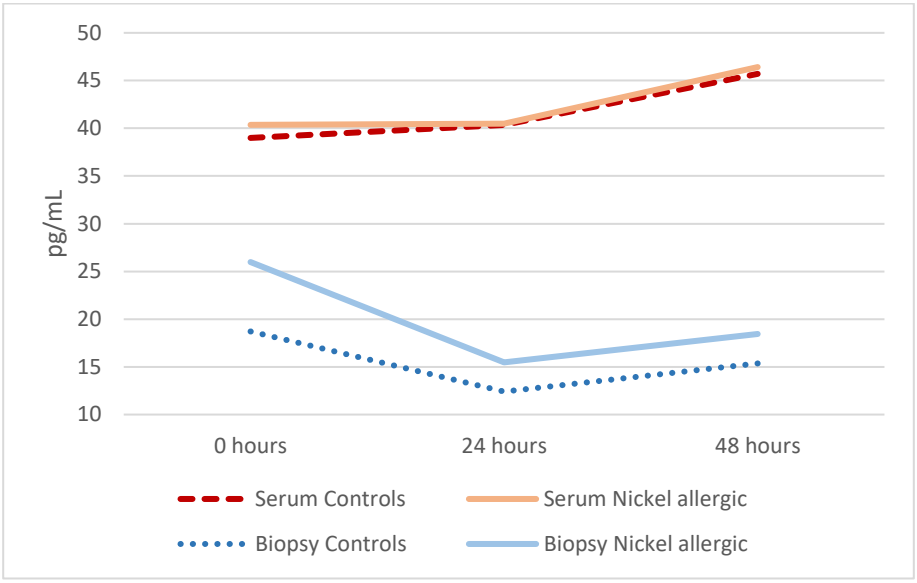


Figure 32. The mean of IL-22 concentrations (pg/mL) in serum and biopsy samples.

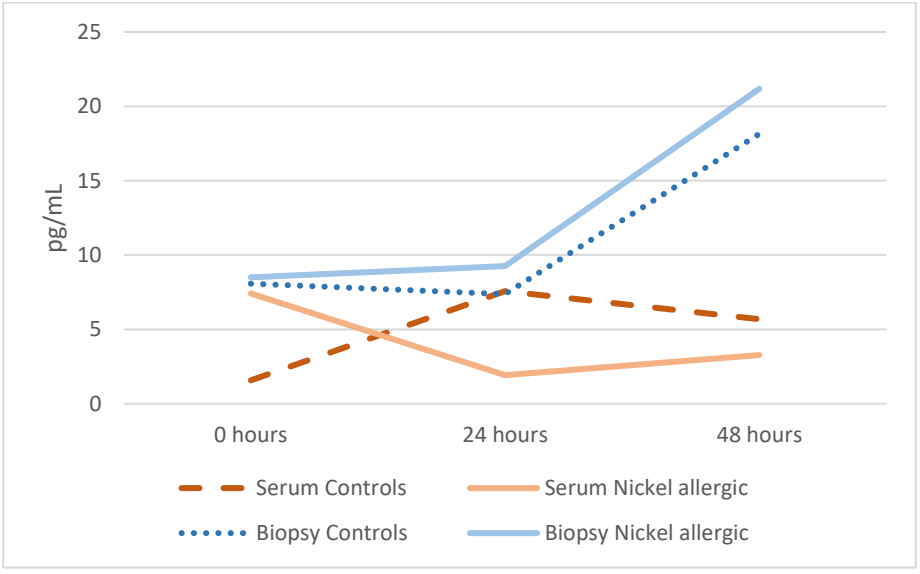


Figure 33. The mean of IL-23 concentration (pg/mL) in serum and biopsy samples.

5. DISCUSSION

5.1. Questionnaire and patch testing

Occupational skin diseases are among the most common occupational diseases in industrial countries, although nowadays, many industries are moving their plants from the EU due to the higher costs and strict requirements [120]. The remaining industrial plants are expected to have implemented better preventive measures, and better health conditions are created for workers. However, this study shows that contact dermatitis is still common in metal plants [121].

Before the survey, just 1.7% of workers had a physician-diagnosed skin disease in our study. Based on the survey self-reported answers, the prevalence of skin problems was noticed to be relatively high and with a significant difference between metalworkers and office staff. Low numbers of physician-diagnosed cases could be explained by the ignorance of the skin symptoms by the workers. It could be a cultural feature of our society where skin problems by males are mostly ignored as too "feminine." High numbers of CD have been noticed in the Swedish study, where metalworkers and staff clerks had dermatitis in 54.8% and 52.6% cases, respectively [23]. Young workers statistically significantly more often complained of having skin symptoms (Figures 22; 23), and this may be explained by the fact that younger age people may be more concerned about their work-related health and are more self-centered compared to older and longer working staff, who are usually used to the working conditions over the years. The healthy worker's effect – the ones who left work early due to skin problems a long time ago are missed among the old workers. This could be one more explanation as to why the old ones had fewer complaints. The Northern Bavarian Germany population-based studies' results are similar to those of our study, where the highest incidence rate of contact allergy was seen at a young age – between 15–24 years. Interestingly, the time between the start of the job and the clinical presentation of CD for metalworkers was about four years [122].

Contact allergy to Ni is very common in the general population [33, 61, 123, 124]. This explains Ni being the most prevalent allergen in women of the control group. Sensitization in the metalworkers to cobalt possibly reflects their exposure. In 2011 a published study by Geier, tracing the presence of Ni, Co, and Cr in the metalworking fluid (MWF), revealed that in hard metal processing, cobalt concentrations up to 300 ppm and in single cases even up to 500 ppm were documented, amounts that are possibly enough even for

induction of sensitization [125]. The elicitation threshold in patients sensitized to cobalt is regarded to be 100–1000 ppm cobalt ions. In damaged skin, allergic reactions could be elicited with 10 ppm of cobalt chloride (2.4 ppm cobalt) [3, 126]. Geier et al. in their study recognized cobalt as an important contact allergen for metal workers and suggested that future investigations on cobalt allergies among metalworkers should describe in detail the kind of metal the patient handles [3]. Our study knows that net, wire, and nails do not contain measurable amounts of cobalt, suggesting that the contact with cobalt is from other work appliances or instruments. We also know that producing these items usually does not involve extensive skin contact with metalworking fluids, necessary for cutting and grinding of metals. Most of the workers did not use gloves at all or used white cotton gloves, which, after the work shift, were black and greasy, leaving their hands dirty. The type of gloves that were used at the time of investigation did not protect hands from contact with oils and lubricants, which are possible contact irritants and allergens. So, it can be speculated that workers without protective gloves have constant skin irritation, increasing penetration of the cobalt into the skin and inducing sensitization.

The IVDK study [121] etrospectively analyzed the data of 2007–2016, where 3411 patients were patch tested, among those 83 were metalworkers. The Swedish study on occupational dermatoses in a metalworking plant producing engines and drivelines for the automotive industry was performed in 1999–2000. In this study, 164 metal workers and 19 office staff were interviewed and patch tested. The prevalence of contact allergy to nickel sulfate, cobalt chloride, and potassium dichromate among metalworkers from Lithuania, Sweden [23], and in the IVDK study [121] members was compatible (Table 18). The Nickel is restricted under the REACH directive, which regulates nickel release from items that have prolonged contact with the skin. Unfortunately, most of the metalworker's work-producing items are not intended to be in prolonged skin contact, so the directive does not impose any regulation in this setting. Denmark, Germany, and Sweden introduced this legislation in 1990 and 1991, which has been the basis for the EU Nickel Directive [121, 127].

We compared our data with the general European population, the EDEN study participants, and found a significant difference in cobalt chloride sensitization between metalworkers and the EDEN study participants from Sweden, Portugal, Italy, and Germany [18]. Furthermore, the sensitization to potassium dichromate statistically significantly differed between metalworkers and the EDEN study's participants from Sweden, The Netherlands, and Italy. The data analysis with significance levels is shown in

Table 18. A similar situation was present when patch testing the general population in the EDEN study. This indicates that sensitization to cobalt chloride and potassium dichromate possibly is occupation-related.

The comparison in Table 19 shows that patch testing patients with suspected CD produce higher positivity rates compared to testing the general population.

One weakness of this study is that workplace materials were not tested, and consequently, we might be missing contact sensitization to other allergens than the ones in the European baseline series.

This study has limitations as not all the workers answered the questionnaire, and not everybody agreed to be patch tested. So, it could be that persons already having skin problems were more prone to enter this study. Besides, we did not patch test with the functional materials. Thus, in some cases, false-negative results can be obtained. Furthermore, we could not assess the influence of atopy due to the small size of our tested population.

Table 18. Comparison of the prevalence of positive reactions to metals in different countries.

	Germany EDEN study [18]		Italy EDEN study [18]		The Nether-lands EDEN study [18]		Portugal EDEN study [18]		Sweden EDEN study [18]		Metal-workers Sweden [33]		Office staff Sweden [33]		Metal-workers IVDK study [121]		Lithuanian study [30]		Metal plant Metal-workers		Metal plant Office staff	
Popu-lation	General population		General population		General population		General population		General population		Metal plant workers		Metal plant workers		Patch tested patients		Patch tested patients		Metal plant workers		Metal plant workers	
Subs-tance	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)	Tested (N)	Positive (n) Rate, % (95% CI)
Nickel chloride	1024	143 13.9 (11.8-16.0)	546	87 16.4 (13.2-19.4)	500	78 15.8 (12.5-18.9)	531	98 18.5 (15.2-21.8)	518	42 8.3 (5.9-10.7)	163	19 11.7 (7.5-17.5)	19	4 21.1 (7.9-43.8)	71	4 5.6 (1.5-13.8)	504	149 29.6 (25.6-33.7)	75	7 9.3 (3.8-18.3)	60	8 13.3 (5.9-24.6)

Cobalt chloride	1024	**24****** **2.3** (1.3-3.2)	546	**5***** **0.9** (0.1-1.7)	500	19 3.8 (2.1-5.5)	531	**13*** **2.5** (1.1-3.8)	518	**6**** **1.1** (0.2-2.1)	163	4 2.5 (0.7-6.3)	19	**0**$^{¥}$ -	73	1 1.4 (0-7.4)	504	44 8.7 (6.4-11.5)	75	**6** **8** (2.9-16.6)	60	**0**$^{¥}$ -
Potas-sium dichro-mate	1024	12 1.1 (0.5-1.8)	546	**2**$^{\&}$ **0.4** (0-0.9)	500	**2**$^{\&}$ **0.4** (0-0.9)	531	7 1.8 (0.4-2.3)	518	**1**§ **0.2** (0-0.7)	163	4 2.5 (0.7-6.3)	19	1 5.3 (0-26.4)	73	1 1.4 (0-7.4)	504	33 6.6 (4.5-9.1)	75	**3**$^{§\&}$ **4** (0.8-11.3)	60	0 -

N – number of tested patients, n – number of positive reactions

CI, confidence interval.

Chi-square or Fisher's exact test (if n≤5).

Significant results compared to our study results (P<.05) are shown in bold.

$^{¥}P$=.03

*P=.02

**P=.001

***P=.0001

****P=.003

$^{§}P$=.007

$^{\&}P$=.01

5.2. Finger immersion test and metal release to artificial sweat

We detected Co in samples with artificial sweat, but no Co presence was listed during an 8-year period when the raw metal specifications were examined. The released amounts were very low (<0.01 μg/cm^2/week), which could be expected from alloys not having Co in the composition specification. There are still no requirements to test Co release from items intended to come in prolonged and/or frequent skin contact, such as with Ni. In our study, the highest amount of Co was on the nail heater's and raw material operators' fingers in concordance to other studies in which the highest quantity was found on hard metal pressing operator [128] and raw material groups [129, 130]. These occupations have the most contact with unprocessed raw goods. Despite the release of Co in the production and the deposited quantity on fingers, the registered amounts are low and possibly with no risk of sensitization or elicitation in already sensitized individuals (Table 19).

Table 19. The released and detected metal levels, their legislation, sensitization, and elicitation properties.

	Released level from production material in our study	Detected level after finger immersion of metalworker in our study	Restriction level	Elicitation levels in sensitized patients	Induction of sensitization level
Ni	0.0257* μg/cm^2/week	0.0297* μg/cm^2	<0.5μg/cm^2/week [131]	0.0075-10 μg/cm^2 [132, 133]	1 μg/cm^2 [133]
Co	<0.01 μg/cm^2/week	<0.01 μg/cm^2	NR	0.066-1.95 μg/cm^2 [134]	2.3-226 μg/cm^2 [135]
Cr	0.0057* μg/cm^2/week	0.0056* μg/cm^2	0.3 μg/cm^2 [21]	5 μg/cm^2 [21]	0.02-0.05 μg/cm^2[21]

*maximum release/detected amount.
NR- not regulated

Contact allergy to Ni is more common than to any other metal. The release of Ni from metal objects, intended to come in direct or prolonged contact with the skin, is restricted to <0.5μg/cm^2/week in artificial sweat and to 0.2 μg/cm^2/week for piercing items in the European Union [131, 136]. It is

a common misconception that regulation only covers things like jewelry, belts etc. that a person could wear. Hence tools and other instruments coming in contact with the skin are also covered by the regulation in REACH [136].

It is also known that the elicitation threshold for contact allergy is significantly lower in skin affected by dermatitis or irritation [137]. In this study the released (Table 15) and detected amount of Ni on fingers (Table 13) indicates a risk of ACD elicitation in already Ni-sensitized MW. Many workers usually work without protective gloves and spend shifts in contact with metal parts, not realizing the risk of sensitization, which was also noticed by Julander et. al. in gas turbines and space propulsion components plan metal workers [44]. Various studies determined the elicitation threshold for Ni in dermatitis patients by performing patch tests on the back [132, 138] (Table 19). Fischer et al. analyzed the reactivity on the palm, which was assessed by single occluded exposure to concentrations 10-fold lower than the elicitation threshold on the back, in order to estimate whether reactivity on palms was increased compared with that on the back. In 2008 workers of different occupations were sampled by the acid wipe technique (17). Our results meet those of cashiers (0.006-0.065 μg/cm^2) and Ni refinery workers (0.0129-0.0130 μg/cm^2) and exceed the amount of Ni detected on department store assistant fingers (<0.0009-0.0076 μg/cm^2). Ni platers had the highest amounts of Ni detected according to Staton et al.[22] (0.0200-7.1596 μg/cm^2). We have found that the IT specialist had high amounts of Ni, possibly because of frequent contact with laptops, phones, and other metal appliances know to release nickel possibly [14, 139, 140]. Our results indicate that if workers are not Ni-sensitized, the released Ni quantity from nails/wire, the deposited amount on their fingers, and the working conditions would probably have no effect on the skin.

Cr is an insoluble metal. However, corrosion in artificial sweat or other fluid can cause salt formation and increase potential sensitization properties, since it is known that hexavalent Cr salts are more soluble and allergenic than trivalent [134]. The risk assessment calculations for the induction of sensitization to Cr was well-discussed by Menné et al. [21] in 2003 and later in 2016 by Bregnbak [141], underlining the need to keep the Cr concentration within 0.02-0.05μg/cm^2 [137]. Note that it is assumed that all Cr is present as hexavalent Cr, which is the form of Cr known to be associated most strongly with skin sensitization. The deposition of Cr on fingers of MW and OS did not differ significantly. The most Cr was detected on raw material operators (0.0056 μg/cm^2) and nail heaters' (0.0033 μg/cm^2) fingers; these workers have direct and prolonged contact with hard metals (Table 15). For comparison, in a study by Julander et al. [44] the highest amount of Cr was found on the hand

of a worker who pulled metal thread through holes in a laser cut profile, performing this without gloves and resulting in high exposure to metals.

There is no published data of Cr and Co deposition on fingers detected by the finger immersion technique. The acid wipe technique and finger immersion method were compared by testing Ni with the finger immersion technique developed by Staton and colleagues [22]. Their study showed a variation in the amount detected, resulting in a conclusion reflecting the inability to precisely control experimental parameters in volunteers, e.g., difficulty in providing a consistent exposure level. Therefore, we chose a cheaper finger immersion method for our study, although it could not detect the total metal amount on hands since metal particles are released passively into the deionized water. Using another method, the acid wipe sampling technique, higher concentrations can be detected. Besides, it is known that an acidic solution generally results in higher amounts of released metals compared with solutions of near-neutral pH conditions [131]. Otherwise, the finger immersion method is less labor intense because samples can be analyzed immediately since there is no extraction step in this method.

Another limitation of our study was the detection of atomic Cr and not trivalent or hexavalent Cr ions. The information of released or detected hexavalent Cr ions could be more helpful in relating detected levels and possible contact outcomes.

5.3. FRANZ CELL DIFUSSION EXPERIMENT

Our study showed that Cr penetrates easily from aq. and pet. preparations, but it seems that the penetration process is quicker with the aq. preparation since no Cr was detected in the wipe samples. That could be expected since Cr salt in pet. is dispersed in a protective hydrophobic matrix, petrolatum, and in this way, Cr has less contact with the skin, surface and limited amounts of Cr ions can reach the skin surface per time unit. This was shown in our study, in which 7–50% (Table 16) of the applied Cr amount in pet. was found in the skin. In the aq. samples chromium salt is dissolved, and the whole amount of Cr applied onto the skin is available for penetration since no Cr was found in the wipe samples where Cr in aq. was placed. Similar results were obtained by Gammelgaard et al. during a 170-hour experiment, when a higher amount of potassium dichromate was applied on human skin and a stripping method was used [142]. The authors noticed that less Cr penetrated the skin in the pet. sample and in the receptor phase, they recovered very small amounts of Cr. Our skin sample results confirm the study by Lidén S et al., where the penetration properties of hexavalent Cr in vivo were studied [143].

We recovered a high amount of Cr in the receptor phase irrespective of the vehicle used in the preparations which were placed on the skin. Thus, there are two main pathways for skin penetration. First, the transepidermal route, when the substances diffuse through the intercellular spaces of the horny layer or the corneal cells; second, the cutaneous annex route where diffusion occurs through the pilosebaceous follicles and sweat glands. Usually, both routes are involved. [144]. Skin areas that are dense in hair follicles (head, armpits, etc.) have higher permeability than those that are less hairy [77]. Furthermore, the cutaneous penetration is rapid for lipophilic molecules and slower for hydrophilic [67]. That could explain why we found less Cr in the skin but more in the receptor fluid using pet. as a vehicle: Cr in pet. easily enters the skin and goes through it. This finding can have an impact on the calculations of the Cr amount, which can induce sensitization or elicitation of contact allergy as these theoretical calculations assume that all Cr placed on the skin penetrates it, but in our experiment, we have shown that this is not the case. So toxicological calculations made for aq. Cr solutions should be more accurate than for pet.

No detected Cr on the skin membrane surface of aq. samples questions the usage of acid wipe sampling or finger immersion testing in certain occupations where chromium salt aqua solutions are used. In our previous study [145], we path-tested 135 metal plant workers: 75 were metal workers and 60 were the office staff. Only metalworkers (4%) were patch tested positive to potassium dichromate and later analyzing the amount of Cr on workers fingers by finger immersion method [146] we have noticed that only two types of workers, that is, raw material operator and nail heaters' who had the highest concentration of Cr left on their fingers. Those occupations involved direct and prolonged contact with hard materials that are usually covered in grease. For a comparison, Julander with colleagues [44] detected most of the Cr on the workers who pulled the metal thread through the holes in a laser-cut profile. The workers also had high exposure to metals. However, Cr is insoluble as metal, but corrosion in contact with sweat, artificial sweat, or other fluids can cause the salt formation and the increase of potential sensitization properties as Cr(VI) is more soluble than Cr(III) [50, 138, 147]. Thus, acid wipe sampling and finger immersion test results might be falsely negative for Cr in occupations involving contact with Cr salts in aq. solutions.

Our study used frozen skin samples, though recent studies evaluated the freezing effect on the skin. Some studies confirmed the stratum corneum's mechanical damage, especially [148], which is most important for a medical device like needles development. Jacques-Jamin C et al. and Barbero AM et al. analyzed the effect of freezing on permeability function, and their studies

suggest that barrier function is maintained, the penetration of metabolically stable chemicals was unaffected by freezing [149, 150].

Limitations of the study. Because of the lack of experimental porcine skin, duplicate samples of 1 ear instead of triplicate were analyzed. The detected differences in duplicate samples B and C and E and F, respectively, raise questions about why and how this happened. It could be that the method used was not able to recover all Cr applied, but the purpose of the study was to find differences in Cr distribution in the skin using different vehicles.

5.4. ALLERGIC CONTACT DERMATITIS CYTOKINE MARKERS

There is plenty of information on the immunological mechanism of ACD, primarily based on various mouse models and *in vitro* studies, but in vivo studies are lacking. Thus we have chosen to investigate cytokines (IFNγ, IL-1α, IL-1β, IL-9, IL-13, IL-17A, IL-22, and IL-23) that were earlier described as possible markers or effectors in ACD in the human material. In this study, we detected low levels of IL-17A in serum and concentration below the analytical value in skin biopsies (Figures 11;12). Contrary to this, Silvestre et al. detected the prevalence of IL-17A in chronic eczema biopsies [102]. The detected levels could be explained by Schmidt et al. results, where they recognized that high levels of IL-17 are detected in the skin when the same site was re-exposed to the contact allergen [100]. High concentrations of IFNγ, as well as IL-1β levels were found at sites where hapten was re-exposed to the previous site of dermatitis [100]. Several in vitro studies, where human keratinocytes and peripheral blood were stimulated with Ni, showed expression of IL-17 in Ni challenged allergic skin biopsies and evidence of Th17 and Th1 in the blood of Ni allergic patients [106, 108, 151]. We obtained similar results of IL-17 in controls and Ni patch test positive participants (Figure 29), and that is in concordance with other authors [100, 102]. We detected almost the same levels of IL-22 in the serum of controls and Ni sensitized participants (Figure 32), although the tendency was that Ni patch test positive had higher concentrations (Table 17). In vitro experiments by Larsen et al. showed IL-22 expression in individuals' Ni-sensitized and Ni-challenged skin without inflammation. Immunohistochemistry found infiltrating cells expressing IL-17 and IL-22 in the inflamed skin of individuals with allergen-challenged skin inflammation [106]. The mouse model studies demonstrated IL-22 dependent Th17-mediated reactions in spontaneous psoriasis-like inflammation. IL-23, IL-17, and IL-22 produced by Th17 cells play a central role in this type of skin inflammation [106, 152]. The detected levels of IL-22 in our study are in concordance with previous

studies showing IL-22 expression when individuals were patch test stimulated with an allergen, but with no difference of their positivity or negativity to a selected contact allergen. It may be that IL-22 is a nonspecific cytokine of epidermal damage as it‘s pathogenic implications are also discussed in atopic dermatitis and psoriasis skin [153].

The proinflammatory cytokine IL-23 is an IL-12 family member, which is expressed by T cells and NK cells [108]. IL-23, which plays a role in the development of Th17 cells, may be involved in the ACD pathogenesis. IL-23 was recognized to be essential for IL-22 expression as well as maturation and proliferation of Th17 [107]. There are in vitro studies with human keratinocytes in which increased levels of IL-23 were observed when these cells were exposed to allergens [79, 108, 109], much the same as we observed in our biopsy samples (Figure 33), but we did not detect any difference between Ni sensitized participants and controls, so this may be a sign that IL-22 is not specific to ACD.

In this study, we detected low levels of IFNγ in serum with a tendency of higher levels after 48 hrs in controls and the presence of one atypical value in Ni sensitized participants (Figure 11). Ulfgren et al. also did not detect IFNγ in Ni skin biopsies from Ni exposed skin areas and controls within 6–72 hrs using the immunohistochemistry method [82]. However, Silvestre et al. found high levels of IFNγ in chronic eczema biopsies [102]. IFNγ is produced by irritation of damaged keratinocytes [90] and activates the inflammatory cells in both ICD and ACD with higher expression levels in ICD [24, 92]. Such tendency is possibly seen in our controls after 48 hrs due to later reaction as Sylvestre et al. [102].

IL-1 dependent pathway of CD was investigated by Bonefeld and colleagues [98] in mice and recently in humans [99]. Numerous other studies have demonstrated that defects in the IL-1β signaling pathway or blocking of IL-1β result in decreased response to contact allergens [89, 99, 154]. We did not find expression of IL-α and IL-β in 3 different time points in Ni allergic individuals compared with control serum samples (Figures 12;13). It is described that the IL-1 family is closely linked to innate immune responses and is primarily associated with acute and chronic inflammation. It plays was analyzed in sensitization, elicitation, and resolution of ACD [89]. Rustemeyer T et al. [84] found the increase of IL-1α and IL-1β as early as 6 hrs after patch testing, so probably at 24 hrs when we took our biopsies blood samples, their function was already expired. Schmidt et al. found high IL-1β and IL-17 levels

in the areas exposed to Ni 21 days before the second patch testing on the same area [100]. In our study, patch testing was performed on the back area, which was not intended to have exposure to Ni lately.

In 2015 Liu et al. studied the IL-9 production in Ni allergic patients' skin biopsy specimens taken at 48 and 72 hrs and found that Ni allergic patients have a significant increase of IL-9 production in response to Ni when compared to non-allergic controls [92]. IL-9 expression in the skin was noticed also by other authors in ACD skin lesions [24, 92]. We investigated IL-9 in serum taken at 3 different time points and detected unmeasurable amounts in Ni allergic participants and controls. So, it could be that there is a local increase of IL-9 in the skin during an acute inflammation without any systemic effect of this cytokine.

IL-13 seems to be an essential mediator regulating, directly and indirectly, skin barrier function formation associated genes, control of skin homeostasis, and innate barrier function [83, 102]. In our study, unmeasurable levels of IL-13 in Ni allergic participants' and controls' serum samples were detected. However, Silvestre et al. detected higher IL-13 levels in chronic than acute eczema patients [102]. The difference in our results might be due to peripheral blood sampling and the sampling period, as the aim of our study was the first 48 hrs of the elicitation phase.

There were some limitations of this study. First, the study enrolled a small number of participants. Only some cytokines based on literature research were analyzed. The study aimed to investigate cytokines only during the first 48 hrs of the effector phase of contact allergy in the skin area with no re-exposure of Ni, contacting with quite a restrictive repertoire of haptens in general, and without chronic inflammation. Thus, the results we obtained can be less influenced by other factors on the one hand, but in real life, probably interaction between different exposures and the baseline status of the skin barrier can influence the elicitation of contact dermatitis. We have chosen the ELISA method for the cytokine analysis due to high sensitivity, specificity, wide analytical range, and reproducibility [119, 155], although a more complex proteomic analysis could give a more representative view on the inflammatory processes.

6. CONCLUDING REMARKS

- Younger metalworkers reported skin symptoms more frequently than older metalworkers. Sensitization to cobalt was more prevalent than in the general population or dermatitis patients, possibly reflecting increased occupational exposure. Education of the workers regarding skin safety and the use protective equipment is still required since it is not self-evident.
- Considerable amounts of Ni, Co, and Cr can be released from nails and wire in different concentrations. Detected Ni and Cr levels can elicit ACD in already sensitized workers. Thus, preventive measures should be employed in the workplace. Co can be present in alloys even if not mentioned in material safety data sheets, so at least theoretically, this could pose a risk for aggravated dermatitis on already compromised skin.
- The finger immersion technique was used for Co and Cr detection on fingers for the first time. It appears to be a simple and reliable method. However, more studies are needed to substantiate this – the method could be an alternative for studies of skin exposure to other metals under standardized experimental conditions, in the general setting, and in the workplace.
- The distribution of Cr in the skin is similar for pet. and aq. preparations, but the concentration of Cr detected in the skin was almost two times lower for pet. comparing aq. preparations. More studies are needed with more samples to determine which vehicle would deliver more consistent dosing results. Acid wipe sampling or finger immersion technique in certain occupations where chromium salts are used in aqua solutions might not be useful as no chromium was detected on the surface. Nevertheless, both vehicles are suitable for patch test preparations since Cr reaches the receptor phase in high amounts for both samples.
- This study presents some data on selected cytokines important in contact allergy and their role in early inflammatory processes. More investigations and real-life studies are needed for a better understanding of the underlying mechanisms since in severe, chronic, difficult-to-treat ACD cases, biologics and small molecules interfering with cytokines and their functions can be used.

7. FUTURE

This research opened interdisciplinary ways to perform exciting studies. In order to better understand the disease that was thought previously known, the collaboration with experienced researchers will continue.

The prevalence of contact allergy to metals will be surveilled for future decades and tendencies analyzed.

Nowadays, during the COVID19 pandemic, the new vision and possibilities for the safety gear are possible. Workers start to experience the positivity of safety recommendations. The communication with factories in need will continue for the future wellness.

The investigation of the ACD pathogenesis just started. This study will be continued for a better understanding of the underlying mechanisms since in severe, chronic and/or difficult-to-treat ACD cases, biologics and small molecules interfering with cytokines and their functions can be used.

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SUPPLEMENTS

Suppl. Table 1. The interwiever-based metal plant worker questionnaire.

I.	General information
	1. Gender o Female o Male 2. Age (fill in) ..... 3. Occupation: o Production house o Office staff 4. Working time in a factory (fill in)..... 5. Have you been diagnosed with a respiratory tract disease? o Yes (if yes, fill in the diagnose.....) o No 6. 5. Have you been diagnosed with a skin disease? o Yes (if yes, fill in the diagnose.....) o No 7. Do you take medicine regularly? o Yes (if yes, fill in the name.....) o No 8. Are you allergic? o Yes (if yes, fill in your allergens.....) o No o I don't know 9. Is there a family history of allergic diseases (asthma, allergic rhinitis, allergic skin diseases)? o Yes (if yes, which one.....) o No o I don't know 10. Do you smoke? o Yes (if yes, how many cigarettes a day.....how many years.....) o No
II.	Signs and symptoms
	1. Do you suffer from respiratory tract symptoms? o Yes o No

2. Mark the frequency of the signs and symptoms (X)

Signs and sypmtoms	Do not bother	Mostly do not bother	Bother a little bit	Moderately bother	Usually bother	Mostly bother	Extremely bother
Itchy nose							
Sneezing							
Runny nose							
Stuffy nose							
Itchy palate							
Itchy eyes							
Tearing							
Shortness of breath							
Wheezing							
Itchy mouth after food consum-ption							

3. The time of nose and eye symptoms and signs
   - Symptoms are usually before work
   - Symptoms bother during work
   - Symptoms bother after work
   - Symptoms disappear while on vacation/dolidays
   - Symptoms depend on the season (Autumn/wonter/spirng/summer)
   - Symptoms do not depend on the season or place
   - I do not have nose and eye symptoms.
4. Do you have skin problems?
   - Yes
   - No
5. The localization of dermatitis/rash?

- Face
- Hands/palms
- Legs/feet
- Abdomen
- Back
- I do not have skin problems

6. Bothering signs and symptoms?
   - Redness
   - Blisters
   - Wounds
   - Fissures
   - Pealing of the skin
   - Itchy skin
   - Burning
   - Stinging
   - Other (fill in.....)
   - I dont have skin symptoms nor signs
7. Have you noticed provoking factors?
   - Yes
   - No
8. What do you think is provoking dermatitis?
   - Cold (cold weather/water)
   - Heat (hot weather/water)
   - The change of the temperature (e.g. from cold weather to warm or opposite)
   - Hygiene products (cleansing products/shampoos, soaps)
   - Wearing of the watch
   - Contact with oils and other working fluids
   - Contact with metals and their products
   - Women: jewelry
   - Leather shoe wearing
   - Rubber shoe wearing
   - Wearing of rubber gloves
9. The use of safety gear:
   - I do not need safety gear for the work I do every day
   - I always use safety gear
   - I usually use safety gear
   - Mostly I use safety gear
   - Sometimes I use safety gear
   - I do not use safety gear, it is impeding my work

Suppl. Table 2. The list the European baseline series contact allergens, their vehicles and concentration.

No.	Substance	Vehicle	Concentration, %
1	Potassium dichromate	Pet.	0.5
2	p-phenylenediamine (PPD)	Pet.	1.0
3	Thiuram mix	Pet.	1.0
4	Neomycin sulfate	Pet.	20.0
5	Cobalt(II)chloride hexahydrate	Pet.	1.0
6	Benzocaine	Pet.	5.0
7	Nickel(II)sulfate hexahydrate	Pet.	5.0
8	Clioquinol	Pet.	5.0
9	Colophonium	Pet.	20.0
10	Paraben mix.	Pet.	16.0
11	N-isopropyl-N-phenyl-4-phenylenediamine (IPPD)	Pet.	0.1
12	Lanolin alcohol	Pet.	30.0
13	Mercapto mix.	Pet.	2.0
14	Epoxy resin, Bisphenol A	Pet.	1.0
15	Peru balsam	Pet.	25.0
16	4-tert-Butylphenolformaldehyde resin (PTBP)	Pet.	1.0
17	2-Mercaptobenzothiazole (MBT)	Pet.	2.0
18	Formaldehyde	Aq.	2.0
19	Fragrance mix I	Pet.	8.0
20	Sesquiterpene lactone mix	Pet.	0.1
21	Quaternium-15	Pet.	1.0
22	2-Methoxy-6-n-pentyl-4-benzoquinone	Pet.	0.01
23	Methylisothiazolinone+Methylchloro isothiazolinone	Aq.	0.02
24	Budesonide	Pet.	0.01
25	Tixocortol-21-pivalate	Pet.	0.1
26	Methyldibromo glutaronitrile	Pet.	0.5
27	Fragrance mix II	Pet.	14.0
28	Hydroxyisohexyl 3-cyclohexene carboxaldehyde	Pet.	5.0
29	Methylisothiazolinone	Aq.	0.2
30	Textile dye mix	Pet.	6.6

SUMMARY

Metal allergy has for a long time been, and is still, the most frequent contact allergy among both dermatitis patients and the general population. Contact with metals is involved in a wide range of occupations, including mechanics, construction workers, welders, assemblers, toolmakers, cashiers, and many others. In this study, the problem of contact allergy was examined from the perspective of the metalworkers' occupation. Firstly one hundred eighty-five metal plant workers (154 metalworkers and 31 from administrative personnel) completed the interviewer-administered questionnaire to provide information about skin symptoms and signs. Working less than 20 years in the factory, metalworkers more often had skin symptoms than with longer working MW. Contact with chemicals at the workplace was often suspected as the main factor provoking skin symptoms by MW. Work-related occupational dermatitis, allergic or irritant, was suspected in 11 out of 75 metalworkers. One hundred thirty-five workers were patch tested with the European baseline series. Out of 135 patch-tested workers, 28.9% had at least one positive patch test reaction. At the time of patch testing, 88 workers agreed to participate in the finger immersion experiment. The passive finger immersion method is accurate and confidently can be used to detect Ni deposition on hands. Its advantages over other methods such as wipe testing and tape stripping in terms of proven extraction efficacy, speed, and ease of technique. One hundred and seventy-six samples of 88 participants were analyzed. Fifty participants were MW, and 38 participants were OS. The samples were analyzed by an inductively coupled plasma sector field mass spectrometer in collaboration with experienced scientists from the Center of Physical Sciences and Technology, Department of Nuclear Research, Vilnius Lithuania. The finger immersion technique was used for Co and Cr detection on fingers for the first time.

The factory was willing to further research and donate raw material (wire) and finished products (nails). It is known that prolonged contact with the skin in already sensitized subjects can elicit ACD. The experiment simulating the prolonged contact with mentioned metal items and artificial sweat was performed. The release of Ni, Co, and Cr was measured using the atomic absorption analysis week in the Department of Occupational and Environmental Dermatology, Lund University, Skåne University Hospital, Malmö, Sweden. The released concentration of Ni statistically significantly increased during a week.

Cr salts in solutions or powder are known to cause skin irritation and even ulcers and known sensitizer causing occupational ACD in industries such as

construction and leather tanning. To understand better the penetration properties Franz cell diffusion experiment with Cr salts in different vehicles was performed. This study was done by the supervision of experienced colleagues in the Department of Occupational and Environmental Dermatology, Lund University, Skåne University Hospital, Malmö, Sweden. Cr was detected in both recipient phases after using aq. and pet. vehicles. No Cr was detected in the wipe samples of the Cr aq. samples. The distribution of the total Cr amount in the skin samples was very similar using pet. and aq. as vehicles in all samples.

The mechanism of ACD was intensively studied during the last two decades, but the underlining immunological mechanisms are still unclear. Keratinocytes and other skin cells produce various cytokines. In most cases, ICD and ACD are clinically and histologically indistinguishable, and no markers have been identified to distinguish between the two. It is known that after the antigen has penetrated the stratum corneum, it exerts cytotoxic effects on the keratinocytes and triggers keratinocytes to release alarming signals, which are cytokines and chemokines. Wanting to contribute to better pathogenesis understanding, our study enrolled 10 volunteers (5 Ni patch test positive and 5 Ni patch test negative). Ten participants were asked not to consume Ni-rich food and canned food throughout the investigation. Blood samples and skin biopsies were taken in 3 different timelines. For the quantitative detection of human IFNγ, IL-1α, IL-1β, IL-9, IL-13, IL-17A, IL-22, and IL-23, the ELISA Invitrogen (Thermo Fisher Scientific, Bender MedSystems GmgH, Vienna, Austria) kits were used. The biopsied skin samples were degraded into liquid solution. Further biopsy analysis due to the small number of samples was performed for IL-17A, IL-22, and IL-23. This study presented data on selected cytokines that may play an important role in inflammatory processes of contact allergy. The results confirm simmilarities between ICD and ACD. More investigations and real-life studies are needed for a better understanding of the underlying mechanisms since in severe, chronic, difficult-to-treat ACD cases, biologics and small molecules interfering with cytokines and their functions can be used.

SANTRAUKA LIETUVIŲ KALBA

Alergija metalams ilgą laiką buvo ir tebėra dažniausia kontaktinė alergija tiek tarp sergančiųjų dermatitu, tiek tarp likusios žmonių populiacijos. Su metalais dažnai kontaktuoja įvairių profesijų atstovai, pvz., mechanikai, statybininkai, suvirintojai, kompiuterinės technikos surinkėjai, įrankių gamintojai, kasininkai. Šiuo tyrimu nagrinėjama, kaip kontaktinė alergija pasireiškia tarp metalo apdirbimo sektoriuje dirbančių žmonių. Darbą sudaro atskiri klinikiniai ir eksperimentiniai tyrimai. Iš pradžių buvo apklausti 185 metalo apdirbimo įmonės darbuotojai (154 darbininkai ir 31 administracijos darbuotojas). Jie atsakė į apklausos rengėjo sudarytą anketą (žr. 1 priedą), pateikdami informaciją apie varginančias odos problemas. Apklausos rezultatai parodė, kad mažiau nei 20 metų darbo stažą turintys metalo apdirbimo sektoriaus darbuotojai dažniau turėjo odos problemų nei ilgiau toje pačioje pozicijoje dirbantieji ir kad odos problemas dažniausiai sukelia kontaktas su cheminėmis medžiagomis darbo vietoje. Tęsiant tyrimą ant 135 metalo apdirbimo sektoriaus darbuotojų (75 darbininkams ir 60 administracijos darbuotojų) nugaros buvo užklijuoti odos lopo mėginiai su Europos bazinės serijos kontaktiniais alergenais. Bent viena teigiama mėginio reakcija pasireiškė 28,9 % tiriamųjų. Įsijautrinimas kobalto chloridui statistiškai reikšmingai skyrėsi tarp darbininkų ir administracijos darbuotojų (teigiami lopo testai/tirti asmenys: 6/75 ir 0/60 atitinkamai, P=.03). Moterys, nepriklausomai nuo atliekamo darbo, buvo statistiškai dažniau įsijautrinusios nikelio sulfatui nei vyrai (18,75 % ir 4.22 % atitinkamai, P=.01). Iš tirtų Europos bazinės serijos kontaktinių alergenų, penki dažniausi nustatyti kontaktiniai alergenais buvo: nikelio sulfatas (11,11 %), Peru balzamas (5,93 %), kobalto chloridas (4,4 %), kvapiųjų medžiagų mišinys I (3,7 %) ir metildibromoglutaronitrilas (2,96 %).

Dirbant įgytas alerginis ar iritacinis dermatitas buvo įtartas 11-ai iš 75 darbininkų. Jaunesni (< 40 m.amžiaus) darbuotojai statistiškai reikšmingai dažniau skundėsi odos simptomais nei, vyresni.(P=.03) viena to priežastis - jaunesnio amžiaus labiau atkreipia dėmesį į sveikatos problemas nei vyresni ilgiau dirbantys asmenys, kurie dažniausiai darbo eigoje pripranta prie darbo sąlygų, atsainiau žiūri į apsaugos ir profilaktikos priemones. Tai pat galimas „sveiko darbuotojo“ efektas, kuomet turintys odos problemų darbuotojai išeina iš darbo, ir lieka odos problemų neturintys arba jas toleruojantys asmenys. Taip galėtų būti viena priežasčių kodėl ilgą darbo stažą turintys asmenys rečiau skundėsi odos problemomis. Lyginant atlikto tyrimo rezultatus su Bavarijos (Vokietija) metalo apdirbimo įmonių darbuotojų duomenimis, didžiausias kontaktinės alergijos dažnis taip pat buvo pastebėtas

jauname amžiuje - 15-24 metų amžiaus grupėje Pažymėtina tai, kad dažniausiai metalo apdirbimo pramonėje kontaktinė alergija pasireiškia per pirmuosius keturis darbo metus.

Lyginant odos lopo mėginių rezultatus su bendra Europos populiacija, EDEN tyrimo dalyviais, tarp kurių buvo ir metalo apdirbimo įmonių darbuotojų, pastebėta, kad metalo įmonių darbuotojai statistiškai reikšmingai dažniau yra įsijautrinę kobalto chloridui ir kalio dichromatui. Tai rodo, kad įsijautrinimas šiems metalams galimai yra susijęs su profesija.

Iš odos lopo tyrime dalyvavusių 135 darbuotojų 88 darbuotojai (50 darbininkų ir 38 administracijos darbuotojai) dar dalyvavo vadinamajame piršto merkimo mėginio eksperimente. Piršto merkimo mėginio metodas naudojamas norint aptikti ant paviršiaus esančius metalų pėdsakus. Palyginti su kitais metodais, pvz., kai mėginiai imami servetėle ar lipnia juosta, šis metodas yra techniškai paprastesnis, o rezultatai tikslūs ir patikimi. Iš viso buvo paimti 176 mėginiai nuo 88 dalyvių smiliaus ir nykščio. Šie mėginiai buvo ištirti induktyviai susietos plazmos masių spektrometru bendradarbiaujant su patyrusiais Fizinių mokslų ir technologijos mokslų centro Branduolinių technologijų skyriaus mokslininkais (Vilnius, Lietuva). Nikelio buvo aptikta visuose mėginiuose, tačiau be statistinio reikšmingumo tarp darbovietės ar lyties. Didžiausi nikelio kiekiai buvo aptikti ant žaliavinės medžiagos operatorių (0,0174 μg/cm^2), vinių atkaitintojų (0,0160 μg/cm^2), informacinių technologijų skyriaus darbuotojų (0,0297 μg/cm^2) ir gamybos kontrolieriaus (0,0153 μg/cm^2) pirštų paviršiaus. Aptikto kobalto kiekio mediana statistiškai reikšmingai skyrėsi tarp darbininkų ir administracijos darbuotojų (0,004 μg/cm^2 ir 0,001 μg/cm^2,P=.04). Pirštų merkimo mėginio metu nustatyto chromo kiekio mediana statistiškai reikšmingai nesiskyrė tarp darbo vietų, tačiau stebėta pokyčio tendencija tarp lyčių: 0,0013 μg/cm^2 vyrams ir 0,0007 μg/cm^2 moterims (P=.06). Šiame tyrime pirmą kartą pirštų merkimo metodas buvo pritaikytas kobalto ir chromo pėdsakams aptikti.

Kontaktinė alergija nikeliui yra dažnesnė nei bet kuriam kitam metalui. Europos Sąjungoje nikelio išsiskyrimas iš metalinių objektų, skirtų tiesiogiai ir ilgesnį laiką liestis su oda, yra ribojamas iki < 0,5 μg/cm^2 per savaitę į dirbtinį prakaitą ir iki 0,2 μg/cm^2 per savaitę bižuterijos papuošalams. Plačiai paplitusi klaidinga nuomonė, kad reglamentas apima tik tokius daiktus kaip papuošalai, diržų sagtys ir kt, kuriuos asmuo gali dėvėti. Tačiau įrankiams ir instrumentams, kurie liečiasi su oda, taip pat yra taikomas REACH reglamentas. Yra žinoma, kad dermatito ar kitaip pažeisto vientiso odos barjero vietoje kontaktinio dermatito išsivystimo slenkstis yra žymiai mažesnis. Mūsų tyrime nustatytas nikelio kiekis ant pirštų paviršiaus (12 lentelė), jau įsijautrinusiam nikeliui asmeniui gali sukelti odos uždegimą,

alerginio kontaktinio dermatito simptomus. Daugelis metalo apdirbimo įmonės darbuotojų dirba be darbo apsaugos priemonių ar pirštinių, pastoviai liesdami metalo gaminius ir įvairius įrankius nesuprasdami įsijautrinimo rizikos. Taigi prevencinis darbas aiškinant darbutojams kaip sumažinti alergijos riziką labai svarbus ir kol kas nepakankamas.

Mūsų tyrime didžiausias kobalto kiekis buvo nustatytas ant vinių atkaitintojų ir žaliavinės medžiagos operatorių pirštų paviršiaus. Panašius rezultatus paskelbė ir kiti autoriai didžiausius kiekius nustatę ant metalo presavimo ir žaliavinės medžiagos operatorių pirštų. Šių profesijų darbuotojai dažniausiai kontaktuoja su neapdorotomis žaliavomis.

Chromas yra netirpus metalas, tačiau jo korozija dirbtiniame prakaite ar kitame skystyje pasireiškia įvairių druskų susidarymu ir taip padidina įjautrinančias savybes. Yra žinoma, kad šešiavalentės chromo druskos yra labiau tirpios ir įjautrinančios nei trivalentės. Chromo įjautrinimo galimybes tyrinėjo Menné su bendraautoriais, vėliau Bregnbak su bendraautoriais. Jie nustatė, kad chromo išsiskyrimo iš galutinio produkto turi neviršyti 0,02-0,05 $\mu g/cm^2$. Tai yra saugi riba, smarkiai sumažinanti įsijautrinimo chromui riziką. Šešiavalentis chromas turi didžiausią įtaką kontktinės alergijos ir alerginio kontaktinio dermatito išsivystymui. Mūsų tyrime chromo kiekis ant pirštų tarp administracijos darbuotojų ir darbininkų nesiskyrė. Daugiausia chromo aptikta ant žaliavinės medžiagos operatorių (0,0056 $\mu g/cm^2$) ir vinių atkaitintojų (0,0033 $\mu g/cm^2$) pirštų paviršiaus. Tai patvirtina ir kitų autorių paskelbtus tyrimų duomenis, kuomet didžiausias chromo kiekis buvo nustatytas tiesioginį ir ilgalaikį kontaktą su metalo lydiniais turintiems darbuotojams.

Yra žinoma, kad jau sudirgintos odos ilgalaikis kontaktas su metalais gali sukelti alerginį kontaktinį dermatitą. Todėl buvo atliktas eksperimentas, imituojantis ilgalaikį – paros ir savaitės trukmės – vielos ir vinių (šias medžiagas suteikė tyrimo dalyvių įmonė) kontaktą su dirbtiniu prakaitu. Į dirbtinį prakaitą iš vielos ir vinių išsiskyrusių metalų (Ni, Co ir Cr) kiekis buvo išmatuotas Lundo universiteto, Profesinės ir aplinkos dermatologijos skyriaus laboratorijoje, Skåne universiteto ligoninėje (Malmė, Švedija) naudojant atominės absorbcijos spektrometrą. Nikelio, kobalto ir chromo koncentracija išsiskyrimas iš vinių, vielos ir žaliavinės medžiagos buvo matuotas praėjus 24 valandos ir po 7 parų. Šis eksperimentas parodė, kad per savaitę iš vinių ir vielos į dirbtinį prakaitą išsiskyrusio Ni kiekio padidėjimas yra statistiškai reikšmingas ($P=.04$). Visi tirti metalai (nikelis, chromas ir kobaltas), išsiskyrė iš tirtų objektų kontaktuojant su dirbtiniu prakaitu, o vidutinis koncentracijos kiekis didėjo ilgėjant kontakto laikui. Tyrimo metu buvo aptiktas kobaltas, nors žaliavinės medžiagos specifikacijos dokumentuose kobaltas sudėtyje

nebuvo nurodytas. Svarbu paminėti, kad vis dar nėra kobalto išsiskyrimą iš objektų, skirtų ilgesnį laiką tiesiogiai liestis su oda, reglamentuojančių teisės aktų.

Chromo druskos yra žinomos kaip įsijautrinimą sukeliantis profesinio alerginio kontaktinio dermatito priežastinis veiksnys. Siekiant geriau suprasti chromo prasiskverbimo per odą ypatybes buvo atliktas Franz tipo difuzinės celės eksperimentas naudojant dvi skirtingas terpes (vazeliną ir vandenį) su chromo druskomis. Eksperimentas atliktas Lundo universiteto Profesinės ir aplinkos dermatologijos skyriaus laboratorijoje, Skåne universitetinės ligoninės (Malmė, Švedija) naudojant kiaulės ausies odą kaip žmogaus odos atitikmenį. Chromas buvo aptiktas abiejų terpių Franz tipo difuzinės celės recipiento fazėse. Chromo pasiskirstymas kiaulės ausies odos sluoksniuose mažai priklausė nuo naudotos terpės ir abiem atvejais buvo panašus. Kiaulės odos donorinis paviršius po eksperimento buvo nuvalytas servetėle siekiant išsiaiškinti, kiek chromo liko neprasiskverbusio. Chromo nebuvo aptikta servetėlės, kuria nuvalytas kiaulės odos paveiktos vandenine terpe paviršius. Tai rodo, kad servetėlių ar kiti odos paviršiaus tyrimo metodai, kai naudojamos vandeninės Cr druskos, gali būti nepatikimi.

Tyrime nustatyta, kad chromas lengvai prasiskverbia naudojant tiek vazeliną, tiek vandeninę terpes, tačiau naudojant vandeninę terpę procesas vyksta greičiau. Tai patvirtina mūsų atlikto paviršiaus nuvalymo mėginio rezultatai, kuriuose chromo neaptikta. To ir galima buvo tikėtis, nes chromas vazelino pagrindo terpėje pasiskirstęs hidrofobinėje aplinkoje, todėl mažiau chromo jonų tiesiogiai kontaktuoja su odos paviršiumi, taip ribojamas chromo jonų kiekis galintis prasiskverbti per odą. Eksperimente naudojant vazelino pagrindo mėginį odos paviršiuje buvo aptiktas nemažas (7-50 %) likutinis atominio chromo kiekis (16 lentelė), tuo tarpu vandeninėje terpėje ištirpusios chromo druskos prasiskverbė per odą ir paviršiuje atominio chromo neaptikta. Panašius rezultatus gavo ir Gammeraard su kolegomis per 160 valandų trukusį eksperimentą, kai ant žmogaus odos buvo užtepta didesnės koncentracijos kalio dichromato druska ir taikytas lipnios juostelės metodas mėginiams nuo odos paviršiaus surinkti.

Atliekant eksperimentą akceptorinėje terpėje aptikome didelį chromo kiekį nepriklausomai nuo pasirinktos terpės (vazelino ar vandens). Yra žinomi du pagrindiniai prasiskverbimo per odą keliai. Pirmasis, transepiderminis kelias, kai medžiagos difunduoja per tarpląstelinius raginio sluoksnio tarpus arba ragines ląsteles; antrasis kelias vyksta per odos priedus, kai difuzija vyksta per plaukų folikulus, riebalines ir prakaito liaukas. Paprastai prasiskverbimas vyksta abiem keliais. Odos sritys, kuriose yra gausu plaukų folikulų (galva, pažastys ir kt.), labiau pralaidžios įvairioms cheminėms

medžiagoms nei kitos, mažiau plaukuotos odos dalys. Be to, lipofilinės molekulės prasiskverbia į odą greitai, o hidrofilinės – lėčiau. Tai gali paaiškinti, kodėl naudojant chromo druskas vazelino pagrindo terpėje odoje radome mažiau chromo, bet didesnį jo kiekį nustatėme receptoriniame skystyje. Tai reiškia, kad lipofilinėje terpėje chromas lengvai patenka į odą ir prasiskverbia per ją. Šie rezultatai gali reikšmingai pasitarnauti apskaičiuojant teorinį atominio chromo kiekį, kuris gali sukelti kontaktinę alergiją arba alerginio kontaktinio dermatito simptomus jau įsijautrinusiems asmenims. Atlikto eksperimento rezultatai rodo, kad toksikologiniai skaičiavimai atliekami vandeniniams chromo druskų tirpalams turėtų būti tikslesni nei lipofilinėms terpėms.

Šiame eksperimente buvo naudota šaldyta kiaulės ausies oda. Yra tyrimų, kurie nagrinėjo šaldymo poveikio įtaką odos raginio sluoksnio vientisumui tiriant įvairius medicininius prietaisus ir įrankius (pvz. chirurgines adatas). Tyrėjai nagrinėję šalčio poveikį odos barjerinei funkcijai ir metaboliškai stabilių cheminių medžiagų prasiskverbimui per odą, nustatė, kad šaldymas barjerinei funkcijai įtakos neturėjo. Taip pat aptikti skirtumai B ir C bei E ir F (16 lentelė) mėginiuose atitinkamai kelia klausimų, kodėl taip atsitiko.

Nors alerginio kontaktinio dermatito mechanizmas aktyviai tyrinėjamas jau kelis dešimtmečius, pagrindiniai alerginio kontaktinio dermatito imunologiniai mechanizmai vis dar neaiškūs. Daugeliu atvejų iritacinis ir alerginis kontaktinis dermatitai yra kliniškai ir histologiškai panašūs, taip pat nebuvo nustatyta jokių biožymenų, leidžiančių juos atskirti serologiškai. Yra žinoma, kad po to, kai antigenas prasiskverbia į raginį odos sluoksnį, jis citotoksiškai veikia keratinocitus ir skatina juos išskirti signalines molekules – citokinus ir chemokinus. Siekiant geriau suprasti alerginio kontaktinio dermatito patogenezę buvo tirti 10 savanorių – 5 įsijautrinę nikeliui ir 5 sveiki. Šių tiriamųjų buvo paprašyta eksperimento metu nevartoti maisto, kuriame gausu nikelio, ir skardinėse konservuotų maisto produktų. Iš tiriamųjų 3 kartus kas 24 val. buvo imami kraujo mėginiai, atliktos odos biopsijos ir naudojant ELISA Invitrogen (Thermo Fisher Scientific, Bender MedSystems GmgH, Viena , Austrija) rinkinius kiekybiškai nustatyti žmogaus IFNγ, IL-1α, IL-1β, IL-9, IL-13, IL-17A, IL-22 ir IL-23. Šių citokinų pasirinkimą lėmė literatūroje aprašytas galimas jų vaidmuo kontaktinio dermatito patogenezėje. Dėl nedidelio mėginių kiekio biopsijose tirti IL-17A, IL-22 ir IL-23.

Šio tyrimo metu praėjus 24 valandoms po odos lopo mėginių užklijavimo visiems penkiems nikeliui alergiškiems tyrimo dalyviams nustatyta teigiamą reakciją (1+) į nikelio sulfatą. Po 48 valandų trims iš penkių tiriamųjų nustatyta 2+ teigiama reakciją ir dviem iš penkių – 3+ teigiama reakcija. Penkiems kontrolinės grupės tiriamiesiems nikelio sulfato mėginys buvo

neigiamas po 48 val. Visuose nikeliui alergiškų ir kontrolinės grupės tiriamųjų kraujo serumo mėginiuose neaptikta IL-1β, IL-9 ir IL-13. Interleukinai (IL) 1α, IL-17A ir IFNγ nustatyti visuose serumo mėginiuose, tačiau jų koncentracija buvo mažesnė nei 4 pg/mL (28, 29, 30 ir 31 pav.). IL-17A koncentracija visose odos biopsijos mėginiuose buvo < 4 pg/mL nepriklausomai nuo mėginio paėmimo laiko. IL-22 ir IL-23 nustatyti didesniais kiekiais lyginant su kitais interleukinais, tačiau statistiškai reikšmingo skirtumo tarp mėginio paėmimo laiko ar įsijautrinimo nikeliui nebuvo (17 lentelė, 32 ir 33 pav.).

Yra nemažai informacijos apie imunologinį alerginio kontaktinio dermatito mechanizmą, tačiau daugelis žinių grindžiamos pelių modeliais ar *in vitro* eksperimentais, todėl trūksta *in vivo* tyrimų. Šio *in vivo* eksperimento metu pasirinkti tirti jau anksčiau kitų autorių minėti kaip galimai patogenetiniai alerginio kontaktinio dermatito citokinai. Šiame eksperimente nustatytas žemas IL-17A kiekis kraujo serume ir odos biopsinėje medžiagoje (28 ir 29 pav.), nors priešingus rezultatus gavo Silvestre su kolegomis, kurie tyrė lėtinio dermatito pažeistos odos mėginius. Šiuos skirtingus rezultatus padeda paaiškinti Schmidt ir kolegų atlikto tyrimo rezultatai, kuriuose didelis IL-17A kiekis nustatytas odos mėginiuose, pakartotinai paveiktuose kontaktinio alergeno. Lygiai taip pat didelės IFNγ ir IL-1β koncentracijos nustatytos tose buvusio dermatito vietose, kuriose oda buvo pakartotinai veikiama kontaktinio alergeno. Keletas *in vitro* tyrimų, kai žmogaus keratinocitai ir periferinis kraujas buvo stimuliuojami nikeliu, parodė IL-17 ekspresiją nikelio paveiktose odos biopsinės medžiagos mėginiuose. Mūsų atlikto eksperimento gauti IL-17A koncentracijos rezultatai (29 pav.) sutampa ir su kitų autorių tyrimų duomenimis.

Nikeliui įsijautrinusių ir kontrolinės grupės tiriamųjų kraujo serume aptikome panašius IL-22 kiekius (32 pav.), tačiau galime įžvelgti koncentracijos didėjimo tendenciją tarp įsijautrinusių nikeliui. *In vitro* eksperimentų, atliktų Larsen su kolegomis, metu nustatyta IL-22 ekspresija įjautrintos nikeliui ir nikeliu paveiktos odos be uždegimo mėginiuose. Tai paaiškintų mūsų eksperimente gautus panašius įsijautrinusiųjų ir kontrolinės grupės rezultatus. Gali būti, kad IL-22 yra nespecifinis epidermio pažeidimo citokinas, nes jis minimas atopinio dermatito ir psoriazės patogeneziniuose mechanizmuose.

Priešuždegiminis citokinas IL-23 yra IL-12 šeimos narys, kurį ekspresuoja T limfocitai ir NK ląstelės. IL- 23 svarbus Th17 ląimfocitų vystymuisi ir galimai dalyvauja alerginio kontaktinio dermatito patogenezėje. Jis būtinas IL-22 ekspresijai, Th17 limfocitų brendimui ir dauginimuisi. Atliktų *in vitro* tyrimų metų stebima padidėjusi IL-23 ekspresija, kuomet

žmogaus keratinocitai buvo veikiamos kontaktiniais alergenais. Tai stebime ir mūsų *in vivo* eksperimento biopsinės medžiagos mėginiuose (33 pav.). Tačiau kas nepastebėjo?nepastebėjo reikšmingo skirtumo tarp nikeliui įsijautrinusiųjų ar kontrolinės grupės asmenų mėginių, todėl tai gali būti ženklas, kad kaip ir IL-22, IL-23 nėra specifinis alerginiam kontaktiniam dermatitui ir yra nespecifinis epidermio pažeidimo citokinas.

Šiame eksperimente nustatėme mažą IFNγ kiekį kraujo serume praėjus 48 val. kai buvo užklijuotas mėginys su nikeliu kontrolinės grupės tiriamiesiems bei radome kiek aukštesnę koncentraciją ir vieną netipinę vertę tarp nikeliui įsijautrinusių tiriamųjų (30 pav.). Atliekant eksperimentus ir naudojant imunohistocheminį metodą Ulfgren su kolegomis taip pat neaptiko IFNγ nikeliu paveiktos odos biopsiniuose mėginiuose tarp įjautrintos nikeliui ir kontrolinės grupės mėginiuose per 6-72 val. nuo eksperimento pradžios. Silvestre su kolegomis tyrinėję lėtinio dermatito pažeistos odos biopsijos mėginius nustatė didelius IFNγ kiekius. Yra žinoma, kad IFNγ gaminamas dirginant pažeistus keratinocitus, jis aktyvina uždegimines ląsteles iritacinio ir alerginio kontaktinių dermatitų atvejais. Tokią tendenciją galima įžvelgti ir mūsų atlikto eksperimento atveju atkreipiant dėmesį į kontrolinės grupės mėginius praėjus 48 valandoms nuo eksperimento pradžios.

Daug dėmesio IL-1 tyrimams skyrė Bonefeld su kolegomis, kurie pradžioje tyrimus atliko su pelėmis ir žmonėmis. Yra tyrimų, kuriuose stebėtas IL-1β koncentracijos padidėjimas alerginio kontaktinio dermatito metu ūminėje fazėje. Nustatyta, kad blokuojant IL-1β mažėja atsakas į kontaktinius alergenus. Mūsų tyrimo metu kraujo serumo mėginiuose nenustatėme IL-1α (31 pav.) ir IL-1β reikšmingos ekspresijos trimis skirtingais laikotarpiais tiek nikeliui įsijautrinusiems, tiek kontrolinės grupės tiriamiesiems. IL- 1 šeima yra susijusi su įgimtu imuninių atsaku, kuris svarbus ūminiu ir lėtiniu uždegimo laikotarpiu. Rustemeyeris su kolegomis nustatė IL-1α ir IL-1β koncentracijos padidėjimą praėjus 6 val. po odos lopo mėginio užklijavimo, todėl tikėtina, kad praėjus 24 val., kai paėmėme biopsiją ir kraujo mėginius, šie interleukinai nebeišskiriami. Schmidt ir kolegų eksperimento metu ta pati odos vieta praėjus 21 dienai pakartotinai buvo paveikta nikelio sulfatu. Išmatavę IL-1β ir IL-17 koncentracijas autoriai nustatė jų padidėjimą.

Šiame eksperimente IL-9 tirtas kraujo serumo mėginiuose trimis skirtingais laiko momentais. IL-9 kiekis buvo neišmatuojamas tiek nikeliui įsijautrinusių, tiek kontrolinės grupės tiriamųjų kraujo serumuose. Todėl vertinant šuos rezultatus, darome prielaidą kad ūminio odos uždegimo metu galimas lokalus IL-9 padidėjimas, be sisteminio šio citokino poveikio.

IL-13 yra vienas iš pagrindinių citokinų tiesiogiai ir netiesiogiai reguliuojantis genus susijusius su įgimta odos barjerine funkcija. Mūsų tyrimo metu kraujo serume buvo neišmatuojamas IL-13 kiekis tiek nikeliui alergiškų, tiek kontrolinės grupės tiriamųjų asmenų mėginiuose. Tačiau Silvestre su kolegomis nustatė didesnius IL-13 kiekius lėtinės egzemos odos biopsijos mėginiuose, nei ūminės egzemos pacientų mėginiuose. Mūsų gauti rezultatai kitokie greičiausiai dėl skirtingos tiriamosios medžiagos, tai yra, atliktas periferinio kraujo mėginio tyrimas, bei mėginių ėmimo laiko, nes mūsų tyrimo tikslas buvo imunologiniaa įvykiai pirmosiomis 48 val. kai vystosi alerginis kontakinis dermatitas .

Šis eksperimentas turi kelis trūkumus. Pirmiausia, tyrime dalyvavo nedidelis tiriamųjų skaičius. Buvo tirti tik kai kurie citokinai, kurių pasirinkimas buvo pagrįstas literatūros duomenimis. Tyrimo tikslas buvo nagrinėti pirmąsias 48 efektorinės alerginio kontaktinio dermatito fazės valandas odoje, kuri prieš tai neturėjo kontakto su tiriamu kontaktiniu alergenu bei nebuvo pažeista lėtinio uždegimo. Taigi, viena vertus, mūsų rezultatams gali turėti mažiau įtakos kiti veiksniai, tačiau realiame gyvenime tikriausiai sąveika tarp skirtingų ekspozicijų ir pradinės odos barjerinės funkcijos būklės gali turėti įtakos alerginio kontaktinio dermatito išsivystymui. ELISA metodas citokinų analizei buvo pasirinktas dėl didelio jautrumo ir specifiškumo, plataus analitinio diapazono ir patvirtinto rezultatų kartotinumo, nors sudėtingesnė proteominė analizė galėtų suteikti platesnį vaizdą apie uždegiminius procesus. Nepaisant minėtų trūkumų atlikto eksperimento rezultatai patvirtino iritacinio ir alerginio kontaktinio dermatito panašumus.

Norint geriau suprasti pagrindinius alerginio kontaktinio dermatito mechanizmus reikia atlikti daugiau eksperimentinių ir kasdienio gyvenimo tyrimų, nes sunkaus, lėtinio ir sunkiai gydomo alerginio kontaktinio dermatito atvejais būtų galimybė pacientų gydymui skirti tikslinius biologinius preparatus.

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THE LIST OF PUBLICATIONS

1. Linauskiene K, Isaksson M, Malinauskiene L. Heavy metals and the skin: Sensitization patterns in Lithuanian metalworkers. *Contact Dermatitis.* 2020;83(6):450-457.
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4. Linauskiene K, Miskinyte M, Dumalakiene I et al. The analysis of selected cytokines in early elicitation phase of allergic contact dermatitis. Submitted to Annals of Allergy, Asthma & Immunology.

SHORT COMMUNICATION ABOUT THE AUTHOR

Kotryna Linauskienė was born in 1984 in Kaunas, where she spent most of her time learning. She graduated from Kaunas Basanavicius secondary school and Kaunas medical university with a master's degree in medicine. She continued her medical studies with an internship in Klaipeda university hospital and residency in allergology and clinical immunology in Vilnius University. Vilnius University is the place where her scientific carrier started. She won the European Academy of Allergology and Clinical Immunology (EAACI) clinical fellowship and spent three months in the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden. Kotryna is the author of 14 articles rated by *Clarivate Analytics Web of Science (CA WoS).* In most of the articles, she is the first author. Most of the publications are with co-authors from different institutions. She has won the best presentation award in the Skin Allergy meeting in Zurich, Switzerland, and was invited as a speaker to talk about contact dermatitis at the European Society of Contact Dermatitis Congress in Milan, Italy. Kotryna also shares her knowledge enthusiastically with younger colleagues and other doctors.

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