From INSTITUTE OF ENVIRONMENTAL MEDICINE
Karolinska Institutet, Stockholm, Sweden

OCCUPATIONAL SKIN EXPOSURE TO COBALT: ORIGIN AND FATE

Jolinde Kettelarij

Karolinska Institutet

Stockholm 2018

Cover image “Kobold” by Jonas Lesser.

Published by Karolinska Institutet
Printed by AJ E-print AB, 2018

ISBN 978-91-7831-109-5

Occupational skin exposure to cobalt: origin and fate

THESIS FOR DOCTORAL DEGREE (Ph.D.)

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet, offentligen försvaras i Atrium, Nobels väg 12b, campus Solna

Fredagen den 15 juni, 2018, kl 09.00

By

Jolinde Kettelarij

Principal Supervisor:
Associate Professor Anneli Julander
Karolinska Institutet
Institute of Environmental Medicine
Unit of Work Environment Toxicology

Co-supervisors:
Assistant Professor Klara Midander
Karolinska Institutet
Institute of Environmental Medicine
Unit of Work Environment Toxicology

Professor Emeritus Carola Lidén
Karolinska Institutet
Institute of Environmental Medicine
Unit of Work Environment Toxicology

Opponent:
Associate Professor Katri Suuronen
Finnish Institute of Occupational Health
Department of Occupational Medicine

Examination Board:
Associate Professor Lina Hagvall
University of Gothenburg
Department of Dermatology and Venereology

Associate Professor Marika Berglund
Karolinska Institutet
Institute of Environmental Medicine
Unit of Biochemical Toxicology

Associate Professor Ingvar Bergdahl
Umeå University
Department of Public Health and Clinical Medicine

And teacher
There are things
That I still have to learn

Georgios Kyriacos Panayiotou

ABSTRACT

Exposure to cobalt is not without risk. Besides adverse health effects on the respiratory system, cobalt is one of the most important sensitising metals with a contact allergy prevalence of 2.2% in the general population. Sources of exposure to cobalt in the general population are diffuse. Because of its specific uses in for example rechargeable batteries, superalloys, and hard metals, presence of cobalt exposure at work is often more obvious. For that reason, adverse health effects of cobalt have often been studied in occupational settings. The research presented in this thesis was performed in the work environment of hard metal workers and dental technicians, which are occupational groups with a recognised exposure to cobalt. The overall aim was to study skin as target organ for occupational exposure to metals, in particular, to cobalt. Dental tools and alloys, handled on a daily basis by dental technicians, were tested for release of cobalt with the cobalt spot test, and for nickel release using the Dimethylglyoxime test. Furthermore, release of cobalt, nickel and chromium was quantified in artificial sweat experiments. Concentrations of nickel and chromium for many dental tools and alloys were considered high enough to pose an allergy risk (paper I). In addition, cobalt, nickel and chromium were detected on skin of all participating dental technicians (n=13) measured by acid wipe sampling. Cobalt was also detected in all ten air samples (0.22-155 $\mu g/m^3$), of which two concentrations exceeded the Swedish Occupational Exposure Limit of 20 $\mu g/m^3$. Despite skin and respiratory exposure to metals, exposure was not reflected in urine samples of dental technicians (paper II). Although this study had a small sample size and a limited amount of work performed with dental alloys made of cobalt-chromium, the results show that dental technicians are exposed to sensitising metals. The studies performed within the hard metal industry (paper III and IV) followed the same protocol as was used for dental technicians, but included a larger cohort of workers (n=76) and only assessed cobalt exposure. Evaluation of surface contamination with the cobalt spot test revealed the presence of cobalt on surfaces in the hard metal facilities, even outside production areas. All hard metal workers were exposed to cobalt on skin. In addition, respiratory cobalt exposure was measured by sampling of the inhalable fraction among hard metal workers at production areas. This showed that all workers were exposed to cobalt through air. Cobalt was found in 72% of the urine samples of hard metal workers. Correlations were found between cobalt in air and cobalt on skin before and at end of shift. No significant change was seen in urinary cobalt concentrations over time during 24h. Quantile regression modelling revealed significant associations between urinary cobalt concentrations as the dependent variable, and cobalt skin and respiratory exposure as independent variables, when each exposure route was modelled independently. When modelling the independent exposure variables together, none of the cobalt skin doses were significantly associated with cobalt in urine. Several theories may explain the observed associations between cobalt skin exposure and concentrations in urine, but from the results in this thesis it was not possible to assess causation. For both dental technicians and hard metal workers, efforts should be made to reduce skin exposure. Examples are the use of disposable gloves, avoiding contamination of other work areas, and no use of private items in work areas.

LIST OF SCIENTIFIC PAPERS

I. **Kettelarij J**, Lidén C, Axén E, Julander A.
Cobalt, nickel and chromium release from dental tools and alloys.
Contact Dermatitis. 2014;70(1):3-10. doi: 10.1111/cod.12111.

II. **Kettelarij J**, Nilsson S, Midander K, Lidén C, Julander A.
Snapshot of cobalt, chromium and nickel exposure in dental technicians.
Contact Dermatitis. 2016;75(6):370-376. doi: 10.1111/cod.12681.

III. **Kettelarij J**, Midander K, Lidén C, Julander A.
Contamination of skin and surfaces by cobalt in the hard metal industry.
[manuscript, submitted]

IV. **Kettelarij J**, Midander K, Lidén C, Bottai M, Julander A.
The neglected exposure route? Cobalt on skin and its associations with urinary cobalt levels.
[manuscript, submitted]

ADDITIONAL PUBLICATIONS

Publications during doctoral studies that were not included in this thesis

Midander K, Julander A, **Kettelarij J**, Lidén C.
Testing in artificial sweat - Is less more? Comparison of metal release in two different artificial sweat solutions.
Regul Toxicol Pharmacol. 2016 Nov;81:381-386. doi: 10.1016/j.yrtph.2016.09.021.

Midander K, **Kettelarij J**, Julander A, Lidén C.
Nickel release from white gold.
Contact Dermatitis. 2016;75(6):370-376. doi: 10.1111/cod.12681.

Gumulka M, Matura M, Lidén C, **Kettelarij J**, Julander A.
Nickel exposure when working out in the gym.
Acta Derm Venereol. 2015 Feb;95(2):247-9. doi: 10.2340/00015555-1917.

Julander A, Lidén C, **Kettelarij J**
Cobalt
In: Kanerva's Occupational Dermatology. John SM, Johansen JD, Rustemeyer Th, Elsner P, and Maibach HI.
[in production] Springer, 2019.

CONTENTS

LIST OF ABBREVIATIONS

A-Co	Cobalt concentration in air sample
AV	Swedish Work Environment Authority (Arbetsmiljöverket)
CEN	European Committee for Standardization
CLP	Classification, Labelling and Packaging
Co	Cobalt
Cr	Chromium
Crea	Creatinine
DMG	Dimethylglyoxime
EC	European Commission
ECHA	European Chemicals Agency
ED	Elicitation dose
IARC	International Agency for Research on Cancer
ICP-MS	Inductively coupled plasma mass spectrometry
In	Indium
IOM	Institute of Occupational Medicine
LOD	Limit of detection
Ni	Nickel
OEL	Occupational Exposure Limit
PAMP	Pathogen-Associated Molecular Pattern
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
Rh	Rhodium
S_B-Co	Cobalt skin dose before start of the work day
S_E-Co	Cobalt skin dose at the end of the work day
SG	Specific gravity
TLR-4	Toll-like receptor 4
U-Co	Urinary cobalt concentration

LIST OF DEFINITIONS

Absorption	"(Dermal, percutaneous and skin absorption): the diffusion of chemicals from the outer surface of the skin to the receptor fluid of an in vitro diffusion cell, or systemic circulation." (Organisation for Economic Co-operation and Development (OECD) 2004)
Alloy	"A metallic material, homogeneous on a macroscopic scale, consisting of two or more elements so combined that they cannot be readily separated by mechanical means" (European Chemicals Agency (ECHA) 2017b)
Biological monitoring	Measuring of biomarker
Biomarker	In this thesis used as biomarker of exposure. It is an "indicator of changes or events in biological systems. Biological markers of exposure refer to cellular, biochemical, analytical, or molecular measures that are obtained from biological media such as tissues, cells, or fluids and are indicative of exposure to an agent." (International Programme on Chemical Safety (IPCS) 2004)
CLP Regulation	A European Union regulation for classification, labelling and packaging of substances and mixtures in the EU, based on the United Nations' Globally Harmonised System (GHS). It has the purpose "to ensure a high level of protection of health and the environment, as well as the free movement of substances, mixtures and articles." (ECHA 2018a)
Elicitation dose	Dose of an allergenic substance at which 10% (ED_{10}) or 50% (ED_{50}) of allergic individuals develop allergic contact dermatitis (Fischer *et al.* 2015)
Occupational contact dermatitis	Contact dermatitis for which a positive relationship has been established between exposure and occupation
REACH	"Regulation of the European union, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the hazard assessment of substances in order to reduce the number of tests on animals." (ECHA 2018b)

1 BACKGROUND

Cobalt was used as a pigment already four millennia ago in Egypt and Persia (Barceloux 1999), but was recognized as an element only as recently as 1735, by the Swedish chemist Georg Brandt (Brandt 1735). The name of this element probably comes from German miners in the 16th century who tried to smelt something they believed was silver ore, but was actually cobalt ore. Toxic arsenic fumes (cobalt arsenide) were released and no silver could be won, which is why they believed the ore was cursed by goblins; in the German language called "kobold" (Emsley 2001).

In fact, exposure to cobalt as an element is not without risk. Besides adverse health effects on the respiratory system, cobalt is one of the most important sensitising metals, and it is an important contact allergen in the general population and among workers (Lidén *et al.* 2011, Pesonen *et al.* 2015). The sources of exposure to cobalt in the general population are diffuse. Because of its specific uses, the presence of cobalt exposure at work is often more obvious. For that reason, adverse health effects of cobalt are often studied in occupational settings.

1.1 OCCURRENCE AND USE

Cobalt ores are mainly sulphides mixed with copper or nickel oxide, but they can also be cobalt arsenides. Cobalt is commonly a by-product from the extraction of nickel and copper. The extraction process varies from ore to ore and companies often manufacture cobalt chemicals direct from ores, concentrates or slag. Electro refining will provide a cathode of pure cobalt metal (99.98%) that is further processed into different types of raw material like powders or ingots (Cobalt Development Institute (CDI) 2015). Cobalt has many favourable properties, including high resistance to wear and oxidation, high conductivity, and ferromagnetic properties. Due to that, it is used in many market segments, with main uses for rechargeable batteries, superalloys (for example used in gas turbine engines), hard metals (cemented tungsten carbides), catalysts, pigments, and magnets (United States Geological Survey (USGS) 2017). Nano-sized (<100 nm) cobalt particles are of increasing interest and examples of uses are in industry for proton exchange membrane fuel cells (Wang et al. 2013), and in medicine for magnetic resonance imaging (MRI), magnetic hyperthermia, and drug delivery (Amiri and Shokrollahi 2013).

Cobalt is a central atom in the structure of vitamin B12 (cobalamin), which is an essential vitamin for mammals (Hodgkin *et al.* 1956). Naturally occurring vitamin B12 is produced by microorganisms and is mostly found in animal products like fish, meat, eggs and milk. The recommended daily intake of vitamin B12 is 2.0 µg for adults in Sweden and between 0.5 and 1.3 µg for children between 0 and 9 years old (Swedish National Food Agency 2018). The information in this thesis will not relate to vitamin B12, but only to cobalt and its compounds, since exposure to the latter is responsible for adverse effects on human health.

1.2 OCCUPATIONAL EXPOSURE TO COBALT

In the area of occupational hygiene, exposure is defined as the "contact between an agent and a target" (International Programme on Chemical Safety (IPCS) 2004). Depending on exposure conditions, like the exposure route, hazard of the substance, and dose, there is a risk of adverse effects after exposure.

High exposure to cobalt might occur in occupations where cobalt-containing materials are produced and used, including hard metal workers, dental technicians, workers in the electronics industry and construction workers (Day *et al.* 2008, Julander *et al.* 2014, Julander *et al.* 2010, Kettelarij *et al.* 2014, Kettelarij *et al.* 2016, Klasson *et al.* 2016, Lee *et al.* 2001, Linnainmaa and Kiilunen 1997, Rystedt and Fischer 1983, Scansetti *et al.* 1994, Shiao *et al.* 2004, Uter *et al.* 2004). Hard metal workers and dental technicians may be exposed on skin or through air, for example when grinding or shaping cobalt-containing alloys. Additionally in the hard metal industry, handling of cobalt powder as a raw material may also result in both skin and respiratory exposure (Fischer and Rystedt 1983).

1.3 COBALT EXPOSURE

1.3.1 Skin exposure

Skin exposure to metals can take place via direct skin contact with metallic surfaces and other metal containing materials in solid or powder form, or by deposition of airborne metal-containing particles onto skin (Schneider *et al.* 1999). A deposited skin dose may become available for permeation or penetration of the skin. The dose and its fate on skin is determined by the character of the contact and the physiological characteristics of the skin. Characteristics include the pH, temperature, and the presence of salts, amino acids, proteins and skin surface lipids that can dissolve, form complex with or oxidise metal atoms present in the naturally oxidised surface of metallic materials (Girod *et al.* 2012, Taylor and Machado-Moreira 2013). After exposure, the metals may be removed from the skin again by washing and abrasion (Schneider *et al.* 1999), or after complete turnover of the stratum corneum (14 days) or epidermis (47 days) (Bergstresser and Taylor 1977), depending on how deep the metals have penetrated the skin.

1.3.2 Respiratory exposure

Respiratory exposure to cobalt occurs when particles become airborne. In occupational settings this may happen for example when creating metal dust by grinding or polishing alloys, when handling powders or other small-scaled particles, or when using techniques like welding and spray-painting. In general, inhaled particles may be transported to different regions of the respiratory tract. This is mainly affected by particle size, but also by particle charge, and parameters like inhalation flow rate, and individual differences like the health state and airway characteristics (Koullapis *et al.* 2015). In humans, the nose or oral pharynx trap most particles that are larger than 10 µm (Leikauf 2013). Most particles between 2.5 and 10 µm stay in the large proximal airways, and smaller particles may be transported to the

lower airways and alveoli. Particles smaller than 0.5 µm are also mostly deposited in the upper airways due to diffusion, a mechanism where these particles collide with gas molecules. However, nanoparticles (1-100 nm) may be deposited in different parts of the respiratory tract. Particles of 1 nm are mainly (90%) deposited in the nose and oral pharynx, whereas about 50% of 20 nm particles deposit in the lower airways and alveoli (Oberdörster *et al.* 2005).

1.3.3 Oral exposure

For the general population, cobalt exposure most likely takes place through ingestion of cobalt-containing food (Kim *et al.* 2006). In occupational settings, unintentional ingestion of cobalt can occur from mucociliary clearance after air exposure, when eating food that contains cobalt naturally or due to contamination from air or skin, when contaminated hands or objects come in contact with the mouth, or when cobalt is deposited around the mouth or in the oral cavity (Cherrie *et al.* 2006). Another way of oral exposure to cobalt is through use of dental materials made of cobalt-chromium alloys (Schalock *et al.* 2012).

1.4 COBALT UPTAKE AND ELIMINATION

Cobalt exposure may take place simultaneously through the respiratory, dermal and gastrointestinal routes. Absorption rate, excretion route and time of excretion are all influenced by the exposure route, but also by factors like the magnitude and duration of exposure, and physicochemical properties of the cobalt compound (Kim *et al.* 2006, Paustenbach *et al.* 2013). In animals, urinary excretion is the primary elimination route of cobalt after respiratory exposure (Kim *et al.* 2006). Depending on the absorption, oral exposure to cobalt may result in faecal or urinary elimination (Paustenbach *et al.* 2013).

Most studies that investigate cobalt kinetics, have focused on cobalt (II) chloride ($CoCl_2$) or cobalt oxides, and not metallic cobalt. It is therefore unknown how comparable the kinetics of $CoCl_2$ and cobalt oxides to the kinetics after exposure to metallic cobalt. Metallic cobalt has very low water-solubility (International Agency for Research on Cancer (IARC) 2006), and it can therefore be speculated that its kinetics in the human body are comparable to that of the insoluble cobalt oxides.

1.4.1 Kinetics after skin exposure

Only few studies have found evidence for systemic uptake of cobalt through skin. Uptake of cobalt through skin is often considered low or non-existent since few studies have found evidence of skin penetration *in vivo* (Linnainmaa and Kiilunen 1997, Scansetti *et al.* 1994). A standardized *in vitro* method to determine skin absorption is the use of diffusion cells (Organisation for Economic Co-operation and Development (OECD) 2004). A piece of human or animal skin is clamped between two compartments of the diffusion cell, with the epidermis facing towards the upper part of the cell that contains the chemical of interest (donor compartment). The lower part (receptor compartment) contains the receptor solution that mimics the salt concentration and temperature of blood. The receptor solution is analysed

to evaluate any skin absorption of the substance at the end of the study period (max. 24 h), or samples are collected at different time points during the experiment to evaluate skin absorption after different time intervals. Skin samples can be analysed after exposure to assess how much of the chemical is retained in the epidermis and dermis.

In vitro diffusion cell studies with human skin have shown skin absorption of cobalt ions (Larese Filon *et al.* 2013, Larese Filon *et al.* 2004). An absorption rate of 0.06% of cobalt ions from cobalt powder (<2 µm) was seen after 24 h on intact skin, whereas absorption rates after exposure to cobalt nanoparticles for 24 h on intact skin and damaged skin were 0.00085% and 0.19%, respectively (calculated from (Larese Filon *et al.* 2013)). Cobalt ions and nanoparticles can also accumulate in the skin and serve as a depot for cobalt ion release (Larese Filon *et al.* 2013, Larese Filon *et al.* 2004).

Due to the scarce number of studies on skin absorption of cobalt, and the fact that most studies only include *in vitro* data, there is no information about the distribution of cobalt in the human body after exposure on skin. Biomonitoring of cobalt exposure in general has been performed in urine and blood, although concentrations in blood may decrease more slowly than in urine when exposure levels are high (Ichikawa *et al.* 1985).

Two studies were performed with healthy volunteers, which showed that urinary cobalt excretion after cobalt skin exposure varied between persons over time. In a study by Linnainmaa and Kiilunen, five healthy volunteers were exposed for 1 hour on skin to a coolant solution containing cobalt (1600 mg cobalt/L) (Linnainmaa and Kiilunen 1997). Urinary cobalt concentrations were elevated in four out of five persons. Two persons reached a peak elimination within 4-6 hours. The other two did not show an increase in urinary cobalt concentration until 10-15 hours after exposure, and concentrations were still increasing 24 hours after exposure. The timing of a peak in urinary concentrations and the declination to baseline level is therefore unknown. Four volunteers in another study were exposed on skin to a powder mixture of 85-95% tungsten carbide and 5-15% cobalt, or to a waste dry powder (cobalt content unknown) on a single occasion (Scansetti *et al.* 1994). Urinary cobalt excretion was followed for three days after a 90 min exposure. Urinary excretion of cobalt was elevated after both exposure conditions, and reached a maximum within 24 h for all volunteers, and levels remained high for up to 48-60 h. It is not known if and how urinary cobalt concentrations are influenced by long term skin exposure. In a recent study by Klasson *et al*., cobalt concentrations in blood were correlated to respiratory and skin exposure (Klasson *et al.* 2016). Linear regression showed an increase of 3-14% in cobalt blood levels for every doubling of cobalt skin exposure, although air exposure had a much greater influence (39-83% increase) on cobalt blood levels.

1.4.2 Kinetics after respiratory exposure

After deposition of insoluble cobalt particles (like cobalt oxide) in the lungs, cobalt may be retained, or it can dissolve and be absorbed into the blood, or transferred mechanically to the gastrointestinal tract (mucociliary clearance) (Kim *et al.* 2006). One animal study has shown

that approximately 30% of the cobalt (cobalt oxide) administrated was absorbed in lungs (Wehner *et al.* 1977). No absorption data for humans are available for cobalt metal or cobalt oxide.

After respiratory exposure, faecal cobalt clearance is initially elevated. Nevertheless, the primary elimination route is via urinary excretion, as shown in animals (Kim *et al.* 2006). In human exposure studies, good correlation has been found between cobalt air exposure and cobalt elimination in urine (Alexandersson 1988, Apostoli *et al.* 1994, Ferdenzi *et al.* 1994, Linnainmaa and Kiilunen 1997, Lison *et al.* 1994, Scansetti *et al.* 1994). Elimination of inhaled insoluble cobalt particles may follow three-phase kinetics: mechanical clearance (2-44 hours after exposure), macrophage-mediated clearance (10-78 days), and long-term clearance (several years) (Kim *et al.* 2006, Leggett 2008, Mosconi *et al.* 1994, Simonsen *et al.* 2012). The third phase of long-term clearance is mostly seen in individuals with high cobalt body-burden, and might be explained by accumulation of cobalt in certain tissues, particularly in the liver (Mosconi *et al.* 1994).

1.4.3 Kinetics after oral exposure

Cobalt absorption rates from the gastrointestinal tract vary much between individuals. This is affected by compound solubility, amount of intake and co-intake with other compounds, and gender and nutritional status (Barceloux 1999, Paustenbach *et al.* 2013). One study showed absorption in healthy humans varying between <5% and >20% with different doses of cobalt (in the form of $CoCl_2$) (Smith *et al.* 1972). A study among individuals with an iron depletion showed absorption rates of $CoCl_2$ of up to 42% (Sorbie *et al.* 1971). Gastrointestinal absorption of non-water-soluble cobalt (cobalt oxide) has been found to be lower compared to water-soluble cobalt compounds, when studied in animals and humans (Paustenbach *et al.* 2013).

After oral exposure to cobalt in humans, unabsorbed cobalt is eliminated via faeces, depending on the health status of the individual (Kim *et al.* 2006). However, cobalt that is absorbed through the gastrointestinal tract is eliminated mainly via urinary excretion, and this elimination is fast, and probably within 24 hours or several days (Kim *et al.* 2006, Paustenbach *et al.* 2013).

1.5 ADVERSE HEALTH EFFECTS OF COBALT EXPOSURE

1.5.1 Contact allergy to cobalt

1.5.1.1 Mechanism

Contact allergy is an important effect of cobalt skin exposure (Lidén and Julander 2012). In general, the induction of contact allergy (sensitisation) can occur when a contact allergen penetrates into the epidermis and binds to peptides on antigen-presenting dendritic cells (Rustemeyer *et al.* 2011). A costimulatory innate immune signal is then needed to activate the dendritic cell and to let it migrate to the draining lymph nodes. In the lymph node, allergen-

specific naïve T-cells are activated, which proliferate and mature, consequently leaving behind allergen-specific memory T-cells (Rustemeyer *et al.* 2011). In the case of cobalt, cobalt ions work as haptens that mimic pathogen-associated molecular patterns (PAMPs) as they interact with parts of Toll-like receptor 4 (TLR-4) expressed on Langerhans cells (dendritic cells in the skin). Activation of TLR-4 is normally responsible for immune responses against lipopolysaccharides from gram-negative bacteria. Thus, this T-cell mediated adaptive immune response is a result of direct activation of the innate immune response (Schmidt and Goebeler 2015). The sensitising potential of a chemical can be tested using in vivo test methods. Cobalt is a potent skin sensitiser, as demonstrated with different *in vivo* animal and human sensitisation test methods (Basketter *et al.* 1999, Kligman 1966, Wahlberg and Boman 1978).According to the European Union Classification, Labelling and Packaging (CLP) Regulation, cobalt and several cobalt compounds are classified as skin sensitising (hazard statement H317) (European Commission (EC) 2008).

The elicitation phase of contact allergy leads to allergic contact dermatitis (Rustemeyer *et al.* 2011). This is the clinical disease, and involves re-exposure to the allergen, after which memory T-cells will be activated. These secrete cytokines to induce an inflammatory reaction at the site of re-exposure and to attract other inflammatory cells that will further increase the reaction. Contact allergy is a life-long condition, which stresses the importance of reduction and prevention of exposure to contact allergens.

The dose that will elicit allergic contact dermatitis in 10% of cobalt allergic individuals (ED_{10}) due to contact with cobalt varies between 0.0663 and 1.95 µg cobalt/cm^2, based on results from several patch test dose-response studies (Fischer *et al.* 2015). Studies have shown that doses within or above this range were deposited on fingers of workers in several occupations while performing their work tasks (Julander *et al.* 2010, Lidén *et al.* 2008a).

A few studies have investigated the ability of alloys to release metals and to induce an allergic skin reaction (Julander *et al.* 2009, Summer *et al.* 2007). The hard metal discs used in these studies released cobalt in artificial sweat. The same discs elicited contact dermatitis in cobalt-sensitised patients, while no reaction was seen in controls without allergy. This reflects good correspondence between release of cobalt from an item and patch test reactivity to the material in cobalt allergic individuals.

1.5.1.2 Diagnosis

Patch testing is the standard method to diagnose contact allergy in humans. Suitable concentrations of substances in test chambers (approximately 8 mm or 12 mm diameter; 0.5 or 1.1 cm^2). are applied to the upper back by adhesive tape for 2 days. If the person has a previously induced allergy to a test substance, this method will elicit allergic contact dermatitis at the test site (Johansen et al. 2015). The most common groups of contact allergens are metals, fragrances, preservatives, and plastic and rubber chemicals (Coenraads et al. 2011). The European baseline series for patch testing includes cobalt (as cobalt (II) chloride hexahydrate) in a concentration of 1% in petrolatum (Andersen et al. 2011). In

addition to this standard concentration, test substances can be used in a serial dilution patch test. With the results from such a test, a dose-response relationship can be established, which can be used to determine elicitation dose (ED) levels in patients with contact allergy (Fischer et al. 2015, Julander 2018).

1.5.1.3 Prevalence of cobalt contact allergy

A recent study showed that the prevalence of contact allergy to cobalt was 2.2% among a sample from the general population in multiple countries in Europe, with fewer men being sensitised to cobalt compared to women (1.1 vs. 3.0%) (Diepgen *et al.* 2016). Cobalt allergy among a birth cohort of adolescents in Sweden has shown a prevalence of 1.2% (Lagrelius *et al.* 2016). This shows that exposure to cobalt probably occurs early in life, but sources are unknown. In patch tested dermatitis patients, prevalence numbers are higher compared to the general population. Between 4.8 and 13.6% of contact dermatitis patients had positive patch test reactions to cobalt chloride, varying between countries (Uter *et al.* 2012). In a multicentre study involving centres from 11 European countries, the prevalence of cobalt allergy was 7.9% among children with atopic dermatitis aged 1-16 years (Belloni Fortina *et al.* 2015). One recent study of patients with occupational contact dermatitis (positive relationship between exposure and occupation) in Europe showed a contact allergy prevalence of 9.3% to cobalt (Pesonen *et al.* 2015). Contact allergy to cobalt is well known among for example construction workers, electronics workers, and dental technicians (Lidén and Julander 2012). A study from the eighties estimated a cobalt allergy prevalence of 2.5% among hard metal workers (Fischer and Rystedt 1983).

1.5.2 Respiratory health effects

Other adverse health effects of cobalt in humans are mostly related to the respiratory system. Cobalt metal and soluble cobalt (II) salts are classified as possibly carcinogenic to humans (group 2B), and the combination of cobalt metal with tungsten carbide (hard metal) is classified as probably carcinogenic to humans (group 2A) (International Agency for Research on Cancer (IARC) 2006). Besides that, cobalt is a respiratory sensitiser (H334) according to EU CLP regulation (EC 2008). Effects of exposure may be irritation in the upper and lower airways, occupational asthma and interstitial lung fibrosis (Barceloux 1999). In a recently published paper, a non-significant, but clear dose–response relationship was seen between cobalt exposure and lung function of Swedish hard metal workers (Rehfisch *et al.* 2012).

1.6 OCCUPATIONAL EXPOSURE LIMITS AND HAZARD STATEMENTS

To prevent adverse health effects, occupational exposure limits (OELs) have been established for many hazardous chemical agents. OELs are set for the inhalable dust fraction in workplace air. The inhalable fraction is “the mass fraction of total airborne particles that is inhaled through the nose and mouth” (International Organization for Standardization (ISO) 2012). Limiting the concentration of a substance in air protects the respiratory system, although the skin is also presumed to be protected by this limit. However, the skin can additionally be exposed through direct contact with substances. There are currently no

Swedish or EU exposure limits to protect workers against direct skin exposure to hazardous substances.

In 2012, the Swedish Work Environment Authority (Arbetsmiljöverket (AV)) reduced the Swedish OEL for cobalt and inorganic compounds in air from 50 µg/m^3 to 20 µg/m^3 (AV 2015). It aims to reduce the risk of lung function impairment and irritation of eyes, nose and throat. OELs for cobalt in workplace air vary between countries and change over time. In Europe, OELs vary between countries, and range from 10 to 100 µg/m^3 (Arbejdstilsynet 2011, Courtois and Cadou 2012, Great Britain Health Safety Commission 2011, Social- och hälsovårdsministeriets publikationer 2014:3 2014, Staatssecretaris van Sociale Zaken en Werkgelegenheid 1997, valid in 2016), whereas the OEL in the United States of America (USA) is 20 µg/m^3 (The American Conference of Governmental Industrial Hygienists (ACGIH) 2001). Reasons for these differences and changes may be the availability of scientific evidence, the interpretation of this evidence by different regulatory bodies, or the feasibility for the industry to comply with new regulations and limits.

Some countries have set occupational urine limit values that are extrapolated from the OEL in air in that country. In Finland, the Biomonitoring Action Limit (BAL) of 130 nmol/L (≈ 7.7 µg/L) is set to correspond with their OEL of 10 µg/m^3 (Kiilunen 2017). In the USA, a Biological Exposure Index (BEI) of 15 µg/L is compatible with an OEL in air of 20 µg/m^3 (The American Conference of Governmental Industrial Hygienists (ACGIH) 2001). Researchers from France have challenged the BEI calculations of the USA, and have proposed a lower Urinary Exposure Threshold (UET) of 5 µg/L corresponding to the USA OEL of 20 µg/m^3 (Martin *et al.* 2010). This lower UET takes into account individual variability and the time dependency between cobalt inhalation and urine excretion. However, limit values in urine usually do not take into account possible skin or oral exposure, and it is difficult to take into account all individual variability. Studies have shown large variability of urinary cobalt in the general population, with levels ranging from of <0.20 to 1.9 µg/L (men) and <0.20 to 2.7 µg/L (women) in Denmark (recalculated from (Kristiansen *et al.* 1997)) and from <0.12 to 2.05 µg/L in the United Kingdom (White and Sabbioni 1998). Exposure limits in urine are therefore not similar for different countries, and direct extrapolation to respiratory exposure is doubtful.

Furthermore, in the EU, the element cobalt should be labelled with hazard statement codes H317 (skin sensitiser), H334 (respiratory sensitiser), and H413 (may cause long lasting hazardous effects to the aquatic environment) (EC 2008). Other cobalt compounds may have additional labelling. For cobalt oxide, dichloride and sulphate it includes H302 (acute oral toxicity), and for cobalt dichloride and sulphate it additionally includes H350i (may be carcinogenic by inhalation) (EC 2008).

1.7 CONCOMITANT EXPOSURE TO OTHER METALS ON SKIN

Patch test reactivity to cobalt in allergic patients may be solitary, or concomitant to other allergens, particularly nickel or chromium. Concomitant reactivity to cobalt and nickel is

considered to be due to sensitisation to both allergens, rather than cross-reactivity (Lidén *et al.* 2016).

1.7.1 Nickel

Nickel is a transition metal that is often used for its ductility and high resistance to oxidation and corrosion. Nickel is mainly used in alloys such as stainless steels, for platings, as catalyst, or in coins and batteries. Examples of consumer products that widely contain nickel are jewellery, electronic equipment and hobby utensils (Lidén 2012, Ringborg *et al.* 2016). Occupational skin exposure to nickel is reported among metal workers and workers in the electronics industry, as well as in professions where hand-held tools or coins are often handled (Lidén 2012).

Nickel is a well-known sensitising metal, and one of the most frequent contact allergens in the general population. The mechanism of developing nickel allergy is similar to that of cobalt (Schmidt and Goebeler 2015). Furthermore, regarding the respiratory system, the IARC has classified nickel compounds as carcinogenic to humans (group 1) (International Agency for Research on Cancer (IARC) 2012).

The prevalence of contact allergy to nickel in the European general population is 14.5%, with a large difference in prevalence between women (22%) and men (5%) (Diepgen *et al.* 2016). The number is higher for dermatitis patients, with approximately 20% of patch tested dermatitis patients in Europe having contact allergy to nickel (Uter *et al.* 2016). Furthermore, the prevalence varies for different countries, among age groups and over time (Ahlström *et al.* 2017). This is likely due to variations in exposure, which are possibly the result of different regulations between countries and over time, and compliance with these regulations. Occupational contact dermatitis to nickel is seen in many professions, including electronics workers, hairdressers, and mechanics (Lidén 2012).

1.7.2 Chromium

Chromium got its name from the Greek word for colour (chroma), because of the colourful appearance of chromium compounds. It is therefore used as pigment in manufacturing of leather and in paints and inks. The main use of chromium is, however, in stainless steel. Just like cobalt, chromium has a high resistance to heat and oxidation (Sethi *et al.* 2012). Exposure to chromium(VI) is often seen in occupational settings. Industries where chromium-containing alloys and compounds are produced and used include stainless steel production, the aerospace industry where chromium-based paints are used, and agriculture where chromium is used in pesticides. The main oxidation states are 0, 2+, 3+ and 6+. The tri- and hexavalent states play the largest role in the development of adverse effects, and have therefore attracted much attention in terms of exposure assessments.

Chromium(VI) is a well-known skin sensitiser (Sethi *et al.* 2012). Compared to cobalt and nickel, less is known about the mechanism of chromium-induced contact allergy. A recent study showed that chromium(VI) activates the innate immune system in a more indirect way

compared to cobalt and nickel (Adam *et al.* 2017). Chromium(III) did not have the same capabilities, and is therefore considered to be less allergenic. Other oxidation states of chromium, including the ground state, are not able to act as haptens and are not considered to be allergens. This is the reason that chromium allergy is referred to as chromate allergy (Lidén *et al.* 2011, Sethi *et al.* 2012). In addition to the skin sensitising capabilities, chromium(VI) is a respiratory sensitiser, and the IARC has classified chromium(VI) as carcinogenic to humans (group 1) when exposed via the respiratory route (European Commission (EC) 2008, International Agency for Research on Cancer (IARC) 2012).

Contact allergy to chromate has a prevalence of 0.8%.in the European general population, with a slightly higher number in men (0.9%) compared to women (0.7%) (Diepgen *et al.* 2016). Among patch tested dermatitis patients in Europe, the prevalence is 4.3% (Uter *et al.* 2016).

Occupational contact dermatitis due to chromium exposure is seen in professions like leather goods workers and cast concrete product workers (Sethi *et al.* 2012). Presence of chromium(VI) in wet cement has been a main cause of chromium contact dermatitis in construction workers (Lidén *et al.* 2011). Since restriction of chromium(VI) content in cement, chromium in leather is a more important cause of chromium allergy.

1.7.3 Nickel and chromium(VI) restrictions

In recent years, it has become clearer from which items and materials people get exposed to nickel and chromium in their daily life. As a consequence, use of nickel in consumer products that come in direct and prolonged contact with the skin is restricted in the EU, as well as presence of chromium(VI) in cement and leather articles (European Commission (EC) 2009).

Nickel release is limited for items inserted into pierced parts of the body (>0.2 µg/cm^2/week) or items intended to come into direct and prolonged contact with the skin (>0.5 µg/cm^2/week) (EC 2009). In 2014, ECHA proposed a definition for "prolonged contact with the skin" to make the restriction clearer for compliance (European Chemicals Agency (ECHA) 2014). A request has been made to ECHA to publish a guideline document with articles that are covered by the new definition (EC 2014b). European Committee for Standardization (CEN) standards shall be used for showing compliance with the restriction, based on testing release of nickel in an artificial sweat solution (ECHA 2017a). Implementation of the nickel restriction is not done as carefully in all EU member states. As a result, the positive effect of the restriction, in terms of less induction and elicitation of nickel contact allergy, is mainly seen in the northern European countries (Ahlström 2017).

Since 2009, chromium(VI) is restricted in cement (<2 mg chromium(VI)/kg) (EC 2009). This has caused a shift in causative factors for chromium allergy. Nowadays, chromium in leather products is an important cause of chromium contact allergy among dermatitis patients (Lidén *et al.* 2011). Chromium(III) in leather, which is used for leather tanning, may oxidise to chromium(VI). Since 2015, the EU restriction therefore also covers leather articles that come into contact with the skin (<3 mg chromium(VI)/kg) (EC 2014a). It is too soon to foresee the

effect of chromium(VI) restriction in leather on the prevalence of contact allergy. At the same time, it has been shown that chromium(III) in leather may also elicit allergic contact dermatitis (Hedberg *et al.* 2018).

2 AIMS

The overall aim of this thesis was to study skin as target organ for occupational exposure to metals - in particular cobalt. This was achieved by examining sources of skin exposure to cobalt and/or nickel and chromium, quantifying occupational skin exposure to these metals, and evaluating the significance of cobalt skin exposure as determinant of urinary cobalt concentrations as biomarker of exposure. The thesis focuses on two occupational groups in which work with cobalt is performed: dental technicians and hard metal workers.

The specific aims were:

- to examine the release of cobalt, nickel, and chromium from dental alloys and tools handled on a daily basis by dental technicians (paper I)
- to quantify skin and air exposure and concentrations in urine of cobalt, nickel, and chromium for dental technicians (paper II)
- to evaluate surface contamination of cobalt within a hard metal industry (paper III)
- to quantify cobalt skin exposure in a hard metal industry (paper III and IV)
- to quantify cobalt air exposure and cobalt concentrations in urine in a hard metal industry (paper IV)
- to evaluate associations between cobalt skin and air exposure, and urinary cobalt concentrations (paper IV)

3 METHODS AND CONSIDERATIONS

The materials and methods used in the different studies of my PhD-project are summarized in this section. For a more detailed description, the reader is referred to the individual papers (paper I-IV). In addition, methodological considerations are described.

3.1 OVERVIEW

Two occupational settings were chosen where cobalt exposure takes place (Figure 3.1). In **project I**, sources of skin exposure to cobalt, as well as chromium and nickel, were examined for dental technicians. In addition, measurement of skin and air exposure, and biomonitoring of these metals in urine was performed. The focus of **project II** was on cobalt skin exposure of hard metal workers. Exposure sources were assessed by evaluating surface contamination. Skin and air exposure to cobalt were quantified and associations of each of these exposure sources with urinary cobalt concentrations were assessed.

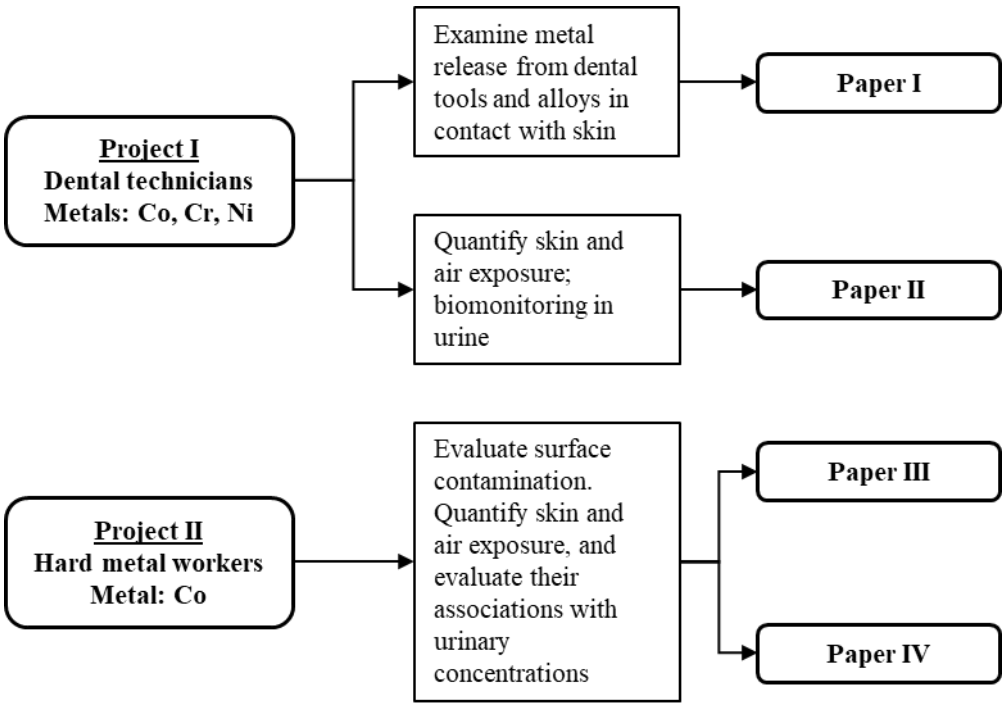


Figure 3.1. Overview of projects, aims and papers included in this thesis

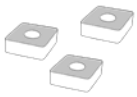
An important aspect when collecting samples and preparing them for chemical analysis of metals is to avoid contamination in the sampling materials. Therefore, throughout the work presented in this thesis, all plastic materials used were washed in 10% HNO_3 (analysis grade) for at least four hours, after which they were rinsed four times in deionized water. After this, materials were left to dry and were then packed into clean, air-tight plastic bags. During all handling of these items, collection of samples, and work in the laboratory, the operators always wore gloves to further reduce the risk of contaminating the samples. In addition, field blanks were collected for each sampling day at work places, and laboratory blanks were also collected and analysed to control for possible contamination.

3.2 STUDY LOCATIONS AND PARTICIPANTS

To reveal sources of cobalt skin exposure for dental technicians, data for paper I were collected at the facility of the dental technology study program of the Department of Dental Medicine, Karolinska Institutet, Sweden. Here, dental technology students learned how to manufacture prostheses, crowns and other dental designs, using tools and alloys equivalent to those used at dental laboratories.

The dental technology program at Karolinska Institutet ceased their activities in the fall of 2013. This is why data for paper II were instead collected at a private dental laboratory, with 21 employees, of which 13 consented to participate. Dental technicians may use cobalt-chromium (CoCr) alloys as casting alloys to make dental prostheses and implants. In the study for paper II, eight dental technicians performed work with CoCr alloys of different compositions. They performed work including grinding, sandblasting and polishing of the material (CoCr-exposed group). Five dental technicians performed this work only with plastics, gypsum and/or porcelain during the study day (non-CoCr-exposed). All participating technicians were considered to be possibly exposed to nickel, since they may use nickel-containing tools during work, as identified in paper I.

Table 3.1. Description of exposure groups in the hard metal facilities with different levels of cobalt exposure. (Figure adapted from manuscript paper III, Fig. 1)

Exposure groups	Work tasks
 Controls	Office work; procurement; machine development
 Raw material	Pressing; press reparations; powder production; powder cleaning and housekeeping; warehouse work at powder storage
 Sintered material	Charge building; work at chemical vapour deposition (CVD) and physical vapour deposition (PVD); product inspection
 Final product	Turning; turning of hard metals; repairing; operating milling and turning machines; production leading

Data for project II (paper III and IV) were collected at different production sites of a hard metal company. In total, 76 workers gave their informed consent to participate. In the hard metal industry, products are made out of cemented tungsten carbide, a hard metal alloy for which cobalt is used as a binding agent in a concentration of 6-30%. A mixture of tungsten carbide and cobalt powder is granulated and pressed into a desired shape. The pressed material is subsequently sintered at 1400-1500°C, by which the material acquires its hardness and strength. The material is then optionally coated by chemical or physical vapour deposition (CVD or PVD). Hard metal workers were assigned to four exposure groups,

depending on the work task that they performed and the stage of that work task within the production process of hard metal tools (Table 3.1).

Workers from production areas (n=58) were categorised as working with raw materials (i.e. hard metal powder, n=24), sintered materials (work with alloys that are hardened under high temperatures, n=16), or work with the alloys in their final form (customer-like use of products, n=18). Eighteen office workers were considered to be non-exposed controls. For paper IV, data from all 76 participants were used. For paper III, data were used from a subgroup of participants (n=40) who were randomly selected to undergo additional skin sampling during their work shift.

Ethical permission for all studies was given by the regional ethical review board in Stockholm, Sweden (ethical permit no. 2012/1802-31/1).

3.3 SAMPLE COLLECTION

For the study in paper I, one dental alloy and 61 metal tools that commonly come in contact with the skin were selected in the stockroom of the Dental Medicine department. Four additional dental alloys that are often used by dental technicians, and that were known to contain cobalt and chromium were purchased elsewhere and tested in our laboratory. The 61 tools were categorised according to their function and use, yielding three categories of tools (grinding tools (n=21), hand-held tools (n=30), other tools (n=10)). The dental alloys were considered as a separate category.

Skin wipe samples, personal air samples, and spot urine samples were collected among dental technicians (paper II) and hard metal workers (Table 3.2). Different parts of the data from the hard metal workers was used to generate paper III and IV. Measuring of oral exposure was outside of the scope of the projects included in this thesis.

Table 3.2. Number of samples collected among 13 dental technicians and 76 hard metal workers (paper II-IV)

		Dental technicians (n=13)	Hard metal workers (n=76)
Skin wipe samples	Before shift	13	76
	2h no hand wash	13	40[A]
	End of shift	13	76
Personal air samples		10[B]	30[C]
Spot urine samples 24 h		89	563

[A]Randomly selected from 76 hard metal workers

[B]Randomly selected from 13 dental technicians

[C]Randomly selected from 58 hard metal production workers (exposed group)

Before the start of the work day, when participants had not changed into their workwear yet, skin wipe samples were collected to measure the baseline level of metals on skin. At that time point, participants were also asked to produce a midstream spot urine sample, and to continue to collect a sample of each void during the next 24 hours. After possible change into

workwear, 10 randomly selected dental technicians and 30 workers from hard metal production areas received an air pump with Institute of Occupational Medicine (IOM) sampling head, which collected the inhalable fraction on a filter during their complete work day. All dental technicians and some randomly selected hard metal workers underwent additional skin sampling at some point during the work day, before and after they had performed their normal work tasks for 2 hours without washing their hands. Skin wipe samples were collected again for all participants at the end of their work day. In addition, participants completed a questionnaire, and spot testing for cobalt and/or nickel was performed in the work environment of participating dental technicians and hard metal workers.

3.4 TEST METHODS FOR EXPOSURE SOURCES

The fact that an item, alloy, or powder contains a metal does not necessarily mean that it releases this metal to a considerable extent (Flint 1998). To assess potential skin exposure, metal ion release from items can be tested with for example a spot test or in artificial sweat. The results of these two methods respectively, give an indication or a concentration of the metals that are potentially available for skin deposition.

3.4.1 Spot tests (paper I and III)

The cobalt spot test and the dimethylglyoxime (DMG) test are validated qualitative colorimetric tests that are used to study the presence of cobalt and nickel, respectively (Julander *et al.* 2011, Midander *et al.* 2013, Thyssen *et al.* 2010a). They are usually used on surfaces that may come in contact with the skin, and are quick and simple, and therefore used by dermatologists, allergic patients, and occupational hygienists. The reagent solution (50 µL) is applied on a cotton wool stick and rubbed against the surface of an item of interest for about 30 seconds. The cobalt spot test is classified as positive (colour change from bright yellow to orange or dark yellow), negative (no colour change), or doubtful (colour change other than an orange or dark yellow colour). In a similar way, the DMG test is classified as positive (colour change from colourless to pink), negative (no colour change), or doubtful (colour change other than a pink).

The DMG test was purchased from Chemotechnique Diagnostics (Chemo-Nickel Test™; Vellinge, Sweden) for the study of metal release from dental tools and alloys (paper I). The cobalt spot test was freshly prepared in our laboratory for the studies of metal release from dental tools and alloys (paper I) and from sources in a hard metal industry (paper III), according to a previously described method (Thyssen *et al.* 2010a). In the study for paper I, cobalt and nickel spot tests were performed in the stockroom of the Dental Medicine department for all tools and one alloy that was in stock, whereas four other commonly used dental alloys were purchased (K.A. Rasmussen AS, Solna, Sweden) and spot tested in our laboratory. In the hard metal industry of project II, the cobalt spot test was used to identify possible sources for cobalt skin exposure (paper III). Items were not cleaned before testing, which was thought to resemble normal use of the items.

3.4.2 Artificial sweat (paper I)

A quantitative method to test metal release *in vitro* is immersion of items in artificial sweat. The artificial sweat used in paper I is a solution described in the reference test method for demonstrating compliance with the EU nickel regulation (EN1811) (CEN 2011). This standardized method is supposed to resemble *in vivo* conditions, when metallic items come in contact with the skin. It aims at testing nickel release from items intended to be in prolonged contact with the skin, but can also be used to test release of other metals. The artificial sweat solution is a simple solution that only mimics the salt content of human sweat, not its biological complexity.

For the study in paper I, a selection of the dental tools (n=21) and all five dental alloys were tested in artificial sweat. Besides selecting tools based on the outcome of the spot test (positive, negative and doubtful tools for both spot tests), selection was also made based on their size, as they should easily fit into a 60 mL plastic container and should not need to be disassembled.

Artificial sweat was freshly prepared on the study day by mixing deionised water with 0.5% (m/m) sodium chloride, 0.1% (m/m) lactic acid, and 0.1% (m/m) urea, and using sodium hydroxide solution to adjust the pH to 6.5 (± 0.05). At least 1 mL artificial sweat per cm^2 surface area of the item was added to each container, or more in case that was not enough to cover the entire surface area. This was taken into account in the final calculations of the release rates. Tools were cleaned in a sodium dodecyl benzene sulphate solution and rinsed with deionized water. Thereafter, they were immersed for one week (exposure duration as described in the standard method), since we only had one specimen of each. Triplicate samples of the dental alloys were immersed in artificial sweat for 2 min, 30 min, 1 hr, 24 hr and 1 week, to also resemble short- and moderate-term exposure durations. After exposure, solutions were transferred to clean plastic containers and acidified with nitric acid to get a pH <2, in order to prevent redeposition of dissolved metal ions. The acidified solutions were stored at 6°C until chemical analysis.

3.5 TEST METHODS FOR EXTERNAL AND INTERNAL EXPOSURE MEASUREMENTS

For the projects in this thesis, skin wipe sampling and air sampling were used to measure external exposure to cobalt, chromium and nickel. Even though oral exposure was not measured directly in these studies, questionnaires did include several questions that were related to possible oral exposure. Urinary concentrations of these metals were used as exposure biomarkers. Participants received their personal results from skin wipe, air and urine sampling after all analysis was performed. The companies only received information on group level, and advice was given on how to reduce exposure. Tape stripping and visualisation of cobalt (particles) on skin

3.5.1 Skin wipe samples (paper II-IV)

Skin exposure was measured by collecting acid wipe samples from the skin. This is an established method to determine a metal skin dose, with a recovery of approximately 93%, assessed for cobalt, nickel and chromium (Lidén *et al.* 2006). The 1% nitric acid used in this method for the sampling of metals from the skin surface, is considered harmless on healthy skin. Sampling areas were marked and then wiped with three consecutive wipes, each moistened with 0.5 mL 1% HNO_3. Each wipe was passed over the area 8-10 times with gentle pressure. The three wipes were pooled in one container and extracted in 1% HNO_3.The extracts were stored at 6°C until analysis.

In all cases, the sampling area for skin wipe sampling was an area of 2 cm^2 on the volar aspect of the non-dominant index finger (Figure 3.2). This area was chosen based on previous experience with acid wipe sampling on multiple skin surfaces in different occupations (Lidén *et al.* 2008a). Similarly, the reference area was an area of 2 cm^2 on the volar aspect of the non-dominant little finger (Figure 3.2). The non-dominant hand was chosen for sampling because in the two studied occupational groups, the materials that contain the metals of interest are mainly held with the non-dominant hand while the dominant hand often holds a tool to work the material. Hence, the skin dose on the non-dominant hand is expected to be the highest dose.

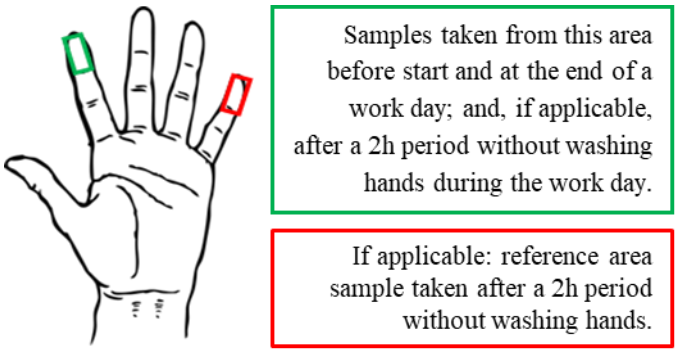


Figure 3.2. Skin wipe sampling areas on non-dominant hand of dental technicians and hard metal workers

For the studies in paper II and IV, skin wipe samples were collected for all participants before the start and at the end of their work day. For the studies in paper II and III, all participating dental technicians and 40 randomly selected hard metal workers underwent additional skin wipe sampling after a 2-hour period during which they were asked not to wash their hands, if possible. This period started either directly at the start of their work day, or sometime during their work day, or 2 h before the end of their work day. Before the start of the 2-h period, both hands were cleaned with 1% HNO_3 and rinsed with deionised water to remove all metals. A reference area was covered with a band aid. Normal work tasks were then performed for two hours, after which the skin was sampled again, including the reference area. This 2-h sampling period was chosen to be able to compare results more easily to other occupational exposure studies using this method (Julander *et al.* 2010, Lidén *et al.* 2008a).

3.5.2 Air sampling

For the studies in paper among dental technicians (paper II) and hard metal workers (paper IV), sampling of the inhalable fraction (also called air sampling in this thesis) was performed according to EN 481 (CEN 1993). An IOM sampling head was used in which a 25-mm filter cassette was mounted. The air flow was set to 2 L per minute, and continuously checked before, during and at end of sampling. Prior to start of measurement and after measurements, the filter mounted in the cassette was placed in a climate chamber of constant temperature and humidity, to acquire a constant weight. In this study the time chosen for this was one week, which should be enough to keep the imprecision in dust weight between samplers as low as possible according to a study by Lidén and Bergman (2001) (Lidén and Bergman 2001). Weighing of the filter and subsequent chemical analysis of the filter yields two different measures. The first is a measurement of the amount of dust collected on the filter (mg/m^3). The second is a quantification of the substance of interest that is present in the dust ($\mu g/m^3$). These amounts can then be compared to an OEL.

It was not practically feasible to monitor respiratory exposure for all participants, because of limited equipment. Ten dental technicians (paper II) and 30 hard metal workers from production areas (paper IV) underwent personal air monitoring to measure airborne metal exposure. In paper IV, no air samples were collected in the control group (office workers), due to noise of the air pumps that could interfere with their work tasks. In addition, the company performs air sampling on a regular basis to monitor exposure of the employees. This routine monitoring is randomly performed among workers in all parts of the facility, including among our control group. The company's own measurements showed no cobalt air exposure among office workers.

3.5.3 Urine sampling

Midstream spot urine samples were collected during 24 hours from the start of the study day for all participants in paper II and IV. Each void was collected separately (up to 250 mL), to be able to study changes in urinary concentrations of metals over time, and to avoid exposure misclassification. All urine samples were stored in a fridge within two days after collection.

Up to 40 mL of each urine sample was transferred to a clean tube, which was stored at -18°C until chemical analysis. A small aliquot of each urine sample was used to measure specific gravity and creatinine content. Eventually, specific gravity was chosen to correct for dilution variations, by adjusting for mean specific gravity of the urine samples in the research populations (1.015 in paper II; 1.016 and 1.019 in paper IV). In studies comparing individuals with large differences in age, gender, muscle mass or meat intake, it is considered to be more appropriate to correct for specific gravity instead of creatinine content in urine samples, because the latter fluctuates more for those variables (Suwazono *et al.* 2005).

Two days before analysis, frozen urine samples were thawed. To get any deposited metal content back into suspension, samples were acidified with 67% HNO_3 to achieve a

concentration of approximately 1% HNO_3. The acidified samples were then stored for 48h in a fridge at 6°C until analysis.

3.5.4 Questionnaires

For the studies in paper II-IV, a questionnaire was composed to be answered by all workers. It included questions about age, gender, employment years, vitamin use, smoking habits, current and one-year eczema prevalence on hands and other parts of the body, work tasks performed on the study day, and use of personal protective equipment like gloves and respiratory protection.

For the study in paper II, data about age, gender, employment years, work tasks, use of protective equipment, and the one-year prevalence of hand eczema among dental technicians were used to answer the specific aims of the study.

Questionnaire data of hard metal workers in project II were used for both paper III and IV. To answer the specific study aims, questionnaire data about workers' age, gender, work tasks, and use of gloves were used (paper III and IV). Data about smoking, vitamin use, employment years, and use of respiratory protection among the participating hard metal workers were only used for paper IV.

3.6 CHEMICAL ANALYSIS

Chemical analysis of skin wipe extracts, digested air filters and diluted urine samples was performed with inductively coupled plasma-mass spectrometry (ICP-MS). This technique allowed for multi-element analysis of metals in a large concentration range in different media.

3.6.1 Instrument information

Artificial sweat samples for the study in paper I were analysed ICP-MS with a hexapole collision cell (XSeries II, Thermo Scientific, Waltham, MA, USA) at the department of Applied Environmental Science, Stockholm University, Sweden. For the studies in paper II-IV, all acid wipe samples were analysed at our laboratory by ICP-MS (iCAP™ Q; Thermo Fisher Scientific, Waltham, MA, USA) with an ASC-520 auto sampler (Teledyne CETAC technologies, Omaha, NE, USA). Urine samples of dental technicians were analysed by ICP-MS in our laboratory as well. Due to technical difficulties with our own instrument at a later stage, urine samples of hard metal workers were analysed by us at the unit of Occupational and Environmental Medicine at Örebro University hospital. An ICP-MS of the same type as ours was used there, but with an SC-4DX auto sampler (Elemental Scientific, Omaha, NE, USA). The laboratory at this unit in Örebro is accredited according to SS-EN ISO/IEC 17025 for measuring elements in air samples. Analysis of air samples from dental technicians and hard metal workers was therefore performed by this certified laboratory, on the same ICP-MS instrument that we used for urine analysis of hard metal workers.

3.6.2 Preparation of samples and calibration standards

All samples, except filter extracts from air sampling, were diluted 10 times in 1% HNO_3 before analysis. Air filter extracts were instead diluted 500 times in 1% HNO_3. Samples were further diluted and re-analysed if values were higher than the highest calibration standard after the first analysis.

For analysis of diluted acid wipe extracts in the studies of paper II-IV, calibration standards of 0, 0.1, 1, 5, 10, 50, 100 and 500 µg/L were prepared from stock solution (Teknolab, Norway, Lot: F2-CO02044, 996 ± 5 µg/mL cobalt in 3% HNO_3 (v/v)) in 1% HNO_3. Samples and calibration standards were spiked with 1 µg/L indium (In) as an internal standard to enable the monitoring of instrument performance during the analysis. A variation of ± 20% in internal standard recovery was generally accepted.

Pooled urine from healthy individuals without exposure to cobalt, was used for preparation of calibration standards for analysis of diluted urine samples. This was done to match the matrix of urine samples as closely as possible. To prepare pooled urine, four volunteers provided two urine samples each, and specific gravity was measured for each sample. From each sample, 180 ml was collected in an acid washed polyethylene bottle and the total was acidified to 1% HNO_3. Undiluted pooled urine was stored at -18°C until use. To serve as a matrix for calibration standards, the pooled urine was diluted 10 times in 1% HNO_3 to match the dilution of the urine samples. ICP-MS analysis of the pooled urine matrix for calibration showed concentrations of cobalt, chromium and nickel of 0.11 µg/l, <LOD, and 0.68 µg/l, respectively.

Standards for calibration; 0, 0.1, 1, 5, 10, 50 and 100 µg/L, were prepared from stock solution (Teknolab, Norway, Lot: F2-CO02044, 996 ± 5 µg/mL cobalt in 3% HNO_3 (v/v)) in diluted pooled urine. All diluted urine samples and calibration standards were spiked with 5 µg/L rhodium (Rh) as internal standard.

The analysis of artificial sweat release experiments (paper I) and all air samples (paper II and IV) was performed at Stockholm University and Örebro University hospital. In all samples and blanks in the study of paper I, Rh was used as internal standard at a concentration of 10 µg/L. For analysis of air samples from dental technicians (paper II), a 7-point calibration curve was used, whereas the air samples from the hard metal facilities (paper IV) were analysed using a 6-point calibration curve. Interpolation of scandium and rhodium recovery was used for internal standard correction in all air samples.

3.6.3 ICP-MS analysis

All analyses were performed in kinetic energy discrimination (KED) measurement mode, which uses helium gas to reduce polyatomic interference. Argon gas was used as cool gas, auxiliary gas and nebulizer gas for all analyses. Statistical certainty was ensured by triplicate analysis of each sample. In case of chromium analysis, no distinction could be made between

different oxidation states. The method limit of detection (LOD) was calculated as three times the mean standard deviation of the blank samples (table 3.3).

Artificial sweat release samples and acid wipe extracts, urine samples and air samples of dental technicians were analysed for their concentration of ^{60}Ni, ^{59}Co, and ^{52}Cr. In all samples of hard metal workers, the concentration of ^{59}Co was analysed only.

Table 3.3. Limits of detection (LOD) and percentage of samples with concentrations above LOD for each ICP-MS analysis performed in the studies for paper I-IV.

Type of sample	Data used in paper	LOD (percentage above LOD)		
		Cobalt	Nickel	Chromium
Artificial sweat (µg/L)	I	0.05 (100)	0.1 (100)	0.04 (100)
Skin wipe (µg/cm^2)	II	0.00016 (85)	0.00018 (100)	0.00021 (94)
	III and IV[A]	0.015-0.27 (98)	-	-
Urine (µg/L)	II	0.092 (70)	0.24 (100)	0.094 (49)
(uncorrected)	IV[B]	0.041-0.098 (72)	-	-
Air (µg/L)	II	0.007 (100)	0.3 (40)	0.11 (90)
	IV	0.007 (100)	-	-

[A] Analysis was performed in 4 batches

[B] Analysis was performed in 3 batches

- Not applicable

For the further evaluation of analysed metal concentrations, blank correction of skin wipe and air samples was performed. This was done by subtracting the mean amount of metal in the field blank from the amount of metal in the sample collected on the corresponding sampling day. Due to the use of pooled urine matrix for the calibration standards of urine sample analysis, the metal concentrations in urine of the workers were corrected for metal content of the pooled urine. For artificial sweat release samples and skin wipe samples, the metal concentration was converted to a dose, expressed in µg/cm^2.

3.6.4 Reference materials

As reference for quality control of the analysis of urine samples, Seronorm™ Trace Elements L-1 (LOT. 1403080) and L-2 (LOT. 1403081) (SERO AS, Billingstad, Norway) were used (paper II and IV). In the study of paper II and IV, STAMI filters (A4-197 (paper II); A4-0089 and A4-0116 (paper IV), National Institute of Occupational Health, Oslo, Norway) were used as reference material for quality control air sample analysis. The mean concentrations of cobalt, chromium and nickel in reference materials for quality control of the urine and air filter analysis were all within the limits provided by the manufacturers.

3.7 STATISTICAL ANALYSIS

No statistical evaluation was applied to the results of paper I. In paper II-IV, we worked with a small number of data points, or with data that were not normally distributed and that contained values below the limit of detection (censored data). We applied non-parametric statistical methods to this data, because those are more robust to outlying values and censored data, and do not assume a normal distribution.

3.7.1 Paper II

Statistical analyses for the study in paper II were performed with IBM SPSS statistics version 22. The difference in median skin doses between CoCr-exposed and non-exposed dental technicians was assessed with the exact version of the Mann–Whitney U-test. Concentrations below the LOD were replaced by LOD/$\sqrt{2}$ for statistical analysis (Hornung and Reed 1990). This was used to reduce bias of the median, instead of setting them to zero or ignoring the censored data, which would lead to a too low or too high estimation of the median, respectively (Hornung and Reed 1990).

3.7.2 Paper III

Statistical calculations of descriptive statistics, and comparison of median skin doses was made using the Wilcoxon's rank-sum test in Stata version 14 (StatCorp, College Station, TX, USA). Graphs and the Kruskal-Wallis test for comparing use of gloves among workers were made using GraphPad Prism version 7.01 (GraphPad Software, Inc., California, USA).

3.7.3 Paper IV

The data on cobalt skin dose before shift (S_B-Co) and at end of shift (S_E-Co), as well as cobalt in air (A-Co) and in urine (U-Co) all contained outliers, and urine data contained values below limit of detection. The median was therefore used to describe the central tendency of these variables.

Correlations between A-Co and either S_B-Co or S_E-Co were evaluated with the Spearman's rank correlation coefficient. Differences in the four variables across the four different groups were verified with the Wilcoxon's rank-sum test.

To verify if there were differences in median U-Co across the four groups, quantile regression was performed on log-transformed U-Co data. Furthermore, the association between the dependent variable U-Co and the independent exposure variables S_B-Co, S_E-Co and A-Co was also assessed using quantile regression on log-transformed variables. Log-transformation of all variables was used, because their distribution was right-skewed. Departures from linearity on the log-scale were tested by introducing splines. Instead of using the natural logarithm ($\log_e$), the logarithm to base 2 ($\log_2$) was used for transformation of the data. By using $\log_2$, the observed ratio (which is the exponentiated regression coefficient) can be interpreted as the ratio or percentage by which the dependent variable (U-Co) changes when the concentration of the covariate(s) (S_B-Co, S_E-Co, and/or A-Co) doubles. Figure 3.3

explains the rationale behind reporting the observed ratio instead of simply stating the regression coefficient, as well as the idea behind using the logarithm to base 2 instead of base e.

Quantile regression is not a conventionally used statistical method in the field of occupational hygiene to describe associations between variables. Instead, linear regression is the more commonly used statistical method. These two methods both estimate the central tendency of the outcome variable (Cade and Noon 2003, Koenker and Bassett 1978). The main difference is that quantile regression estimates any quantile of interest (e.g. median, quartiles) of the outcome variable, while linear regression estimates the mean of the outcome variable. The regression coefficients of these two methods can also be interpreted in a comparable way. A regression coefficient in quantile regression describes the change in the quantile that is being estimated (for example the median) that is associated with a unit-change in the corresponding covariate. On the other hand, the regression coefficient in linear regression represents the change in the mean instead of the quantile. In the study of paper IV, log-transformation of the data normalised the distribution of the data. However, linear regression of the data would be a bad choice, since it does not take into account censored data (i.e. measurements below the LOD). The quantile regression method permitted inclusion of all the available concentrations, without having to impute measures below the LOD. Besides that, it was robust to the outlying values and marked skewness of the outcome variable.

We estimated five quantile regression models separately: in model 1.1 and 1.2, the logarithm of S_B-Co was the only covariate, whereas in model 2.1 and 2.2 the only covariate was logarithm of S_E-Co. In model 3 logarithm of A-Co was the only covariate. In model 4, both the logarithm of S_B-Co and A-Co were included as covariates, and model 5 included the logarithm of S_E-Co and A-Co as covariates. In model 1.2 and 2.2, workers with high A-Co were excluded, thereby keeping the influence of air exposure on U-Co in these models as low as possible. Ninety-five percent confidence intervals were reported along with the point estimates of the median. Because the measures were taken repeatedly on the same individuals, we estimated the standard errors of the regression coefficient with 500 design-matrix bootstrap samples. All the analyses were performed in Stata version 14 (StatCorp, College Station, TX, USA).

Consider the following equation that describes our independent variable:

$$\mathrm{M}\,(\log\,(\text{U-Co})) = \beta_0 + \beta_1 \log(x) \qquad (1)$$

where the median (M) of the logarithm of urinary cobalt ($\log\,(\text{U-Co})$) is described by the Y-intercept (β_0), and the coefficient of the covariate (β_1) multiplied by the logarithm of the covariate of interest x (S_B-Co, S_E-Co or A-Co)[A]. Due to the use of the median it is possible to re-write equation (1) as

$$\log\,(\mathrm{M}\,(\text{U-Co})) = \beta_0 + \beta_1 \log(x) \qquad (2)$$

From equation (2) we learn that every time $\log(x)$ goes up by 1 unit, $\log\,(\mathrm{M}\,(\text{U-Co}))$ goes up by β_1.

The usefulness of the logarithm of 2 can be explained by a simple decomposition. If you take two concentrations of $\log(x)$ that are 1 unit apart, this can be formalised and decomposed like

$$\begin{aligned} \log(x_2) &= 1 + \log(x_1) \\ \log(x_2) - \log(x_1) &= 1 \\ \log\frac{x_2}{x_1} &= 1 \\ \frac{x_2}{x_1} &= 2^1 \\ x_2 &= 2 \cdot x_1 \end{aligned} \qquad (3)$$

From equation (3) we can conclude that one unit increase of $\log(x)$ is the same as a doubling of the concentration of x. Combined with the information in equation (2), this means that when concentration x doubles, then $\log\,(\mathrm{M}\,(\text{U-Co}))$ goes up by β_1.

So why do we report a ratio (observed ratio) to describe the outcome variable? This can be explained by visualising what it means when $\log\,(\mathrm{M}\,(\text{U-Co}))$ goes up by β_1. Consider the following equation

$$\begin{aligned} &\log\,(\mathrm{M}\,(\text{U-Co}_2)) - \log\left(\mathrm{M}\,(\text{U-Co}_1)\right) = (\beta_0 + \beta_1 \log(x_2)) - (\beta_0 + \beta_1 \log(x_1)) \\ &\log\,(\mathrm{M}\,(\text{U-Co}_2)) - \log\left(\mathrm{M}\,(\text{U-Co}_1)\right) = \beta_1 \log(x_2 - x_1) \end{aligned} \qquad (4)$$

For a doubling of concentration x (in other words $\log\,(x_2 - x_1) = 1$) it follows that

$$\begin{aligned} \log\,(\mathrm{M}\,(\text{U-Co}_2)) - \log\left(\mathrm{M}\,(\text{U-Co}_1)\right) &= \beta_1 \\ \log\left(\frac{\mathrm{M}\,(\text{U-Co}_2)}{\mathrm{M}\,(\text{U-Co}_1)}\right) &= \beta_1 \\ \frac{\mathrm{M}\,(\text{U-Co}_2)}{\mathrm{M}\,(\text{U-Co}_1)} &= 2^{\beta_1} \\ \mathrm{M}\,(\text{U-Co}_2) &= 2^{\beta_1} \cdot \mathrm{M}\,(\text{U-Co}_1) \end{aligned} \qquad (5)$$

This says that for a doubling of concentration x, the median urinary cobalt concentration is multiplied by 2^{β_1}, which is the observed ratio reported in paper IV. Multiplication by 2^{β_1} can be expressed as a percentage change in median U-Co (multiplication by e.g. 1.70 is the same as a 70% increase in median).

Figure 3.3. Explanation of the use of the logarithm to base 2 and the observed ratio in paper IV.

[A] S_B-Co: cobalt on skin before shift; S_E-Co: cobalt on skin at end of skin; or A-Co: cobalt in air

4 RESULTS AND DISCUSSION

A summary of the main findings of paper I-IV is described and discussed in this section. For a more detailed description, the reader is referred to the respective papers (paper I-IV).

4.1 COBALT, NICKEL AND CHROMIUM RELEASE FROM DENTAL TOOLS AND ALLOYS (PAPER I)

4.1.1 Release of cobalt and nickel was shown with spot tests

Of the 61 tested dental tools in the study of paper I, 23 were positive when tested with the cobalt spot test, whereas 20 tested positive for nickel release with the DMG test. Four out of five dental alloys were tested positive for cobalt release, and none for nickel release. The spot tests are simple and quick tools to screen for release of cobalt and nickel. However, it is difficult to quantify a limit of detection for these tests.

A previous study showed that the DMG test is able to detect nickel from items that release approximately 0.5 µg/cm^2/week in artificial sweat solution at specific test conditions (Thyssen *et al.* 2010b). Yet, the sensitivity and specificity of this test have been studied various times, including in paper I. When comparing the results of the DMG test with nickel release in artificial sweat (Paper I, Table 3 and 4), it is shown that even if the DMG test is negative or doubtful, nickel release can still be significantly higher than the 0.5 µg/cm^2/week threshold. Taken together with results from other studies, sensitivity of the DMG test ranged between 59.3 and 75% and specificity between 67 and 97.5% (Kettelarij *et al.* 2014, Thyssen *et al.* 2010b). This shows that there is a wide variation in performance of the DMG test among different studies.

The difficulty with the cobalt spot test is that the colour of the test is bright yellow, and the colour change is gradual with increasing concentrations of cobalt (Midander *et al.* 2013). This means that at low cobalt concentrations, the colour change may be difficult to see as it goes from clear yellow to slightly darker yellow. In paper I, it was therefore difficult in some cases to assess if a cobalt spot test result was positive or negative. Grading of the spot test result was therefore done by two persons, who did not know from which item the spot test came and how the spot test was graded by the other person.

4.1.2 Dental tools and alloys released metals in artificial sweat

Metal release from 21 out of 61 dental tools and from 5 dental alloys was tested quantitatively in artificial sweat. This demonstrated that all tested tools released nickel and chromium, whereas all but one released cobalt. Released amounts of metal after one-week immersion in artificial sweat were in the range of 0.0047-820 µg/cm^2 for cobalt, 0.0051-10 µg/cm^2 for nickel and 0.010-160 µg/cm^2 for chromium. All dental alloys released cobalt (0.0010-17 µg/cm^2) and nickel (0.0046-0.024 µg/cm^2), and all but one released chromium (0.0054-0.066 µg/cm^2).

In the case of nickel, the released amount can be compared to the migration limit of 0.5 µg/cm^2/week under REACH for articles "coming into direct and prolonged contact with skin" (ECHA 2014, EC 2009). This limit value was exceeded for 13 out of 26 examined products. No such release limits are available for cobalt and chromium. Released concentrations of cobalt and chromium were therefore simply compared to concentrations corresponding to the amounts that are known to cause the development of allergic contact dermatitis in sensitised patients (elicitation dose (ED)). A recent study revealed that cobalt can elicit contact dermatitis already at concentrations of 0.0663-1.95 µg/cm^2 in 10% of cobalt allergic patients (ED_{10}), based on patch test results (Fischer *et al.* 2015). The lowest concentration in that range was exceeded for 18 out of 26 dental items in the study of paper I. Similarly, chromium-allergic patients may react to chromium(VI) (potassium dichromate) in concentrations as low as 1.04 µg/cm^2 (Fischer *et al.* 2011). Seven out of 26 items tested in artificial sweat released higher concentrations of chromium. However, the different oxidation states of chromium were not analysed, so it is unknown what the clinical significance of the chromium release is in paper I.

4.1.3 Limitations

Dental tools and alloys were not cleaned before spot testing. Before immersion in artificial sweat, all materials were cleaned. Comparison of spot test results with release of cobalt and nickel in artificial sweat is therefore not straightforward. Nickel and cobalt on the surface of dental tools and alloys may have been contaminants during spot testing. In addition, the release takes place under different conditions in the two methods: stagnant immersion in artificial sweat versus rubbing of a surface during spot testing, and thereby applying a force to induce release into the reagent solution.

Furthermore, comparing metal release in artificial sweat to the elicitation threshold dose is not so simple. First, the metal concentration that will be released during skin contact is influenced by many variables. These include contact duration and frequency, presence of skin components like sweat or sebum, and material characteristics (Midander *et al.* 2016). When it comes to contact duration and frequency, the contact that dental technicians and students have with the tools in paper I will mainly be short and repeated (1-2 minutes per contact). Contact with dental alloys may be longer and more intensive, with an estimated contact duration of up to 1 hour per contact. However, even short and repeated contact with metal items that release metal in high concentrations may lead to a large deposition of sensitising metals on skin, which is high enough to elicit an allergic response (Erfani *et al.* 2015, Lidén *et al.* 2008a, Lidén *et al.* 2008b, Thyssen *et al.* 2013). The data in paper I also suggest that release rates of cobalt, nickel and chromium from dental alloys are highest after a short exposure time, and decrease over time (Paper I, Figure 1). Other variables that may influence the release of metals during skin contact are material characteristics, like the condition of the material surface and type of material (Midander *et al.* 2016). In the study of paper I, tools were often irregularly shaped, making it challenging to measure the exact surface area. The dental alloys were studied in their crude form, which has two disadvantages. First, there were

differences in the amount of surface irregularities between the crude alloys, which were not feasible to take into account when measuring the surface area. Second, dental technicians will not use the alloys in their crude form, but will instead melt, shape and grind them. This will also change the surface area, and thereby the release of metals from the material. Another assumption that should be kept in mind is that the elicitation threshold is derived from patch test data, in which the exposed skin area is small. It may not be likely that all of the released metal in artificial sweat will be deposited on such a small skin area.

For the above reasons, the study in paper II was performed to get an indication of the actual skin dose of cobalt, nickel and chromium after working with these dental tools and alloys.

4.2 EXPOSURE TO COBALT, NICKEL AND CHROMIUM IN DENTAL TECHNICIANS (PAPER II)

4.2.1 Dental technicians were exposed to metals on skin

Cobalt, nickel and chromium were detected on the skin of all participating dental technicians in the study in paper II. After two hours without hand washing and at the end of the work day some cobalt skin doses in the CoCr exposed group exceeded the lowest ED_{10} for cobalt (0.0663 µg Co/cm^2; Figure 4.1). Skin doses of the three metals built up during the day, as can be seen in Figure 4.1A and 4.1C, and Figure 2 in paper II. This confirms that metal exposure took place during working hours. None of the participating dental technicians reported having hand eczema currently or during the last 12 months.

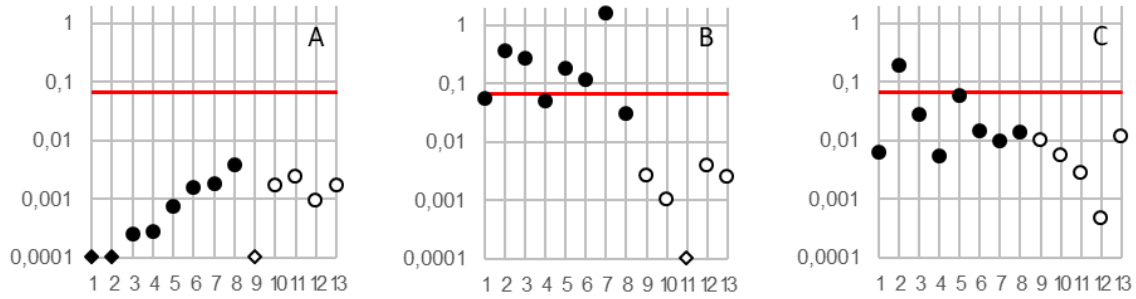


Figure 4.1. Individual concentrations of cobalt on skin (µg/cm^2) before work (A), after a 2-h period without hand washing (B), and at the end of the work day (C). The red line indicates the lowest elicitation dose (ED_{10}; 0.0663 µg/cm^2) for cobalt described in a study by (Fischer *et al.* 2015). X-axes show participants 1-13: filled black circles: CoCr exposed (1-8); open circles: non-CoCr-exposed (9-13); diamonds: value below limit of detection. Y-axes are displayed on a logarithmic scale. (Figure adapted from Paper II, Fig. 2).

4.2.2 Dental technicians were exposed to metals through air

Cobalt was detected in all ten air samples (0.22-155 µg/m^3). In two of these samples, cobalt concentrations exceeded the Swedish OEL of 20 µg/m^3. The OEL for nickel (500 µg/m^3 (AV 2015)) was not exceeded for the six participants that had nickel in their air sample (0.48-3.7 µg/m^3). Chromium was found in nine air samples (0.43-71 µg/m^3). However, we did not analyse the different oxidation states of chromium, so the air levels cannot be compared to OELs for chromium(VI) (5 µg/m^3) and chromium(II/III) (500 µg/m^3) (AV 2015).

4.2.3 Metal exposure was not reflected in urine of dental technicians

Detectable levels of cobalt and chromium were found in 62 (70%) and 42 (47%) of the 89 urine samples, respectively. Nickel was found in all urine samples. Median concentrations, before and after correction for dilution variation with specific gravity, in all 89 samples are shown in Table 4.1. For chromium, more than 50% of the samples were below LOD, which means that the median is below LOD as well. Because of the small sample size, no statistical tests were performed to look for an association between exposure through air and on skin, and excretion of the metals in urine. Instead, urinary metal concentrations were compared to levels found in a previous study among dental technicians, and in a Danish reference

population (Kristiansen *et al.* 1997, Leghissa *et al.* 1994). In this thesis, data of a Belgian reference population were added for comparison (Hoet *et al.* 2013). Urinary concentrations of cobalt, chromium and nickel in these three populations are shown in Table 4.1.

Table 4.1. Median urinary concentrations (uncorrected or corrected for specific gravity; µg/L) of cobalt (Co), chromium (Cr) and nickel (Ni) of all participants (n=13) from the study in paper II. Comparison was made with mean concentrations (samples taken post shift at the end of a work week) from a CoCr-exposed population (n=31) (Leghissa *et al.* 1994); and with median or geometric mean concentrations, respectively, from non-exposed reference populations from Denmark (n=189, recalculated concentrations) and Belgium (n=1001) (Hoet *et al.* 2013, Kristiansen *et al.* 1997).

		Paper II			Leghissa *et al*.		Kristiansen *et al*.		Hoet *et al*.	
			Median (range)			**Mean** (range)		**Median** (range)		**GM** (95% CI)[A]
		N[B]	Uncorrected[C]	SG corrected[D]	N	Uncorrected	N	Uncorrected	N	Uncorrected
Co	Total	89	**0.15** (<LOD-6.0)	**0.14** (<LOD-4.5)	31	**1-91** (0.80-5.7)	-	-	1001	**0.15** (0.14-0.17)
	Women		-	-		-	97	**0.39** (<0.20-2.7)	541	**0.18**
	Men		-	-		-	89	**0.22** (<0.20-1.9)	460	**0.12**
Cr	Total	89	**<LOD** (<LOD-0.96)	**<LOD** (<LOD-0.58)	31	**0.69** (0.10-2.0)	186	**0.22** (<0.20-1.3)	1001	**0.10** (0.10-0.11)
Ni	Total	89	**1.8** (0.26-52)	**1.8** (0.51-32)	31	-	118	**0.88** (<0.12-5.7)	1001	**1.7** (1.6-1.8)

[A] GM= Geometric mean; only a 95% confidence interval (95% CI) was provided for these data

[B] N=Number of samples

[C] Limit of detection (LOD) for uncorrected data is 0.092 µg/L for Co, 0.094 µg/L for Cr, 0.24 µg/L for Ni

[D] Corrected for mean specific gravity (SG) of the urine samples in the study population (1.015). Consequently, LOD for corrected data is determined by the correction factor of each individual sample, and can therefore not be shown.

Leghissa *et al*. reported mean urinary concentrations for dental technicians producing cobalt-chromium prostheses for 4 different moments during a week (Leghissa *et al.* 1994). The ranges of individual cobalt values were comparable to the ranges found in the study of paper II, but mean cobalt values in the study by Leghissa *et al*. (1.13-1.91 µg/L) differed slightly from the median values in our study (0.15 µg/L). However, the use of the mean in occupational exposure studies is questionable, since most occupational exposure data are skewed to the right. In general, skewness of occupational exposure data is due to random variability in exposures between and within workers, which causes a wide range of exposure levels, and due to the fact that exposure measurements cannot be below zero (Waters *et al.* 2015). These data are therefore best described by a lognormal distribution, for which the median or geometric mean is best to describe the central tendency of the data.

Furthermore, the median urinary metal concentrations in paper II were lower than median concentrations in a Danish reference population (recalculated from (Kristiansen *et al.* 1997)) and comparable to geometric mean concentrations in a Belgian reference population (Hoet *et al.* 2013).

Urinary metal concentrations can also be compared to occupational urinary limit values, which are available in some countries. These limit values correspond with the occupational exposure limit values for air in those countries. The cobalt urine levels for participants in the study of paper II are all below the USA biological exposure index (BEI, 15 µg/L) and the Finnish biomonitoring action limit (BAL, 130 nmol/L ≈ 7.7 µg/L) for cobalt (6, 7). The USA BEI for total chromium is 25 µg/L, which is not exceeded in this study. In Finland, a biomonitoring action limit exists also for nickel (0.2 µmol/L ≈ 12 µg/L), which is exceeded for one participant. One should bear in mind that when calculating these limits in urine possible metal skin uptake or intake via food, as well as individual differences in exposure and metabolism are to some extend overlooked. The limits are therefore not similar in all countries, and direct extrapolation from air exposure to urine excretion is doubtful.

When looking at individual urinary metal concentration in our study, one dental technician had slightly higher urinary cobalt levels (median 3.1 µg/L), and another one had high urinary nickel levels (median 30 µg/L) compared to the other participants. One can speculate that the increased nickel level could be due to the fact that that participant had a vegetarian diet, and since it is known that many vegetables and nuts have high nickel content (Veien *et al.* 1993), elevated consumption of these foods may increase nickel intake and elimination. No explanation could be found for increased cobalt levels in another participant, since that person's skin and air exposure was comparable to that of the other exposed participants.

Altogether, median urinary concentrations of cobalt, chromium and nickel in paper II were comparable to observed concentrations in reference populations. I therefore conclude in this thesis that the concentration in urine in the study of paper II did not reflect occupational exposure to cobalt, nickel or chromium on skin or through air.

4.2.4 Limitations

It is difficult to generalize the results of the study in paper II, because of the small sample size of this study, and because the CoCr exposed dental technicians did not work exclusively nor on a daily basis with these materials.

Limitations of the comparison of skin doses with the ED_{10} are discussed in section 4.4.3.

4.3 SURFACE CONTAMINATION OF COBALT IN THE HARD METAL INDUSTRY (PAPER III)

4.3.1 Cobalt was present on surfaces in the hard metal facilities

Cobalt spot testing within the hard metal facilities revealed the presence of cobalt on surfaces in the entire work area, even outside production areas (Fig 4.2). Due to these results, the company became more aware of the transfer of cobalt through the work areas, and changed cleaning routines to reduce surface contamination.

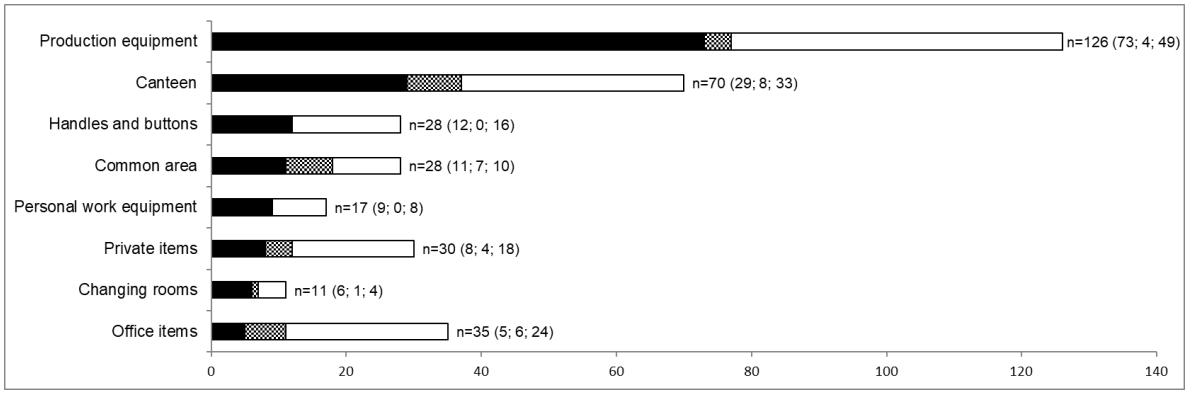


Figure 4.2. Cobalt spot tests (n=358) to assess contamination of cobalt through different areas at the hard metal industry (total (positive ■; doubtful ▩; negative □)) Positive: orange colour; doubtful: colour other than orange; negative: clear yellow, no colour change. Production equipment: e.g. machines, tools, computers, Canteen: e.g. coffee machines, furniture; Handles and buttons: e.g. door handles, light switches, soap dispensers in office and production area; Common area: e.g. toilets, floors in production and office areas; Personal work equipment: e.g. safety goggles, inside of gloves, key cards; Private items: e.g. private phones and glasses; Changing rooms: e.g. lockers for private- and work clothes, benches; Office items: e.g. computers, furniture in office area

4.3.2 Limitations

The results of cobalt spot testing cannot be used to quantify to what extent contaminated surfaces contributed to the cobalt skin dose. They rather point out that cobalt is transferred from the production area to other areas, where it may also contaminate skin. However, in the study of paper III, we were actively looking for cobalt-contaminated surfaces that were expected to come in contact with the skin. It is therefore likely that the frequency of positive tests is not representative of all surfaces within the company.

4.4 COBALT SKIN EXPOSURE IN THE HARD METAL INDUSTRY

4.4.1 Hard metal workers were exposed to cobalt on skin (paper III and IV)

Cobalt was detected on skin of all 76 hard metal workers before shift and at the end of the shift, and on skin of 37 out of 40 workers after a 2-h period without hand washing (Table 4.2). Results were compared with the elicitation dose needed for 10% or 50% of cobalt allergic patients to develop dermatitis (ED_{10} or ED_{50}). These elicitation doses for cobalt were found to be ranging from 0.0663 to 1.95 μg/cm^2 (ED_{10}) and from 1.45 to 17 μg/cm^2 (ED_{50}) based on different patch test studies (Fischer *et al.* 2015). For 84 out of 189 (44%) of the skin doses of hard metal workers sampled in this project, levels were higher than the lower bound of the ED10 (0.0663 μg/cm^2). The lower bound of the ED_{50} (1.45 μg/cm^2) was even exceeded for 18 out of 189 skin doses (9.5%). Control groups showed little or no cobalt on skin; 4 out of 42 skin dose measurements (range 0.068-0.43) in this group were just above the lower bound of the ED_{10} concentration.

Within the hard metal industry, it is generally believed that only the raw materials in powder form pose a risk for exposure. However, from results seen in this project, it should be stressed that even after sintering of the hard metal materials, the products are not safe from a skin exposure perspective. Skin protection should therefore be used even when handling the finished products.

Table 4.2. Median (range) cobalt skin dose (μg/cm^2) for workers of four exposure groups in the hard metal industry (Table adapted from tables paper III and IV)

Exposure group	Before shift (n=76)	End of shift (n=76)	After 2h without hand washing (n=40[A])
Control	0.012 (0.0024-0.086)	0.012 (0.00059-0.43)*	0.011 (0.0019-0.047)*
Raw material	0.096 (0.0090-0.76)**	0.86 (0.065-135)**	1.51 (0.25-28)***
Sintered material	0.013 (0.0030-0.035)	0.046 (0.015-0.99)	0.12 (0.024-9.0)
Final product	0.014 (0.0036-0.038)	0.12 (0.0091-2.9)	0.42 (0.017-24)

[A]Skin doses of 2 controls and 1 sintered material worker were below LOD, and were not included in this range.

Median skin dose statistically significantly *lower than the dose in all other groups ($p<0.01$), or higher than the dose in **all other groups or ***only the control and sintered material group at that time point ($p<0.001$) using Wilcoxon's rank-sum test.

4.4.2 Frequency of glove use varied widely among workers (paper III)

Differences in glove use were seen between hard metal workers from the four groups. None of the office workers wore gloves, whereas all raw material workers wore gloves all or most of the time. Glove use for sintered material and final product workers varied much. The cobalt skin dose was plotted for production workers that wore gloves all the time, some of the time, and not at all. This revealed that the cobalt skin dose for workers that wore gloves all the time is not statistically significantly lower than the dose of workers that sometimes or never wore gloves. This may be due to factors like contamination of the inside of re-usable

gloves, incorrect use of gloves, or inadequate awareness of exposure sources. In addition, in some areas, workers wore gloves mainly to protect the material and final products, rather than protecting their hands.

4.4.3 Limitations

Some assumptions are made when making a comparison between skin wipe samples and elicitation doses. First, the ED_{10} values are calculated from patch test studies performed with $CoCl_2$ as test substance. Absorption rates may be different for this soluble cobalt compound, compared to the less soluble metallic cobalt in skin doses of the studies in paper III and IV. Second, patch testing is performed under occluded conditions, whereas the skin of hard metal workers was not occluded, or only occluded for shorter periods of time when using gloves. This may also result in differences in absorption between the two methods. Third, acid wipe sampling is a method that provides a "total metal skin dose", since metal in any form present on the skin will be sampled by this method. This dose, available for skin absorption, is considered to be of dynamic character, including short- and long-term contact events that result in the deposition and removal of metals on skin. This is for example due to hand washing routines, friction, individual skin properties, and differences in exposure during the work day. The skin dose used for patch testing is a static dose, although absorption may result in reduction of the dose.

4.5 COBALT RESPIRATORY EXPOSURE AND URINARY CONCENTRATIONS IN THE HARD METAL INDUSTRY (PAPER IV)

4.5.1 Hard metal workers from production areas were exposed to cobalt in air

Cobalt concentrations in air were significantly higher among raw material workers, compared to concentrations in the other two production area groups. Two workers in the raw material group were exposed to cobalt concentrations (23 and 24 µg/m^3) above the Swedish OEL of 20 µg/m^3.

4.5.2 Cobalt was found in urine samples of many hard metal workers

Out of 563 urine samples, 28% contained cobalt concentrations below the LOD. The median urinary cobalt concentration (corrected for specific gravity) in the raw material group was significantly higher compared to the median in other groups (Table 4.3). The medians in the other three groups were not significantly different from each other. With the exception of the raw material group, median urinary cobalt concentrations in the hard metal workers were in the same range as the median concentrations found in dental technicians (Table 4.1).

Table 4.3. Median cobalt concentration in urine; uncorrected, corrected for specific gravity (SG), and corrected for creatinine content (crea); of 76 workers in the hard metal industry, divided over 4 exposure groups

		Median cobalt concentration in urine (range)		
Exposure group	**n above LOD (% of total)**	**Uncorrected (µg/L)**	**SG corrected (µg/L)**	**Crea corrected (µg/g crea)**
Controls	73 (55.7)	0.11 (<LOD-1.1)	0.094 (<LOD-2.1)	0.080 (<LOD-3.1)
Raw material	160 (92.5)	1.4 (<LOD-26)	1.5 (<LOD-31)*	1.3 (<LOD-38)
Sintered material	83 (66.9)	0.15 (<LOD-5.5)	0.12 (<LOD-4.4)	0.13 (<LOD-3.5)
Final product	90 (66.7)	0.14 (<LOD-8.3)	0.19 (<LOD-7.2)	0.18 (<LOD-6.8)

*Median urine level statistically significantly different from the other groups, p<0.001, using design-matrix bootstrapped quantile regression.

4.5.3 Limitations

Two air samples of raw material workers were excluded from statistical analysis, because these workers wore respiratory protection during the time that the air sample was taken. It would have been interesting to calculate their exposure, using the measured value in the air sample, and the protection factor of the mask that they wore. Unfortunately, we did not collect information about the type of respiratory protection that the workers used.

4.6 ASSOCIATIONS BETWEEN COBALT ON SKIN AND IN AIR, AND URINARY CONCENTRATIONS (PAPER IV)

In paper IV, we describe the associations between skin doses and respiratory exposure, as well as the associations between these exposures and urinary cobalt concentrations. This was performed to assess the significance of cobalt skin exposure as determinant of urinary cobalt concentrations as biomarker of exposure.

4.6.1 Cobalt in air was correlated to cobalt on skin before and at end of shift.

Spearman's rank correlation showed that cobalt skin doses on hands of the workers at the end of shift correlated well with respiratory cobalt exposure during the shift (r_s=0.801; p<0.001). Interestingly, the skin doses on the hands before start of shift also correlated with respiratory cobalt exposure during the following shift, although to a lower degree (r_s=0.448; p<0.001). If respiratory cobalt exposure would be considered to be similar on all work days throughout the year, comparison with cobalt on skin before shift would be suitable. This is however a bold assumption. It is therefore unknown what the significance and meaning of this correlation is.

4.6.2 No significant change in urinary cobalt over time during 24h

The workers participating in this study collected spot urine samples during 24 hours from the start of the shift. Using quantile regression, we evaluated if there was a change in urinary cobalt concentration over time, related to the measured exposure during that day. In this group of workers, we could not find a significant change in urinary cobalt over time during the 24-hour period (Figure 4.3). Results were the same when the association was examined for each group separately. This means that in our study, it does not seem to matter when during the day you measure cobalt in urine to predict exposure.

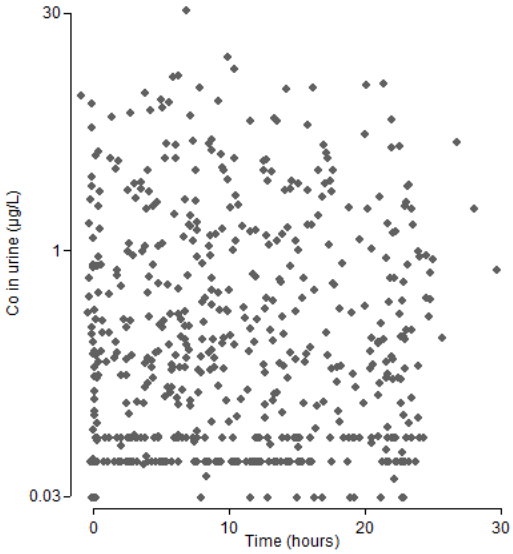


Figure 4.3. Association between time of urination and the concentration of cobalt in urine for 76 hard metal workers in Sweden. Concentrations below limit of detection (LOD) were treated as LOD/√2.

4.6.3 Associations between cobalt exposure and urinary excretion

Efforts have been made to find the optimal time point for urine sampling that reflects occupational respiratory exposure, and to find a limit for cobalt in urine that corresponds with a certain OEL in air (Martin *et al.* 2010, Mosconi *et al.* 1994). With this information as support, the study in paper IV focused on quantifying the association between urinary elimination of cobalt and both skin and respiratory exposure to cobalt.

Quantile regression was performed to evaluate the association between the dependent variable (cobalt in urine; U-Co) and the independent exposure variables (cobalt skin dose before shift (S_B-Co) or at end of shift (S_E-Co) and/or cobalt in air (A-Co)). The results of the five different regression models are shown in Table 4.4.

The observed ratio is the exponentiated regression coefficient (2^{β_1}) (see method section, Figure 3.3). For every doubling of the concentration of the independent variable, the median urinary cobalt concentration is multiplied by the observed ratio. Multiplication by 2^{β_1} can be expressed as a percentage change in median U-Co.

Table 4.4. Quantile regression models showing the associations of different independent variables with the dependent variable (median urinary cobalt concentration) (Adapted from paper IV, Table S3).

Model[a]	Independent variable(s)	Amount of clusters available for regression	Observed ratio	95% confidence interval		Bootstrap standard error	z	P>|z|
1.1	S_B-Co	76	1.70	1.51	1.91	0.086	8.82	0.000*
1.2	S_B-Co	52	1.33	1.04	1.70	0.179	2.31	0.021*
2.1	S_E-Co	76	1.32	1.17	1.49	0.088	4.55	0.000*
2.2	S_E-Co	52	1.17	1.06	1.30	0.074	3.14	0.002*
3	A-Co	46	1.38	1.25	1.54	0.076	6.19	0.000*
4	S_B-Co	46	1.27	0.94	1.71	0.219	1.56	0.118
	A-Co		1.31	1.13	1.52	0.110	3.52	0.000*
5	S_E-Co	46	0.98	0.84	1.14	0.116	-0.27	0.784
	A-Co		1.42	1.19	1.70	0.131	3.88	0.000*

[a] Model 1.1: logarithm of skin dose before shift (S_B-Co) as the only independent variable; model 1.2: S_B-Co as the only independent variable, excluding workers with high air exposure (*raw material* group); model 2.1: logarithm of skin exposure at end of shift (S_E-Co) as the only independent variable; model 2.2: S_E-Co as the only independent variable, excluding *raw material* group; model 3: logarithm of air exposure (A-Co) as the only independent variable; model 4: both log S_B-Co and A-Co as covariates; model 5: both S_E-Co and A-Co as covariates. All variables are log-transformed to base 2. *Statistically significant association of independent variable with median U-Co.

From the quantile regression modelling, it is observed that the association between S_B-Co and U-Co was significant (model 1.1). The same was true for the association between S_E-Co and U-Co (model 2.1). The observed ratios of 1.70 and 1.32, can be explained as an increase of 70 and 32 percent in median U-Co for every doubling of S_B-Co and S_E-Co, respectively.

The associations were still significant when data of raw material workers were not used in the regression models. By excluding these workers in the analysis, the model was built on

workers with cobalt skin exposure and relatively low respiratory exposure (below 3% of the Swedish OEL). In these models (1.2 and 2.2), the median U-Co increased with 33 and 17 percent for every doubling of S_B-Co and S_E-Co, respectively.

Model 3 included 28 A-Co concentrations measured in this study, as well as 18 imputed A-Co concentrations for the control group (0.010 µg/m^3). Imputation of A-Co values for the control group was done to increase the amount of clusters available for the regression model. The value of 0.010 µg cobalt/m^3 was selected based on comparison with cobalt concentrations measured in air samples of the production workers in this study. The value was lower than the lowest cobalt concentrations measured in production areas, and was equal to 1/2000 of the Swedish OEL. According to our judgement, this represents a negligible respiratory exposure. The association between A-Co and U-Co was significant. For every doubling of respiratory cobalt concentration, median urinary concentrations would increase with 38 percent.

Hereafter, the independent exposure variables were modelled together. Model 4 shows the association between the dependent variable U-Co and independent variables S_B-Co and A-Co. In model 5, S_E-Co and A-Co are modelled as independent variables. In both models, skin exposure was not associated with an increase of urinary cobalt. The association between air exposure and urinary elimination was still significant.

Considering all exposure routes and variations in elimination routes, times and rates, it is difficult to establish a general half-life of cobalt in the human body. Few studies have used urine as biomarker for skin exposure, and only one has used blood as biomarker for skin exposure (Klasson *et al.* 2016, Linnainmaa and Kiilunen 1997, Scansetti *et al.* 1994). In two studies with healthy volunteers, urinary cobalt concentrations after skin exposure varied much (Linnainmaa and Kiilunen 1997, Scansetti *et al.* 1994). In most volunteers, peak elimination was seen within 4-24 hours. After 24 hours, urinary concentrations remained elevated, or were still increasing for some persons. Skin exposures were relatively high in these studies, although volunteers were only exposed during a single event. Possible influence of long term skin exposure on urinary cobalt concentrations has never been studied. Elimination of cobalt in urine after air exposure may vary from hours to weeks or even years, depending on the clearance mechanism (Kim *et al.* 2006, Leggett 2008, Mosconi *et al.* 1994). Oral exposure to cobalt mainly leads to elimination via urinary excretion of absorbed cobalt and faecal elimination of unabsorbed cobalt. Elimination rate after oral exposure is not often studied in human, but it is thought to be rapid; probably within 24 hours or several days (Kim *et al.* 2006, Paustenbach *et al.* 2013).

Based on this information, it is not straight-forward to determine the cause of elevated urinary cobalt concentrations in the study of paper IV. Both short and long-term cobalt exposure from the three described exposure routes may contribute to urinary elimination.

When evaluating the associations between the independent variables and dependent variable in this study, one should keep in mind that an association does not imply causation. Different

hypotheses can be proposed to explain the significant associations between cobalt skin exposure and urinary elimination in models 1 and 2. First, the associations may be explained by cobalt skin uptake on the study day and previous days. A lack of association with skin in model 4 and 5 may in that case be explained by a lack of sufficient statistical power, due to a limited data-set. Only 46 pairs of samples were available to evaluate the effect of cobalt skin dose and air exposure simultaneously. A second hypothesis is that the correlation between air exposure and skin exposure is a confounding factor in the association between skin exposure and urinary excretion. Third, there may be another confounding variable that distorts the association. Oral exposure, in terms of hand- or object-to-mouth hygiene, smoking, and consumption of cobalt-containing products, may have contributed to the urinary cobalt concentrations (Cherrie *et al.* 2006, Hutter *et al.* 2016). A fourth hypothesis may be a combination of the three hypotheses above. From the data in the study of paper IV, it is not possible to determine which hypothesis is most likely to be true.

Even though data were gathered on smoking and vitamin use, this was not used for correction in the regression models. The data set was judged to be too small to correct for such factors in this study. Furthermore, Linnainmaa and Kiilunen showed that the use of multivitamins only had a small influence on urinary cobalt concentrations, and the use of vitamin B12 did not increase cobalt concentrations in urine. Smoking may have interfered with the association between cobalt exposure and urinary excretion (Hutter *et al.* 2016).

Interestingly, the association between S_B-Co and U-Co was stronger than the association between S_E-Co and U-Co. One hypothesis could be that a cobalt depot may build up on and in the skin, with S_B-Co reflecting a continuous low-dose skin exposure. In addition, we had no information about whether and how long before the end of shift workers had washed their hands. S_E-Co may therefore be less reliable for estimating skin exposure in the study of paper IV.

4.6.4 Strengths and limitations

The optimal sampling moment for biological monitoring of cobalt has been proposed to be a spot urine sample at the end of a shift taken at the end of a work week. This was based on several studies performed in the 80ies and 90ies (Linnainmaa and Kiilunen 1997, Lison *et al.* 1994, Scansetti *et al.* 1994, Scansetti *et al.* 1985). In the exposure study among hard metal workers (paper IV), multiple spot urine samples collected during 24 hr for all workers were used for statistical analysis. Using quantile regression modelling we were able to use all collected samples to evaluate the contribution of the different exposure routes, and as a result reduce the risk of exposure misclassification of our exposure biomarker (Wang *et al.* 2016).

In the 1980s, hand eczema and contact allergy to cobalt were common among workers in hard metal facilities in Sweden (Fischer and Rystedt 1985). Since then, no study has investigated the prevalence of hand eczema, although it has been shown by patch test that hard metal alloys are able to elicit allergic contact dermatitis (Julander et al. 2009). A few studies revealed that respiratory exposure has decreased compared to the 1980ies (Hutter *et*

al. 2016, Westberg *et al.* 2017). Pictures of skin exposure in the Swedish hard metal industry in the 1980ies (personal communication with Torkil Fischer, April 2018) were compared to skin exposure that we have seen during our study in the Swedish hard metal industry. In the 1980ies, workers performed many work tasks manually and without gloves. Similar work tasks are nowadays automated or performed with use of protective gloves. Even though skin exposure was not quantified back in the 1980ies, work conditions were very different, and it is highly likely that skin exposure has decreased. I therefore speculate that the prevalence of hand eczema among hard metal workers has decreased as well.

A limitation of this study is that we did not collect air samples in the control group. The use of imputed values in the quantile regression analysis may have influenced the result, for example due to possible erroneous assumptions of the exposure of control workers, or due to a lack of variability in the imputed data.

4.7 IMPLICATIONS FOR DENTAL TECHNICIANS (PROJECT I)

To reduce or eliminate the occupational exposure to metals, we suggest that local process ventilation is improved to rule out respiratory exposure to these metals. Local ventilation is however not intended to protect against skin exposure, which emphasizes the need for other protective measures. Disposable gloves should be used where possible, and tools and other equipment should not be shared between workbenches and for different work tasks. Hand washing after work with metal materials may also reduce the metal skin dose.

Generalisation of the results of the study in paper II is not straight-forward, because of the small sample size and the limited amount of work performed with dental alloys made of CoCr. It would have been interesting to study exposure in dental technicians that worked exclusively with CoCr alloys all the time. However, no dental laboratory in Sweden was found with this requirement.

4.8 IMPLICATIONS FOR HARD METAL WORKERS (PROJECT II)

To protect the hard metal workers from skin exposure to cobalt, there is a need for improvement of work conditions and routines, as well as improved awareness of the exposure of skin to cobalt in the work environments. The workers should improve their skin care, in terms of hand washing routines before entering lunch rooms, and improve their use of protective gloves.

Fact is that urinary cobalt concentration for workers in the raw material group were higher than those in the other groups, and higher compared to concentrations found in the general population. However, it is difficult to determine what the observed significant association between cobalt skin exposure and urinary cobalt concentrations actually means. The implications of these elevated concentrations for the health of workers are unknown. I would advise that urinary cobalt concentrations should be monitored and preferably kept below occupational limit values that are available in some countries, since they are validated to protect the health of exposed workers.

An increased awareness about occupational skin exposure to cobalt among workers and managers could result in better hygiene at work. This may include avoiding contamination of areas outside the production areas and hand-to-mouth transfer, more frequent washing and more careful handling of work clothes, storage of private items during the work day.

5 CONCLUSIONS

Taken together, the results of this thesis show that dental technicians and hard metal workers are exposed to sensitising metals, in particular cobalt. Specifically, the results demonstrated that:

Dental tools and alloys released cobalt, nickel and chromium. The short and repeated contact that dental technicians and students of the dental technology program usually have with dental tools and alloys, may result in the deposition of sensitising metals on their skin.

Dental technicians were exposed to cobalt, nickel and chromium on skin due to their occupational activities. Some cobalt skin doses were in a range known to elicit allergic contact dermatitis in sensitised persons. For two participating dental technicians, cobalt levels in air samples were above the Swedish OEL. Occupational exposure to cobalt, nickel or chromium on skin or through air was not reflected in urinary concentrations of these metals.

All hard metal workers were exposed to cobalt on skin. Contact with raw materials, but also with sintered materials, leads to cobalt skin doses that are high enough to be able to elicit allergic contact dermatitis. Unintentional transfer of cobalt from production areas to other areas in the hard metal facilities may be a source for cobalt skin exposure for the hard metal workers. Respiratory exposure, mainly below OEL, was shown for all workers in hard metal production areas. Cobalt was found in 72% of the urine samples collected among the participating hard metal workers.

No significant change was seen in urinary cobalt concentrations over time during 24h, when analysing samples of 76 hard metal workers. Significant associations were revealed between urinary cobalt concentrations, and cobalt skin and respiratory exposure, when each exposure route was modelled independently. When modelling skin and respiratory exposure together, neither of the cobalt skin doses were significantly associated with cobalt in urine. Several theories may explain the observed associations between cobalt skin exposure and concentrations in urine, but from the results in this thesis it was not possible to assess causation.

For both dental technicians and hard metal workers, efforts should be made to reduce skin exposure, to protect workers from possible skin related problems, and to minimise the risk of contribution to systemic dose by possible skin penetration and hand-to-mouth behaviour. Strategies for reduction could be to increase correct use of gloves, avoiding contamination of other work areas, and no use of private items in work areas.

.

6 FUTURE PERSPECTIVES

Given the relatively high prevalence of cobalt allergy among dermatitis patients, and in the general population, including adolescents, it is necessary to understand sources of exposure to cobalt. As we know very little about cobalt exposure, the studies in this thesis can help to fill this knowledge gap.

Data from some European countries suggest that occurrence of cobalt allergy among dental technicians is low, compared to allergy to other sensitising materials, like (meth)acrylates. In contrast, it was reported that cobalt allergy is prevalent among dental technicians in Korea. It can be speculated that this geographical difference in occupational exposure to cobalt is related to preferential use of CoCr alloys due to favourable costs. Moreover, standards of cleanliness, exhaust possibilities and overall work procedures might vary between laboratories and different countries, which may affect the exposure patterns. Results from the study among dental technicians in this thesis are not generalizable. In order to assess the risk of metal allergy, further research is needed and the skin exposure to metals should preferably be studied in a larger group of dental technicians that use materials releasing cobalt, nickel and chromium on a daily basis.

At present, it is difficult to determine what the observed significant association between cobalt skin exposure and urinary cobalt concentrations in paper IV of this thesis means. It would therefore be interesting to design a study of controlled skin exposure to cobalt that can answer the question whether cobalt can be systemically absorbed via the skin exposure route. It is challenging to design such a study *in vivo* in humans, as it may involve ethical issues, like sensitisation of research persons, or practical issues like avoiding exposure through the other exposure routes or collecting samples at the right time points.

The work in this thesis was designed to be followed up by studies that investigate skin penetration of cobalt, and such studies are already initiated by our research group. *In vitro* skin absorption will be tested, using Franz diffusion cells and cobalt particles of different sizes. Furthermore, skin absorption will be studied *in vivo* in cobalt allergic patients that undergo patch testing, by monitoring of cobalt in blood and urine. Cobalt particles of different size will be used for skin exposure in the patch test, in order to understand the influence of particle size on allergic skin reactions. This will be achieved by scoring of the reactions from their external appearance and by cytokine profiling.

Finally, it would be interesting to examine the dynamics of occupational skin exposure to metals. The skin dose before shift may be a reflection of previous exposure, although the dynamics behind this dose are unknown. It is important to better understand how much metal we can “carry” on our skin, and for how long it stays there. Hence the fate of the metal skin dose under the influence of washing or friction, differences in exposure during work or in leisure time, as well as the influence of individual skin properties, need to be further investigated. Besides that, all the results presented indicate that short and repeated contact should get more attention, as that is what happens in real life.

7 ACKNOWLEDGEMENTS

To my dear supervisors **Anneli Julander**, **Klara Midander**, and **Carola Lidén**. **Anneli** - just before the start of my PhD, you told me that there will be a moment that I will be angry and fed-up with you (and maybe vice versa?). I think we tried our best, but that moment never came. You even learned to recognize my sarcasm after some practice. Over the years, you have always been pro-active and engaged in my studies. It has been an exciting journey for both of us: for you as first-time main supervisor, for me as a PhD student. Thank you for your support, patience, friendship and sometimes motherly advice, and for sharing your great knowledge and enthusiasm. I could not have wished for a better supervisor during my PhD studies. **Klara** - I have learned a lot from your time-optimism, and positive and practical view on research and life. I loved the open door policy and appreciated the 24/7 availability. And let's not forget that beautiful stork you made! Thank you for being the perfect counterpart of "the mini-boss" during my PhD. **Carola** - thank you for giving me the opportunity to join your (former) research unit during my masters' internship and PhD studies. You have always been available for advice, with sharp and punctual insights, and your inspiring affection for research.

To my other co-authors **Emmy Axén**, **Sara Nilsson**, **Matteo Bottai, Martin Gumulka and Miháli Matura** - thank you for your input and feedback on manuscripts. **Matteo** - thank you in addition for the crystal clear and extensive explanations of the statistical methods.

To my mentor **Paulina Nowicka** – thank you for the supportive and motivating talks over lunch and fika. Your enthusiasm and passion are very inspiring.

Thanks to all the past and present members of the Unit of **Work Environment Toxicology** and **Lung and Airway Research**, and the former Unit of **Occupational and Environmental Dermatology** and the clinic for the fun conversations during lunch and fika time and the friendly and open atmosphere.

To all the **EFH PhD students** at IMM - thank you for the nice events, movies in the afternoon, and retreats including Swedish skinny dipping. **Anda Gliga** – in addition, thank you for all the fun lunch and staircase conversations.

To my toast master and friend, **Antero Silva**, and his amazing side-kick **Adina Tenie**. Thank you for being such great, hospitable, caring, and generous friends over the years. I hope you will honour us with many visits to the Netherlands.

To my other toast master and Dutchie-in-crime, **Eva Herweijer**. We seem to have followed a similar path over the last years, both work and private. Thank you for the fun lunches, dinners, Sinterklaas (sorry, I still owe you a long poem), game nights, Eurovision, and of course our baby/toddler meet-ups and making me feel better when talking about our parental "crises". I hope our paths will keep on crossing!

To my dear friend **Eline Bazuin** – thank you for all the times you came to Sweden and made Stockholm feel like home. Your unconditional support and encouragements have kept me motivated over the years. I will give you ten minutes of my time more often from now on!

To my sweet high-school friends - **Marlene Kleinbloesem** and **Joyce Boom-Krajenbrink** – thank you for the fun reunions in Stockholm and the Netherlands. We should plan another day with all of our girls soon!

To my university friends – **Ideke Lamers**, **Sharon Smits** and **Loes Soetens** – thank you for your support, friendship, and short but valuable meet-ups. I hope we can meet more often when we move back to the Netherlands.

To my long-time friend from good-old Brummen - **Thijmen Bruijnderink** and his sweet girlfriend **Eva Vermeulen** – thank you for being there for me over the years, even though we did not always find the time to meet. The long phone conversations did make up for some of that time, but we should really get together soon.

To the other fellow Dutchies that I met in Stockholm - **Linda Labberton**, thank you for making Stockholm feel like the Netherlands every now and then, especially with being gekleurde Piet and drinking cheap beers at the Dutch embassy. Now we might have to start a little Swedish club in the Netherlands! - **Judith Brand**, thank you for organising fun nights with movies, games and dinner. – **Daniëlle Vaartjes** – thank you for the good lunch talks and for the mental support in the final stages of my PhD. We are (hopefully) graduating on the same day; congratulations to you!

To the people I met at Jägargatan, and other good friends I got to know via Karolinska Institutet - thank you for the memorable times.

To my loving family – my sweet sisters **Irma** and **Myrthe** and their great families; and to my sweet family-in-law - thank you for supporting us during this great journey abroad and for the fun visits to Stockholm. You mean the world to me, and I can't wait to live close to you again.

To my great examples and biggest supporters in life – my oudertjes **Wilco** and **Ina** – thank you for always being there for me. You have always encouraged me to find my own way, with your unconditional support and love. I could not have done this without you!

And finally, to the love of my life, **Pim** – thank you for joining me on this Swedish adventure, and for the endless support and patience. Together with our beautiful daughter **Saga**, you make me the happiest person in the world. You always give me the courage and the strength I need. Schat, ik hou van je!

8 REFERENCES

Adam, C., Wohlfarth, J., Haussmann, M., Sennefelder, H., Rodin, A., Maler, M., Martin, S. F., Goebeler, M. and Schmidt, M. (2017). "Allergy-Inducing Chromium Compounds Trigger Potent Innate Immune Stimulation Via ROS-Dependent Inflammasome Activation." J Invest Dermatol **137**(2): 367-376.

Ahlström, M. G., Thyssen, J. P., Menné, T. and Johansen, J. D. (2017). "Prevalence of nickel allergy in Europe following the EU Nickel Directive - a review." Contact Dermatitis **77**(4): 193-200.

Alexandersson, R. (1988). "Blood and urinary concentrations as estimators of cobalt exposure." Arch Environ Health **43**(4): 299-303.

Amiri, S. and Shokrollahi, H. (2013). "The role of cobalt ferrite magnetic nanoparticles in medical science." Mater Sci Eng C Mater Biol Appl **33**(1): 1-8.

Andersen, K. E., White, I. R. and Goossens, A. (2011). "Allergens from the European Baseline Series." In: Contact Dermatitis (5th edition). J. D. Johansen, P. J. Frosch and J.-P. Lepoittevin. Berlin and Heidelberg, Springer-Verlag. pp. 545-590.

Apostoli, P., Porru, S. and Alessio, L. (1994). "Urinary cobalt excretion in short time occupational exposure to cobalt powders." Sci Total Environ **150**(1-3): 129-132.

Arbejdstilsynet (2011). "Bekendtgørelse om grænseværdier for stoffer og materialer." Beskæftigelseministeriet.

Barceloux, D. G. (1999). "Cobalt." J Toxicol Clin Toxicol **37**(2): 201-206.

Basketter, D. A., Lea, L. J., Cooper, K. J., Ryan, C. A., Gerberick, G. F., Dearman, R. J. and Kimber, I. (1999). "Identification of metal allergens in the local lymph node assay." Am J Contact Dermat **10**(4): 207-212.

Belloni Fortina, A., Cooper, S. M., Spiewak, R., Fontana, E., Schnuch, A. and Uter, W. (2015). "Patch test results in children and adolescents across Europe. Analysis of the ESSCA Network 2002-2010." Pediatr Allergy Immunol.

Bergstresser, P. R. and Taylor, J. R. (1977). "Epidermal 'turnover time'--a new examination." Br J Dermatol **96**(5): 503-509.

Brandt, G. (1735). "Differtatio de Semi-Metallis." Acta Literaria et Scientiarum Sveciae. Upsaliae et Stockholm (Olavi D. Wikblad) **4**.

Cade, B. S. and Noon, B. R. (2003). "A gentle introduction to quantile regression for ecologists." Front Ecol Environ **1**(8): 412-420.

Cherrie, J. W., Semple, S., Christopher, Y., Saleem, A., Hughson, G. W. and Philips, A. (2006). "How important is inadvertent ingestion of hazardous substances at work?" Ann Occup Hyg **50**(7): 693-704.

Cobalt Development Institute (CDI). (2015). "Cobalt Supply and Demand." Cobalt facts. Last accessed: 8 December 2016, from http://www.thecdi.com/cdi/images/documents/facts/Cobalt%20Facts%20-%20Supply%20%20Demand%20-%2014.pdf.

Coenraads, P., Uter, W. and Diepgen, T. (2011). "Contact Dermatitis" (5th edition). J. Johansen, P. Frosch and J. Lepoittevin, Springer, Berlin, Heidelberg. pp. 193-211.

Courtois, B. and Cadou, S. (2012). "Valeurs limites d'exposition professionnelle aux agents chimiques en France." L'Institut national de recherche et de sécurité (INRS).

Day, G. A., Virji, M. A. and Stefaniak, A. B. (2008). "Characterization of exposures among cemented tungsten carbide workers. Part II: Assessment of surface contamination and skin exposures to cobalt, chromium and nickel." J Expos Sci Environ Epidemiol **19**(4): 423-434.

Diepgen, T. L., Ofenloch, R. F., Bruze, M., Bertuccio, P., Cazzaniga, S., Coenraads, P. J., Elsner, P., Goncalo, M., Svensson, A. and Naldi, L. (2016). "Prevalence of contact allergy in the general population in different European regions." Br J Dermatol **174**(2): 319-329.

Emsley, J. (2001). "Nature's Building Blocks: An A-Z Guide to the Elements." Oxford University Press.

Erfani, B., Lidén, C. and Midander, K. (2015). "Short and frequent skin contact with nickel." Contact Dermatitis **73**(4): 222-230.

European Chemicals Agency (ECHA). (2014). "Prolonged contact with the skin - definition building for nickel." Last accessed: 12 December 2017, from https://echa.europa.eu/documents/10162/13641/nickel_restriction_prolonged_contact_skin_en.pdf.

European Chemicals Agency (ECHA) (2017a). "Commission Communication in the framework of the implementation of Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)." Official Journal of the European Union **C11**: 13.

European Chemicals Agency (ECHA) (2017b). "Guidance on labelling and packaging in accordance with Regulation (EC) No 1272/2008." **Version 3.0**.

European Chemicals Agency (ECHA). (2018a). "Understanding CLP." Last accessed: 8 May 2018, from https://echa.europa.eu/regulations/clp/understanding-clp.

European Chemicals Agency (ECHA). (2018b). "Understanding REACH." Last accessed: 8 May 2018, from https://echa.europa.eu/regulations/reach/understanding-reach.

European Commission (EC) (2008). "Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006." Official Journal of the European Union **Volume 51**(L353).

European Commission (EC) (2009). "Commission Regulation (EC) No. 552/2009 of 22 June 2009 amending Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XVII." Official Journal of the European Union.

European Commission (EC) (2014a). "Commission Regulation (EU) No 301/2014 of 25 March 2014 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards chromium VI compounds." Official Journal of the European Union **L 90/1**.

European Commission (EC). (2014b). "Ref: Request made to ECHA for the elaboration of guidelines in support to the implementation of existing restrictions on Nickel (entry 27), Polycyclic Aromatic Hydrocarbons in consumer articles (entry 50) and of the upcoming restriction of lead in consumer articles." Last accessed: 8 May 2018, from https://echa.europa.eu/documents/10162/13641/echa_commissions_request_en.pdf.

European Committee for Standardization (CEN) (1993). "Workplace atmospheres - Size fraction definitions for measurement of airborne particles." **EN 481**.

European Committee for Standardization (CEN) (2011). "Reference test method for release of nickel from all post assemblies which are inserted into pierced parts of the human body and articles intended to come into direct and prolonged contact with the skin." **EN 1811:2011**.

Ferdenzi, P., Giaroli, C., Mori, P., Pedroni, C., Piccinini, R., Ricci, R., Sala, O., Veronesi, C. and Mineo, F. (1994). "Cobalt powdersintering industry (stone cutting diamond wheels): a study of environmental-biological monitoring, workplace improvement and health surveillance." Sci Total Environ **150**(1-3): 245-248.

Fischer, L. A., Johansen, J. D., Voelund, A., Lidén, C., Julander, A., Midander, K., Menné, T. and Thyssen, J. P. (2015). "Elicitation threshold of cobalt chloride: analysis of patch test dose-response studies." Contact Dermatitis **74**(2): 105-109.

Fischer, L. A., Menné, T., Voelund, A. and Johansen, J. D. (2011). "Can exposure limitations for well-known contact allergens be simplified? An analysis of dose-response patch test data." Contact Dermatitis **64**(6): 337-342.

Fischer, T. and Rystedt, I. (1983). "Cobalt allergy in hard metal workers." Contact Dermatitis **9**(2): 115-121.

Flint, G. N. (1998). "A metallurgical approach to metal contact dermatitis." Contact Dermatitis **39**(5): 213-221.

Girod, A., Ramotowski, R. and Weyermann, C. (2012). "Composition of fingermark residue: a qualitative and quantitative review." Forensic Sci Int **223**(1-3): 10-24.

Great Britain Health Safety Commission (2011). "EH40/2005 Workplace Exposure Limits: Containing the List of Workplace Exposure Limits for Use with the Control of Substances Hazardous to Health Regulations (As Amended)", HSE Books.

Hedberg, Y. S., Erfani, B., Matura, M. and Liden, C. (2018). "Chromium(III) release from chromium-tanned leather elicits allergic contact dermatitis: a use test study." Contact Dermatitis **78**(5): 307-314.

Hodgkin, D. C., Kamper, J., Mackay, M., Pickworth, J., Trueblood, K. N. and White, J. G. (1956). "Structure of Vitamin B12." Nature **178**(4524): 64-66.

Hoet, P., Jacquerye, C., Deumer, G., Lison, D. and Haufroid, V. (2013). "Reference values and upper reference limits for 26 trace elements in the urine of adults living in Belgium." Clin Chem Lab Med **51**(4): 839-849.

Hornung, R. W. and Reed, L. D. (1990). "Estimation of Average Concentration in the Presence of Nondetectable Values." Applied Occupational and Environmental Hygiene **5**(1): 46-51.

Hutter, H. P., Wallner, P., Moshammer, H. and Marsh, G. (2016). "Dust and Cobalt Levels in the Austrian Tungsten Industry: Workplace and Human Biomonitoring Data." Int J Environ Res Public Health **13**(9).

Ichikawa, Y., Kusaka, Y. and Goto, S. (1985). "Biological monitoring of cobalt exposure, based on cobalt concentrations in blood and urine." Int Arch Occup Environ Health **55**(4): 269-276.

International Agency for Research on Cancer (IARC) (2006). "Cobalt in hard metals and cobalt sulfate, gallium arsenide, indium phosphide and vanadium pentoxide." IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Monogr Eval Carcinog Risks Hum **86**: 1-294.

International Agency for Research on Cancer (IARC) (2012). "Arsenic, Metals, Fibres and Dusts." IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Monogr Eval Carcinog Risks Hum **100 C**.

International Organization for Standardization (ISO) (2012). "ISO 15202-1:2012. Workplace air - Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry - Part 1: Sampling". Geneva, Switzerland, ISO.

International Programme on Chemical Safety (IPCS) (2004). "IPCS Risk Assessment Terminology". Geneva, World Health Organization (WHO).

Johansen, J. D., Aalto-Korte, K., Agner, T., Andersen, K. E., Bircher, A., Bruze, M., Cannavó, A., Giménez-Arnau, A., Gonçalo, M., Goossens, A., John, S. M., Lidén, C., Lindberg, M., Mahler, V., Matura, M., Rustemeyer, T., Serup, J., Spiewak, R., Thyssen, J. P., Vigan, M., White, I. R., Wilkinson, M. and Uter, W. (2015). "European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice." Contact Dermatitis **73**(4): 195-221.

Julander, A. (2018). "Metal Allergy: Cobalt" In: Metal Allergy: From Dermatitis to Implant and Device Failure. J. K. Chen and J. P. Thyssen, Springer International Publishing. pp. 365-372.

Julander, A., Hindsén, M., Skare, L. and Lidén, C. (2009). "Cobalt-containing alloys and their ability to release cobalt and cause dermatitis." Contact Dermatitis **60**(3): 165-170.

Julander, A., Lundgren, L., Skare, L., Grandér, M., Palm, B., Vahter, M. and Lidén, C. (2014). "Formal recycling of e-waste leads to increased exposure to toxic metals: an occupational exposure study from Sweden." Environ Int **73**: 243-251.

Julander, A., Skare, L., Mulder, M., Grandér, M., Vahter, M. and Lidén, C. (2010). "Skin deposition of nickel, cobalt, and chromium in production of gas turbines and space propulsion components." Ann Occup Hyg **54**(3): 340-350.

Julander, A., Skare, L., Vahter, M. and Lidén, C. (2011). "Nickel deposited on the skin-visualization by DMG test." Contact Dermatitis **64**(3): 151-157.

Kettelarij, J., Lidén, C., Axén, E. and Julander, A. (2014). "Cobalt, nickel and chromium release from dental tools and alloys." Contact Dermatitis **70**(1): 3-10.

Kettelarij, J., Nilsson, S., Midander, K., Lidén, C. and Julander, A. (2016). "Snapshot of cobalt, chromium and nickel exposure in dental technicians." Contact Dermatitis.

Kiilunen, M. (2017). "Biomonitoring of exposure to chemicals: Guideline for specimen collection." Last accessed: 9 May 2018, from https://www.ttl.fi/wp-content/uploads/2017/11/Biomonitoring-of-exposure-to-chemicals-Guideline-for-specimen-collection.pdf.

Kim, J. H., Gibb, H. J., Howe, P. D. and Sheffer, M. (2006). "Cobalt and Inorganic Cobalt Compounds", World Health Organization.

Klasson, M., Bryngelsson, I. L., Pettersson, C., Husby, B., Arvidsson, H. and Westberg, H. (2016). "Occupational Exposure to Cobalt and Tungsten in the Swedish Hard Metal Industry: Air Concentrations of Particle Mass, Number, and Surface Area." Ann Occup Hyg **60**(6): 684-699.

Kligman, A. M. (1966). "The identification of contact allergens by human assay. 3. The maximization test: a procedure for screening and rating contact sensitizers." J Invest Dermatol **47**(5): 393-409.

Koenker, R. and Bassett, G. (1978). "Regression Quantiles." Econometrica **46**(1): 33-50.

Koullapis, P. G., Kassinos, S. C., Bivolarova, M. P. and Melikov, A. K. (2015). "Particle deposition in a realistic geometry of the human conducting airways: Effects of inlet velocity profile, inhalation flowrate and electrostatic charge." J Biomech.

Kristiansen, J., Christensen, J. M., Iversen, B. S. and Sabbioni, E. (1997). "Toxic trace element reference levels in blood and urine: influence of gender and lifestyle factors." Science of The Total Environment **204**(2): 147-160.

Lagrelius, M., Wahlgren, C. F., Matura, M., Kull, I. and Lidén, C. (2016). "High prevalence of contact allergy in adolescence: results from the population-based BAMSE birth cohort." Contact Dermatitis **74**(1): 44-51.

Larese Filon, F., Crosera, M., Timeus, E., Adami, G., Bovenzi, M., Ponti, J. and Maina, G. (2013). "Human skin penetration of cobalt nanoparticles through intact and damaged skin." Toxicol In Vitro **27**(1): 121-127.

Larese Filon, F., Maina, G., Adami, G., Venier, M., Coceani, N., Bussani, R., Massiccio, M., Barbieri, P. and Spinelli, P. (2004). "In vitro percutaneous absorption of cobalt." Int Arch Occup Environ Health **77**(2): 85-89.

Lee, J. Y., Yoo, J. M., Cho, B. K. and Kim, H. O. (2001). "Contact dermatitis in Korean dental technicians." Contact Dermatitis **45**(1): 13-16.

Leggett, R. W. (2008). "The biokinetics of inorganic cobalt in the human body." Sci Total Environ **389**(2-3): 259-269.

Leghissa, P., Ferrari, M. T., Piazzolla, S., Caironi, M., Parigi, P. C. and Lebbolo, E. (1994). "Cobalt exposure evaluation in dental prostheses production." Sci Total Environ **150**(1-3): 253-257.

Leikauf, G. D. (2013). "Chapter 15: Toxic responses of the respiratory system." In: Casarett & Doull's toxicology: the basic science of poisons (8th edition). C. D. Klaassen, L. J. Casarett and J. Doull. New York, McGraw-Hill.

Lidén, C. (2012). "Nickel" In: Kanerva's Occupational Dermatology. T. Rustemeyer, P. Elsner, S. John and H. I. Maibach. Berlin, Heidelberg, Springer. pp. 485-494.

Lidén, C., Andersson, N., Julander, A. and Matura, M. (2016). "Cobalt allergy: suitable test concentration, and concomitant reactivity to nickel and chromium." Contact Dermatitis **74**(6): 360-367.

Lidén, C., Bruze, M., Thyssen, J. P. and Menné, T. (2011). "Metals" In: Contact Dermatitis (5th edition). J. D. Johansen, P. J. Frosch and J.-P. Lepoittevin. Berlin and Heidelberg, Springer-Verlag. pp. 643-679.

Lidén, C. and Julander, A. (2012). "Cobalt" In: Kanerva's Occupational Dermatology. T. Rustemeyer, P. Elsner, S. John and H. I. Maibach. Berlin, Heidelberg, Springer. pp. 505-510.

Lidén, C., Skare, L., Lind, B., Nise, G. and Vahter, M. (2006). "Assessment of skin exposure to nickel, chromium and cobalt by acid wipe sampling and ICP-MS." Contact Dermatitis **54**(5): 233-238.

Lidén, C., Skare, L., Nise, G. and Vahter, M. (2008a). "Deposition of nickel, chromium, and cobalt on the skin in some occupations - assessment by acid wipe sampling." Contact Dermatitis **58**(6): 347-354.

Lidén, C., Skare, L. and Vahter, M. (2008b). "Release of nickel from coins and deposition onto skin from coin handling--comparing euro coins and SEK." Contact Dermatitis **59**(1): 31-37.

Lidén, G. and Bergman, G. (2001). "Weighing imprecision and handleability of the sampling cassettes of the IOM sampler for inhalable dust." Ann Occup Hyg **45**(3): 241-252.

Linnainmaa, M. and Kiilunen, M. (1997). "Urinary cobalt as a measure of exposure in the wet sharpening of hard metal and stellite blades." Int Arch Occup Environ Health **69**(3): 193-200.

Lison, D., Buchet, J. P., Swennen, B., Molders, J. and Lauwerys, R. (1994). "Biological monitoring of workers exposed to cobalt metal, salt, oxides, and hard metal dust." Occup Environ Med **51**(7): 447-450.

Martin, A., Bois, F. Y., Pierre, F. and Wild, P. (2010). "Occupational exposure to cobalt: a population toxicokinetic modeling approach validated by field results challenges the biological exposure index for urinary cobalt." J Occup Environ Hyg **7**(1): 54-62.

Midander, K., Julander, A., Kettelarij, J. and Lidén, C. (2016). "Testing in artificial sweat - Is less more? Comparison of metal release in two different artificial sweat solutions." Regul Toxicol Pharmacol **81**: 381-386.

Midander, K., Julander, A., Skare, L., Thyssen, J. P. and Lidén, C. (2013). "The cobalt spot test--further insights into its performance and use." Contact Dermatitis **69**(5): 280-287.

Mosconi, G., Bacis, M., Vitali, M. T., Leghissa, P. and Sabbioni, E. (1994). "Cobalt excretion in urine: results of a study on workers producing diamond grinding tools and on a control group." Sci Total Environ **150**(1-3): 133-139.

Oberdörster, G., Oberdörster, E. and Oberdörster, J. (2005). "Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles." Environ Health Perspect **113**(7): 823-839.

Organisation for Economic Co-operation and Development (OECD) (2004). "Test guideline 428: Skin Absorption: *in vitro* Method", OECD Publishing.

Paustenbach, D. J., Tvermoes, B. E., Unice, K. M., Finley, B. L. and Kerger, B. D. (2013). "A review of the health hazards posed by cobalt." Crit Rev Toxicol **43**(4): 316-362.

Pesonen, M., Jolanki, R., Larese Filon, F., Wilkinson, M., Kręcisz, B., Kieć-Świerczyńska, M., Bauer, A., Mahler, V., John, S. M., Schnuch, A., Uter, W. and and on behalf of the ESSCA network (2015). "Patch test results of the European baseline series among patients with occupational contact dermatitis across Europe – analyses of the

European Surveillance System on Contact Allergy network, 2002–2010." Contact Dermatitis **72**(3): 154-163.

Rehfisch, P., Anderson, M., Berg, P., Lampa, E., Nordling, Y., Svartengren, M., Westberg, H. and Gunnarsson, L. G. (2012). "Lung function and respiratory symptoms in hard metal workers exposed to cobalt." J Occup Environ Med **54**(4): 409-413.

Ringborg, E., Lidén, C. and Julander, A. (2016). "Nickel on the market: a baseline survey of articles in 'prolonged contact' with skin." Contact Dermatitis **75**(2): 77-81.

Rustemeyer, T., van Hoogstraten, I. M. W., von Blomberg, B. M. E., Gibbs, S. and Scheper, R. J. (2011). "Mechanisms of Irritant and Allergic Contact Dermatitis" In: Contact Dermatitis. J. D. Johansen, P. J. Frosch and J.-P. Lepoittevin. Berlin, Heidelberg, Springer Berlin Heidelberg. pp. 43-90.

Rystedt, I. and Fischer, T. (1983). "Relationship between nickel and cobalt sensitization in hard metal workers." Contact Dermatitis **9**(3): 195-200.

Scansetti, G., Botta, G. C., Spinelli, P., Reviglione, L. and Ponzetti, C. (1994). "Absorption and excretion of cobalt in the hard metal industry." Sci Total Environ **150**(1-3): 141-144.

Scansetti, G., Lamon, S., Talarico, S., Botta, G. C., Spinelli, P., Sulotto, F. and Fantoni, F. (1985). "Urinary cobalt as a measure of exposure in the hard metal industry." Int Arch Occup Environ Health **57**(1): 19-26.

Schalock, P. C., Menné, T., Johansen, J. D., Taylor, J. S., Maibach, H. I., Lidén, C., Bruze, M. and Thyssen, J. P. (2012). "Hypersensitivity reactions to metallic implants - diagnostic algorithm and suggested patch test series for clinical use." Contact Dermatitis **66**(1): 4-19.

Schmidt, M. and Goebeler, M. (2015). "Immunology of metal allergies." J Dtsch Dermatol Ges **13**(7): 653-660.

Schneider, T., Vermeulen, R., Brouwer, D. H., Cherrie, J. W., Kromhout, H. and Fogh, C. L. (1999). "Conceptual model for assessment of dermal exposure." Occupational and Environmental Medicine **56**(11): 765-773.

Sethi, G., Belum, V. R., Burrows, D., Maibach, H. I. and Hostynek, J. J. (2012). "Chromium" In: Kanerva's Occupational Dermatology. T. Rustemeyer, P. Elsner, S. John and H. I. Maibach. Berlin, Heidelberg, Springer. pp. 495-504.

Shiao, J. S., Sheu, H. M., Chen, C. J., Tsai, P. J. and Guo, Y. L. (2004). "Prevalence and risk factors of occupational hand dermatoses in electronics workers." Toxicol Ind Health **20**(1-5): 1-7.

Simonsen, L. O., Harbak, H. and Bennekou, P. (2012). "Cobalt metabolism and toxicology--a brief update." Sci Total Environ **432**: 210-215.

Smith, T., Edmonds, C. J. and Barnaby, C. F. (1972). "Absorption and retention of cobalt in man by whole-body counting." Health Phys **22**(4): 359-367.

Social- och hälsovårdsministeriets publikationer 2014:3 (2014). "HTP-värden 2014: Koncentrationer som befunnits skadliga." Helsingfors, Social- och hälsovårdsministeriet.

Sorbie, J., Olatunbosun, D., Corbett, W. E. and Valberg, L. S. (1971). "Cobalt excretion test for the assessment of body iron stores." Can Med Assoc J **104**(9): 777-782.

Staatssecretaris van Sociale Zaken en Werkgelegenheid (1997, valid in 2016). "Lijst van wettelijke grenswaarden op grond van de artikelen 4.3, eerste lid, en 4.16, eerste lid, van het Arbeidsomstandighedenbesluit." Ministerie van Sociale Zaken en Werkgelegenheid. **Artikel 4.19, eerste lid. Bijlage XIII**.

Summer, B., Fink, U., Zeller, R., Rueff, F., Maier, S., Roider, G. and Thomas, P. (2007). "Patch test reactivity to a cobalt–chromium–molybdenum alloy and stainless steel in metal-allergic patients in correlation to the metal ion release." Contact Dermatitis **57**(1): 35-39.

Suwazono, Y., Åkesson, A., Alfvén, T., Järup, L. and Vahter, M. (2005). "Creatinine versus specific gravity-adjusted urinary cadmium concentrations." Biomarkers **10**(2-3): 117-126.

Swedish National Food Agency. (2018). "Vitamin B12." Last accessed: 9 May 2018, from http://www.livsmedelsverket.se/livsmedel-och-innehall/naringsamne/vitaminer-och-antioxidanter/vitamin-b12/.

Swedish Work Environment Authority (AV). (2015). "Occupational Exposure Limit Values, AFS 2015:7." Last accessed: 9 May 2018, from https://www.av.se/globalassets/filer/publikationer/foreskrifter/hygieniska-gransvarden-afs-2015-7.pdf.

Taylor, N. A. and Machado-Moreira, C. A. (2013). "Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans." Extrem Physiol Med **2**(1): 4.

The American Conference of Governmental Industrial Hygienists (ACGIH) (2001). "Cobalt, Elemental and Inorganic Compounds: TLV(R) Chemical Substances 7th Edition Documentation."

Thyssen, J. P., Gawkrodger, D. J., White, I. R., Julander, A., Menne, T. and Liden, C. (2013). "Coin exposure may cause allergic nickel dermatitis: a review." Contact Dermatitis **68**(1): 3-14.

Thyssen, J. P., Menné, T., Johansen, J. D., Lidén, C., Julander, A., Møller, P. and Jellesen, M. S. (2010a). "A spot test for detection of cobalt release - early experience and findings." Contact Dermatitis **63**(2): 63-69.

Thyssen, J. P., Skare, L., Lundgren, L., Menne, T., Johansen, J. D., Maibach, H. I. and Liden, C. (2010b). "Sensitivity and specificity of the nickel spot (dimethylglyoxime) test." Contact Dermatitis **62**(5): 279-288.

United States Geological Survey (USGS) (2017). "2015 Minerals Yearbook: Cobalt [advance release]". Washington DC.

Uter, W., Aberer, W., Armario-Hita, J. C., Fernandez-Vozmediano, J. M., Ayala, F., Balato, A., Bauer, A., Ballmer-Weber, B., Beliauskiene, A., Fortina, A. B., Bircher, A., Brasch, J., Chowdhury, M. M., Coenraads, P. J., Schuttelaar, M. L., Cooper, S., Czarnecka-Operacz, M., Zmudzinska, M., Elsner, P., English, J. S., Frosch, P. J., Fuchs, T., García-Gavín, J., Fernández-Redondo, V., Gawkrodger, D. J., Giménez-Arnau, A., Green, C. M., Horne, H. L., Johansen, J. D., Jolanki, R., Pesonen, M., King, C. M., Kręcisz, B., Chomiczewska, D., Kieć-Świerczyńska, M., Larese, F., Mahler, V., Ormerod, A. D., Peserico, A., Rantanen, T., Rustemeyer, T., Sanchez-Perez, J., Sansom, J. E., Silvestre, J. F., Simon, D., Spiewak, R., Statham, B. N., Stone, N., Wilkinson, M. and Schnuch, A. (2012). "Current patch test results with the

European baseline series and extensions to it from the 'European Surveillance System on Contact Allergy' network, 2007-2008." Contact Dermatitis **67**(1): 9-19.

Uter, W., Larese Filon, F., Rui, F., Balato, A., Wilkinson, M., Kręcisz, B., Chomiczewska-Skora, D., Kieć-Świerczyńska, M., Schuttelaar, M. L., Frosch, P. J. and Bircher, A. J. (2016). "ESSCA results with nickel, cobalt and chromium, 2009-2012." Contact Dermatitis **75**(2): 117-121.

Uter, W., Ruhl, R., Pfahlberg, A., Geier, J., Schnuch, A. and Gefeller, O. (2004). "Contact allergy in construction workers: results of a multifactorial analysis." Ann Occup Hyg **48**(1): 21-27.

Wahlberg, J. E. and Boman, A. (1978). "Sensitization and testing of guinea pigs with cobalt chloride." Contact Dermatitis **4**(3): 128-132.

Wang, D., Xin, H. L., Hovden, R., Wang, H., Yu, Y., Muller, D. A., DiSalvo, F. J. and Abruña, H. D. (2013). "Structurally ordered intermetallic platinum–cobalt core–shell nanoparticles with enhanced activity and stability as oxygen reduction electrocatalysts." Nat Mater **12**(1): 81-87.

Wang, Y. X., Feng, W., Zeng, Q., Sun, Y., Wang, P., You, L., Yang, P., Huang, Z., Yu, S. L. and Lu, W. Q. (2016). "Variability of Metal Levels in Spot, First Morning, and 24-Hour Urine Samples over a 3-Month Period in Healthy Adult Chinese Men." Environ Health Perspect **124**(4): 468-476.

Waters, M., McKernan, L., Maier, A., Jayjock, M., Schaeffer, V. and Brosseau, L. (2015). "Exposure Estimation and Interpretation of Occupational Risk: Enhanced Information for the Occupational Risk Manager." J Occup Environ Hyg **12 Suppl 1**: S99-111.

Wehner, A. P., Busch, R. H., Olson, R. J. and Craig, D. K. (1977). "Chronic inhalation of cobalt oxide and cigarette smoke by hamsters." Am Ind Hyg Assoc J **38**(7): 338-346.

Veien, N. K., Hattel, T. and Laurberg, G. (1993). "Low nickel diet: an open, prospective trial." J Am Acad Dermatol **29**(6): 1002-1007.

Westberg, H., Bryngelsson, I. L., Marsh, G., Buchanich, J., Zimmerman, S., Kennedy, K., Esmen, N. and Svartengren, M. (2017). "Mortality Among Hardmetal Production Workers: The Swedish Cohort." J Occup Environ Med **59**(12): e263-e274.

White, M. A. and Sabbioni, E. (1998). "Trace element reference values in tissues from inhabitants of the European Union. X. A study of 13 elements in blood and urine of a United Kingdom population." Science of The Total Environment **216**(3): 253-270.