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# **OCCUPATIONAL IMMEDIATE SENSITIZATION TO CHEMICALS**

- WITH SPECIAL REFERENCE TO DIAGNOSTIC SKIN  
TESTS AND CONTACT URTICARIA

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ACADEMIC DISSERTATION

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# ABSTRACT

A large number of chemicals are used in work life, and a substantial number of workers come into contact with chemicals in their daily work. Some of the chemicals have sensitizing properties and can cause long-term health effects in exposed workers; at worst even loss of work ability. The most effective measures for reducing the risk of health disorders due to sensitizing substances are the prevention of exposure and early recognition of emerging symptoms.

The lack of knowledge regarding the diagnostics and prevention of immediate sensitization to chemicals in the occupational environment remains substantial. The main aim of this thesis was to assess the diagnostic procedures of immediate allergic diseases with an occupational background and evaluate the feasibility of skin tests in the diagnostics. Occupational contact urticaria, a rare and not so well-known occupational disease, was given particular attention. The concomitant occurrence of immediate allergic skin and airway diseases was also investigated.

The study was based on a retrospective review of the patient material of the occupational medicine clinic at the Finnish Institute of Occupational Health (FIOH) in Helsinki for the period 1.1.1990–31.5.2011. FIOH's occupational medicine clinic is a tertiary-level referral clinic for patients with suspected occupational diseases. The most common indications for referral to the clinic are work-related respiratory and skin symptoms. As the substudies had different aims, the populations of the individual studies were not identical. For Study I, FIOH's patient and test files for the period 1990–2006 were reviewed to identify all patients who had been diagnosed with occupational contact urticaria due to organic acid anhydrides. A total of 21 such patients were found. For Study II, patient and test files for the period of 2001–2011 were reviewed, and 11 patients diagnosed with occupational immediate allergic diseases associated with oxidative hair dyes were analysed. Study III was based on all the skin prick tests performed with chemicals at FIOH during the period 1.1.1991–31.5.2011. In Study IV, FIOH's patient and test files for the period 1.1.1995–31.5.2011 were retrospectively reviewed. Occupational contact urticaria or protein contact dermatitis (another skin disease mediated by immediate allergy), was found in 291 patients.

During the study period, positive skin prick tests were noted for organic acid anhydrides, isocyanates, epoxy resins, persulfates, chloramine T, chlorhexidine, and aziridine. Amine hardeners, formaldehyde, glutaraldehyde, paraphenylene-diamine, methacrylates and colophonium all induced sporadic, small prick test reactions with doubtful clinical relevance. Ethanolamines, pyrocatechol, ammonium thioglycolate and glyoxal did not induce positive prick test reactions during the study period.

Of the 291 diagnoses of immediate allergic skin diseases, contact urticaria was diagnosed in 232 cases (80%) and protein contact dermatitis in 59 cases (20%). Flour, grains and animal feed were the most common cause (21%), followed by cow dander (18%) and natural rubber latex (15%). Exposure to chemicals was the cause of contact urticaria in 41 (14%) of the patients. Exposure to acid anhydrides was the most common causative agent in chemical-induced contact urticaria.

A concomitant airway disease caused by the same work-related agent was diagnosed in as many as 134 (46%) of the patients with contact urticaria or protein contact dermatitis. Of these, 111 patients (38%) were diagnosed with occupational rhinitis, and 60 patients (21%) with occupational asthma caused by the same substance that caused the skin reaction.

*Conclusions:* Diagnostics of immediate allergic diseases induced by the occupational environment are challenging and there is a strong need for standardized allergen extracts, established test methods and confirmed guidelines for diagnostics.

IgE-mediated sensitization seems to play a significant role in the development of occupational allergic diseases for a selected group of chemicals, and for these chemicals skin prick tests can be of substantial value in the diagnostics. The results should still be interpreted cautiously, and data on exposure, clinical symptoms and results in other clinical tests performed should be taken into account. For a considerable amount of chemicals, the role of specific IgE in the development of allergic symptoms remains uncertain, and the value of skin prick testing in the diagnostics is limited.

Early identification of potential immediate sensitizers in the workplace is important to prevent long-lasting health effects and the loss of work ability. Immediate sensitization to chemicals can be prevented by substituting harmful agents with safer ones when possible, appropriate working methods, educating workers, and adequate protective equipment. Concurrent allergic airway diseases are quite common in patients with occupational contact urticaria and protein contact dermatitis, and preventive measures should comprise both skin and airway protection.

# TIIVISTELMÄ

Työelämässä käytetään suuria määriä erilaisia kemikaaleja ja merkittävä joukko työntekijöitä kohtaa kemikaaleja päivittäisessä työssään. Eräillä kemikaaleilla on herkistäviä ominaisuuksia ja ne voivat aiheuttaa pitkäaikaisia terveysvaikutuksia ja pahimmillaan työkyvyn menettämistä altistuneissa työntekijöissä. Tehokkaimmat toimenpiteet herkistävien aineiden haitallisten terveysvaikutusten estämiseksi ovat altistumisen ennaltaehkäisy ja alkavien oireiden varhainen tunnistaminen.

Välittömän työperäisen kemikaaliherkistymisen diagnostiikassa ja ennaltaehkäisyssä on edelleen huomattavia osaamisen puutteita. Tämän väitöskirjatyön päätavoitteena oli arvioida työperäisten välittömien allergisten sairauksien diagnostisia käytäntöjä ja ihopistokokeiden soveltuvuutta yhtenä diagnostisena keinona. Ammattitautina verrattain tuntemattomaan kosketusurtikariaan kohdistettiin erityistä huomiota. Tämän lisäksi tutkimme välittömien allergisten iho- ja hengitystiesairauksien samanaikaista esiintymistä.

Tutkimus perustui Työterveyslaitoksen (TTL) Helsingin työlääkietieteen klinikan potilasaineiston retrospektiiviseen tarkasteluun ajanjaksolta 1.1.1990–31.5.2011. Työterveyslaitoksen työlääkietieteen klinikka on erityistason läheteclinikka henkilöille, joilla on epäily ammattitaudista. Yleisimmät lähettämisen indikaatiot ovat työhön liittyvät hengitystie- ja iho-oireet. Tutkimusosioiden erilaisten tavoitteiden johdosta tutkimusaineisto ei ollut identtinen kaikissa osatutkimuksissa. Tutkimuksessa I tarkastelimme TTL:n potilas- ja testitiedostoja ajanjaksolta 1990–2006 löytääksemme kaikki tutkittavat, joilla oli todettu orgaanisten happoanhydridien aiheuttama kosketusurtikaria ja identifioimme yhteensä 21 henkilöä, joilla kriteerit täyttyivät. Tutkimuksessa II kartoitettiin potilas- ja testitiedostoja ajalta 2001–2011 niiden yhdentoista henkilön identifioimiseksi, joilla oli todettu välitön allerginen ammattitauti hapetettaviin hiusväreihin liittyen. Tutkimus III perustui kaikkien TTL:lla ajanjaksolla 1.1.1991–31.5.2011 kemikaaleilla suoritettujen ihopistokokeiden tulosten analysointiin. Tutkimuksessa IV tarkasteltiin TTL:n potilas- ja testiaineistoja ajanjaksolta 1.1.1995–31.5.2011 niiden 291 henkilön löytämiseksi, joilla oli todettu kosketusurtikaria tai proteiinikosketusihottuma ammattitautina.

Tutkimusjakson aikana positiivisia ihopistokokeita todettiin orgaanisille happoanhydrideille, isosyanaateille, epoksiresiineille, persulfaateille, Kloramin T:lle, klooriheksidiinille ja atsiridiinille. Amiinikovetteet, formaldehydi, glutaraldehydi, parafenyleenidiamiini, metakrylaatit ja kolofoni aiheuttivat kaikki satunnaisia, pieniä ihopistokoereaktioita, joiden kliininen merkitys jäi epävarmaksi. Etanoliamiinit, pyrokatekoli, ammoniumtioglykolaatti ja glyoksaali eivät aiheuttaneet lainkaan positiivisia ihopistokoereaktioita tutkimusjakson aikana.

Kaikista 291 tutkittavista, joilla oli todettu välitön, allerginen ihosairaus kosketusurtikaria todettiin 232 henkilöllä (80 %) ja proteiinikosketusihottuma 59 henkilöllä (20 %). Jauhot, jyvät ja rehut olivat yleisin aiheuttaja (21 %), ennen lehmän hilsettä (18 %) ja luonnonkumia (15 %). Altistuminen kemikaaleille aiheutti työperäisen kosketusurtikarian 41 henkilölle (14 %), yleisin aiheuttaja kemikaalien joukossa oli happoanhydritit.

Samanaikainen hengitystiesairaus saman työperäisen altisteen aiheuttamana todettiin 134 tutkittavalla (46 %), joilla oli todettu tutkimusjakson aikana kosketusurtikaria tai proteiinikosketusihottuma. Heistä 111 henkilöllä (38 %) todettiin ammattinuha ja 60 henkilöllä (21 %) ammattiastma saman työssä esiintyvän tekijän aiheuttamana, joka oli aiheuttanut ihosairauden.

*Johtopäätökset:* Välittömien työperäisten allergisten sairauksien diagnostiikka on vaativaa ja standardoiduille allergeeniuutteille, vakiintuneille testausmenetelmille ja yleisesti hyväksytyille toimintaohjeille on huomattava tarve.

IgE-välitteinen herkistyminen vaikuttaa olevan merkittävässä osassa välittömien allergisten ammattitautien kehittämisessä valikoituneissa kemikaaliryhmissä ja näiden ryhmien kohdalla ihopistokokeista voi olla ratkaisevaa hyötyä diagnostiikassa. Tuloksia tulee kuitenkin tulkita varovasti, huomioiden myös altistumistieto, kliiniset oireet ja muiden suoritettujen tutkimusten tulokset. Huomattavalla osalla kemikaaleja spesifin IgE:n rooli allergisten oireiden kehittämisessä jää edelleen epäselväksi ja ihopistokokeista saatava hyöty diagnostiikassa on rajallinen.

Potentiaalisten herkistäjien varhainen tunnistaminen työssä on tärkeää pitkäaikaisten haitallisten terveysvaikutusten ja työkyvyn menettämisen ehkäisemiseksi. Välitön herkistyminen kemikaaleille on ehkäistävissä korvaamalla mahdollisuuksien mukaan haitalliset aineet turvallisemmilla, käyttämällä asianmukaisia työmenetelmiä ja suojarusteita ja kouluttamalla työntekijöitä. Samanaikaiset allergiset hengitystiesairaudet ovat varsin yleisiä potilailla, joilla todetaan ammattitautina kosketusurtikaria tai proteiinikosketusihottuma ja ennaltaehkäisevien toimenpiteiden tulisi käsittää sekä ihon että hengitysteiden suojaamisen.

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# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, referred to in the text by Roman numerals I to IV. The original articles have been reprinted with the permission of the copyright holders.

- I Helaskoski E, Kuuliala O, Aalto-Korte K. Occupational contact urticaria caused by cyclic acid anhydrides. *Contact Dermatitis*. 2009 Apr;60(4):214-21.
- II Helaskoski E, Suojalehto H, Virtanen H, Airaksinen L, Kuuliala O, Aalto-Korte K, Pesonen M. Occupational asthma, rhinitis, and contact urticaria caused by oxidative hair dyes in hairdressers. *Ann Allergy Asthma Immunol*. 2014 Jan;112(1):46-52.
- III Helaskoski E, Suojalehto H, Kuuliala O, Aalto-Korte K. Prick testing with chemicals in the diagnosis of occupational contact urticaria and respiratory diseases. *Contact Dermatitis*. 2015 Jan;72(1):20-32.
- IV Helaskoski E, Suojalehto H, Kuuliala O, Aalto-Korte K. Occupational contact urticaria and protein contact dermatitis: causes and concomitant airway diseases. *Contact Dermatitis*. 2017 Dec;77(6):390-396.

# ABBREVIATIONS

APC	antigen-presenting cell
CA	chlrendic anhydride
CLP	Classification, Labelling and Packaging
CU	contact urticaria
ECHA	European Chemicals Agency
ELISA	enzyme-linked immunosorbent assay
FENO	fractional exhaled nitric oxide
FIOH	Finnish Institute of Occupational Health
FROD	Finnish Register of Occupational Diseases
GINA	Global Initiative for Asthma
GHS	Globally Harmonised System
HDI	hexamethylene diisocyanate
HHPA	hexahydrophthalic anhydride
HI	hazard index
HMW	high molecular weight
HSA	human serum albumin
ICU	immunological contact urticaria
IgE	immunoglobulin E
kDa	kilodalton
LMW	low molecular weight
MA	maleic anhydride
MDI	diphenylmethane diisocyanate
MHHPA	methyl hexahydrophthalic anhydride
MSDS	material safety data sheet
MTHPA	methyl tetrahydrophthalic anhydride
NRL	natural rubber latex
OA	occupational asthma
OEL	occupational exposure limits
OR	occupational rhinitis
PA	phthalic anhydride
PCD	protein contact dermatitis
PEF	peak expiratory flow
PPD	para-phenylenediamine
QSAR	quantitative structure-activity relationship
RAST	radioallergosorbent test
SIC	specific inhalation challenge
SPT	skin prick test
TDI	toluene diisocyanate
WER	work-exacerbated rhinitis



# 1 INTRODUCTION

Regardless of today's fast-changing work life, work-related exposure to chemicals is still quite common in Finland. New chemicals are constantly being introduced onto the market and not all their potential health effects are known in detail. It is estimated that over one million Finnish workers come into contact with different chemicals, dusts and gases in their work environment to some extent, and that about 40 000 workers are exposed to chemicals in their daily work (Vainio et al. 2005). Exposure to chemicals occurs in a wide range of professional fields, not only in industrial environments, but also in, for example, health care, cleaning and hairdressing.

Some chemicals are able to induce allergic sensitization in humans. Low molecular weight (LMW) chemicals typically induce delayed, cell-mediated Type IV sensitization, while IgE-mediated immediate Type I sensitization is more commonly caused by high molecular weight (HMW) proteins (Maestrelli et al. 2012). Nevertheless, some groups of chemicals are also known to induce immediate allergic reactions similar to the allergic reactions caused by proteins. The mechanisms of immediate sensitization to chemicals are still mainly unknown, but in the case of some chemical groups at least, IgE-mediated mechanisms seem to play a role (Kimber et al. 2009).

Immediate sensitization to a work-related agent can evolve into a symptomatic occupational allergic disease such as asthma, rhinitis or contact urticaria (CU), and in very rare cases anaphylaxis. The present summary refers to these diseases as *occupational immediate allergic diseases*, although all the cases referred to by this definition are not necessarily mediated by a Type I immediate allergic mechanism. The symptoms of the diseases are usually immediate, and immediate IgE-mediated allergy is at present the only allergic mechanism that can be investigated in clinical practice.

Occupational allergic diseases often have a marked influence on work ability and the future working career of a patient. Prevention and early recognition of potential sensitizers are therefore essential. Prevention of occupational diseases and improvement of workplace health and safety are among the most important tasks of the occupational health care system. It is therefore crucial that occupational health care professionals are aware of the risks involved with handling sensitizing substances, and that they recognize potential cases of sensitization and occupational diseases and know how exposure and sensitization can be prevented at the workplace.

There are still large gaps in the knowledge regarding immediate sensitization to chemicals and the related occupational skin and airway diseases. The aim

of the present set of studies was to evaluate the diagnostic procedures of occupational immediate allergic diseases caused by chemicals and to provide guidance and recommendations for health care professionals. The occupational medicine unit at the Finnish Institute of Occupational Health (FIOH) has a long tradition in exposure assessment at the workplace and diagnostics of occupational diseases, especially diseases related to chemical exposure. Skin prick tests (SPTs) and open application tests of the skin have been used extensively at FIOH, and there was an obvious need for a thorough evaluation of the feasibility of these test methods for the diagnostics of occupational diseases.

## 2 REVIEW OF THE LITERATURE

### 2.1 IMMEDIATE SENSITIZATION IN OCCUPATIONAL MEDICINE

Immediate sensitization is classified as a Type I hypersensitivity reaction mediated by allergen-specific *immunoglobulin E* (IgE) in an individual who has previously been sensitized to the allergen. Sensitization usually occurs via the respiratory or gastrointestinal tracts, but sensitization through the skin can also occur. Immediate allergic reactions are the result of the production of specific IgE against external antigens, which are typically proteins, but in some cases also chemicals (Maestrelli et al. 2012).

Immediate sensitization as such is very common. Atopy, or the tendency to produce IgE antibodies to environmental allergens, affects up to 30%–40% of the population in developed countries (Oettgen and Broide 2012). Most epidemiological studies define atopy as a positive test result of specific IgE to any common food or inhalant allergen (Gruchalla et al. 2012). The World Allergy Organization (WAO) defines atopy as “a personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins” (Johansson et al. 2004). Genetic as well as environmental factors play an important role in the development of atopy (Barnes 2011).

Immediate sensitization is associated with the development of allergic diseases including allergic rhinitis, asthma, urticaria, atopic dermatitis and anaphylaxis. In occupational settings, the most common manifestations of immediate sensitization are asthma, allergic rhinitis and CU. Atopy has been linked to an increased risk of sensitization to various occupational HMW agents (Bourrain 2006).

Immediate sensitization, however, is not the same as a clinical allergic disease. A person might not experience any allergic symptoms despite laboratory results showing that the mechanisms of immediate sensitization have been activated in the body. Nevertheless, sensitization may evolve into a symptomatic allergic disorder if the exposure continues. In an occupational setting this is of particular importance as regards preventive measures (Cox et al. 2008).

Immediate sensitization to occupational agents and the possible occupational diseases following sensitization are significant threats to workers' health and can lead to permanent impairment and loss of work ability. At worst, an employee may be forced to leave the occupation for which



he is trained and find a new career, which can be challenging in many ways. In addition, the symptoms and clinical findings of an allergic occupational disease can in some cases persist even after the worker is removed from the causative agent, as has been shown for several substances causing occupational asthma (OA) (Tarlo and Lemiere 2014).

Some studies have reported the risk of developing IgE-mediated sensitization at work to be directly related to the level and duration of exposure, as in, for instance, sensitization to laboratory animals (Nieuwenhuijsen et al. 2003), flour (Cullinan et al. 2001) and enzymes (Cullinan et al. 2000). Exposure seems to be an even more important determinant of the risk of developing specific IgE to these substances than personal and genetic factors. Other studies, however, have shown a more complex relationship between exposure and sensitization, in which the risk of sensitization declines at high exposure levels (Jones 2008). This might be explained by the healthy worker effect, which is when the ratio of sensitized workers is modified because symptomatic workers have been removed from employment. This has been demonstrated among, for example, bakery workers by a bell-shape exposure-response relationship (Jacobs et al. 2008). Nevertheless, reducing exposure to potential sensitizers is crucial for the prevention of immediate sensitization and occupational allergic diseases.

## **2.2 MECHANISMS OF IgE-MEDIATED SENSITIZATION**

The immunological cascade reaction leading to an immediate hypersensitivity response is quite complex and not yet fully understood. The chain of events begins with exposure to an antigen that acts as an allergen. The allergen is taken up by antigen-presenting cells (APCs), typically macrophages, dendritic cells or B cells, which present the allergen to helper T cells. The activated helper T cells secrete cytokines that make the B cells proliferate to plasma cells and produce antigen-specific IgE. Antigen-dependent activation of the mast cells starts with IgE antibodies binding to FcεRI, CD23 and other receptors on the surface of the mast cells and other hematopoietic cells (Galli and Tsai 2012). Subsequent allergen exposure causes the antigen-specific IgE to become crosslinked by the allergen, which activates the cells to release both preformed and newly synthesized mediator substances such as histamine, proteoglycans, proteases, cytokines and growth factors, resulting in an immediate allergic reaction (Galli et al. 2008).

The presence in serum of a factor that causes allergy was proposed already in 1919, when a case of allergic asthma was reported to have been transferred by blood transfusion. In 1921, Praunitz and Kustner succeeded in transferring positive skin test reactivity from human to human. IgE was finally discovered by two independent research teams in the United States and in Sweden in the

1960s and the World Health Organization Immunoglobulin Reference Centre in Lausanne officially declared a new immunoglobulin in 1968 (Johansson 2011). IgE is thought to have originally emerged as a defence mechanism against parasites. Compared to the other four classes of antibodies in serum (IgA, IgG, IgM, IgD), the serum concentration of IgE is normally quite low, only around 50 ng/ml, or approximately 0.0005% of total serum immunoglobulins in adults. IgE has a short half-life in plasma, but in the tissues, where it is tightly bound to mast cells, IgE may persist for several weeks (Hamilton 2010).

IgE is synthesized and secreted by B cell lymphocytes. Mature B cells leave the bone marrow-producing IgD and IgM, but they can modify the isotype they produce into IgG, IgA, or IgE, in a transition process called 'class switching'. After completing this process, the B cells are irreversibly 'committed to the production of IgE antibodies and can further develop into IgE-synthesizing plasma cells (Burton and Oettgen 2011).

## **2.3 DIAGNOSTIC TESTS**

Immediate allergic diseases are quite common in the population, and in clinical practice many cases of allergy are diagnosed solely based on typical clinical symptoms and a favourable response to allergy medication, without employing diagnostic laboratory tests. However, especially in the work environment, identifying the exact causal connection between exposure and symptoms is important in order to take preventive measures at the workplace. Clinical laboratory tests can be used to establish immediate sensitization to a specific agent and to help confirm the correct diagnosis of an allergic disease (Hamilton 2010). Immediate sensitization can be investigated by SPTs or by determination of specific IgE in serum. It is important to notice that a positive allergy test result only indicates the presence of allergen-specific IgE and does not by itself establish a clinical allergic disease (Cox et al. 2008).

Standardized test preparations for SPTs and IgE assays are commercially available for mainly common environmental allergens such as pollen and animals. The use of purified recombinant allergens has further improved the identification of the relevant proteins that induce sensitization in each individual case. In contrast to environmental allergens, only very few standardized test methods are available for occupational exposure agents (van Kampen et al. 2013b).

In occupational medicine, selecting the most appropriate diagnostic test methods in a case of suspected immediate allergic occupational disease requires good knowledge of the exposure at work and assessment of the possible sensitizers involved in the symptoms of the patient. A positive test

result indicates Type I sensitization to the tested substance, but without specific work-related clinical symptoms upon exposure, this finding alone is not sufficient to establish a connection between symptoms and work. A negative test result, on the other hand, does not rule out an allergic disease, since *in vivo* and *in vitro* tests for specific IgE can also give false-negative test responses. The likelihood of finding a true clinical allergy increases considerably when the focus of testing is based on the clinical history of the patient (Roberts et al. 2016). If the test results and the history of the patient are contradictory, a different test method or material might be needed to further evaluate the connection between symptoms and causative agents (Mahler 2012). An expert assessment by an occupational hygienist can help to identify potential sensitizers in the work environment, as shown by a recent report, in which an occupational hygienist identified the causative agent in almost all cases, including those that clinicians had failed to identify (de Olim et al. 2015).

### **2.3.1 ALLERGY SKIN TESTING**

SPTs are widely used to demonstrate immediate IgE-mediated sensitization. When a relevant allergen is introduced into the skin, specific IgE bound to the surface receptors on mast cells become crosslinked and the histamine and other mediators released produce a wheal and flare reaction that can be measured (Heinzerling et al. 2013). SPTs require suitable facilities and trained health professionals, but when available, they provide a cost-effective, fast and safe method for confirming sensitization (van Kampen et al. 2013b). SPTs provide the opportunity to screen a large number of substances at the same time. Although the principles of SPT still largely follow the original methods, there is a wide range of modifications and interpretations in the literature, which makes it difficult to compare the results of different studies (Heinzerling et al. 2013). It is important to remember that a positive SPT is not definite proof of the existence of specific IgE to the tested agent. SPT is an indirect test method that measures the effect of the suspected allergen and the test result can be influenced by many factors such as incomplete or contaminated test material, incorrect technique, dermatographism, or irritant reactions (Bousquet et al. 2012).

Correct technique is crucial for the reliability of the SPT. According to the European standards for skin prick testing, one drop of each antigen test solution should first be applied on the intact skin of the volar forearm, with a distance of at least 2 cm between two SPTs to avoid false-positive reactions. A disposable single-head metal lancet is passed through the drop and inserted into the surface of the epidermis for at least one second and then gently lifted without causing bleeding. All SPTs require a positive control (normally 0.1% or 1.0% histamine dihydrochloride) and a negative control (usually the diluent used in the test extracts). Excess allergen solution is removed with tissue

paper, and after 15–20 minutes the size of the test reaction is measured. A wheal diameter of at least 3 mm is usually classified as positive in the absence of a reaction to the negative control (Heinzerling et al. 2013).

The skin reaction following the SPT can result in a variety of wheal sizes and shapes, and the wheal surface area can be difficult to measure exactly. The mean value of the largest diameter in millimetres and the diameter perpendicular to this is the commonly used surrogate of wheal size (van Kampen et al. 2013b). Some studies, on the other hand, conclude that the longest wheal diameter alone seems to correlate better with the wheal surface (Konstantinou et al. 2010). New, more accurate methods to determine the wheal area are being developed, such as the ‘scanned area method’, which is so far mainly used in academic research (van der Valk et al. 2015). The GA<sup>2</sup>LEN SPT study data from 14 European countries were used to assess the relationship between wheal size in SPT and reported clinical symptoms and diagnosed allergic diseases. Depending on the allergen, positive SPTs (wheal size of  $\geq 3$  mm) were associated with clinical symptoms in 40%–89% of the cases. The probability of allergic symptoms increased significantly with larger wheal sizes for all 18 tested allergens, except for *Aspergillus*. The study results have been used for developing reading keys to help clinicians interpret SPT results (Haahtela et al. 2014).

Histamine reactivity in the skin varies among individuals and wheal size is not solely due to histamine release. Consequently, when assessing the significance of the reaction, the SPT results for allergens should not be strictly compared to the positive control histamine reaction. The main function of the positive control wheal is to ensure that the test is correctly performed and no interfering medication has been taken. Antihistamine medication can interfere with the test result and should, if possible, be paused at least five days before the tests. Other medications that can influence the test results are, for example, certain antidepressants, corticosteroids and calcineurin inhibitors. Severe eczema in the test area and dermographism can make it difficult to interpret the SPTs, so alternative test methods such as measurements of serum IgE should be considered. Pregnancy is a relative contraindication for SPTs, mostly due to the remote possibility of a systemic reaction (Heinzerling et al. 2013).

Ideally, allergen extracts for SPTs should be standardized in respect of their major and minor allergen content, and they should be comparable when using extracts of the same allergen from different manufacturers. This is very difficult to achieve, since the extracts are mixtures of many different allergens. Quantification of the major allergens in the individual extracts and the development of recombinant allergen extracts have been introduced to improve standardization. Tight regulation of SPT extracts has made their

production and registration difficult, which has led to gaps in the availability of certain extracts (Heinzerling et al. 2013).

Commercial SPT solutions are available for only a limited number of relevant occupational allergens such as flour, natural rubber latex (NRL) and cow. While the specificity of these SPT solutions for occupational agents has been shown to be high (80%–100%), sensitivity is very diverse, and for several SPT solutions markedly low (21%–89%), reflecting the high variability in the protein and allergen content of the extracts (van Kampen et al. 2013a). Since commercial test substances are seldom available for occupational agents, SPTs often have to be performed with non-standardized solutions. It is very difficult to develop stable test extracts for certain allergens such as fruits and vegetables. In these cases, the prick-prick technique is recommended, in which the lancet first pricks the fresh food and then pricks the skin (Heinzerling et al. 2013). The prick-prick technique can also be used for dry foods such as flour, grains or nuts, if the test substance is first diluted in saline.

Intracutaneous testing is considered a more sensitive method, but these tests are technically more demanding to perform and also induce more false-positive reactions than SPTs. Studies have demonstrated that SPT results correlate better with clinical allergy than the responses of intradermal tests. Intracutaneous tests also pose an increased risk of systemic response than SPTs, since the allergen is delivered deeper into the skin (Oppenheimer and Nelson 2006). In Finland, intracutaneous tests are now seldom used in clinical practice. The skin scratch test, originally introduced in 1873, is no longer recommended due to difficulties in interpretation and standardization and a higher risk of systemic reactions (Heinzerling et al. 2013).

Severe, systemic reactions are rare, but it is important to consider the potential risks. SPTs should only be performed by trained staff and emergency medicaments and equipment must be available. In a large study of 31 000 patients in the United Kingdom, 24 (0.077%) had systemic reactions, the most common allergen being peanut. Most of the patients (19 out of 24) had a clinical history of severe allergic reactions before the SPTs (Sellaturay et al. 2015). In a study in an allergy clinic in Florida, of 1456 patients who underwent skin tests during one year, systemic reactions occurred in 0.4% of the performed SPTs and 3.2% of the intradermal tests (Bagg et al. 2009). If concern regarding an increased risk of systemic reactions arises, the SPTs should be started at very low concentrations and the number of tested substances reduced to only the most important ones, or the SPTs should be replaced with *in vitro* measurements of specific IgEs.

### **2.3.2 IN VITRO DETERMINATION OF ALLERGEN-SPECIFIC IgE IN SERUM**

Serological assessment of specific IgE can also be used to demonstrate immediate sensitization. Whereas SPTs detect cell-bound specific IgE by eliciting a local allergic response, in vitro tests identify circulating (unbound) specific IgE. In this way the test methods complement each other. In vitro tests are recommended especially when patients have severe generalized symptoms, dermographism, or medications that interfere with the SPT result.

The first technique used to measure serum-specific IgE was the radioallergosorbent test (RAST) introduced in 1967. In RAST, the allergen is bound to a paper disc that is incubated with the serum of the patient. Specific IgE in the serum binds to the allergen and unbound IgE antibodies are washed away. Radioactively labelled anti-IgE is added, and the radioactivity can be quantified as a measure of specific IgE. Later, new autoanalyser-based techniques were developed, such as ELISA (enzyme-linked immunosorbent assay) and the most commonly used system to date, ImmunoCAP. These have now replaced the RAST method, but the main principle of the procedure remains the same. Specific IgE can also be determined by Western blotting (Jensen 2012) and by immunospot methods (Mäkinen-Kiljunen 1994). More recently, new methods based on allergen microarrays have been introduced, which make it possible to identify a large number of allergens at the same time and to assess cross-reactivity between structurally similar antigens (Hamilton 2010). Serologic IgE antibody results of 0.35 kU/l or more in the presence of related allergic symptoms are usually considered significant.

Commercial standardized allergen extracts for determining specific IgE are available for only a few significant work-related substances. When in-house antigen preparations are used, quality control is of particular importance (Mahler 2012).

Serological measurements of specific IgE are considered less sensitive and less specific than SPTs, depending on the methods used. In studies, concordance between specific IgE antibody assays and SPT results has been between 85% and 95%, depending on the tested allergen and the methods used (Heinzerling et al. 2013).

## **2.4 IMMEDIATE SENSITIZATION TO CHEMICALS**

Low-molecular weight (LMW) chemicals typically induce delayed cell-mediated Type IV allergic reactions, which can lead to the development of allergic contact dermatitis (ACD). Although immediate Type I sensitization is more common for high molecular weight (HMW) proteins, and IgE-mediated responses to LMW chemicals have been considered rare, some chemicals are

capable of inducing IgE-mediated sensitization (Dykewicz 2009). Chemical allergens have displayed heterogeneity with respect to the form of the allergic diseases to which they are primarily associated. Most chemicals that are known to cause skin sensitization by a cell-mediated mechanism have never been connected to immediate IgE-mediated sensitization and, respectively, several chemical allergens shown to induce IgE-mediated sensitization are not considered strong contact allergens to the skin (Kimber et al. 2009). Data from animal studies suggest that contact allergens and immediate allergens preferably induce different kinds of immune responses. Whereas contact allergens are primarily associated with T helper (T<sub>H</sub>)<sub>1</sub>-type responses, chemicals causing immediate sensitization to the airways are related to T<sub>H</sub><sub>2</sub>-type responses. These subsets are not, however, entirely exclusive; several chemicals have been associated with both immediate and delayed allergy (Basketter and Kimber 2016).

Protein substances with molecular weights of at least 10 000 kDa are recognized by the immune system and may trigger immediate sensitization by themselves. Chemical molecules with molecular weights typically less than 1000 kDa are generally too small to induce an IgE-mediated immune response on their own, but reactive chemicals can react with nucleophilic groups on amino acids and form covalent linkages with serum proteins. By binding to a carrier protein and forming an immunogenic hapten protein conjugate they can stimulate a specific immediate immune reaction (North et al. 2016). Analyses of the chemical structures of LMW compounds have shown that sensitizing chemicals are more likely to contain functional groups with heteroatoms (nitrogen or oxygen) and form covalent bonds with native proteins such as human serum albumin (HSA) (Seed and Agius 2017).

Several factors have shown to have an impact on an individual's susceptibility of immediate sensitization to chemicals. Dose, route, frequency and duration of exposure as well as environmental factors affect the development of allergy. Genetic factors have a strong, complex influence on the immune response, but in contrast to HMW agents, atopy does not seem to be a predisposing factor for immediate allergy to LMW chemicals (Corsini and Kimber 2007).

Immunologically active chemicals can in some cases be linked to immediate allergic diseases such as asthma, allergic rhinitis and CU. On the other hand, a large group of chemicals is linked to immediate-type hypersensitivity diseases such as asthma and rhinitis, without any evidence of specific IgE, and the underlying mechanisms in these cases remain mainly unknown. The role of specific IgE in chemical respiratory sensitization is still unclear as regards many chemicals associated with OA and rhinitis (North et al. 2016). Therefore, with chemicals, IgE-mediated sensitization and respiratory sensitization cannot be used as synonyms.

#### 2.4.1 CHEMICALS LINKED TO IgE-MEDIATED SENSITIZATION

The list of chemicals shown to induce IgE-mediated immediate sensitization is constantly growing. It can sometimes be difficult to identify the possible specific causative agent in a complex work environment. Material safety data sheets (MSDS) can help identify potential sensitizers, but the reliability of the information in MSDSs is considerably limited, since manufacturers are allowed to exclude key information on the sensitizing potential of the substance and ingredients classified as non-hazardous (Bernstein 2002). The European Chemicals Agency (ECHA) provides guidance on the legislation and regulations for chemical safety in Europe. REACH is a European Union regulation that applies to all chemical compounds and is intended to protect human health and the environment from chemical risks. The CLP (Classification, Labelling and Packaging) regulation in turn attempts to ensure that the hazards caused by chemicals are clearly communicated to workers and consumers through the classification and labelling of products. CLP classification is based on the United Nations' Globally Harmonised System (GHS). GHS classification has its own labelling categories for suspected respiratory sensitizers (H334) and skin sensitizers (H317).

Expert consultations with chemists or occupational hygienists can also be quite helpful in the evaluation of potential sensitizers, when available.

*Diisocyanates*, used as hardeners in polyurethane products such as paints and adhesives are well-known for their ability to cause OA, but the role of specific IgE in the sensitization of the respiratory tract in asthma cases remains controversial (Wisnewski and Jones 2010). Specific IgE to isocyanates can be demonstrated in only a minority of patients with OA linked to isocyanate exposure, but the presence of specific IgE is a relatively precise marker for isocyanate-induced asthma and supports diagnosis (Budnik et al. 2013). Some studies have associated specific IgE in patients diagnosed with OA caused by isocyanates with better outcome and shorter duration of symptoms (Piirila et al. 2000). In vitro tests are commercially available to demonstrate specific IgE for hexamethylene diisocyanate (HDI), diphenylmethane diisocyanate (MDI) and toluene diisocyanate (TDI).

*Ammonium and potassium persulfates* are mainly used in hairdressing as hair bleaching agents, but also in various industrial settings. Earlier studies provide controversial data on the role of IgE in the immediate allergy symptoms induced by persulfates. Some studies have clearly found IgE and positive SPTs to persulfates among some patients (Aalto-Korte and Makinen-Kiljunen 2003; Munoz et al. 2004), while others have found no specific IgE or positive SPTs to persulfates among hairdressers, despite the patients' exposure-related symptoms (Diab et al. 2009; Moscato et al. 2010).



Ingredients in permanent hair dyes, *p*-phenylenediamine (PPD) and its derivatives, such as *p*-toluenediamine sulphate (TDS), have occasionally been reported to induce positive SPTs. Some of the described allergic reactions have been quite strong, and have included anaphylactic symptoms (Pasche-Koo et al. 1998; Sahoo et al. 2000). Positive SPTs have also been described for Basic Blue 99 and Basic Brown 17 in semipermanent hair dyes (Vanden Broecke et al. 2014; Wigger-Alberti et al. 1996).

Several reports have reported immediate allergic reactions to *reactive dyes* used in the textile industrial and other occupational settings (Alanko et al. 1978; Jin et al. 2011). Reactive dyes contain a chromogen group and reactive functional groups that form covalent irreversible bonds with the fibres in textiles. The IgE epitopes of reactive dyes and HSA seem to be heterogeneous, which makes diagnostics of immediate allergy to reactive dyes quite complex (Park et al. 2001a).

The literature contains numerous reports on anaphylactic reactions linked to the use of *chlorhexidine* as a disinfectant, mainly in patients in connection with invasive medical procedures (Krautheim et al. 2004). Several of these reports include demonstrations of specific IgE to chlorhexidine (Aalto-Korte and Makinen-Kiljunen 2006; Garvey et al. 2007). IgE-mediated allergy to chlorhexidine is uncommon in an occupational setting, but has been described (Ibler et al. 2016).

*Chloramine T* (sodium-N-chlorine-p-toluene sulphonamide) is a derivative of chlorine that has been widely used as a disinfectant, especially in dental and medical facilities. Chloramine T is well known for its ability to induce IgE-mediated sensitization and occupational allergic skin and airway diseases (Kujala et al. 1995; Piirila et al. 2002).

Despite the widespread use of *formaldehyde* in different settings, descriptions of specific IgE to formaldehyde are rare in the literature, and reports of positive SPTs are scarce. Although anaphylaxis connected to formaldehyde exposure has been reported (Modre and Kranke 2001), the clinical relevance of the positive SPTs and specific IgE findings remains somewhat uncertain (Braun et al. 2003; Wantke et al. 2000).

*Glutaraldehyde* is primarily used for sterilizing heat-sensitive medical devices, and has been reported as inducing several cases of IgE-mediated sensitization (Curran et al. 1996; Di Stefano et al. 1999; Vyas et al. 2000). Glutaraldehyde has recently been largely replaced by ortho-phthalaldehyde, which has also been reported as an IgE-inducing sensitizer (Pala and Moscato 2013).

*Sodium hypochlorite* used as a disinfectant and bleaching agent has sporadically been reported as inducing Type I hypersensitivity. The first occupational case of sensitization and positive SPT to sodium hypochlorite was only recently reported (Chia Shi Zhe et al. 2016).

*Epoxy resins* are mainly used in paints, flooring materials and other protective coatings, and are a significant cause of allergic contact dermatitis. Diglycidyl ether of bisphenol A (DGEBA) resin is the most widely used epoxy resin. Immediate allergy to epoxy chemicals is rare: only a few case reports of specific IgE have been published (Hannu et al. 2009; Kanerva et al. 1991).

*Cyclic (organic) acid anhydrides* are synthetic, highly reactive LMW chemicals that are widely used as curing agents for epoxy resins and in the production of polyester and alkyd resins. Acid anhydrides are well known for their ability to induce IgE-mediated sensitization and allergic occupational diseases such as asthma, allergic rhinitis and CU (Nielsen et al. 2001; Venables 1989). Commercial tests are available for determining specific IgE to acid anhydrides.

*Polyfunctional aziridine* is used as a highly reactive crosslinking hardener for surface coatings. A few cases of positive SPTs to aziridine have been reported (Kanerva et al. 1995b; Sartorelli et al. 2003).

*Colophony* or rosin is a complex mixture of resin acids derived from pine trees and has a broad scope of use in industry. It is also used for soldering, printing inks and on the bows of string instruments, for instance. Contact allergy to colophony is common, but positive SPTs or specific IgE have only rarely been reported. The ability of colophony to induce immediate sensitization is uncertain (Elms et al. 2005; Rivers and Rycroft 1987).

Several *metals*, or predominantly *metal salts*, are able to induce immediate sensitization. The salts of the platinum metal group (platinum, iridium, ruthenium, palladium, osmium, and rhodium) in particular, used in catalysis and other sophisticated technical procedures, have been connected to IgE-mediated allergy (Cristaudo et al. 2005; Merget et al. 2010). Nickel and chrome are quite extensively used and frequently cause delayed sensitization, but immediate occupational allergy with positive test results for specific IgE has also been reported (Estlander et al. 1993; Fernandez-Nieto et al. 2006). Positive SPTs and specific IgE to cobalt have been described (Kusaka et al. 1996), but the relevance of cobalt-specific IgE remains unclear: for example, none of 22 patients with cobalt asthma in a Finnish study had a positive SPT to cobalt (Sauni et al. 2010). Positive SPTs have also occasionally been reported for zinc (Malo et al. 1993).

Immediate allergic reactions to *drugs* are mostly connected with patients' therapeutic use of medicaments. Work-related sensitization following the handling of drugs is much rarer, but occupational IgE-associated immediate sensitization to antibiotics in particular, including penicillin and cephalosporins (Whitaker 2016), piperacillin (Moscato et al. 1995), thiamphenicol, ceftoram and 7-ACSA (Pralong et al. 2012) has been described, as well as sporadically to other drugs: for example, piperazine citrate (Quirce et al. 2006) and sodium alendronate (Pala et al. 2008).

#### **2.4.2 SKIN PRICK TESTS WITH CHEMICALS**

Since hardly any commercial SPT substances for LMW chemicals exist, SPTs usually have to be performed on in-house test substances. Diagnostic guidelines are scarce, and examples of testing procedures in the literature usually only describe single cases or small case series.

Practice has shown that some chemicals can be used for SPTs as water solutions. There are several examples of positive SPTs performed with water solutions of chemicals such as persulfates (Aalto-Korte and Makinen-Kiljunen 2003), chlorhexidine (Aalto-Korte and Makinen-Kiljunen 2006) and chloramine T (D'Alo et al. 2012). However, some chemicals, for example, acid anhydrides and diisocyanates, cannot be tested as water solutions, since they immediately react with water and form the corresponding acids and amines, which no longer correspond with the original exposure agent. In these cases, SPTs performed with hapten-protein conjugates should be preferred. Hapten-protein conjugates can be prepared *in vitro* in a laboratory. The suitable methods vary according to the reactivity and solubility of the chemical, and temperature, pH and reaction time also influence the conjugation reaction. HSA has been the most used carrier protein. It seems to be the main protein to form adducts with hexahydrophthalic anhydride (HHPA) *in vivo* (Johannesson et al. 2001). Batches of test substance are usually prepared by simply mixing the chemical and the protein in phosphate-buffered saline or another suitable buffer solution. After incubation, the unconjugated, free hapten is dialyzed off. The ratio of chemical molecules bound per molecule of protein can be studied by spectrometric analysis or gas chromatography, but in most cases the hapten-protein conjugates are not properly characterized or standardized (Bernstein and Zeiss 1989). In a study on phthalic anhydride (PA) conjugates, the most active IgE-binding conjugates had a PA:HSA molar ratio of 80:1, and in the optimal conjugates the average number of PA haptens per HSA carrier molecule was 14–16 (Pakarinen et al. 2002).

HSA conjugates were initially used for *in vitro* determination of specific IgE in RAST tests, but since the 1980s some centres, including FIOH, have also used HSA conjugates for SPTs with chemicals.

Severe systemic reactions or anaphylaxis are rare in SPTs with chemicals, but such cases have been described, and the same caution should be applied as that for other SPTs (Hoekstra et al. 2012).

### **2.4.3 DETERMINATION OF SPECIFIC IgE TO CHEMICALS**

Commercial specific IgE determinations are available for only a limited group of chemicals used in the work environment. Commercial methods are found for chlorhexidine, chloramine T, formaldehyde, ethylene oxide, diisocyanates, and cyclic acid anhydrides. In addition, specific IgE assays exist for several drugs such as antibiotics, but they are mainly used for diagnosing drug reactions in patients.

Some centres and studies have successfully used in-house methods for determining specific IgE to chemicals. At the Helsinki University Central Hospital, specific IgE to persulfates was demonstrated using in-house immunospot and RAST methods with HSA conjugates of ammonium and potassium persulfates (Aalto-Korte and Makinen-Kiljunen 2003).

Specific IgE determinations and SPTs with chemicals have generally had a good correlation in previous studies (Garvey et al. 2007). A study that compared SPTs performed with reactive dyes to specific IgE measured with ELISA found a higher sensitivity (76.2% vs. 53.7%), specificity (91.4% vs. 86.0%), positive predictive value (80.0% vs. 62.9%), and negative predictive value (89.5% vs. 80.8%) for the SPTs (Park et al. 2001b).

## **2.5 OCCUPATIONAL SKIN DISEASES ASSOCIATED WITH IMMEDIATE SENSITIZATION**

### **2.5.1 GENERAL ASPECTS**

*Contact urticaria* refers to a wheal and flare reaction that appears immediately (within 30 min) after skin contact with an eliciting substance, and usually clears completely in a few hours (Giménez-Arnau and Maibach 2015). The term was introduced by Fisher et al. in 1973 (Fisher 1973) and was defined by Maibach and Johnson as contact urticaria syndrome (CUS) (Maibach and Johnson 1975).

Immunological CU (ICU) or allergic CU is a Type I allergic reaction, mediated by allergen-specific IgE (Wakelin 2001). It occurs in a previously sensitized individual. In the immunologic response leading to a CU reaction, skin contact results in allergen penetration through the epidermis to the dermis and binding of the allergen to specific IgE on the mast cells, causing

degranulation and the release of histamine, prostaglandins and leukotrienes. This in turn induces a wheal and flare reaction in the contact area. ICU is more frequent in someone with previous atopic symptoms (Le Coz 2012).

Non-immunological contact urticaria (NICU), is an immediate contact reaction of the skin, and occurs without previous sensitization. The pathomechanism is not completely understood, but it is an irritant rather than immunological reaction. Skin contact with the eliciting substance leads to the release of prostaglandins and other vasogenic mediators. Histamine does not seem to be the main mediator of the reaction, since antihistamines do not inhibit NICU. The classic example of NICU is the stinging nettle (*Urtica dioica*), which induces transient wheals and itching following skin contact. Some chemicals, including benzoic acid and sorbic acid, can also induce NICU reactions. In occupational dermatology, NICU reactions are seldom noted or diagnosed. They represent an immediate-type temporary irritant reaction of the skin that does not normally require medical attention.

*Protein contact dermatitis* (PCD) is an allergic skin reaction induced by skin contact with proteins of animal or plant origin. The clinical presentation of protein contact dermatitis is a recurrent, itching eczema, which appears quite quickly following skin contact with the causative substance, typically affecting the hands and fingertips. The exact mechanisms behind PCD are still unclear, but several authors have proposed a combination of Type I and IV allergic reactions (Amaro and Goossens 2008; Levin and Warshaw 2008). PCD was first described by Hjorth and Roed-Petersen, who in 1976 reported a series of 33 food caterers with immediate itching, erythema and vesiculation within 30 minutes of handling meat, fish or vegetables (Hjorth and Roed-Petersen 1976). Numerous organic substances, typically foodstuffs such as vegetables, fruits, meat, and seafood have been reported as causative agents of PCD (Amaro and Goossens 2008; Doutre 2005). In contrast to CU, the eczematous lesions of PCD require several days to heal and may also become chronic (Vester et al. 2012).

## **2.5.2 EPIDEMIOLOGY**

The general prevalence and incidence of occupational CU and PCD is not known in detail (Aalto-Korte and Suomela 2015). Most publications found in the literature are case reports or small series, and epidemiological studies are rare. It is likely that some mild and transient cases of CU remain unnoted, as patients do not report them to their physician.

Not many countries provide statistics on occupational CU or PCD. The figures of existing reports vary to some extent between countries, depending on their registration policy regarding occupational diseases. At a tertiary-level occupational dermatology clinic in Australia, 9.9% of the 1443 patients with a

work-related dermatosis were diagnosed with CU, the most prevalent cause being NRL (52% of CU cases), followed by foods (35% of CU cases) and ammonium persulfate (figure not given) (Williams et al. 2008). According to the United Kingdom's national surveillance systems, NRL was the dominating exposure (75%) causing occupational CU (Turner et al. 2007). NRL was also the predominant cause (49%) of occupational CU according to nationwide surveillance in France, but the number of NRL cases declined significantly during the study period (Bensefa-Colas et al. 2015).

According to the Finnish Register of Occupational Diseases (FROD) 411 cases of CU or PCD were notified between 2005 and 2013 in Finland. This is 9.9% of the total amount of 4148 notified cases of occupational skin diseases during the same period. The most prevalent causes of CU/PCD were cow dander (48%), and flour, grain, and animal feed (18%). In the Finnish data, NRL was the causative agent in only 8.5% (35 cases) of all occupational CU/PCD cases during this period. In an earlier study based on FROD, during the period of 1990–1994, the most prevalent cause of CU/PCD was also cow dander (44.4%), followed by NRL (23.7%), flour, grains and feed (11.3%) and handling of foodstuffs (3.1%) (Kanerva et al. 1996).

The list of reported causes of CU or PCD comprises numerous different agents and is constantly growing. Most of the agents can also occur in the work environment (Amaro and Goossens 2008; Bourrain 2006; Doutre 2005). Of all individual exposure agents, the prevalence of NRL allergy has been studied the most. High rates of NRL sensitization in health care during the 1980s and 1990s led to preventive measures that resulted in a rapid decrease of sensitized workers (Larese Filon et al. 2014; Vandenplas and Raulf 2017). This development can also be noted in the Finnish figures. In addition to health care workers, immediate skin reactions are common among bakers, food handlers and hairdressers (Williams et al. 2008).

### **2.5.3 DIAGNOSTIC TOOLS**

The diagnostic examinations of a patient with suspected occupational CU or PCD should always begin with a full medical history including work-related symptoms and exposure at work to potential causative agents. Physical examinations of the skin and a detailed clinical history of the location and occurrence of skin symptoms and possible symptoms from other organs are important. Diagnostic laboratory tests are planned on the basis of this evaluation. The recommendations for diagnostic procedures for CU and PCD show some variation in the literature (Doutre 2005).

The diagnostic tests usually start with SPTs and/or serum IgE measurements to assess whether a Type I allergic reaction is involved. In a clear case, with typical work history and work-related immediate skin

symptoms combined with the demonstration of IgE-mediated sensitization, further examinations are not always necessary to confirm the diagnosis of CU or PCD (Mortz and Andersen 2015).

If the SPTs/specific IgE are negative or the diagnosis is still unclear, the next recommended step is an *open application test*. This is typically performed by applying the test substance to a 3 x 3 cm area (sometimes larger test areas are recommended) of normal skin on the forearm, extensor site of the upper arm or the upper back. The recommended quantity is usually 0.1 ml of the test substance, but some substances require larger amounts. The test is normally read after 20, 40, and 60 minutes. An immunological reaction usually appears within 15–20 minutes, but the delay can be up to 60 minutes (Basketter and Lahti 2011).

Other test procedure guidelines recommend open application on healthy skin as the first step, followed by open application to slightly or previously affected skin and then occlusive application, if the first test remains negative. Rubbing the test material on the skin has also been recommended to provoke a reaction. SPTs and intradermal tests are only suggested in these recommendations if the open applications are negative, since invasive skin tests are believed to increase the risk of systemic allergic reactions (Gimenez-Arnau et al. 2010). These test methods can, however, lead to false positive reactions, and difficulties in distinguishing a true positive reaction from an unspecific reaction due to irritation.

Some exposure agents are also suitable for other provocation tests or use tests to establish the connection between the agent and the symptoms. For example, a cook could handle foodstuffs under observation for skin symptoms, or a patient with suspected NRL allergy could be exposed by first wearing one finger of a latex glove, and if no symptoms develop, the whole glove. A non-latex disposable glove should be used as a negative control to exclude glove-related hand urticaria due to the pressure caused by a tight-fitting glove (Sheeran et al. 2014).

All tests involving direct skin contact with the suspected causative allergen should be performed under anaphylaxis surveillance, although severe generalized reactions are rare.

#### **2.5.4 CONTACT URTICARIA DUE TO CHEMICALS**

Occupational ICU due to chemicals has been considered relatively rare as chemicals do not typically induce IgE-mediated sensitization. Non-immunological CU (NICU), on the other hand, typically occurs following exposure to chemicals such as sorbic and benzoic acid. NICU is not covered here in detail.

However, many chemicals have been shown to induce immediate contact skin reactions that are indistinguishable from allergic reactions and new agents are constantly being discovered. The literature provides extensive lists and case reports of chemical agents that have been linked to ICU (Basketter and Lahti 2011; Bourrain 2006; Giménes-Arnau 2015; Gimenez-Arnau et al. 2010).

Several chemical groups used in industry have been associated with occupational ICU. Organic acid anhydrides are quite strong sensitizers, and have been linked to occupational CU in a number of reported cases (Gutierrez-Fernandez et al. 2007; Kanerva et al. 1999a; Tarvainen et al. 1995; Yokota et al. 2001). Diisocyanates are well known for their ability to induce OA, but ICU caused by diisocyanates has also occasionally been described (Kanerva et al. 1999b; Stingeni et al. 2008; Valks et al. 2003). Other chemicals in industrial settings that have been reported as causative agents of ICU are epoxy resins (Kanerva et al. 2002; Sasseville 1998; Stutz et al. 2008), polyfunctional aziridine hardener (Kanerva et al. 1995a), colophony (Rivers and Rycroft 1987), HBTU ((*o*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate) (Hannu et al. 2006) and triphenyl phosphite (Torresani et al. 2003).

Metals and metallic salts can also induce CU, and occupational CU cases caused by platinum, palladium, iridium and rhodium salts (Bergman et al. 1995; Cristaudo et al. 2005; Pesonen et al. 2014), chromium, cobalt (Krecisz et al. 2009), nickel (Estlander et al. 1993), and aluminium (Helgesen and Austad 1997) have been reported. Positive SPTs or specific IgE were not reported in all these cases.

Hairdressers are at an increased risk of CU (Foss-Skiftesvik et al. 2017) as they are exposed to several chemicals that can cause immediate allergy, the most prevalent being persulfates (Leino et al. 1998). Permanent and semipermanent hair-dye ingredients, such as phenylenediamine (PPD) and Basic Blue 99 and Basic Brown 17, have also been linked to ICU (Birnie and English 2007; Davari and Maibach 2011; Vanden Broecke et al. 2014). Many cosmetic products also contain chemical ingredients and fragrances that can induce immediate hypersensitivity skin reactions, which in some cases evolve into generalized reactions (Verhulst and Goossens 2016).

Health care, dental and laboratory workers may be exposed to a number of CU-inducing chemical compounds in their work environment. Skin contact with antibiotics or other drugs can provoke allergic CU (Gimenez-Arnau et al. 2010). Chloramine T used in disinfectants is a well-known sensitizer, and has been linked to CU (Kanerva et al. 1997), as have chlorhexidine (Nagendran et al. 2009; Wittczak et al. 2013) and formaldehyde (Dean et al. 2016). A rare



case of ICU caused by sodium hypochlorite solution in an operating theatre technician was recently reported in Singapore (Chia Shi Zhe et al. 2016). Methacrylates in dentistry are a well-known cause of occupational contact dermatitis, but no cases of allergic CU due to methacrylates can be found in the literature.

## **2.6 OCCUPATIONAL AIRWAY DISEASES ASSOCIATED WITH IMMEDIATE SENSITIZATION**

### **2.6.1 OCCUPATIONAL ASTHMA**

Asthma is a heterogeneous disease, which develops as a result of multiple various genetic, environmental, and behavioural factors. The current Global Initiative for Asthma (GINA) characterizes asthma as chronic airway inflammation, causing different respiratory symptoms such as coughing, wheezing, shortness of breath, and chest tightness (GINA. 2017). Asthma can be induced by both immunological and non-immunological mechanisms (Maestrelli et al. 2009).

The workplace environment can both induce the development of new asthma and worsen pre-existing asthma. If work-related asthma is caused by a specific agent or substance at work, the term occupational asthma (OA) is used to separate it from asthma exacerbated by the work environment. OA can result from either immunologically mediated sensitization or from exposure of the airways to high concentrations of irritant compounds (Vandenplas et al. 2017). It is one of the most common forms of occupational lung disease in industrialized countries and it has been estimated that 15%–20% of all new cases of asthma in adulthood are due to work-related exposure (Toren and Blanc 2009). Available data still show that OA often remains unrecognized and inadequately investigated (Fishwick et al. 2007; Sauni et al. 2009). OA should be considered in every adult case of new-onset asthma.

HMW and LMW sensitizers can both be causes of immunological asthma. For HMW agents, an IgE-mediated mechanism is usually behind the development of immunological asthma. The intensity of exposure to the airway sensitizer is the most important determinant of OA (Tarlo and Lemiere 2014). The respiratory tract is considered the main route of exposure and site of initiation of the immune response, but the relevance of dermal exposure in inducing sensitization of the airways has recently gained attention (Redlich 2010). Asthma symptoms usually appear after a latency period of weeks to months, sometimes years, after the first exposure to the sensitizer.

The diagnosis of sensitizer-induced OA is based on a confirmed asthma diagnosis and an association of the symptoms with the work environment. The

symptoms typically appear at work or after workdays and improve during weekends and holidays. Clinical and work history are combined with immunological tests, lung function tests, assessment of non-specific bronchial hyper-responsiveness, serial measurements of peak expiratory flow (PEF) at and off work, and an assessment of airway inflammation by measurements of fractional exhaled nitric oxide (FeNO) or induced sputum eosinophils. A specific inhalation challenge (SIC) is considered the most reliable test for diagnosing OA, but is not always necessary in cases when the diagnosis can be confirmed by other test methods. If serial PEF measurements at and off work, combined with an immunological sensitization assessment, confirm the OA diagnosis with sufficient certainty, SIC is not usually required (Vandenplas et al. 2017).

A wide range of occupational agents causing OA have been detected. Baur et al. identified 372 different causes of allergic OA (Baur 2013). Typical HMW agents that induce immunologic OA are flour, animals, enzymes and NRL. Chemicals linked to OA include diisocyanates, acid anhydrides, metal salts, hairdressing chemicals, and reactive dyes (Tarlo and Lemiere 2014).

## **2.6.2 ALLERGIC OCCUPATIONAL RHINITIS**

Work-related rhinitis includes both work-exacerbated rhinitis (WER) and occupational rhinitis (OR). OR is defined as rhinitis induced by a specific substance at the workplace, while WER appears in workers with pre-existing rhinitis (perennial allergic rhinitis, vasomotor rhinitis) in whom agents with irritant properties provoke nasal symptoms. The European Academy of Allergy and Clinical Immunology defines OR as “an inflammatory disease of the nose, which is characterized by intermittent or persistent symptoms (i.e. nasal congestion, sneezing, rhinorrhoea, itching), and/or variable nasal airflow limitation and/or hypersecretion due to causes and conditions attributable to a particular work environment and not to stimuli encountered outside the workplace” (Moscato et al. 2009). OR is commonly associated with IgE-mediated sensitization to HMW protein allergens, and in some cases chemical sensitizers at the workplace (Sublett and Bernstein 2010). Allergic OR is characterized by a latency period of months to years during which sensitization to the causative agent develops (Stevens and Grammer 2015). The level of exposure is the most important determinant of IgE-mediated sensitization to occupational agents and OR (Moscato et al. 2009).

OR is reported as being quite common in occupations with high exposure to organic agents. Among laboratory animal workers, the prevalence of allergic OR ranged from 10% to 33%. OR was two to four times more common than OA in this group (Siracusa et al. 2000). OR has been reported as occurring in 18% to 29% of all bakers (Sublett and Bernstein 2010).

The diagnosis of allergic OR is based on a detailed medical history including exposure at work, symptoms associated with work exposure and improvement of symptoms when away from the work environment. Clinical evaluation includes nasal examination, complemented by objective methods to assess nasal patency (openness), such as rhinomanometry, acoustic rhinometry and peak nasal inspiratory flow, if available. Immunological testing with SPTs and/or quantification of specific IgE levels are used to confirm sensitization. Nasal provocation tests are a valuable tool for verifying the connection between sensitization to the exposure agent and the work-related rhinitis symptoms (Airaksinen et al. 2007).

The development of OA and OR is closely linked and they often coexist in the same patient. The prevalence of OR in workers with confirmed OA has been 76% to 92% in different studies (Sublett and Bernstein 2010). In a Finnish national register study from the 1980s and 1990s, 11.6% of all workers reporting OR were subsequently confirmed to have developed OA. The crude relative risk of OA was 4.8 in workers with OR in this study (Karjalainen et al. 2003). Since workers with OR are at risk of developing OA, exposure to the causative agent should be avoided if possible (Sublett and Bernstein 2010).

### **2.6.3 OCCUPATIONAL AIRWAY DISEASES CAUSED BY SENSITIZATION TO CHEMICALS**

Although organic HMW agents are more frequent causes of immunologic OA and OR, LMW chemicals are still a central subset of etiologic agents, and include over 100 different chemical substances shown to induce OA and/or OR. The knowledge of the mechanisms of allergic airway diseases caused by chemicals is limited, and for a large group of chemicals the mechanisms remain unknown (Maestrelli et al. 2009).

There is uncertainty regarding the role of IgE in the development of respiratory sensitization to most chemicals. Specific IgE has not been detected in all patients with OA or OR linked to chemical exposure, despite the diseases behaving like immediate allergic diseases, that is, showing an induction phase and an elicitation phase with allergic symptoms following exposure (Kimber et al. 2009).

Chemicals that are able to induce synthesis of specific IgE can cause asthma or rhinitis in a similar way to that in which HMW agents do. Acid anhydrides are well known for their connection with OA and OR. Other examples of LMW agents, where an IgE-mediated mechanism is considered the main cause of OA or OR, are platinum and rhodium salts and reactive dyes (Tarlo and Lemiere 2014).

Diisocyanates are probably the most rigorously studied group of chemicals that cause occupational airway diseases. Diisocyanates are able to induce the production of specific IgE, but the role of IgE-mediated sensitization in the development of asthma is still a matter of debate in the literature (Wisniewski and Jones 2010).

The list of chemical agents causing OA and OR is constantly growing. A review of new LMW agents that cause OA, reported during the period 2000–2010, revealed 41 new causative LMW agents. Immunological tests (SPTs and/or specific IgE) were performed for 22 of the agents and provided evidence of specific IgE in eight cases (20% of all agents): the drugs 7-ACSA, thiamphenicol, ceftoram, wood dust from Cedroarana, Angelim pedra, Ipe and Antiaris and rhodium metal (Pralong et al. 2012).

New causes are usually identified through patient cases and systematic health surveillance, reliable methods for predicting the sensitizing potential of chemicals are needed. Computer-based models for evaluating the relationship between chemical structures and the potential to cause asthma in humans have been successfully developed. For example, the quantitative structure-activity relationship (QSAR) model, originally developed in the pharmaceutical industry for predicting the adverse effects of drugs, offers a tool to predict the risk of chemical induced asthma. By comparing the molecular structure of the suspected asthmagen with a set of control and asthmagenic compounds, the model generates a hazard index (HI) as an estimate of the asthmagenic potential of the compound of interest. These models have associated nitrogen- and oxygen-containing functional groups in particular with an elevated risk of asthma (Jarvis et al. 2005; Jarvis et al. 2015).

## **2.7 SIMULTANEOUS SKIN AND AIRWAY DISEASES ASSOCIATED WITH IMMEDIATE SENSITIZATION**

Immediate skin and airway diseases can be expected to occur simultaneously, as an IgE-mediated mechanism is usually associated with both. Nevertheless, limited data exist on this issue in the literature, if we exclude the numerous case reports on singular patients presenting simultaneous skin and airway symptoms caused by the same IgE-inducing allergen.

Larger studies are usually limited to separate branches or exposure agents. In a study on laboratory animal workers, one fourth of the respondents reported both respiratory and skin symptoms (Ruoppi et al. 2004). Professional cleaners with work-related skin symptoms were significantly more likely to have work-related asthma than cleaners without skin symptoms (Lynde et al. 2009). A study on OA due to platinum salts, found that 53% of its

participants also had skin complaints, but did not specify the character of the skin symptoms in detail. In the follow-up, when the majority of the participants had been transferred to jobs with very low or no exposure to platinum salts, the skin symptoms and rhinitis and conjunctivitis symptoms decreased significantly, whereas the asthma symptoms persisted among the majority, regardless of cessation of exposure (Merget et al. 2017). Over two-thirds of veterinarians in California reporting skin symptoms also reported respiratory symptoms, but IgE-mediated allergy was not investigated (Susitaival et al. 2003).

The possible connection between skin exposure and the development of asthma has recently received significant attention, especially from a preventive point of view. Evidence that skin exposure contributes to the development of asthma is still limited, but the role of skin exposure in the sensitization of the airways needs to be further explored (Heederik et al. 2012).

## **2.8 PREVENTION OF OCCUPATIONAL ALLERGIC DISEASES CAUSED BY CHEMICALS**

Accurate identification and diagnosis of occupational sensitization and occupational allergic diseases is important to prevent the continued exposure of a symptomatic worker and their colleagues. The main benefits of linking work-related exposure to an occupational disease are gained from subsequent interventions at the workplace to reduce exposure (Cherrie and Semple 2010).

Occupational exposure limits (OELs) have been set for many occupational agents. In Finland, the Ministry of Social Affairs and Health confirms and maintains a list of concentrations of impurities in workplace air known to be harmful (HTP values). These standards do not, however, necessarily take into account the risk of sensitization, and they cannot be used as a general safe limit to prevent sensitization at work. For most sensitizing substances, the exposure levels below which the risk of sensitization can be completely excluded are so low, that OELs are difficult to set considering technical feasibility and knowledge (Rijnkels et al. 2008).

Reduction or elimination of exposure is still the most important measure for reducing the risk of immunologic sensitization and development of allergic occupational diseases. Exposure at the workplace can be reduced by adequate ventilation, local exhaust ventilation systems, using less hazardous substances, and creating isolated or closed manufacturing processes. Workers should be provided with adequate personal protective equipment, such as protective gloves, protective clothing and respiratory protection if the exposure cannot otherwise be avoided. Workers should also be educated about how best to avoid exposure through adequate working methods. If the

exposure cannot be eliminated and there is still a residual risk of sensitization and the development of an occupational disease, health surveillance, preferably organized by occupational health care, might be needed to identify early signs of sensitization or disease (Cullinan et al. 2017).

A Cochrane systematic review based on 21 controlled studies, conducted to evaluate the effectiveness of workplace interventions on OA, concluded that total removal from exposure improved asthma symptoms to a greater extent than reduction of exposure, but the effect on lung function did not differ between the groups. It was also noted that removal from exposure resulted in a much higher risk of unemployment and loss of income than reduction of exposure (de Groene et al. 2011).

Individuals with particularly severe symptoms, such as anaphylaxis, should preferably be removed entirely from any further exposure to the causative agent. The most common triggers of occupational anaphylaxis have been *hymenoptera* stings (wasps, bees, sawflies and ants) and NRL. Other less frequently reported triggers are foodstuffs, animals, medications and some chemicals, such as chlorhexidine, insecticides, dyes and bleach, metals, and MDI. Immunotherapy is recommended as treatment only for hymenoptera stings; for other agents avoidance is the primary preventive measure (Siracusa et al. 2015).

### 3 OBJECTIVES

The aim of the present study was to evaluate all patients with a suspected work-related immediate allergic skin disease referred to the national occupational medicine outpatient clinic, and the test methods used in their diagnostics.

This thesis consists of four separate studies. The specific objectives of each study were:

1. To characterize patient cases and evaluate a specialized occupational medicine clinic's diagnostic procedures for occupational contact urticaria associated with exposure to organic acid anhydrides (Study I).
2. To characterize patient cases and evaluate a specialized occupational medicine clinic's diagnostic procedures for occupational immediate skin and airway diseases associated with exposure to oxidative hair dyes (Study II).
3. To evaluate the relevance and usefulness of SPTs in the diagnosis of occupational immediate allergic diseases caused by chemicals based on the experience of a tertiary-level occupational medicine clinic (Study III).
4. To characterize patients diagnosed with occupational contact urticaria or protein contact dermatitis at a specialized occupational medicine clinic and to assess concurrent occupational asthma and occupational rhinitis caused by the same agent (Study IV).

## **4 MATERIALS AND METHODS**

### **4.1 PATIENTS**

The studies were based on material on patients referred to the occupational medicine clinic at the Finnish Institute of Occupational Health (FIOH) in Helsinki during the period 1.1.1990–31.5.2011. FIOH's occupational medicine clinic is a tertiary-level referral clinic that receives patients with suspected occupational diseases from throughout the country. The most common indications for referral are work-related respiratory and skin symptoms.

In Study I we retrospectively reviewed the FIOH patient and test files for the period 1990–2006 to identify all those who had been diagnosed with occupational CU due to exposure to organic acid anhydrides. We found 21 such patients, 16 (76%) of them male. From the medical records, we collected data on occupation, work tasks, exposure to acid anhydrides, work-related symptoms, results of clinical examinations and immunological tests, and diagnoses of occupational diseases.

In Study II we retrospectively reviewed the FIOH medical files for the period 1.1.2001–31.5.2011 to find patients diagnosed with occupational CU, OR and/or OA associated with oxidative hair dyes. We found 11 such patients. From the medical records, we gathered data on occupational history, exposure to hairdressing chemicals, symptoms related to work, any association between symptoms and specific substances used at work, the duration of exposure before onset of symptoms, the duration of symptoms before diagnosis, results of clinical examinations and immunological tests, and diagnosed occupational diseases.

Study III was based on the SPTs performed with chemicals at FIOH during the period 1.1.1991–31.5.2011. We reviewed the SPT files of the clinic to find all patients with at least one positive SPT result in the SPT series performed with chemicals. From the medical records of the patients, we collected data on occupation, work tasks, exposure, the results of clinical examinations and immunological tests, and diagnosed occupational diseases.

In Study IV we retrospectively reviewed FIOH's patient and test files for the period 1.1.1995–31.5.2011 to find all patients who had been diagnosed with occupational CU or PCD. We identified 291 patients with these diagnoses. From their medical records, we obtained data on occupation, work tasks and occupational exposure, the type of symptoms related to work (cutaneous, bronchial and nasal), the order of appearance of the symptoms, the results of



clinical examinations and immunological tests, and diagnosed occupational diseases.

## 4.2 DIAGNOSTIC METHODS

### 4.2.1 SKIN PRICK TESTS

SPTs with common environmental allergens were performed with standardized allergen extracts of birch, alder, timothy, meadow fescue, mugwort, cat, dog, horse, cow, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, *Cladosporium herbarum* (ALK, Copenhagen, Denmark), and NRL (Stallergenes SA, Antony, France). The tests included histamine hydrochloride (10 mg/ml) and a diluent control. A prick test reaction of 3 mm and at least half the size of the histamine control reaction, in the absence of a reaction to the negative control, was considered positive.

Commercial allergen extracts for work-related agents were used for SPTs when available. Prick-prick testing was used primarily for SPTs with fresh food, vegetables and plants. Flour, grains, animal feed, spices, and other dry substances were diluted in a potassium phosphate buffer and then used for prick-prick testing. In addition, several in-house test series for chemicals and enzymes were used during the study period.

#### 4.2.1.1 Skin prick testing with chemicals

Many chemicals (e.g. acid anhydrides, diisocyanates, and epoxy resin) were tested as hapten conjugates of the chemical and HSA. The conjugation between, for example, acid anhydrides and HSA was carried out essentially as described by Howe et al. (Howe et al. 1983). The diisocyanate-HSA conjugates were prepared according to Wass and Belin (Wass et al. 1989).

Hapten chemicals dissolved in a suitable solvent were allowed to react with 1% HSA (ALBUMIN SPR 200mg/ml, Finnish Red Cross Blood Transfusion Service, Finland) in a mild alkaline buffer and ice bath for about one hour. The solvents and buffers were selected according to the haptens. After filtration through filter paper, the hapten-HSA conjugate was concentrated to a 2% HSA solution and the buffer was changed to coca solution (0.5% sodium chloride, 0.3% sodium hydrogen carbonate, 0.4% phenol) by ultrafiltration through an Ultracel 10 kDa membrane (Millipore, USA). Control HSA solution was prepared in the same manner without the hapten. A 2.5 ml solution of hapten-HSA was passed through a Millex GV filter with a 0.22µm membrane (MerckMillipore Ltd, Ireland) into a sterile vial containing 2.5 ml glycerol, to yield a final HSA concentration of 10 mg/ml (1%).

For some chemicals (e.g. persulfates, chlorhexidine), solutions with water or coca/glycerine (NaCl 0.5%, NaHCO<sub>3</sub> 0.275%, phenol 0.4% and glycerol 1:1) as a vehicle were used parallel to or instead of the HSA conjugates.

#### **4.2.2 DETERMINATION OF TOTAL AND SPECIFIC IgE**

In most cases, serum total immunoglobulin E (IgE) and specific IgE were measured to confirm sensitization, if available. Serum total IgE was measured using the Phadia UniCAP System (Phadia, Uppsala, Sweden). A total IgE of < 110 kU/l was regarded as normal. For determining specific IgE, several different commercial methods and in-house radioallergosorbent tests (RAST) were used during the study period.

#### **4.2.3 OPEN APPLICATION (SKIN PROVOCATION)**

Open application was performed in some cases to confirm the diagnosis of CU or PCD. In open application, the test substance was applied thinly to a 5 x 5 cm area of healthy skin on the forearm. Protein substances were usually tested as is, minced if needed, and applied to the skin. Chemicals were tested either as is or diluted to the desired concentration in water, petrolatum or ethanol. NRL was tested using latex gloves or parts of gloves, for example, a finger.

A dermatologist inspected the skin after 20 minutes and the application was repeated on the same area. After 40 minutes, the test was reread and the test material removed. The test was considered positive if a reaction of one or several urticarial wheals appeared in the application area. Alternatively, for PCD, an immediate eczematous reaction was regarded positive.

If the clinical symptoms were typical and sensitization was established by other test methods, open application was not always considered necessary to confirm the diagnosis.

#### **4.2.4 LUNG FUNCTION TESTS**

Flow-volume spirometry and bronchodilation tests were performed in accordance with the American Thoracic Society criteria (ATS 1995), using a standard spirometer (Spirostar USB Medikro, Finland), and the predicted values for the Finnish population. The bronchial challenge with histamine was carried out according to the method described by Sovijärvi et al. (Sovijarvi et al. 1993). We measured the provocative dose that caused a 15% reduction (PD<sub>15</sub>) in forced expiratory volume in 1 second (FEV<sub>1</sub>). Bronchial hyper-responsiveness was graded as severe if PD<sub>15</sub> < 0.1 mg, moderate if PD<sub>15</sub> 0.1–

0.4 mg, and mild if PD<sub>15</sub> 0.4–1.6 mg. A PD<sub>15</sub> of > 1.6 mg indicated no hyper-responsiveness.

Fractional exhaled nitric oxide was measured using an online chemiluminescence analyser (NIOX, Aerocrine AB, Solna, Sweden) and was interpreted in compliance with current recommendations (American Thoracic and European Respiratory 2005; Dweik et al. 2011).

PEF measurements during work days and days off were performed according to the method of Burge (Burge 1982).

#### **4.2.5 SPECIFIC INHALATION CHALLENGE TESTS**

Patients with suspected OA or OR underwent SICs in a 6 m<sup>3</sup> challenge chamber or with commercial allergen extracts using a dose dosimeter. The test was considered positive for OA when there was a sustained fall in FEV<sub>1</sub> of 20% or more from the pre-challenge value, in the absence of significant ( $\geq 10\%$ ) changes in the control test (Vanhanen et al. 2000). The positive bronchial reactions were classified into three patterns: early reactions occurring within an hour after the end of exposure, late reactions within 1–8 hours, and dual reactions consisting of both early and late reactions.

The degree of rhinorrhoea and nasal blockage of patients with suspected OR was evaluated by anterior rhinoscopy before the SIC, and approximately 20 minutes after the end of the SIC. Rhinorrhoea and nasal blockage were scored on a range of 0 (dry or thin mucosa) to 3 points (dripping mucus or swelling of the mucosa). We also measured the amount of nasal secretion running out of the patient's nose and to the vestibulum of the nostrils. OR was considered if the control SIC test was negative and the score changed by  $\geq 4$  points in both nostrils in the SIC (Hytonen and Sala 1996). Nasal secretion of > 200 mg supported the scoring positivity (Airaksinen et al. 2008). Acoustic rhinometry was performed before and after the challenge.

#### **4.2.6 NASAL CHALLENGE TESTS**

Local nasal provocation tests, according to the methods described earlier (Airaksinen et al. 2007), were used in cases when the patients had only upper airway symptoms and the causative agent was not considered particularly irritating to the mucous membrane.

## **4.3 DIAGNOSTIC CRITERIA OF OCCUPATIONAL IMMEDIATE SKIN DISEASES**

### **4.3.1 OCCUPATIONAL CONTACT URTICARIA**

A confirmed diagnosis of occupational allergic (immunological) CU was based on typical immediate symptoms (itchy visible skin changes) after skin contact with a work-related agent and demonstration of immediate sensitization to the same substance by SPTs and/or specific IgE. A positive result in open application performed on healthy skin further supported the diagnosis, but was not a definite requirement for diagnosing CU if the work-related symptoms and immunological tests were clear.

### **4.3.2 OCCUPATIONAL PROTEIN CONTACT DERMATITIS**

The diagnosis of occupational PCD required demonstration of immediate sensitization to a protein-containing substance in SPTs or by determining specific IgE. It also required considerable skin exposure and direct skin contact with the substance at work, resulting in eczematous changes on exposed skin areas that healed when skin contact with the material was avoided, as well as a positive result from open application to the same material (clear wheals or minute papules). In our experience, vesicles are only exceptionally noted during the short test duration of 40-60 minutes.

## 5 RESULTS

### 5.1 OCCUPATIONAL CONTACT URTICARIA AND PROTEIN CONTACT DERMATITIS (STUDY IV)

During the 16-year study period, 291 patients were diagnosed with occupational CU or PCD. The majority (62%) of the patients were female, but the gender distribution varied according to the exposure group. The mean age at the time of diagnosis was 38 years (range 20–62 years). CU was diagnosed in 233 cases (80%) and PCD in 59 cases (20%). Flour, grains and animal feed were the most common cause (21%), followed by cow dander (18%) and NRL (15%). Table 1 presents the most common occupations in the study group and the respective most frequent causative agents.

**Table 1** *Most common occupations and causes of CU and PCD in present material 1995–2011.*

Occupation	n	Most common causes
Food service workers (chefs, bakers)	93	Flour (n = 43), vegetables (n = 18), fish and shrimps (n = 10), meat (n=6), NRL (n = 4), egg (n = 3)
Farmers	60	Cow dander (n = 46), grains and animal feed (n = 12)
Health care workers (nurses, dentistry)	32	NRL (n = 27), Chloramine T (n = 4), chlorhexidine (n = 1)
Gardeners, florists	25	Ornamental plants (n = 19), vegetables (n = 3)
Electrical equipment assemblers	17	Organic acid anhydrides (n = 17)
Researchers, laboratory workers	15	Rat and mice (n = 8)
Hairdressers	8	Persulfates (n = 5), permanent hair dyes (n = 3)
Other occupations	37	NRL (n = 12), animal dander (n = 8), chemicals (n = 6), enzymes (n = 5)
<b>Total</b>	<b>291</b>	

NRL = natural rubber latex

## **5.2 OCCUPATIONAL CONTACT URTICARIA CAUSED BY CYCLIC ACID ANHYDRIDES (STUDY I)**

Of the 21 patients diagnosed with occupational CU caused by organic acid anhydrides during the study period 1990–2006, 12 had worked in the manufacture of electrical machines and were exposed to an epoxy hardener containing methyl hexahydrophthalic anhydride (MHHPA). The largest prick test reaction was in most cases noted for the same acid anhydride the patient had been exposed to at work, but cross-reactions with other acid anhydrides were frequent. The specific IgE results were generally in line with the prick test reactions. Specific IgE to PA was found in 19 out of 21 patients, regardless of what type of anhydride had been used at the workplace.

Open applications were performed on 11 patients, all of which yielded a positive test result. In five cases, a positive test result in the open application required the use of the undiluted hardener as is, because open application with a diluted product remained negative.

In seven patients, the CU symptoms were limited to the hands and forearms, five patients had CU symptoms only in the face and neck region, six patients had more widespread CU symptoms on the bare skin areas of the face and upper limbs, and in two patients the symptoms had also spread to covered skin areas.

Concomitant airway symptoms appeared in 18 patients, but no cases of severe generalized symptoms were reported. The detailed results of the clinical tests are presented in the original article.

## **5.3 OCCUPATIONAL CONTACT URTICARIA CAUSED BY OTHER CHEMICALS (STUDY IV)**

Exposure to chemicals was the cause of CU in 41 (14%) of the 291 patients in the study group. In this subgroup, the majority were male (59%). The largest group of chemicals that triggered CU was organic acid anhydrides – in 21 cases. Other chemicals associated with occupational CU were persulfates, oxidative hair dyes, chloramine T, diisocyanates, epoxy resins, chlorhexidine, formaldehyde, HBTU (o-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate) and tetrachloroisophthalonitrile. We detected positive SPTs or specific IgE to the causative chemical in all cases except for two, in which CU was caused by oxidative hair dyes (Table 2).

**Table 2** Occupational CU and positive test reactions caused by chemicals in patients referred to FIOH 1995–2011 (acid anhydrides excluded).

No	Substance	SPT	Open application	OR	OA
1	Persulfates	+	Ammonium persulfate 2%, 5% in aqua pos.	+	+
2	Persulfates	+	Ammonium persulfate 2% in aqua pos.	-	-
3	Persulfates	+	Ammonium persulfate 2%, 5% in aqua pos.	-	-
4	Persulfates	+	NT	+	-
5	Persulfates	+	NT	+	-
6	Permanent hair dyes	PPD-HSA + TDS-HSA +	Undiluted hair dye pos.	-	-
7	Permanent hair dyes	-	Undiluted hair dye pos.	-	+
8	Permanent hair dyes	-	Undiluted hair dye pos.	-	-
9	Chloramine T	+	2% in aqua neg, 100% pos.	+	-
10	Chloramine T	+	1% in aqua pos.	-	-
11	Chloramine T	+	NT	-	-
12	Chloramine T	+	NT	-	-
13	HDI	+	Product used at work 0.01%, 0.1%, 1% neg.	-	-
14	MDI	+	2% in petrolatum pos. HSA-MDI SPT-substance pos.	-	-
15	MDI	+	NT	-	+
16	Epoxy resin (DGEBA)	+	Product used at work diluted to 2%, 4% in petrolatum pos.	-	-
17	Chlorhexidine	+	NT	-	-
18	Formaldehyde	Dermografis mus (Spec.IgE 1.9 kU/l)	1% in aqua neg.	-	-
19	HBTU	+	0.1%, 0.5% neg. 1% pos.	-	-

## Results

20	Tetrachloroisophthalo nitrile	+	Product used at work 0.1%, 1% in aqua pos. Tetrachloroisophthalonitrile 0.02%, 0.0002% in aqua pos.	-	-
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SPT = skin prick test; OR = occupational rhinitis; OA = occupational asthma; NT = not tested; HSA = human serum albumin; PPD = *p*-phenylenediamine; TDS = toluenediamine sulphate; HDI = hexamethylene diisocyanate; MDI = diphenylmethane diisocyanate; DGEBA = diglycidyl ether of bisphenol A; HBTU = (o-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate)

### 5.4 OCCUPATIONAL IMMEDIATE ALLERGIC DISEASES CAUSED BY OXIDATIVE HAIR DYES (STUDY II)

During the ten-year study period of 2001-2011 used in this study we identified 11 patients diagnosed with occupational immediate-type skin and airway diseases due to oxidative hair dyes. All the patients were hairdressers, ten of them were female. The mean age at the time of diagnosis was 34 (range 21-52 years). The mean exposure time before the symptoms connected to work appeared was nine years, and the duration of the work-related symptoms before diagnosis of an occupational disease was 4.5 years.

During the study period, three cases of CU were diagnosed using open skin tests to confirm the diagnoses. One of the patients, who had experienced an anaphylactic reaction connected to the use of oxidative hair dyes, had repeated positive SPTs to PPD and toluene-2, 5-diamine sulphate (TDS) tested as HSA conjugates. This was the first positive SPT reaction to PPD seen at our institute.

SICs with oxidative hair dyes were performed on 52 patients during the study period: five of them (9.6%) had a positive bronchial test reaction and five (9.6%) had a positive nasal reaction to a hair-dye product, confirming the diagnoses of OR and OA in these cases. The test results are presented in detail in the original report.

### 5.5 SKIN PRICK TESTING WITH CHEMICALS IN THE DIAGNOSIS OF OCCUPATIONAL DISEASES (STUDY III)

During the study period of 1991–2011, positive SPTs were noted for organic acid anhydrides, isocyanates, epoxy resins, persulfates, chloramine T, chlorhexidine, and aziridine. Amine hardeners, formaldehyde, glutaraldehyde, phenylenediamine, methacrylates and colophonium each induced sporadic, small reactions, which were all smaller than the test reaction



to the histamine control. Ethanolamines, pyrocatechol, ammonium thioglycolate, and glyoxal did not induce any positive SPT reactions during the study period.

Table 3 shows the total number of SPTs performed with chemicals and the positive reactions in each chemical group during the study period.

**Table 3** *Routinely tested chemicals at FIOH during 1991–2011.*

<b>Group of chemical</b>	<b>Chemicals in the group</b>	<b>Test substance</b>	<b>Patients tested N</b>	<b>SPT positive N (%)</b>
Organic acid anhydrides	PA, MA, TMA, MHHPA, HHPA (since 1994), CA (since 1999), MTHPA (until 2004)	Hapten-HSA conjugates	695; HHPA:572 MTHPA: 501 CA: 376	93 (13)
Isocyanates	HDI, MDI, TDI	Hapten-HSA conjugates	1320	20 (1.5)
Chloramine T	Chloramine T	a) 1% solution (coca/glycerine or aq.) b) HSA conjugate (1998-2010)	597 580	a) 11 (1.8) b) HSA 10 (1.7)
Epoxy resin	DGEBA	HSA conjugate	1268	19 (1.5)
Amine hardeners	EDA, DETA, TETA, MDA, IPDA, tris-DMP, MXDA, TMD	a) 1% solution (coca/glycerine) b) HSA conjugate	796	25 (3.1)
Persulfates	Ammonium persulfate Potassium persulfate	2% (aq.)	803 806	17 (2.1)
Other hairdressing chemicals	PPD Pyrocatechol	HSA conjugate HSA conjugate	166 112	1 (0.6) 0
	Ammonium thioglycolate	10% (aq.)	191	0
Chlorhexidine	Chlorhexidine digluconate	0.5% (aq.)	337	1 (0.3)

Results

Group of chemical	Chemicals in the group	Test substance	Patients tested N	SPT positive N (%)
Aziridines	Polyfunctional aziridine	a) 0.1% and 1% (aq.) b) HSA conjugate	46	4 (8.7)
Methacrylate	2-HEMA MMA PEGDMA	HSA conjugate	1158	1 (0.08)
Ethanolamines	Ethanolamine Diethanolamine Triethanolamine	a) 1% solution (coca/glycerine) b) HSA conjug.	386	0
Aldehydes	Formaldehyde	a) 1% solution (aq. or coca/glycerine) b) HSA conjug.	a) 2703 b) 1053	21 (0.78)
	Glutaraldehyde	a) 1% solution (coca/glycerine) b) HSA conjug.	1214	2 (0.16)
	Glyoxal	a) 1% solution (coca/glycerine) b) HSA conjug.	1051	0
Colophony-related substances	Colophonium	a) HSA conjugate	1553	2 (0.13)
	Tall oil rosin Abietic acid	b) 10 and 100 mg/ml extracts (coca/glycerine)	Abietic acid 608	

aq. = aqua; PA = phthalic anhydride; MA = maleic anhydride; HHPA = hexahydrophthalic anhydride; MHHPA = methyl hexahydrophthalic anhydride; MTHPA = methyl tetrahydrophthalic anhydride; CA = chlorendic anhydride; HSA = human serum albumin; HDI = hexamethylene diisocyanate; MDI = diphenylmethane diisocyanate; TDI = toluene diisocyanate; DGEBA = diglycidyl ether of bisphenol A; EDA = ethylene diamine; DETA = diethylenetriamine; TETA = triethylenetetramine; MDA = diaminodiphenylmethane; IPDA = isophorone diamine; tris-DMP = 2,4,6-Tris-dimethylaminomethylphenol; MXDA = m-Xylylenediamine; TMD = trimethylhexamethylene diamine; PPD = *p*-phenylenediamine; 2-HEMA = 2-hydroxyethyl methacrylate; MMA = methyl methacrylate; PEGDMA = poly(ethylene glycol) dimethacrylate.

High proportions of verified occupational allergic diseases connected to the demonstration of positive SPT reactions were noted, especially for organic acid anhydrides (74 of 93; 80%), isocyanates (17 of 20; 85%) and persulfates (11 of 17; 65%). Occupational allergic diseases were diagnosed in only a minority of the patients with positive SPTs to chloramine T (5 of 11; 45%) and epoxy resins

(2 of 19; 11%). Amine hardeners and aldehydes induced several SPT reactions that fulfilled the criteria of a positive test result, but they were all smaller than the control wheal and were not associated with immediate allergic diseases. Three cases of OA were diagnosed among the patients with SPTs classified as positive to formaldehyde.

No serious systemic reactions requiring medical attention were noted in connection with the SPTs performed during the study period.

## **5.6 CONCOMITANT SKIN AND AIRWAY DISEASES (STUDIES I, II AND IV)**

Of the 291 patients diagnosed with CU or PCD during the study period (Study IV), 134 (46%) had a concomitant airway disease caused by the same work-related agent. Of these, 111 patients (38%) were diagnosed with OR, and 60 patients (21%) with OA caused by the same substance that caused the skin reaction.

Flour, grains and animal feed caused simultaneous allergic rhinitis in 67% and asthma in 25% of the patients diagnosed with CU/PCD. Other exposure groups with a large portion of concomitant airway diseases were ornamental plants (75%), enzymes (60%), cow dander (45%), and other animals (46%). In contrast, vegetables and fruit, fish and shrimps, and other foodstuffs rarely caused simultaneous respiratory diseases.

Chemicals induced concomitant airway diseases in 54% of the patients with CU, the most common agents being acid anhydrides (81%) and persulfates (60%). One of the patients with CU due to diisocyanates also had OA, and OR was diagnosed in one of the patients with CU related to chloramine T.

Of the 134 CU patients, 71 (53%) reported that the skin and airway symptoms had occurred simultaneously, 37 (28%) that the skin symptoms appeared before the respiratory symptoms, and 26 (19%) that the respiratory symptoms appeared first.

Concurrent skin and airway diseases were also common in the study that focused on cyclic acid anhydrides (Study I), in which 18 of the 21 patients with occupational CU caused by acid anhydrides had a simultaneous airway disease (16 OR and 5 OA). In five cases, the patients reported that the rhinitis symptoms were the first symptoms to appear, and in three cases the CU symptoms had occurred first. In the rest of the cases (n = 10) the airway and skin symptoms were reported to have occurred simultaneously.

## *Results*

For oxidative hair dyes (Study II), simultaneous airway diseases occurred in two of the 11 patients with occupational CU caused by hair dyes. One of these was diagnosed with OA, confirmed by SIC performed as a work simulation by mixing hair-dye ingredients, where a late bronchial reaction ( $FEV_1 - 34\%$ ) was observed seven hours after the challenge. The other patient had experienced recurrent coughing and dyspnoea when handling oxidative hair dyes at work, and was diagnosed with asthma, but due to a history of anaphylaxis connected to dying her own hair, SIC was not performed, and the diagnosis of OA could not be established.

## 6 DISCUSSION

### 6.1 IMMEDIATE SENSITIZATION TO CHEMICALS IN OCCUPATIONAL MEDICINE

Exposure to chemicals is an important factor to consider when evaluating working conditions and potential health risks at many workplaces. Many employees in different occupational fields encounter chemicals in their daily work. Several chemicals have sensitizing abilities and can induce allergic diseases in exposed workers.

Chemicals are typically associated with delayed, Type IV hypersensitivity and the development of allergic contact dermatitis, whereas immediate, Type I sensitization, is usually connected to protein agents. However, especially in work environments, several chemical substances are known for their ability to induce immediate-type sensitization and allergic occupational diseases, such as asthma, allergic rhinitis and CU.

Diagnostics of immediate sensitization to chemicals is challenging. The occurrence and relevance of IgE-mediated sensitization is still a matter of debate, and the test methods are diverse and unstandardized (Mahler 2012). The lists presented in the literature of chemicals known or suspected to induce immediate sensitization in occupational settings are primarily based on case reports, and the convincingly confirmed examples of immediate chemical sensitizers are sparse. For occupational airway diseases, a wide range of LMW agents have been linked to the development of OR and OA, but an IgE-mechanism has been shown for only a minority of them (Chan-Yeung et al. 2013).

Cyclic acid anhydrides are an exceptional group of chemicals, which has long been known to induce IgE-mediated sensitization and respiratory occupational diseases (Venables 1989). Acid anhydrides are quite reactive and can induce sensitization at very low concentrations (Nielsen et al. 2001). Reports on occupational allergic airway diseases following sensitization to acid anhydrides are frequent in the literature, but descriptions of CU induced by acid anhydrides are found mainly as single case reports. The total amount of nearly 100 patients sensitized to acid anhydrides in the present study, most of them diagnosed with a respiratory occupational allergic disease, is yet another demonstration of the strong sensitizing properties of these agents. A significant proportion of the patients exposed to acid anhydrides in the present study came from a large company producing electrical machines for industrial use, in which successful measures have been conducted to diminish exposure,

but where highly volatile and sensitizing acid anhydrides remain a challenge for the protection of workers' health.

The role of IgE-mediated sensitization in occupational allergic diseases caused by diisocyanates and persulfates has been debated and the controversy is ongoing (Moscato et al. 2010; Wisnewski and Jones 2010). This present study material supports the role of IgE, at least in some cases, in immediate allergy to these substances, although other mechanisms cannot be ruled out.

Previous studies have linked chloramine T to IgE-mediated sensitization, and the present study also found several cases of immediate allergy to chloramine T. The findings show that IgE-mediated mechanisms also seem possible, in at least some cases of immediate hypersensitivity symptoms, for chlorhexidine, epoxy resin, aziridine, formaldehyde, glutaraldehyde, PPD and TDS in oxidative hair dyes, HBTU and tetrachloroisophthalonitrile. On the other hand, this study found no additional support for methacrylates, amine hardeners, ethanolamines, glyoxal, or colophonium-related substances being inducers of immediate sensitization.

In this series of studies, the diagnostics of immediate sensitization to chemicals at work and related occupational diseases were scrutinized from a specialized clinic's point of view. The occupational medicine clinic at FIOH has been the main centre for diagnostics of immediate allergic occupational diseases in Finland, especially those caused by chemicals, for several decades, consequently building up wide-ranging material to evaluate.

At the same time, a clear limitation of this study is that it was based solely on the patient material of a tertiary-level referral clinic in which uncommon and challenging diagnostic cases are concentrated. Thus, conclusions regarding the general prevalence and incidence of immediate sensitization to chemicals and related occupational diseases cannot be made on the basis of these results. Due to the retrospective nature of the study and the long study period, the clinical methods also varied substantially. However, this study material enables a thorough analysis of infrequent symptoms and causative agents that usually appear only as single cases in clinical work.

## **6.2 SKIN PRICK TESTING WITH CHEMICALS**

The proportion of positive test results in SPTs performed with chemicals was quite small for all the chemical groups in the study material. SPTs have been used extensively at FIOH, and patients who have only a mild suspicion of an occupational disease caused by a chemical also undergo SPTs, which probably influences the results. The total number of tested individuals for each chemical consequently does not directly correspond to the group of individuals with

proven significant work-related exposure or symptoms clearly related to this substance.

The present study confirmed the findings of earlier studies that SPTs with organic acid anhydrides are a reliable tool for diagnosing allergic occupational diseases in this exposure group (Venables 1989). The SPT results in the present study showed a good correlation with occupational exposure; the largest wheal was usually caused by the acid anhydride the patient had been exposed to at work. The SPT results also correlated well with specific IgE levels to the same acid anhydrides.

For diisocyanates, 20 positive SPTs were noted during the study period. All the patients with a positive SPT also showed specific IgE to diisocyanates. All except three of these patients were diagnosed with an isocyanate-related occupational disease. This corresponds well with other studies, in which the presence of specific IgE is highly predictive and supportive of a diagnosis of OA caused by isocyanates, although specific IgE antibodies cannot be detected in all cases of isocyanate-induced OA (Budnik et al. 2013; Wisnewski 2007). Nevertheless, in the majority of OA cases related to isocyanate exposure, no indications of specific IgE can be found, and a negative SPT does not exclude an occupational respiratory disease caused by isocyanates (Jones et al. 2006).

The significance of specific IgE and the value of SPTs in the diagnosis of persulfate-related allergic diseases has been a controversial issue. While some studies have found no positive SPTs in patients with OA associated with persulfates (Diab et al. 2009; Moscato et al. 2010), others have reported positive SPTs as a frequent finding (Aalto-Korte and Makinen-Kiljunen 2003; Munoz et al. 2004). The present study found several positive SPTs to persulfates, most of them associated with a clinical occupational disease, which supports the relevance of SPTs performed with persulfates. These SPTs were performed with water solutions and thus we were unable to evaluate the feasibility of SPTs performed with HSA conjugates of persulfates in this study.

Epoxy chemicals are quite a common cause of occupational ACD, but positive SPTs or specific IgE to epoxy resins is a very rare finding in the literature. During the study period, we found 19 patients with a positive SPT to DGEBA-HSA conjugate, but only two of these were diagnosed with an occupational disease caused by epoxy resin. It is worth noting that nine of the patients were diagnosed with an occupational disease (OA, OR or CU) caused by simultaneous exposure to organic acid anhydrides, commonly used as curing agents for epoxy resins.

Several of the chemical agents examined in this study induced small-sized reactions, still fulfilling the criteria of a positive SPT reaction. These were particularly common for formaldehyde and amine hardeners. Although

specific IgE to formaldehyde has been described as a rare finding (Wantke et al. 2000), the role of IgE in occupational diseases related to these substances remains unclear, and SPTs cannot be recommended as a reliable diagnostic tool. An irritant reaction is also a possible explanation for small reactions. Moreover, the study results do not support the regular use of SPTs for diagnosing occupational diseases connected to methacrylates, colophonium-related substances, ethanolamines, glutaraldehyde, glyoxal, pyrocatechol, or ammonium thioglycolate. This is also in line with a review of the literature, which found hardly any reports on positive SPTs or specific IgE to these agents.

There were no severe systemic reactions among this large quantity of SPTs performed with different kinds of chemicals over a 20-year period. We can thus conclude that the risks connected to performing SPTs with chemicals are quite small. However, appropriate safety measures are naturally required, and the tests must be performed with caution, especially in cases of a history of severe or systemic symptoms.

### **6.3 OCCUPATIONAL CONTACT URTICARIA**

ICU is typically triggered by direct skin contact with a protein substance to which the patient has been previously sensitized. Epidemiological studies from other countries have found that the predominant cause of occupational CU to be NRL, although the share of NRL allergy has declined significantly in recent years due to successful preventive measures (Bensefa-Colas et al. 2015; Williams et al. 2008). In Finland, cow dander has traditionally been the most prevalent registered cause of occupational CU, due at least partly to the long winters when cattle is kept indoors, resulting in increased exposure among farmers (Kanerva et al. 1996). Such high proportions of CU due to cow dander are not seen in reports from other countries.

In the present study, the most common causative agents for CU or PCD were flour, grains and animal feed. The proportion of CU/PCD induced by these agents in this study is relatively larger than that in studies from other centres. This is probably a consequence of the great number of flour-exposed workers referred to the occupational medicine clinic at FIOH due to work-related airway symptoms, and the fact that skin symptoms also get noticed among these workers during the examinations. Commercial test substances for SPTs with wheat and rye flour have been recognized as having generally low sensitivities (van Kampen et al. 2013a). The use of prick-prick testing with flour instead of commercial test solutions has probably also led to improved identification of flour-sensitized workers.



Exposure to cow dander was the second and NRL the third most common cause of CU in the present material. The number of NRL cases has also declined sharply at FIOH in recent years, and only five new cases of CU related to NRL exposure have been diagnosed since 2004, which corresponds well with findings from other centres (Vandenplas and Raulf 2017).

ICU induced by chemicals has generally been considered relatively rare. In the present study, chemicals were the causative agent of CU in 14% of the cases. Organic acid anhydrides were the dominant reason: a total of 51% of the cases were attributable to chemical exposure. CU induced by acid anhydrides is considered rather unusual and mainly found in the literature as single case reports. To our knowledge, our finding of 21 cases of CU induced by acid anhydrides is the largest number reported in the literature, suggesting that CU caused by acid anhydrides might be more common in exposed individuals than previously reported.

Other chemicals that induced several cases of occupational CU in this study were persulfates, permanent hair dyes, diisocyanates and chloramine T, which earlier studies have all also described as causative agents of occupational CU (Giménes-Arnau 2015). Formaldehyde and epoxy resins induced only single cases of CU during the study period, which is quite little in proportion to the widespread use of these substances in the work environment. Moreover, in the literature, occupational CU caused by these agents are mainly reported as single uncommon cases. Chlorhexidine also induced only one case of occupational CU in the present study. Numerous reports exist on serious allergic reactions in patients connected to the use of chlorhexidine in invasive medical procedures (Aalto-Korte and Makinen-Kiljunen 2006; Garvey et al. 2007), but occupational allergic diseases caused by chlorhexidine are rare. Direct skin and airway exposure to chlorhexidine is probably usually insignificant in the work environment, especially when adequate protective gloves are used. The risk of sensitization is rather low, but should be kept in mind.

Open application served as a suitable tool for verifying the CU diagnosis in several cases of this study. Open application is a sensitive and convenient diagnostic method that gives an immediate test result, but reliable performance and interpretation of the test requires adequate facilities and trained personnel. It is notable that many of the open application tests, especially those performed with chemicals, required the test to be performed with the undiluted substance used at work to obtain a positive test reaction, as the tests results with diluted test substances remained negative. Despite this, starting with diluted products is still recommended before applying the undiluted substance, to avoid overly strong reactions. Guidelines for diagnosing occupational CU vary significantly in the literature. Some of the guidelines recommend open application as the first diagnostic method, before

SPTs, to minimize the risk of hazardous generalized reactions induced by invasive skin tests (Gimenez-Arnau et al. 2010), but in practice the diagnostic tests usually begin with SPTs and/or in vitro tests (Mortz and Andersen 2015). We noted no severe reactions in either the SPTs or the open applications in this study material, and the conclusion of these results is that immediate sensitization, confirmed by SPTs or specific IgE, combined with a reliable history of exposure and symptoms, is sufficient in clear cases to confirm the CU diagnosis. Open tests are recommended if the history of exposure and symptoms and results of immunological tests do not verify the diagnosis reliably, as is often the case for chemical exposure agents.

## **6.4 SIMULTANEOUS SKIN AND AIRWAY DISEASES**

Concurrent skin and airway diseases were quite common in this study, as 46% of the patients with CU or PCD also had an allergic airway disease caused by the same work-related agent. Concomitant skin and airway diseases were particularly common for dusty exposure agents such as flour, grains and animal feed, animal dander, ornamental plants, and enzymes, which is probably due to airborne exposure of the airways and skin. Chemicals also induced simultaneous skin and airway diseases in over half of the CU cases (54%), the most significant causative agent being acid anhydrides, as 81% of the patients with occupational CU also had a concomitant airway disease. For persulfates also, the proportion of patients with concurrent airway diseases was high (3 out of 5), but this number is too small to draw definite conclusions regarding the frequency of simultaneous skin and airway diseases. Vegetables and fruits, fish and shrimps and other foodstuffs seldom caused any related airway diseases, which is in line with the fact that exposure to these agents occurs primarily through skin contact.

Not many studies can be found in the literature with which to compare these findings. In a recent study on workers with OA due to platinum salts, 53% of the respondents had simultaneous skin complaints (Merget et al. 2017). In a study of laboratory animal workers, 25% of the participants had both skin and airway symptoms (Ruoppi et al. 2004). For acid anhydrides and persulfates, the literature provides only single case reports on combined skin and airway symptoms. More extensive reports cannot be found with which to compare the results of this study to evaluate how commonly concurrent skin and airway symptoms follow occupational chemical exposure. As acid anhydrides are highly volatile and reactive, a high degree of both skin and airway exposure can be expected.

The role of the occupational medicine clinic at FIOH as a tertiary-level referral clinic that examines both occupational skin and airway diseases probably increases the proportion of diagnosed concurrent diseases. During

the clinical examinations, the patients are questioned about both skin and airway complaints, which means that mild and transient symptoms can also be found. However, it is important in clinical work to recognize this connection and ask patients about skin as well as airway symptoms.

## **6.5 PREVENTION OF IMMEDIATE SENSITIZATION AND RELATED OCCUPATIONAL DISEASES AT THE WORKPLACE**

The most effective way to prevent sensitization and any ensuing occupational allergic diseases is to eliminate exposure to the sensitizing agents. Occupational health care plays a key role in the identification, assessment and reduction of possible health risks in the work environment. This requires good knowledge regarding the working conditions and products used at work, well-functioning collaboration with the employer, and preferably also with the manufacturers of the products.

Accurate identification of occupational immediate sensitizers is important to prevent long-lasting health effects and loss of work ability. The potential sensitizers should be identified as early as possible before any adverse health effects have appeared. It is essential that the occupational health physician is familiar with the most important sensitizing occupational agents in the work environment and knows how to deal with them. Occupational health care professionals should also be aware that MSDSs often provide insufficient information regarding the sensitizing properties of many substances and that the information gained from these may not exclude the possibility of sensitization (Nicol et al. 2008). A specialized clinic such as that at FIOH, which can consult chemists and occupational hygienists when needed, improves the chances of identifying the true sensitizing agents at the workplace. This supports the centralization of at least complicated cases of chemical exposure and related symptoms in expert units.

Methods for reducing exposure may include total or partial substitution of the agent, adjustments of the processes, isolation or enclosure of the source, improvements in general hygiene at the workplace, and the use of personal protective equipment. A combination of different interventions is often required to achieve optimal reduction of exposure (Heederik et al. 2012). Complete removal from exposure is the most effective way to control work-related symptoms in patients who have developed an occupational allergic disease, but for some individuals this can have negative consequences on future employment and income (de Groene et al. 2011).

## **7 SUMMARY AND CONCLUSIONS**

The aim of this thesis was to evaluate the clinical methods, especially skin testing, used in the diagnostics of occupational immediate allergic diseases, through a retrospective analysis of the patients examined at the national occupational medicine outpatient clinic at FIOH.

Below are the conclusions of the thesis:

- Diagnostics of OA, OR and CU caused by chemicals is challenging, and the number of patients is relatively small. Therefore, it is advisable to concentrate the activity into centres with experience in the diagnostic procedures and in assessing exposure.
- SPTs performed with chemicals can be of significant value in the diagnostics of occupational allergic diseases for selected chemical groups, but the results should be interpreted cautiously, taking into account data on exposure, clinical symptoms and results of other clinical tests. Positive allergy test results alone are not sufficient for the diagnosis of an occupational allergic disease, without suitable work-related symptoms and exposure history.
- IgE-mediated sensitization seems to play a significant role in the development of occupational allergic diseases from acid anhydrides, chloramine T, chlorhexidine, aziridine, reactive dyes, and some metals and drugs. As regards isocyanates, persulfates, epoxy resins, formaldehyde, glutaraldehyde, and permanent hair dyes, the role of specific IgE still remains uncertain, although positive SPTs have been observed in some patients. For many chemicals, including (meth)acrylates, amine hardeners, ethanolamines, and colophonium, no convincing evidence has so far been found of an IgE-mediated mechanism in occupational immediate allergic diseases.
- There is a great need for standardized allergen extracts, established test methods and confirmed guidelines for the diagnostics of chemical-induced immediate allergic diseases in occupational medicine.
- Concurrent allergic airway diseases are quite common among patients with occupational CU and PCD, and preventive measures should comprise both skin and airway protection.
- Immediate sensitization to chemicals can be prevented at the workplace by exact identification of sensitizing substances, substitution of harmful

agents with safer ones when possible, appropriate working methods, educating workers, and adequate protective equipment.

- Since our study population consisted of the patient material of a tertiary-level referral clinic, the conclusions cannot be directly generalized to the whole working population.

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## **ORIGINAL PUBLICATIONS I-IV**