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The impact of airway-irritating exposure and wet work on subjects with allergy or other sensitivity

– epidemiology and mechanisms

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…och en viss allergi får man finna sig i
om man önskar en stark industri.

Allan Edvall

ABSTRACT

The prevalence of asthma and allergies is increasing, which means that the proportion of workers on the labour market with allergy or other sensitivity also increases. It is as of yet not known to what extent these persons with an elevated sensitivity in the airways or the skin are affected by exposure to irritating substances at work.

The main aims of this thesis were to investigate how subjects with allergy or other sensitivity are exposed at work places compared to healthy subjects and consequences of an airway diagnosis early in life.

A job-exposure matrix (JEM) for Swedish conditions, for estimation of airway irritating exposure and wet work was developed in Study I. The JEM was applied on two large population cohorts, the Conscription cohort and the People Health Survey. In both cohorts, subjects with asthma had exposed jobs to nearly the same extent as healthy subjects, while subjects with allergic rhinitis rarely had jobs assessed as exposed. Wet work was more associated to symptoms caused by occupational exposure (hand eczema) than to symptoms appearing in childhood (atopic eczema).

In Study II the consequences of an airway diagnosis for health was studied in the Conscription cohort, which revealed that asthmatic subjects had an increased risk for morbidity and mortality compared to healthy subjects and subjects with allergic rhinitis.

In Study III-V the exposure to inhaled particles was studied, especially the lung deposition and permeability of ultrafine combustion particles. In collaboration with a German research group we modified a method for the production of ultrafine radiolabelled combustion particles. Asthmatics, smokers and healthy subjects inhaled the particles. The modified method yielded particles with a stable radioactive label and a stable particle size, making it a suitable method for the study of inhaled particles. We found that the translocation from the lungs to the circulation was negligible for both 100 nm and 35 nm particles. The dose to the lung was however higher in subjects with asthma and in smokers, compared to in healthy subjects.

In conclusion, an increasing proportion of the workers today is sensitive and reacts with symptoms already at low levels of airway-irritating exposure. To be able to give appropriate information and develop prevention, we have to know where efforts are needed. With a JEM for airway-irritating agents it is possible to find associations between sensitive groups and exposure, something that we have poor knowledge of today. This thesis has revealed some sensitive groups that do not voluntarily avoid health detrimental exposures, groups that are known to have poor health, high unemployment and absence due to sickness. We found little evidence for the hypothesis that the vulnerability of susceptible individuals should be a consequence of a higher direct dose of particles to target organs. It is, however, possible that a higher particle dose to the lung contribute to systemic effects. More research is needed to establish if a minimal fraction of translocated particles is sufficient to cause harmful effects.

SAMMANFATTNING

Förekomsten av astma och andra allergier ökar, vilket betyder att andelen av arbetare på arbetsmarknaden med allergi eller annan överkänslighet också ökar. Det är inte känt på vilket sätt dessa personer med en ökad känslighet i luftvägar och hud påverkas av exponeringar för irriterande ämnen på arbetsplatsen.

Huvudsyftet med den här avhandlingen var att undersöka hur personer med allergi och annan överkänslighet är exponerade på arbetet i jämförelse med friska personer, och vad hälsoeffekterna blir för personer som får en luftvägs- eller huddiagnos tidigt i livet.

En jobbexponeringsmatris (JEM) för svenska förhållanden, innehållande bedömningar av luftvägsirriterande exponering och våtarbete utvecklades i Studie I. JEMen applicerades på två stora populationskohorter, Värnpliktskohorten och Folkhälsoenkäten. I båda kohorterna hade personer med astma exponerade arbeten nästan lika ofta som friska personer, medan personer med allergisk rinit mer sällan hade jobb bedömda som exponerade. Våtarbete var vanligare hos arbetare med symptom som vanligtvis orsakas av yrkesexponering (handeksem), än hos arbetare med symptom som utvecklas i barndomen (atopiskt eksem). I Studie II studerades hälsokonsekvenserna av att ha en luftvägsdiagnos i Värnpliktskohorten, vilket visade att personer med astma hade en ökad risk för sjuklighet och dödlighet jämfört med friska personer och personer med allergisk rinit.

I Studie III-V studerades exponering för inhalerade partiklar, speciellt dos till lungan och lungans genomsläpplighet för ultrafina förbränningspartiklar. I samarbete med en tysk forskargrupp modifierade vi en metod för att kunna framställa radioaktiva ultrafina kolpartiklar. Partiklarna inhalerades av personer med astma, rökare och friska personer. Med den modifierade metoden fick vi fram partiklar med en stabil storlek och med en stabil bindning mellan partikeln och radiomärkningen, vilket är en förutsättning när man följer partiklar inuti kroppen under en lägre tid. Det fanns ingen mätbar translokering av de ultrafina partiklarna från lungorna till andra delar av kroppen. Dosen till lungan var däremot högre hos personer med astma och hos rökare, jämfört med hos friska personer.

Sammanfattningsvis är en ökande andel av arbetskraften idag extra känslig och reagerar med symptom redan vid låga nivåer av exponeringar i arbetsmiljön. För att kunna ge bra information och utveckla preventionen måste vi först veta var insatserna behövs. Med hjälp av en JEM för luftvägsirriterande ämnen kan man hitta samband mellan känsliga grupper och exponeringar, något vi har dålig kunskap om idag. Denna avhandling har visat att det finns känsliga grupper som vi vet har hög sjukfrånvaro och arbetslöshet, och ofta har arbetsbyten och psykosociala problem, men som trots det inte självmant undviker exponeringar som påverkar hälsan negativt. Resultaten i denna avhandling stödjer inte hypotesen att känsliga grupper kan få en högre dos av partiklar till målorgan på grund av att fler partiklar translokerar. Det är däremot möjligt att den högre dos av partiklar till lungan som vi såg hos känsliga försökspersoner kan medverka till systemiska effekter. Mer forskning krävs för att utreda om även en minimal translokering av partiklar är tillräcklig för att orsaka skadliga effekter.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-V).

I. **Wiebert P**, Svartengren M, Lindberg M, Hemmingsson T, Lundberg I, Nise G. Development of a Job-exposure matrix for airway-irritating agents and wet work and its application on two Swedish cohorts.

II. **Wiebert P**, Svartengren M, Lindberg M, Hemmingsson T, Lundberg I, Nise G. Mortality, morbidity and occupational exposure to airway irritating agents among men with a respiratory diagnosis in adolescence. Occup Environ Med. 2007 Aug 6; (Epub ahead of print)

III. Möller W, Felten K, Seitz J, Sommerer K, Takenaka S, **Wiebert P**, Philipson K, Svartengren M, Kreyling WG.
A generator for the production of radiolabelled ultrafine carbonaceous particles for deposition and clearance studies in the respiratory tract. *J Aerosol Sci* 2006; 37: 631-644

IV. **Wiebert P**, Sanchez-Crespo A, Seitz J, Falk R, Philipson K, Kreyling WG, Möller W, Sommerer K, Larsson S, Svartengren M.
Negligible clearance of ultrafine particles retained in healthy and affected human lungs. *Eur Respir J* 2006; 28: 286-290

V. **Wiebert P**, Sanchez-Crespo A, Falk R, Philipson K, Lundin A, Larsson S, Möller W, Kreyling WG, Svartengren M.
No significant translocation of inhaled 35 nm carbon particles to the circulation in humans. *Inhal Tox* 2006; 18: 741-747

CONTENTS

LIST OF ABBREVIATIONS

Introduction

IgE	Immunoglobuline E (antibody)
ISCO	International Standard Classification of Occupations
NYK	Nordisk Yrkesklassificering, the Nordic modification of the International Standard Classification of Occupations
OPCS	Office of Population Censuses and Surveys

Study I-II

ETS	Environmental tobacco smoke
ICD	International Classification of Diseases
ISA	The Swedish Information System on Occupational Accidents and Work related diseases
JEM	Job Exposure Matrix
HR	Hazard ratio
OEL	Occupational Exposure Limit
OR	Odds ratio
RR	Risk ratio

Study III-V

^{192}Ir	^{192}Iridium
^{99m}Tc	^{99m}Technetium
$^{99m}TcO_4^-$	Pertechnetate
CMD	Count median diameter
CPC	Condensation particle counter
DF	Deposition fraction
DMPS	Differential mobility particle sizer
DTPA	Diethylenetriaminepentaacetic acid
FEV_1	Forced expiratory volume during the 1st second of exhalation
FVC	Forced vital capacity
GSD	Geometric standard deviation
HPGe-detector	High-purity germanium detector
MBq	Mega Becquerel (10^6)
NaI	Sodium iodide
µm	Micrometer 1 µm=1x10^{-6} m
Nm	Nanometer, 1 nm=1x10^{-9} m
ROI	Region of interest
SSI	Swedish Radiation Protection Authority

1 INTRODUCTION

Today we go to our work place expecting a health promoting work environment, and do not expect to get ill due to work. Still, in a developed country like Sweden with a broad knowledge of occupational hygiene and with resources to make improvements in the work place if necessary, many employees experience problems due to exposures at work [1]. During 2004, 1,500 work related cases of allergy or other sensitivity were reported to the Swedish social security according to ISA – The Swedish Information System on Occupational Accidents and Work related diseases. Most cases of airway problems came from the trade "Manufacture of wood and wooden products". Inhalation of dust, gases, fumes etc. are known to cause irritation in the airways. The highest prevalence of skin problems were reported from the trade including "Washing of textile and fur products, hairdressing, physical well-being activities etc". Wet work, i.e. tasks where the skin is in contact with water, is a common skin irritating exposure.

For a person of good health, there is a great chance that he or she can tolerate exposures at work without any problems. If that person, however, were to have an enhanced sensitivity in the airways or in the skin, even low levels of exposure or a short exposure event may be sufficient to induce symptoms [2]. Persons with allergy and other hypersensitivity typically react at lower levels of irritating substances than healthy persons do, and repeated exposure can lead to aggravated symptoms and even chronic conditions [3, 4]. There is also a risk for achievement of an elevated unspecific sensitivity.

Work disability due to a respiratory disease is common and costly in the working population [5] and asthmatics have been found to change jobs more often than non-asthmatics [6]. Also upper respiratory tract complaints that are generally considered to be non-disabling, such as allergic rhinitis, have been associated with prominent decrements in work productivity [7]. Hand eczema is the most frequent occupational skin disease and is more prevalent in high-risk occupations [8]. Consequences of hand eczema are sick-leave, change of job and psychosocial effects [4]. Overall, airway and skin disorders influence health in many ways.

In the western world there is a high and increasing prevalence of allergy and other hypersensitivity. Asthma and allergies are common chronic diseases affecting all age groups [9]. About one child in four in Europe [10] has an ongoing allergic disease. Among adults, the prevalence of both respiratory symptoms [11] and atopy (positive Phadiatop) [12-14] is 20-30%. The most substantial increase in allergy has been in children and young adults [15]. This means that an increasing part of the population has heredity for, or have already developed allergy, when entering the labour market. Although it is known that persons with allergy and other hypersensitivity are more affected by irritating exposures, the long-term consequences of allergy in working life are not well studied.

Associations between exposures to increased concentrations of ambient particles and adverse health effects in susceptible individuals have been indicated in epidemiological studies [16-22]. It has been suggested that ultrafine particles may be more toxic than

larger particles [23, 24]. The mechanisms underlying the effects are largely unknown, and further particle exposure studies are needed. One hypothesis is, however, that the health effect of the particles originate from inhaled particles translocating from the lungs to the blood circulation, and the particles can cause inflammation in the organs they reach, i.e. the heart [25].

1.1 ALLERGY AND OTHER SENSITIVITY

In a group of subjects with an increased sensitivity in the airways or the skin, the underlying cause of the sensitivity may be of different origins (allergic or non-allergic) even though the symptoms may be similar.

The term hypersensitivity includes both allergic and non-allergic conditions. Hypersensitivity is defined as *"objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons"* [26]. When no immunologic mechanisms are involved, the hypersensitivity is termed non-allergic, or unspecific.

Allergy, or allergic hypersensitivity, is a reaction initiated by specific immunologic mechanisms. It can be antibody-mediated (IgE) or cell-mediated. Most subjects with allergic asthma and allergic rhinitis have an IgE-mediated allergy. In cell-mediated allergy the inflammation can be mediated by allergen-specific lymphocytes, as in allergic contact dermatitis, or by IgG antibodies, as in allergic alveolitis.

Atopy is a personal and/or familial tendency to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens. As a consequence, these persons can develop typical symptoms of asthma, rhinoconjunctivitis or eczema.

The following symptoms were studied in this thesis; asthma, bronchitis, rhinitis, atopic eczema, nickel eczema and hand eczema.

Asthma and bronchitis result in symptoms from the lower airways. Asthma is a chronic inflammatory disorder that is characterized by reversible airway obstruction. The obstruction is caused by swelling of and increased mucus production in the airways. Asthma is of allergic origin in about 80% of childhood asthma and in about 40-50% of the adult form [26].

The main symptom of bronchitis is productive cough. In its chronic form, bronchitis has been associated with many years of smoking or exposure to other airway irritants, and is therefore less common in younger years [27].

Rhinitis is characterised by symptoms from the upper airways (nasal catarrh or congestion) and the eyes (conjunctivitis). In the Swedish population, the prevalence of both allergic and unspecific rhinitis is 15-20% [28].

Atopic eczema is associated with a gene mutation affecting the skin barrier [29], where allergy may be one of several factors influencing the eczema. An irritative eczema (non-allergic) is also easier to develop when the skin barrier is affected.

Hand eczema, including nickel eczema, implies an inflammation of the skin that is confined to the hands and may be allergic or irritative. It is a common skin disease affecting about 10% of the general population of working age in Sweden [8]. Risk factors are atopic eczema, contact allergy and wet work [30]. The major part of hand eczema, 70-80%, is irritative, caused by contact with water, detergents etc [31]. A combination of allergic and irritative eczema is however common [32]. Nickel eczema is caused by skin contact with nickel and is more prevalent in young women (30%) than in men (6%) [33]. Nickel is an important risk factor for hand eczema [34]. Hand eczema may develop into a chronic eczema if the exposure is repeated [4].

1.2 WORK PLACE EXPOSURE

At the work place, many employees daily encounter a mixture of agents that may have an irritating effect on the respiratory system such as dusts, gases, smoke and organic solvents. The skin may be irritated by contact with water, dust, chemicals, mechanic abrasion and thermal trauma. Some of these agents, such as isocyanates, acrylates, enzymes, metals and furred animals may also act as allergen and have an allergic effect. A subject sensitised to an allergen will react to very low concentrations of that specific substance and work place concentrations of exposure agents are often higher than ambient levels.

1.2.1 The job exposure matrix (JEM)

The ideal method for assessing the occupational or environmental exposures of subjects in epidemiological studies is to measure the dose in the body, preferably in the target organ, or second best to measure the concentration in the air or on the skin. Unfortunately this can be difficult or even impossible to achieve, especially if the purpose is to estimate exposure in the past.

A JEM is an exposure estimation technique, which can be used as a surrogate to exposure measurements. JEMs have been used in epidemiological studies in many different areas, for example to find associations between air pollutants and lung cancer [35-37]. In a JEM, occupations or industries are listed on one axis, exposure substances on the other, and the cells of the matrix indicate the presence, intensity, frequency and/or probability of exposure to a specific agent in a specific job [38].

In large population studies with data on occupation and allergy or other sensitivity, a JEM for airway irritating agents and wet work can provide information about how the subjects choose occupation with regard to exposure.

Three asthma specific JEMs have been described in the literature. Kennedy et al. [39] have developed an asthma specific matrix that contain risk factors known to cause occupational asthma. Although the same factors can be a problem to subjects that

already experience symptoms due to allergy or other sensitivity, the JEM lacks data on agents that aggravate symptoms, such as irritants, which do not necessarily cause asthma. The second JEM is described by Blanc et al. [6] and includes an asthma matrix and a dust matrix partly based on self-reported job exposure instead of objective assessments. Finally, Sunyer et al. [40] constructed a population specific JEM based on an ad hoc JEM and on self-reported exposure to vapours, gas, dust and fumes. A drawback of all these JEMs is that the occupations are based on international job codes (ISCO: International Standard Classification of Occupations [41] and OPCS: Office of Population Census and Surveys [42]) and a translation into Swedish job codes (NYK: Nordic modification of ISCO [43]) or vice versa would lead to loss of information.

Hence, it was necessary to develop a new JEM for airway irritating exposure for Swedish conditions, relevant for allergy and other sensitivity. At the department of Occupational and Environmental Medicine in Stockholm there is a long tradition of development of JEMs. Publicized studies from the department include JEMs covering exposure to organic solvents [44-46], man-made vitreous fibres [47], chemicals [48], car exhausts [49], magnetic fields [50] and exposure correlated to cancer [37, 51, 52].

1.3 PARTICLE EXPOSURE

Particle exposure studies can bring important knowledge to the understanding of adverse reactions to exposure in susceptible subjects. The lungs of these individuals may in some way be primed – for example by inflammation – to hyper-respond to particle exposure in a way that normal lungs do not.

Particulate matter is a mixture of solid particles and liquid droplets, and the sizes of ambient particles range from 0.005 μm (5 nm) to 100 μm. Particles with an aerodynamic diameter less than 100 μm are inhalable, and those <10 μm are small enough to reach the conductive airways and lower respiratory system. These particles are often divided in a coarse fraction (2.5-10 μm), a fine fraction (<2.5 μm), and finally an ultrafine fraction (<0.1 μm or <100 nm, also sometimes called nanoparticles).

Coarse particles mainly originate from natural sources, such as dust and pollen, but also mechanical processes, e.g. mining and tire-road wear. Fine and ultrafine particles are usually formed from gases, mainly as a result of fossil fuel combustion. Diesel particles, for example, consist of agglomerates where the primary particles range in size from 10 to 30 nm [53]. Exposures to ultrafine particles have increased over the years [54]. Earlier, such particles were mostly a work place phenomenon with workers' exposures to metal fumes consisting – at least initially – of ultrafine particles, which could induce symptoms of metal fume fever. Now, with the increased road traffic density and emissions from automotive combustion engines, environmental exposures have become more widespread in the general population.

Work place exposures to metal fumes can be very high, in the mg/m^3 range, which implies that the initially ultrafine particle size will quickly aggregate into larger particles [55]. Environmental exposures, in contrast, are at much lower concentrations, and consequently the particle aggregation is slower.

Ultrafine particles are found to a large number in urban air, both as singlet and aggregated particles, although they contribute modestly to total mass [54]. There is evidence that ultrafine particles behave differently from the larger respirable ones, and a number of factors suggest that a particle which is non-toxic in the micrometer size may be toxic in the nanometre range [19, 23, 56-59].

The most important of these factors is the *surface properties* of the ultrafine particles. Their surface area per given mass is very large and might be able to act as a catalyst for specific reactions with cells. The increased area can also act as a carrier for co-pollutants such as gases and chemicals [60]. It has also been shown that short time health effects of particle exposure, such as lung function, are correlated to the surface area [61]. Another factor is the *composition* of the particles. Normally, ultrafine particles are produced in combustion processes, and are very reactive [62]. Finally, the *deposition and disposition* of ultrafine particles is different from that of larger particles [63, 64]. Ultrafine particles are very small compared with the cellular structures, which may be important in the apparent problems they present to the lung [55]. Deposited particles can translocate into the lung epithelium and to extra-pulmonary organs to a higher extent than larger particles [59, 65, 66].

1.3.1 Exposure dose and deposition

The dose of inhaled particles depends on the size, density, shape and hygroscopy of the particles, as well as breathing pattern [63, 67]. The dose will also be dependent on the anatomy of the respiratory tract, and on the susceptibility of the subject. Data suggest that affected lungs receive an increased dose from inhaled particles, relative to healthy lungs [68-71]. In healthy lungs particles deposit in the airway and alveolar region, with an area of 100 m^2, while obstruction of the airways force particles to deposit in the airways in an area of 0.5 m^2, giving a high local dose, which is primarily important for airway diseases [72].

Particles will be deposited by *impaction* when the air-stream changes direction while the particles continue in their original direction due to momentum. Impaction is of importance for particle deposition in the upper airways and in the larger airways of the lung. Its effect increases with air velocity and particle size, and affects particles down to 1 or 2 µm. *Sedimentation* is particle deposition due to gravitational force. This is primarily important for the alveolar region, where the dimensions of the airways are small and the transition time long. Sedimentation increases with particle size and decreasing air velocity, and affects particles down to around 0.5 µm in size. Thus, breath-holding enhances deposition due to sedimentation. *Diffusion* denotes the mechanism by which particles move randomly as they collide with gas molecules. Deposition by this mechanism is important only for particles less than 0.5 µm, and increases as the particle size decreases. Therefore there is a minimum deposition in the respiratory tract around the size of 0.5 µm. Diffusion is important in the entire respiratory tract. [67]

1.3.2 Lung retention and clearance

The airways transport approximately 10,000-20,000 L air per day, air that is contaminated with a variety of particles, viruses and bacteria. Therefore, the airways need to be a highly effective filter to protect the sensitive alveolar region. Multiple defence mechanisms cooperate to remove material deposited in the airways.

The most important is *mucociliary clearance*. Material is trapped in the mucus covering the ciliary cells present in the airway walls, and is propelled upward by ciliary strokes. Deposited material is normally cleared within 24 h with this mechanism. *Cough* is another important defence mechanism, although it is not a significant factor for clearance of inhaled material from the airways of healthy subjects.

Insoluble particles that have reached the alveoli are mainly cleared by phagocytosis by macrophages and subsequent transport to the mucociliary escalator. This *alveolar clearance* mechanism is extremely slow and might take years [73]. The clearance of ultrafine particles deposited in the alveolar region seems to be less macrophage-mediated than that of larger particles, and ultrafine particles also have a deleterious effect on phagocytosis [74], possibly due to increased oxidative stress on macrophages from the large surface area [55].

Insoluble particles can also translocate form the airways to other parts of the body. Deposited particles have been shown to translocate into the epithelium and interstitium of the lung [58, 66]. From the interstitium, uptake into the blood circulation or lymphatic pathways can occur. Translocation to the central nervous system via neuronal axons has also been shown specifically for ultrafine particles [75].

The importance of the translocation of particles into the circulatory system has been disputed. Some results claim that the translocation of ultrafine particles into the circulation is low and that it is a very slow process [65, 66, 76], while others describe an immediate process of significant quantity [77, 78]. Human studies on particle translocation into the circulation is limited [76, 78] and it have been suggested that the difference in conclusions could originate from methodological limitations.

1.3.3 Health effects

For a long time, the associations between mortality and particle concentrations were met with scepticism, partly because the concentrations of particles at which effects seem to occur are low compared to concentrations to which people are exposed in industrial work places without apparent harm. Epidemiological studies provide however evidence that air pollution contributes to mortality [19, 20], systemic [16] as well as pulmonary diseases [18], and reactive airway effects [22, 79]. The mechanisms underlying the effects are largely unknown, but autonomic regulation of the heartbeat, inflammation and systemic coagulation effects, and direct metal toxicity to the heart muscle are proposed mechanisms [60, 80, 81].

1.3.3.1 Mechanisms

Hypotheses have focused on both direct and indirect cardiac effects [25]. Direct effects occur when inhaled particles translocate from the lungs to the circulation. Particles can cause inflammation in the target organs and the particles per se or the metal compounds bound to the particles can have a toxic effect [58]. Particles can also enter into the nervous system with subsequent irritation of the autonomous nervous system [75]. Indirect effects occur when the inhaled particles cause an inflammation in the lung epithelium, releasing inflammatory mediators into the blood stream inducing increased blood viscosity or increased blood coagulability [81].

2 AIMS OF THE THESIS

When investigating the effect of work place exposure, most studies focus on healthy subjects and the effect is measured as new cases of, for instance, asthma or eczema. While these studies provide important information of the cause of disease, several related questions remain to be answered. One such question is whether persons with allergy or other sensitivity are exposed to the same agents as healthy subjects, or if they avoid certain exposures. And further, what are the consequences of such exposure for persons with enhanced sensitivity? Since an increasing part of the labour market consists of persons that already have an increased sensitivity, this thesis focuses on these subjects.

The main aim of this thesis is to study the impact of allergy or other sensitivity on work life and health.

Firstly, work place exposure was studied to assess if subjects with allergy or other sensitivity had airway irritating exposure and wet work to the same extent as healthy subjects.

Secondly, the impact of disease was studied to see if there were differences in health outcome, measured as morbidity and mortality, between subjects with airway diseases and healthy subjects.

Thirdly, the fate of inhaled material in affected and healthy lungs was examined.

Specific aims of the thesis include:

- Development of a JEM for airway irritating agents and wet work for Swedish conditions in order to determine which job families involve exposure.
- To study if the JEM is applicable on cohorts with different properties.
- To examine if men with an airway diagnosis avoid occupations with high risk of being exposed to airway irritating substances.
- To investigate if an airway diagnosis is a risk factor for overall mortality.
- To study if airway diagnoses are associated with inpatient care in general and for inpatient care for respiratory diseases in particular.
- To determine the translocation of inhaled ultrafine combustion particles, 100 and 30 nm in size, in subjects with affected or healthy lungs.

The results of this thesis will give information on to what extent groups with allergy or other sensitivity are exposed in work life. It will improve the knowledge about how a diagnosis for allergy early in life affects health factors, and which groups that can benefit from an improved occupational guidance. Further it will be important for the understanding of the mechanisms behind the observed adverse health effects due to particle exposure.

3 GENERAL APPROACH IN THE STUDIES

This thesis consists of both epidemiological and mechanistic studies complementing each other.

3.1 EPIDEMIOLOGICAL STUDIES I-II

The first two studies are epidemiological. In Study I the development of a JEM for occupational airway irritating exposure and wet work is described. The JEM was applied on two large Swedish cohorts with different properties, the Conscription cohort, with a prospective design, and the People Health Survey 2002, a cross-sectional cohort. The work place exposure in subjects with asthma, allergic rhinitis or eczema was compared with that of healthy subjects.

In Study II a more careful investigation of the men in the Conscription cohort was performed. Men with asthma, rhinitis and healthy subjects were studied during a follow-up period of 30 years, in relation to work place exposure, morbidity and mortality.

3.2 EXPERIMENTAL STUDIES III-V

Study III describes the development of a method used in the experimental studies. Basically ultrafine carbonaceous particles are labelled with a radiotracer in order to be able to follow the inhaled particles inside the human body. The method was developed in collaboration with a German research group, GSF National Research Center and InAMed.

In Study IV, healthy subjects, asthmatics and smokers inhaled 100 nm particles, produced with the improved method. In Study V, healthy and asthmatic subjects inhaled 35 nm particles. The initial lung deposition was measured, and retention and translocation of the particles were monitored for several days.

4 SUBJECTS AND METHODS

4.1 EPIDEMIOLOGICAL STUDIES I-II

This chapter describes the development of the JEM, as well as the two cohorts that the JEM is applied to.

4.1.1 The job exposure matrix (JEM)

A JEM was developed for occupational airway-irritating exposure and wet work in the Swedish labour market in 1970-1980 (JEM-75). The exposure axis includes 18 agents known to have an irritating effect on the respiratory system, as well as exposure to wet work. The exposure agents are presented in paragraph 4.1.2.

An additional version of the JEM was developed to cover the exposure situation during the period 1990-2000 (JEM-95). The occupational axis of each JEM was based on the appropriate version of the Nordic occupational classification (NYK-74 and NYK-83 respectively)[43, 82]. This means that the two JEMs cannot be compared directly, since the composition of the job families differs slightly.

For each job family and exposure agent, three assessments were made; the *probability* for exposure, the *air level* and *short time exposure*. For the exposure agent "cold air" an assessment of exposure level was not applicable, and *duration* of the exposure during the year was assessed instead. Further, for the agent "environmental tobacco smoke (ETS)", we found no application for short time exposure. Wet work was assessed for the probability to be exposed and the frequency of water contact at work. A section of the JEM is presented in Table 1 and exposure categories in Table 2.

Table 1. *A section of JEM-75, with occupations listed on the y-axis and exposure substances on the x-axis. In the cells are indicated the probability (P), the air level (L) in relation to the Occupational exposure level, the frequency of short time exposure (S) and the duration (D) of the exposure.*

	Exposure axis →										
	Organic solvents			Irritating gases			ETS*		Cold air		
Occupation	P	L	S	P	L	S	P	L	P	S	D
Wood workers and log-drivers	0	0	0	3	3	0	0	0	3	0	2
Miners, quarrymen	0	0	0	3	2	0	0	0	0	0	0
Well drillers, diamond drillers	0	0	0	0	0	0	0	0	2	0	1
Ore dressers	0	0	0	2	0	2	0	0	2	2	0
Other mining and quarrying work	7	7	7	7	7	7	7	7	7	7	7

* Environmental tobacco smoke

Table 2. *The probability refers to the proportion of workers within a job family being exposed. The level is the air concentration of the exposure agent in reference to the Occupational Exposure Limit (OEL). The same categories were also used for duration of exposure during the year (used for the agent "cold air"). Frequency of short time exposure and wet work were assessed using the same categories. In addition to the exposure categories 0-3, category 7 was used when exposure was not possible to assess, and category 9 when none of the exposure agents occur in the work place. In category 0 exposure may occur, but since few in the job family (<1/10) are assessed as exposed, it is considered to be negligible.*

Exposure category	Probability (P)	Level (L)/ Duration (D)	Short time (S)/ Frequency (F)
0 / Negligible	<1/10	<1/30	<1/month
1 / Low	1/10-1/3	1/30-1/10	1/month
2 / Medium	1/3-2/3	1/10-1/3	1/week
3 / High	>2/3	>1/3	≥1/day

4.1.2 Agents with an irritating effect

Below is a description of the groups of airway-irritating substances included in the JEM.

Gases and vapours

Organic solvents are carbon-based substances capable of dissolving other substances and are used in paints, lacquers, adhesives, glues, degreasing/cleaning agents, disinfections, etc. Exposure to organic solvents is irritating to the mucous membrane of the airways. Workers involved in painting and paint manufacturing, as well as floor layers and rotogravure printers may have heavy exposure to organic solvents.

Some *gases*, such as car exhausts, acids, ammoniac and ozone, have an irritating effect already at low concentrations. Transport workers and workers in production and workshop industries are often exposed to irritating gases.

Repeated exposure to *environmental tobacco smoke* (ETS) lead to the same health risks as smoking. Workers at restaurants, was the most important exposed group before smoking in restaurants was prohibited in Sweden in 2005.

Cold air is both irritating and dehydrating to the mucus membranes in the airways. Further, work in cold air is often associated to heavy manual work, with increased breathing volume and frequency as a consequence, for example in forestry and agriculture.

Animals or animal products such as dander, hair, scales, fur, saliva, and body wastes not only have an irritating effect, but also act as powerful allergens. Also materials such as bedding and feed are irritating to the airways. Workers at risk include laboratory animal and veterinary technicians, researchers and veterinarians.

Living *plants*, such as potted plants and bulbs, can irritate both airways and skin. Horticultural workers, greenhouse workers and florists are examples of exposed groups.

Organic dust

Paper dust consists of cellulose fibres and particles. Upper respiratory symptoms are common and long-term exposure may impair respiratory function. Exposure occurs when paper is processed and handled, as in packaging, recycling, printing and bookbinding.

Inhaled dust from *plastic and rubber* may lead to an impaired lung function and cause allergy. Acrylates and isocyanates are often related to airway problems. Exposures occur in dentistry, painting and work with car tires.

Dust from *textile, hide and leather* is irritating and may induce airway inflammation. Occupations with exposure are tailors, workers in textile manufacturing, and launderers.

Wood dust is irritating to the upper airways and may cause asthma. Exposure occurs mainly in sawmill work, paper pulp industry and in bench carpenters and cabinet makers etc.

Mould and fungus contain mycotoxins that can cause allergic alveolitis or exacerbate asthma and rhinitis. Exposure may occur in contact with wood dust, hay, grain, provisions and in buildings. Exposures occur in agriculture and the wood and construction trades.

Flour dust may cause problems as irritation in the airways, asthma, runny nose and itching eyes. Bakers and pastry cooks are exposed but also workers in production and packaging of provisions and animal feed may be exposed.

Other organic dust that may irritate the lungs is e.g. soot. Inhalation may induce chronic bronchitis, cough and mucus production. Chimney-sweeps and fire fighters are occupationally exposed to soot.

Inorganic dust

Inhalation of dust from *rock and concrete* is irritating and may impair the lung function. Mining, street-sweeping, tunnel work, construction, foundering and ceramic industry are exposed occupations.

Welding and soldering fumes are irritating to the airways and may cause asthma. The fumes are a mixture of gas and particles including ozone, phosgene and metals as chromium and nickel, depending on the method and material applied. Welders, flame cutters and blacksmiths are exposed groups.

Metal dust from e.g. cadmium, cobalt, aluminium, manganese and stainless steel can irritate and be detrimental to the airways. Exposure occurs mainly in production and workshop industry, i.e. metal casters and moulders, at surface treatment, etc.
Other inorganic dust with an irritating effect on the airways is e.g. asbestos, insulation material, plaster and talc. Insulators, plumbers and pipe fitters as well as painters are exposed workers.

Metal working fluids are oils, emulsifiers, anti-weld agents, corrosion inhibitors and buffers that are aerosolized in working processes. Further, the aerosol may be contaminated by biocides and microbes and products from the metal. Exposure can induce asthma and chronic bronchitis. Workers in industrial machining and grinding operations are exposed groups.

Wet work is dehydrating to the skin and may cause and worsen eczema. Wet work is here defined as tasks where the skin is in contact with water. Exposure is common among cleaners and workers in dental and health care, among hair dressers and cooks.

4.1.3 Study bases

Study I
The JEM was applied on two large Swedish cohorts with different population properties. The Conscription cohort have a prospective design and is based on data from a nation wide survey of 49,321 Swedish males, born in the years 1949-51, who were conscripted in 1969/70 [83]. At conscription all conscripts were seen by a physician, who diagnosed formal disorders. The Military register is further linked to several nation-wide registers, providing follow-up information about occupation (1970-1990), morbidity (1971-2003) and mortality (1969-2002). The study base consists of 45,512 subjects (42,638 healthy; 2,874 with a diagnosis). The following groups of diagnoses were studied: i) asthma; ii) asthma and allergic rhinitis; iii) allergic rhinitis and iv) atopic eczema and other eczema. Subjects without these diagnoses formed a healthy group.

The second database, the People Health Survey 2002, is a cross-sectional cohort including 25,410 men (44.6%) and women (54.1%) (1.3% without information on sex) in the age 18-64 years randomly selected from the population in Stockholm County. The study base consists of 17,663 subjects (7,873 healthy; 9,790 with a diagnosis). The following groups were studied: i) asthma; ii) asthma and allergic rhinitis; iii) allergic rhinitis; iv) unspecific rhinitis; v) atopic eczema; vi) hand eczema and vii) nickel eczema. Subjects not reporting any of the symptoms formed a healthy group.

In the Conscription cohort self reported smoking habits was reported by 98.4% of the men at the time for conscription. In the People Health Survey smoking habits was reported by 95.0% of the subjects. Ex-smokers were included in the non-smoker group.

Since the diseases in the Conscription cohort probably developed during childhood (diagnosed at age 18-20 years), the father's socioeconomic position was chosen to represent the conditions during which the conscripts were brought up. Five childhood

socio-economic groups were employed; children to i) farmers, ii) manual workers, iii) low and intermediate non-manual employees, iv) higher non-manual employees, v) company owners and academics. In the People Health Survey, the subjects own socioeconomic position was used, since the symptom debut was unknown. The same five categories of socioeconomic position were used.

Study II
In Study II, the following diagnoses from the Conscription cohort were used: i) total asthma, with the subgroups severe asthma and mild asthma; ii) allergic rhinitis, including subjects with allergic rhinitis without concurrent asthma. Subjects without any of these diagnoses formed a healthy group. The study base consist of 47,671 subjects (45,470 healthy; 2,201 with a diagnosis). Four childhood socio-economic groups were employed; children to i) farmers, ii) manual workers, iii) low and intermediate non-manual employees, iv) higher non-manual employees.

In Study I and II, smoking and childhood socioeconomic position were controlled for in the analyses, being correlated to both airway disorders, morbidity and mortality [84, 85].

4.1.4 Data analysis

Study I
The proportion of subjects with allergy or other sensitivity in jobs assessed as exposed was compared to the proportion of healthy subjects in exposed jobs. Crude odds ratios (OR) were calculated as well as ORs controlled for a socioeconomic position and smoking.

Study II
Differences in mortality between healthy, asthmatics and subjects with allergic rhinitis were tested, giving a hazard ratio (HR). The proportion of subjects with allergy or other sensitivity in jobs assessed as exposed was compared to the proportion of healthy subjects in exposed jobs, resulting in an OR. Further, differences in years spent in an exposed occupation was analysed for healthy subjects and subjects with a diagnosis. The results were controlled for smoking and childhood socio-economic position.

4.2 EXPERIMENTAL STUDIES III-V

A modified method suitable for the study of translocation of inhaled particles is described in this chapter. The volunteers who inhaled the particles are described and it is explained how the particles could be followed inside the body.

4.2.1 Technegas – ultrafine radiolabelled particles

To generate ultrafine particles we used a Technegas generator, which is a commercial device used in clinical routine to investigate the ventilation of the human lung. The Technegas method is non-invasive, which is an advantage in human studies.

In the standard method, technetium solution (99mTc sodium pertechnetate) is loaded into a graphite crucible, which is heated until the solution has evaporated. When the crucible is burned in an argon atmosphere, an aerosol of radiolabelled carbonaceous particles is formed. As saline is present in the technetium-solution the carbonaceous particles are formed in conjunction with sodium chloride (NaCl) particles. After several minutes of storage of the aerosol, it can be directly inhaled from the generator chamber.

The standard method has several drawbacks. Firstly, because of the high particle number concentration in the chamber, particle coagulation is very rapid [86-88], and aggregates of larger particles are created. Secondly, both the NaCl in the technetium-solution and the presence of oxygen in the generation chamber atmosphere will contribute to the production of pertechnetate during particle generation, which is soluble in water and will not give stable radiolabelling of the carbon particles. This means that when the aerosol is inhaled, labels in pertechnetate will dissolve in body fluids, a phenomenon called leaching, in contrast to particle bound labels. The position of the radiolabel inside the body is monitored with a gamma camera, and therefore it is important that the aerosol consists of insoluble radiolabelled particles. Several modifications to the method had to be performed.

To guarantee formation of insoluble radiolabelled particles:

- the PVC tubes leading gas from the argon tube to the generator chamber was replaced by metal tubes to ensure a pure argon atmosphere in the generation chamber
- the atmosphere (argon gas) of the generator chamber was purified from oxygen and other impurities
- the generator chamber was flushed with argon gas for 15 minutes before aerosol formation (instead of normally 5 minutes) for a more thorough remove of oxygen
- sodium was eliminated from the technetium-solution in an ion exchange column

To guarantee formation of particles with a stable particle size:

- burning time and temperature was modified to produce particles of the desired size
- the particle aerosol was diluted with purified air into a 70 L flexible bag to prevent particle aggregation

The experimental set-up of the system is shown in Figure 1.

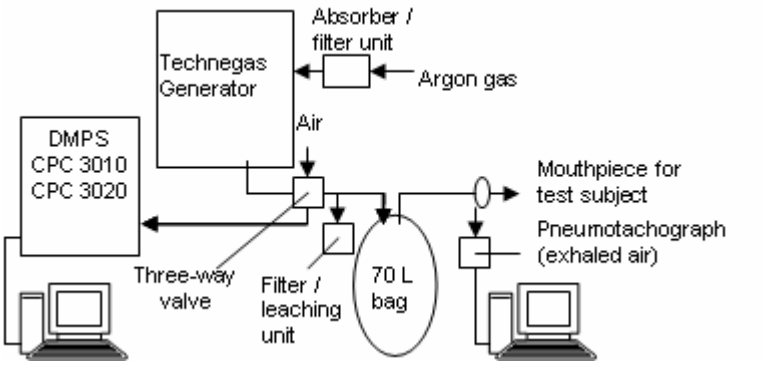

Figure 1. *Experimental set-up. The concentrated aerosol in the Technegas Generator is diluted into the flexible bag. Size distribution and number concentration of the diluted aerosol is monitored before and after inhalation (differential mobility particle sizer (DMPS), condensation particle counter (CPC)). The test subject inhales aerosol via a mouthpiece connected to the flexible bag.*

4.2.2 Exposure

A fresh aerosol of radiolabelled carbonaceous particles of 100 nm diameter (Study IV) or 35 nm particles (Study V) was produced for every subject using the modified Technegas method. The subjects were instructed to inhale the aerosol in continuous deep and slow breaths, with a brief breath-hold after each inhalation. Mean deposited activity was 26 MBq in the 100 nm study and 3 MBq in the 35 nm study. The specific activity of each aerosol was calculated as activity deposited on filters divided by the aerosol volume sampled through the filter.

4.2.3 Measurement of retention and clearance

Study IV
Activity from the 100 nm particles deposited in the chest region was measured immediately after aerosol inhalation and after 2, 24, 46 and 70 hours. The first three measurements were performed with a gamma camera, but as the activity is rapidly decreasing with time the subsequent measurements had to be made with a more sensitive whole-body scanner [89]. The deposition fraction (DF), indicating how much of the inhaled activity that stayed in the lung, is defined as deposited activity divided by inhaled activity. The deposited activity is the inhaled activity minus exhaled activity.

If pertechnetate is present in the aerosol, the dissolved radiolabels will be visible on the gamma camera scans, since technetium accumulates in the thyroids [90, 91]. Particle bound radiolabels, on the other hand, will accumulate in the liver [92].

Study V
A gamma camera was used for activity measurements of the 35 nm particles in the chest region at all time points. Activity was measured immediately, and after 60 and 100 min and 24 h after aerosol inhalation.

4.2.4 Estimates of leaching

Activity leached from the particles was estimated by four methods.

4.2.4.1 Filter sandwich method (Study IV)

After exposure, the particles of the remaining aerosol in the flexible bag were collected on a filter. In Study IV, leaching studies of each aerosol were performed using the filter with the collected particles, mounted between two membranes forming a sandwich tightly closed at its perimeter by a filter holder. The filter sandwich was submersed in 0.9% NaCl solution (Fig. 2). Particle bound activity stays on the filter while free activity can pass through the filters and go into the solution. The filter sandwich was temporarily removed for activity measurement in the solution.

Figure 2. *The filter sandwich method. Separation of free activity from particle bound activity in filter with particles.*

4.2.4.2 Dialysis method (Study V)

In Study V, particles were collected on a filter from each aerosol. Particle bound activity on each filter was separated from dissolved activity with the dialysis method. The filter was transferred into a dialysis tube, and 1 ml of 0.9% NaCl was added before the tube was sealed and submerged into 0.9% NaCl (Fig 3.). Particle-bound activity stays inside the tube, while free activity can pass through the membrane. Activity was measured in samples from the solution surrounding the dialysis membrane.

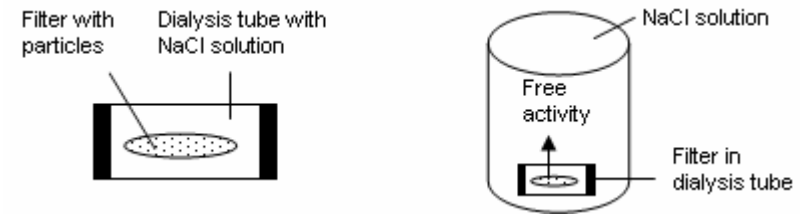

Figure 3. *The dialysis method. Separation of free activity from particle bound activity in filter with particles.*

4.2.4.3 Leaching in blood (Study V)

Dialysis was performed on blood samples taken at 80 min and 24 h after exposure. One millilitre of the blood sample was transferred into a dialysis tube, which was sealed and submerged in 0.9% NaCl solution (Fig. 4). After 1 h of dialysis, the free activity was measured in the NaCl solution.

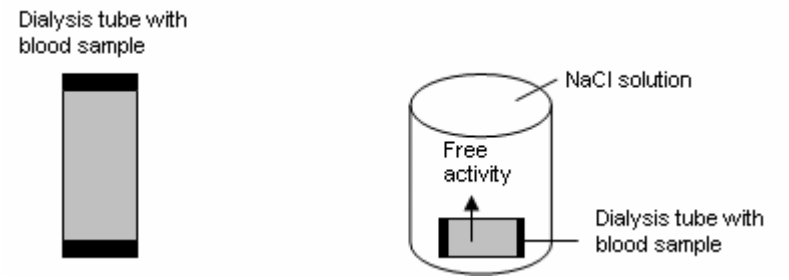

Figure 4. *The dialysis method. Separation of free activity from particles bound activity in blood samples.*

4.2.4.4 Leaching in urine (Study IV and V)

The fourth estimate of leaching was performed by measuring activity in urine from the subjects sampled during the first 24 h after exposure. About 30% of dissolved technetium is excreted with urine within 24 h in humans [90].

4.2.5 Subjects

Study IV
Fifteen subjects (nine males and six females), including six healthy non-smokers, five subjects with asthma symptoms and four asymptomatic smokers participated in the study. There were no significant differences in pulmonary function between healthy, asthmatic or smoking subjects.

Study V
Fourteen subjects (six males and eight females), including nine healthy subjects and four subjects with mild asthma but negative tests for non-specific hyper reactivity. One additional subject served as a positive control for leaching particles. All subjects were non-smokers. There were no significant differences in lung function between healthy or asthmatic subjects.

5 RESULTS WITH COMMENTS

5.1 EPIDEMIOLOGICAL STUDIES I-II

All results are controlled for smoking and socio-economic position. Hazard ratios (HR) and odds ratios (OR) are presented with 95% confidence interval (CI).

5.1.1 Study I

In JEM-75, 47.7% of the job families were assessed as exposed (low-high probability) to airway-irritating agents, and 17.4% had a high probability for exposure. No exposure occurred in 41.2% and in the remaining 11.0%, exposure was not possible to assess. Wet work occurred in 9.6% of the job families and the probability for exposure was high in 7.1%.

In JEM-95, 43.7% of the job families were exposed and 19.0% had a high probability for exposure. In 43.0% no exposure occurred and in the remaining 13.4% no exposure assessment was feasible. Wet work occurred in 9.1% of the job families and 6.9% had a high probability for wet work.

A high probability for airway-irritating exposures occurred in heavy industries such as mining, quarrying construction and metal processing work. Other exposed job families were farmers, wood workers, textile workers, fire fighters and hairdressers.

Jobs with a high probability for wet work were often found in health care jobs; physicians, nurses, midwifes, dentists, veterinarians etc. Food preparation was also associated with wet work with job families such as chefs, bakers and canning workers.

Jobs held by the men in the Conscription cohort in 1985 were coded both according to the job codes in JEM-75 and in JEM-95, which made it possible to compare the two matrices. The probability for exposure was more or less unchanged, while the exposure level was decreased and the short time exposure was slightly increased. Men exposed to wet work were uncommon in the Conscription cohort and decreased slightly during the period.

In the Conscription cohort, 8.6% had a high probability for airway-irritating exposure in 1990. Subjects with asthma had jobs with a high probability for exposure almost as prevalent as healthy subjects (OR 0.91, 95% CI 0.74-1.13). Those with allergic rhinitis, on the other hand, had less often jobs with a high probability for exposure (OR 0.58, 95% CI 0.47-0.69). Subjects with both asthma and allergic rhinitis had exposed jobs to the same extent as subjects with allergic rhinitis.

In the People Health Survey, more men (13.2%) than women (3.8%) had jobs with a high probability for airway-irritating exposure. Again, subjects with asthma had exposed jobs almost as prevalent as healthy subjects, while subjects with allergic rhinitis less often had exposed jobs. The differences were however more pronounced in

the Conscription cohort. Subjects with unspecific rhinitis had exposed jobs as often as healthy subjects.

Subjects with allergic rhinitis avoided short time exposure in both cohorts, when comparing with healthy and asthmatic subjects. Furthermore, the risk for a subject with allergic rhinitis to have a job with short time exposure decreased with increasing exposure frequency, an association not seen in asthmatics.

Eczema diagnoses were uncommon in the Conscription cohort, why all subjects with eczema were analyzed as one group. Wet work turned out to be more common among subjects with an eczema diagnosis at conscription (4.8%), compared to healthy subjects (3.5%).

In the People Health Survey, wet work was more common among women (16.5%) than among men (5.0%). Subjects with hand eczema had wet work more often than healthy subjects, both women (20.4%) and men (7.3%). Also nickel eczema tended to be more prevalent (women 17.2%; men 5.7%) while atopic eczema tended to be less prevalent in occupations with a high probability for wet work (women 14.3%; men 4.0%).

5.1.2 Study II

In the conscription cohort, subjects with a diagnosis for asthma or allergic rhinitis at conscription were compared with healthy subjects regarding health outcomes and work place exposure.

Subjects with asthma had higher mortality than healthy subjects, (HR 1.49, 95% CI 1.00-2.23), during the follow-up period 1969-2002. The lowest mortality was seen in subjects with allergic rhinitis, (HR 0.52, 95% CI 0.30-0.91) (Fig. 5A).

During the follow up period 1971-2003, 61.4% of the men needed inpatient care. Asthmatics had an increased risk for inpatient care, especially subjects with severe asthma (OR 1.38, 95% CI 1.04-1.85) compared to healthy subjects. Subjects with allergic rhinitis, on the other hand, tended to have less inpatient care (OR 0.92, 95% CI 0.82-1.03) compared to healthy subjects. In the cohort 0.36% needed inpatient care with the primary diagnosis asthma, and subjects with severe asthma had a high risk for care (OR 40.56, 95% CI 24.90-66.06) compared to healthy subjects. Also allergic rhinitis was a risk factor for asthma care (OR 2.20, 95% CI 1.02-4.73).

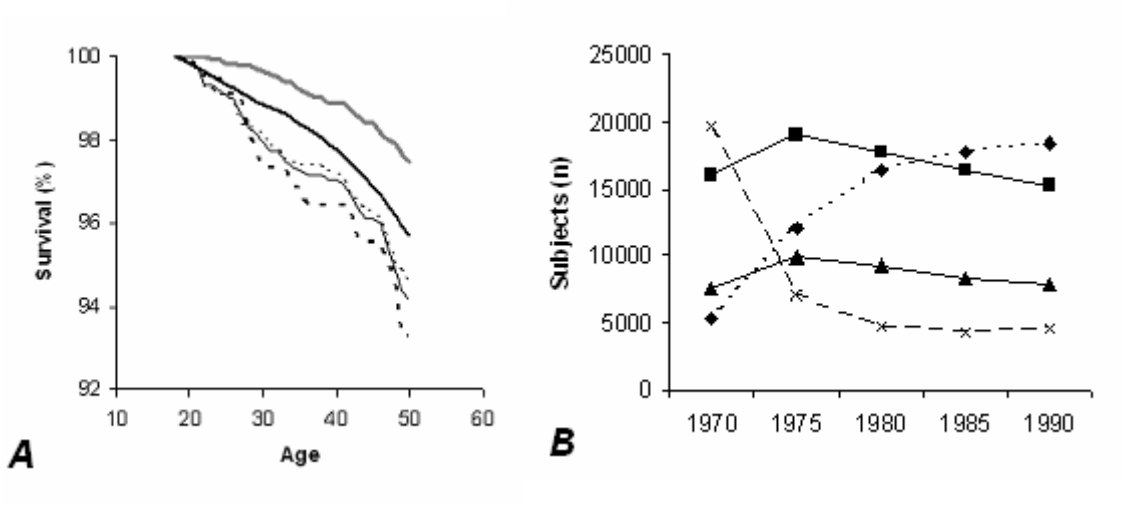

Figure5. *(A) Survival of subjects with allergic rhinitis (——), healthy (▬▬), mild asthma (·····), total asthma (——) and severe asthma (– – –) in the Conscription cohort. (B) Number of subjects in occupations with different probability for exposure; no occupation (– × – –), unexposed (···♦·····), low to medium probability for exposure (—■——) and high probability for exposure (—▲——).*

By applying the JEM on the Conscription cohort, occupations during the follow-up period 1970-90 were graded for exposure. Figure 5B demonstrate that the prevalence of exposed jobs dominated in the beginning and were fairly stable during the whole period, whereas unexposed jobs were few in the beginning and increased steadily during the period.

During the follow-up period 1970-90, the healthy group spent on average 13.2 years in exposed occupations, asthmatics 12.6 years ($p=0.010$), the subgroup severe asthma 12.4 years ($p=0.10$) and subjects with allergic rhinitis 11.7 years ($p<0.01$). The group that received inpatient care for asthma spent the longest period in exposed jobs, 14.1 years ($p=0.11$).

Asthmatics tended to avoid jobs with a low to medium probability for airway-irritating exposure and they avoided jobs with high probability for exposure to the same degree, com pared to the healthy group (Fig. 6A). Asthma severity had no influence on the results. Subjects with allergic rhinitis had less often exposed jobs compared to healthy and asthmatic subjects, and the likelihood for subjects with allergic rhinitis to have a job with a high probability for exposure was even lower (Fig. 6B).

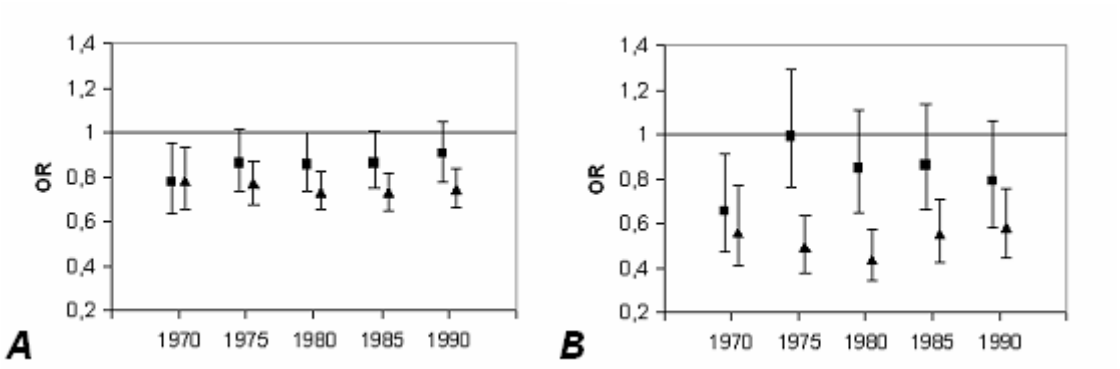

Figure 6. *OR with 95% CI for asthmatics (A) and subjects with allergic rhinitis (B) to have a job with low to medium probability for exposure (squares) respectively high probability for exposure (triangles). Healthy subjects form the reference group.*

There were no differences in unemployment between healthy subjects and subjects with asthma or allergic rhinitis over the whole period, but proportionally fewer subjects with allergic rhinitis had a job in 1970 and 1975, which is in agreement with their higher educational level. In 1990, 40.4% of subjects with allergic rhinitis had a higher education than upper secondary school compared to 29.4% in total asthma and 26.2% in healthy subjects.

5.2 EXPERIMENTAL STUDIES III-V

5.2.1 Study III (Method study)

The Technegas method was modified to produce leaching free particles with a stable particle size.

With increasing heating temperature of the graphite crucible, the size (CMD in Fig. 7A) increased linearly. With increasing aging time the particles coagulate further and the particle size increases, followed by a decrease in particle number concentration. Figure 7B shows typical particle size distributions of the Technegas aerosol, directly after dilution into the flexible bag (15 L) and after 13 min aging within the bag.

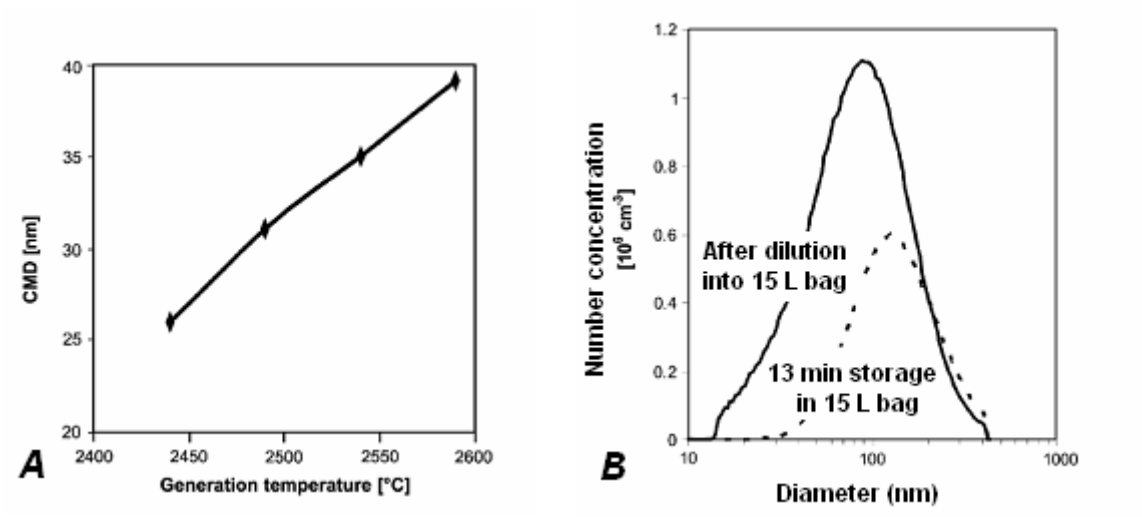

Figure 7. *(A) Correlation between particle size (CMD) and generation temperature. (B) Particle size distribution after 13 min storage in 15 L bag due to coagulation of particles. The median particle size (diameter) increased from 90 nm to 150 nm.*

Dilution of the aerosol into a larger container reduced the coagulation of particles. Particle number concentration and particle size distribution were compared between the 5 L generator chamber, and after dilution into a 30 L flexible bag. Figure 8 shows the CMD during a 15 min period after particle generation, together with the estimation of the total mass of the aerosol (right axis). Within the generator chamber, the particles undergo a rapid increase in CMD and a decrease in particle number concentration (data not shown), together with a significant decrease in total aerosol mass. When diluting the particles into the 30 L bag, the particle size increase is less pronounced, and the mass of suspended particles stays constant.

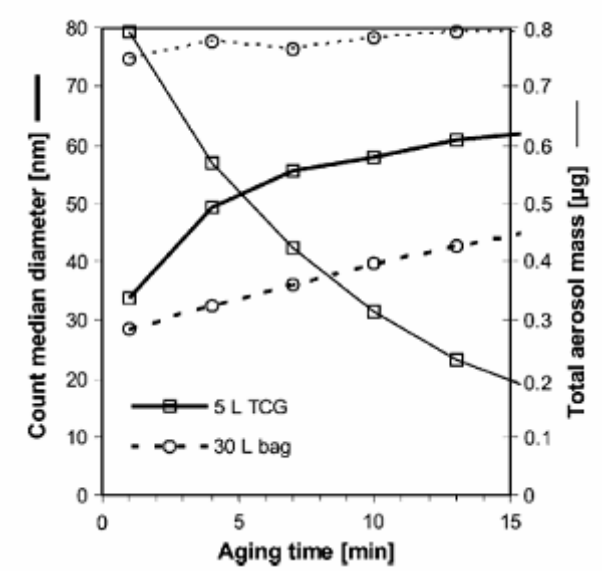

Figure 8. *Results of measurements of count median diameter (CMD, bold lines) with aging time together with the estimated total suspended mass (thin lines) (TCG, Technegas Generator Chamber).*

5.2.2 Study IV (100 nm particles)

The aerosols produced in this study had a count median diameter (CMD) of 98 nm (Fig. 9A). Lung retention at 24 h was 99±3.0% (mean±SD) (Fig. 9B). There were no significant differences in retention when comparing affected and healthy lungs. No activity could be detected in the liver or thyroid. The DF in the 100 nm study was higher in asthmatics (48%) and smokers (56%) compared to healthy subjects (32%).

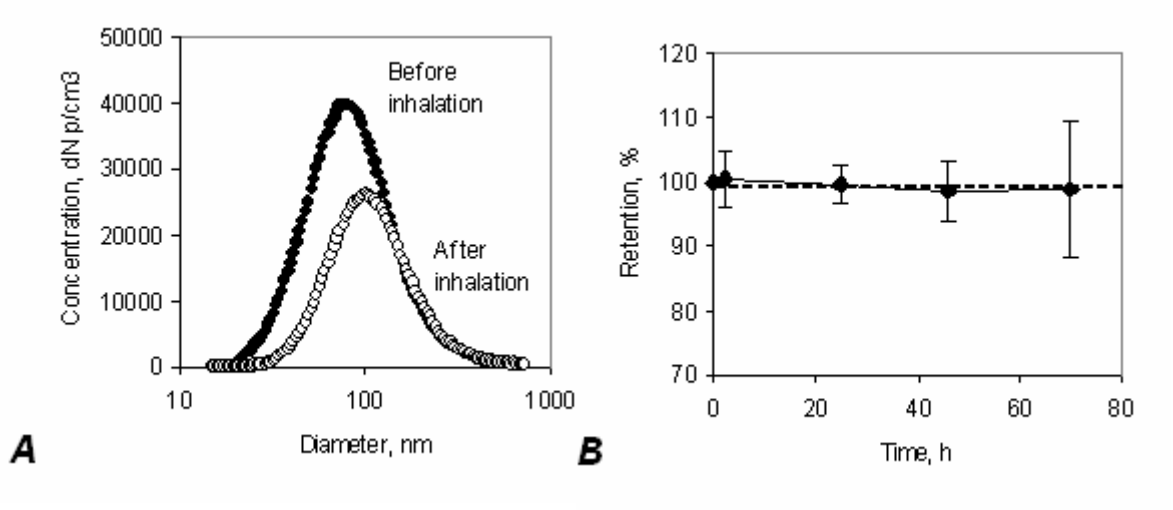

Figure 9. *(A) Particle size distribution before and after inhalation. (B) Lung retention of 100 nm particles.*

Cumulative leaching from particles (the filter sandwich method) at 70 h was 2.6±0.96% (Fig. 10). Activity leaching in urine was 1.0±0.55% during the first 24 h. In contrast, leaching from particles produced with the standard Technegas Generator method was 11% within 24 h (fig. 4). Individual leaching did not correlate to individual retention.

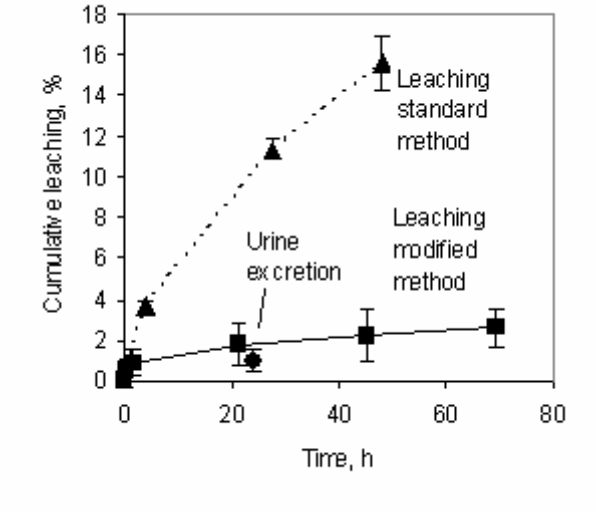

Figure 10. *Leaching from particles generated by the modified method (■) compared with the standard method (▲). Twenty-four hour cumulative urine excretion after particle exposure (•) is also shown (modified method).*

5.2.3 Study V (35 nm particles)

In this study CMD of the aerosols was 37 nm (Fig. 11A). Lung retention was 102±4.7% (mean±SD) for the 14 subjects exposed to the aerosol with stable labels (Fig. 11B). As in Study IV, there were no significant differences in the retention when comparing affected and healthy lungs. And again, no activity could be detected in the liver or thyroid. A larger fraction of the 35 nm particles were deposited in the lungs compared to the 100 nm particles, 60±17% respectively 41±10%. Mean DF tended to be higher in healthy subjects (63%) than in asthmatics (51%). This result is in contrast to the 100 nm study where healthy subjects tended to have a lower DF than asthmatics. However, in the 35 nm study, DF was correlated to tidal volume during exposure ($p = 0.009$) (Spearman).

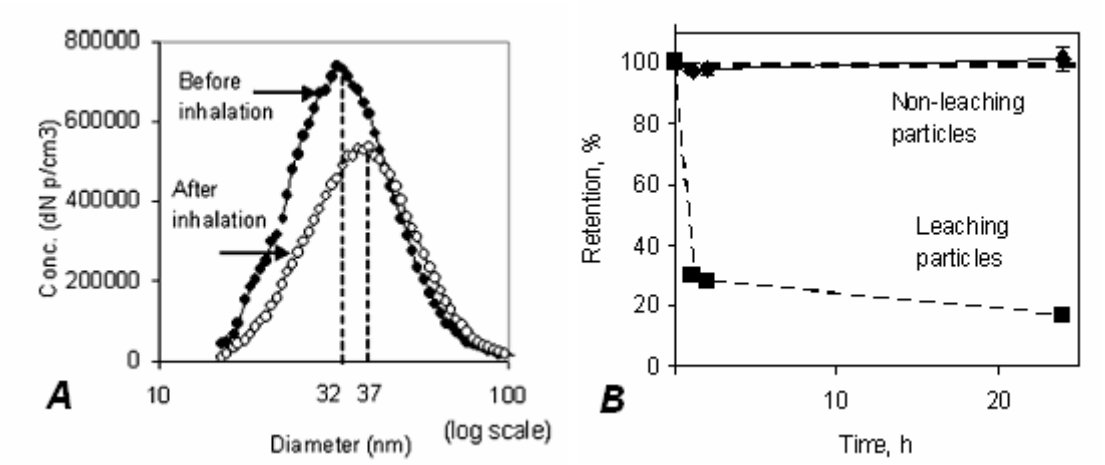

Figure 11. *(A) Particle size distribution of 35 nm aerosol before and after inhalation. (B) Lung retention of non-leaching and leaching 35 nm particles.*

Cumulative leaching from particles and urine is presented in Figure 12A. Leaching after 45 h was 2.1±1.1% of initial activity on the filter. Activity in urine sampled during the first 24 h was 3.6±0.9% of deposited activity.

Total activity in the blood is shown in Figure 12B. In blood samples taken 20 and 80 min and 24 h after exposure, 0.9±0.6%, 1.1±0.4%, and 1.5±0.5% respectively, of initially deposited activity was detected. In the 1.1% of activity found in the blood at 80 min, 90% was dissolved activity.

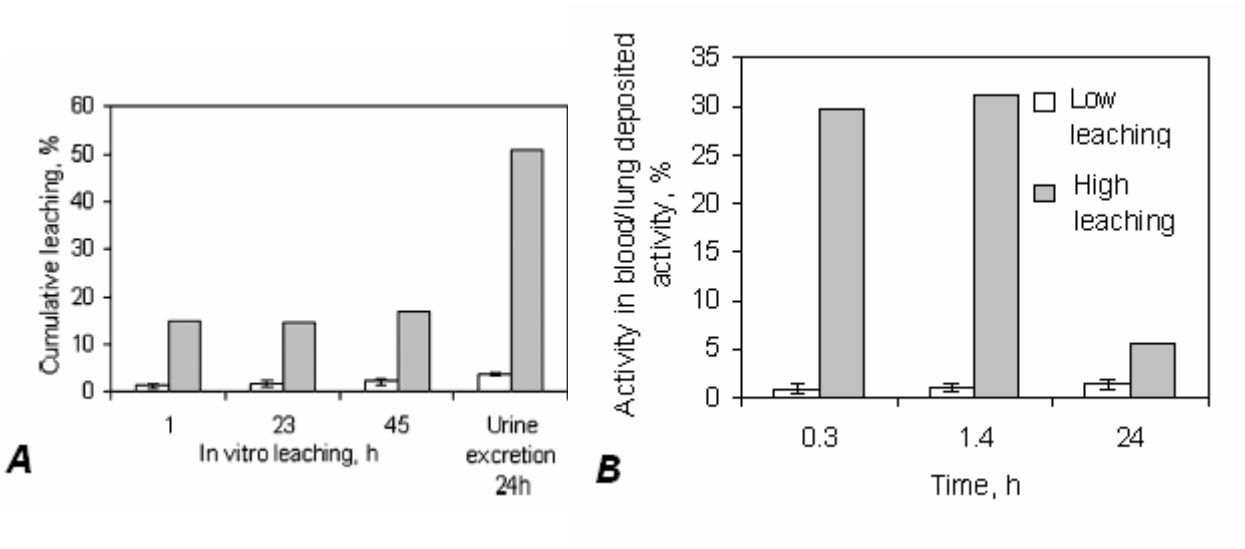

Figure 12. *(A) Leaching from particles (in vitro) and urine (low leaching particles; high leaching particles). (B) Activity in blood.*

In the subject exposed to the leaching aerosol, retention was 30% after 1 h (Fig. 11B). Total activity in the blood samples taken at 20 and 80 min was about 30% of lung deposited activity (Fig. 12B). Total activity levels in the blood then declined to a few percent in day 2. Of the total activity in the blood, 80% was dissolved, that is, not bound to particles. Within 24 h, 50% of the deposited activity was excreted with the urine (Fig. 12A). This is a higher fraction than expected and might be an effect of underestimation of deposited activity in the lung.

Figure 13 illustrates radioactivity distribution in the thoracic region of two exposed subjects. When particles are leaching, the activity is visible in the thyroid and the intestines after a short period of time (Fig. 13d).

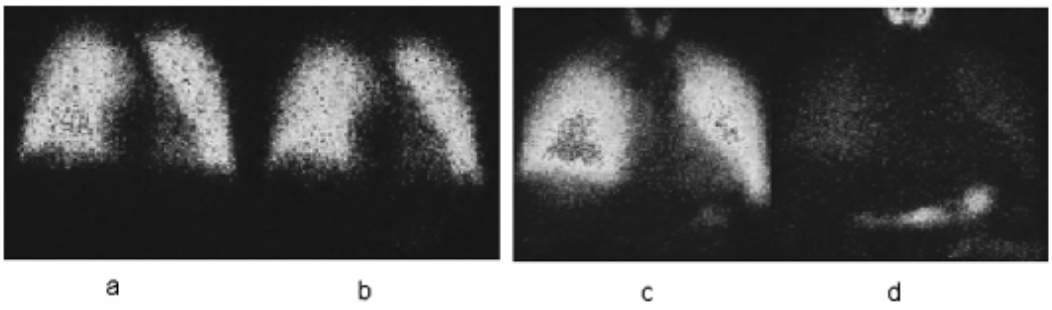

Figure 13. *Lung images of subject exposed to non-leaching aerosol after a) 10 min and b) 100 min. Lung images c) and d) represent the corresponding time points of the subject exposed to the leaching aerosol.*

6 DISCUSSION

Considering the successful progress in working conditions in the past decades, one would think the work place today is a health-promoting area, at least in countries with high living conditions. Occupational exposures affecting the human health have been regulated and levels have decreased considerably, hazardous substances are exchanged to less harmful ones and many tasks with special hazards are performed by means of robots and computers. This progress has indeed resulted in an improved work place environment, and for many workers the occupation constitutes no special threat to their health. However, even low levels or a short exposure event of an irritating substance can pose a risk to workers with an enhanced sensitivity, in some cases making it impossible to continue in the occupation [6, 7].

Therefore, this thesis focuses on describing occupational exposures and their impact on subjects with an enhanced sensitivity. How is the working life for a subject with an enhanced sensitivity? Are they less exposed compared to other workers, and is there a difference in where the deposited material end up in the body?

Who is exposed to what?

The advice given to individuals with allergy often relates to how to avoid allergen-induced symptoms. Unfortunately, this information is not very helpful when the symptoms are caused by other airway irritating agents than allergens, such as dust and gases. To be able to extend the information to include also these, it is crucial to find out which agents workers with an enhanced sensitivity expose themselves to. The new JEM, described in this thesis, can be used to study the exposure to 18 airway-irritating agents and to wet work in populations with information on occupations and diseases or symptoms, and hence reveal if subjects with allergy or other sensitivity have airway-irritating exposure and wet work.

The JEM was applied on two large Swedish cohorts, the Conscription cohort, and the People Health Survey. The most interesting finding was that an asthma diagnosis in adolescence only marginally influenced the choice of job and airway-irritating exposure. Subjects with allergic rhinitis had however less often exposed jobs compared to healthy and asthmatic subjects, and they particularly avoided jobs with a high probability for exposure.

Considering that asthma can be a severe disease with possibly life threatening symptoms, this finding is puzzling, and especially in the light of the results on allergic rhinitis, which is generally considered to be non-disabling. Yet, it is possible that the results can be explained by a difference in how the symptoms are experienced. The acute irritation in nose and eyes of a subject with allergic rhinitis may be more difficult to endure, while easier to connect to a specific exposure, than the non-acute symptoms experienced by a subject with asthma, such as heavier breathing, that may appear several hours after exposure [3, 93].

In the literature, it has been reported that asthmatics change jobs more often than non-asthmatics [6] and work disability is common among adults with asthma receiving specialist care [94]. Estimates of the occupational impact on rhinitis are limited. The occupational form of rhinitis may however have a profound effect on the worker, resulting in performance deficits, reduced productivity and psychosocial problems [95]. In a study by Blanc et al. [7], subjects with asthma were less likely to be employed at all while among those remaining at the job, rhinitis was a more potent cause of decreased job effectiveness.

In a study by Radon et al. [96] from 2002, teenagers without previous occupational exposure were asked about the type of job they would like to have in the future. Subjects with allergic rhinitis tended to avoid highly exposed jobs, while this association was not found in subjects with asthma. These results agree well with how the men in the Conscription cohort chose their occupation in 1970, although the two cohorts differ by almost 30 years. The knowledge about work-life exposure and its impact on asthma was limited in the early seventies, and occupational guidance did not include information about risk-jobs for asthmatics. The similar findings from the more recent teenager study indicate that occupational guidance has not improved sufficiently, or that subjects with asthma do not follow the advice they are given.

Methodological issues

The finding that an asthma diagnosis has less influence on exposure compared to allergic rhinitis was found in both cohorts, but the association was more pronounced in the Conscription cohort, where diagnoses were set before most of the 18-20 year old men had entered work-life. Thus, very limited occupationally derived diseases should be included in the Conscription cohort. In the People Health Survey however, part of the symptoms may be a result of occupational exposure, increasing the symptom cases in exposed jobs.

The prevalence of diagnoses was significantly lower in the Conscription cohort compared to the self-reported symptoms in the People Health Survey. The most important difference is probably that self-reported symptoms normally result in higher prevalence than doctor's diagnosis [97]. There has also been an increase in the prevalence of allergic diseases during the time period since the diagnoses/symptoms were registered [85, 98]. Further, there is a difference in age and sex composition between the cohorts.

Eczema

We found that hand eczema was more associated with wet work than atopic eczema was. Since atopic eczema typically appears in childhood, in contrast to hand eczema, which may be a result of occupational exposure to wet work, this was an expected result.

Atopic eczema was uncommon in the Conscription cohort, and hence, was not analysed separately. Subjects with any eczema at conscription, however, had more often wet

work compared to healthy subjects. In the People Health Survey, workers reporting atopic eczema only tended to avoid wet work compared to healthy workers. Since atopic eczema is a risk factor for hand eczema in wet work [30], we had expected a more apparent avoidance of wet work in this group. Our results agree however with earlier findings, showing that childhood atopic dermatitis does not seem to influence the choice of job, nor hazardous occupational skin exposure [99]. Subjects with childhood atopic dermatitis was also found to have an increased risk for job changes, sick leave and medical consultation mainly due to the increased risk of symptoms from hand eczema.

Methodological issues of the matrix

As previous research has showed that job exposure matrices are optimised when specificity is favoured over sensitivity [100], a job family was classified with a high probability for exposure only if more than two thirds of the job tasks were expected to be exposed. An important drawback of JEMs is that within-job exposure variation is not taken into account, meaning that there are many tasks in a job family, each with its own exposure situation. This results in non-differential misclassification [39]. In other words, it is possible for a subject to shift from an exposed task to a less exposed task within the same job family. This distinction is not possible to make when applying the JEM, since the exposure in the job family is a mean of all exposed tasks included.

Impact of disease on morbidity and mortality

Another aim was to study the impact of disease, measured as differences in morbidity and mortality between subjects with allergy or other sensitivity and healthy subjects.

An asthma diagnosis at conscription was associated to a higher overall mortality and higher consumption of inpatient care, both when comparing with healthy subjects and subject with allergic rhinitis. Mortality caused specifically by asthma could not be studied due to limited number of deaths in this still young cohort.

The size of the increased mortality risk for the asthmatics (HR 1.49, 95% CI 1.00-2.23), is comparable to those reported in previous studies [101-103]. Vandentorren et al. [103] found that impaired lung function increased the risk of death, and the authors implicated that mortality in asthmatics could be diminished by avoiding lung function decrease. We know from earlier studies that hazardous occupational exposure is correlated to an enhanced disability risk for asthmatics [1, 6, 7, 94, 104]. Therefore, it is possible that there is an association between the higher mortality rates in asthmatic subjects found in this thesis, and job exposure.

From the results of our study it is evident that subjects with asthma have a deprived health. Others have reported that young subjects with asthma have poor knowledge of the disease, and that many do not receive adequate treatment [105, 106].

How does it happen?

We have shown that many workers with asthma are exposed in their work place. Next step was to investigate if there is a difference in the fate of inhaled particles in the lungs of healthy and asthmatic subjects.

First we had to develop a method that could be used for studies of inhaled ultrafine particles. Under standard conditions, the Technegas generator does not produce ultrafine particles with a stable radiolabel. Several modifications resulted in aerosols of radiolabelled ultrafine particles with a stable particle size and with a strong binding between the particle and the radiolabel. The system is suitable for human inhalation and clearance studies of ultrafine particles, where different radiolabels can be used, such as technetium with a half-life of 6h, or indium with a half-life of 2.6 days.

The deposition fraction of 100 nm particles was higher in the lungs of asthmatics and in smokers compared to in the lungs of healthy. This is in agreement with earlier studies [68-70], and has been suggested to be one of the mechanisms behind the harmful effect of ultrafine particles in susceptible groups [60]. A recent study [79] of asthmatic responses to urban diesel traffic exposure in London, showed greater effects in subjects with asthma compared to healthy subjects. Walking for two hours through Oxford Street induced reduction in lungfunction and changes in biomarkers which were associated most consistently with ultrafine particle and elemental carbon exposures. The conclusion from the study is that diesel traffic exposures are associated with asthma severity.

When subjects inhaled the particles produced by the modified method, we could show that the majority of the deposited particles stayed inside the lungs and did not translocate to the circulation or other organs. As a consequence, there was no evidence for an increased translocation of particles in subjects with affected lungs, i.e. subjects with asthma or smokers, compared to healthy subjects. It is possible that there was a translocation of particles into the pulmonary interstitium [66] although other methods are required to make this distinction in particle location.

Our results are in conflict with those of Nemmar et al. [78] who used the standard Technegas method and reported important translocation of particles into the circulation. However, presence of oxygen during particle generation produces pertechnetate, which instantaneously after inhalation dissolve in body fluids [107-109]. Pertechnetate assembles in the thyroid and is excreted with urine within days [90, 91], while particle-bound technetium accumulates in the liver and only a limited amount is found in the bladder [92]. Since the thyroid glands and bladder were visible in the whole body scan in the Nemmar study, it is possible to assume that what they interpreted as translocation was actually leached radiolabels.

Another human study reporting declining retention values assessed lung retention in healthy and chronic obstructive pulmonary disease patients [68]. Lung retention was 86% one day after deposition, but because leaching from the particles was substantial (23%), 24-h retentions were corrected for leaching. Furthermore, the subjects inhaled particles under realistic resting breathing conditions (i.e. not deep breathing with

breath-holding), which may have resulted in a greater fraction of particles depositing on bronchial airways, i.e. particles might clear by mucociliary clearance. These particles are eventually swallowed and may translocate from the gastro-intestinal tract. In order to avoid deposition in the upper airways, the subjects participating in our studies were instructed to inhale particles in a slow breathing pattern with deep breaths. A slow velocity of the air stream gives the particles more time to deposit in the smaller airways [110].

The results of studies in this area are conflicting; some claiming that there is a high translocation of particles into the blood, while others state the opposite. Our modified method may be too insensitive to measure very small uptake of the deposited particles from the lungs to the circulation. On the other hand it is possible that studies that have found uptake of particles have used particle that have dissolved in the lungs [92, 111]. And in whole-body exposure studies of rats, particles deposited on the fur may have entered the gastrointestinal tract when animals lick their fur, implicating that particles found in the extra-pulmonary organs could have translocated from the gastro-intestinal tract instead of from the lungs [92].

Two studies are in agreement with our findings. An animal study showed that the fraction of translocated particles from the lung to extrapulmonary organs was only <0.002 and 0.001 for deposited 15-nm and 80-nm particles, respectively [66]. In a study of human volunteers [76], retention was 95.6% after 6 h. The authors used the standard Technegas method and in vitro leaching from the particles was 5% after 6 h (compared to 2.6% after 70 h in our 100 nm study). Besides retention, the authors also measured blood activity using thin layer chromatography, and found the small amount of blood-borne 99mTc to be unbound and thus concluded that translocation of particles from the lungs to the circulation is negligible.

7 CONCLUSIONS AND FUTURE PERSPECTIVES

The prevalence of asthma and other allergies have increased for several decades, which mean that an increasing proportion of the workers today are sensitive and react with symptoms also to low levels of airway-irritating exposure at the work place. To be able to give appropriate information and develop prevention, we have to know where efforts are needed. With a JEM for airway-irritating agents it is possible to find associations between sensitive groups and exposure, something that we have poor knowledge of today. This thesis has revealed some sensitive groups that do not voluntarily avoid health detrimental exposures, groups that are known to have poor health, high unemployment and absence due to sickness.

We found little evidence for the hypothesis that the vulnerability of susceptible individuals should be a consequence of a higher direct dose of particles to target organs. It is, however, possible that a higher particle dose to the lung contribute to systemic effects. More research is needed to establish if a minimal fraction of translocated particles is sufficient to cause harmful effects.

And future perspectives…

My next assignment will be to develop the JEM further and apply it on populations with information on cardiovascular diseases. The most important application of the JEMs is to screen for groups in large populations, and assess their exposure. In order to specify the exposure, more precise methods than the JEM have to be used, for example collecting information on self-assessed exposure or performing work place measurements.

The JEMs design also open up the possibility to study the importance the air level of the exposure is compared to short time exposure. Further, in populations with follow-up information on occupations, associations between new cases of diseases and duration of the exposure can be studied. It is also possible to improve the specificity of the exposure information. If the cohort that the JEM is applied to includes some information about the tasks involved in the occupation, this would give a more precise assessment of the exposure the subject experience at work. With the JEM it is also possible to study each of the 18 groups of airway-irritating substances on the exposure axis, either separately or in smaller groups, to investigate the association with diagnoses/symptoms. The design also makes in possible to study the combination of airway-irritating exposure and wet work.

The studies of inhaled particles have generated several new ideas. A longer study period of several months can add to the knowledge of long term kinetics of inhaled particles. It is possible that an accumulation of particles in target organs outside the lungs can be detectable if the subjects are followed for a longer period of time. It would also be valuable to study the extent and kinetics of translocation of ultrafine particles into the lung interstitium. This can be determined with lung lavage, a method that will wash out only the part of the deposited particles that have not translocated into the

interstitium. An examination of the cells in the lavage fluid could also shed light on the fate of the deposited particles. Further, it would be interesting to study other sensitive groups with airway diseases, for example individuals with more severe asthma or COPD (Chronic obstructive pulmonary disease); it is feasible that severely impaired lungs are more permeable to particles. Combined with a longer measurement period the probability to detect a possible particle translocation will increase.

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