Analysis of respiratory impairment due to occupational exposure

ANALYSIS OF RESPIRATORY IMPAIRMENT DUE TO OCCUPATIONAL EXPOSURE

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PROEFSCHRIFT

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Voor mijn ouders

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CHAPTER 1

Introduction

1.1. GENERAL INTRODUCTION

Chronic diseases of the airways, in particular those associated with airflow limitation, constitute a growing health problem and have been the focus of international (Murrary,1989) and national concern (Stuurgroep Toekomstscenario's Gezondheidszorg, 1990). Chronic airflow obstruction is a multifactorial determined condition. The conditions are not confined to industrialized countries (Murrary, 1989; Yunginger, 1992) and tobacco smoking has been identified as an important environmental risk factor (Woolcock, 1992).

There is scattered evidence, collected over many years, that some occupational exposures make a significant contribution to acute or chronic airflow limitation. This is definite in occupational asthma (for example from exposure to diisocyanates), is probable in exposure to some mineral dusts (coal, silica) and organic dust (cotton, grain), and is uncertain in long term exposure to irritant chemical vapours and gases (for example chlorine) (Weill, 1993).

Prevention of adverse pulmonary effects from occupational exposures can be attained in several ways. Finding specific culprits or excluding them from a workers' environment may be very challenging but is often very difficult, because many agents appear in an extraordinarily diverse range of materials and processes, often as unlabeled minor constituents or contaminants (Cullen 1990-a,b). Being unlabeled makes it hard to recognize the presence of these minor constituents and contaminants. Furthermore workers are usually exposed to multiple agents and interaction of exposure constituents may not be unlikely.

Detection of mechanical and pathophysiologic changes offers an alternative opportunity to prevent adverse pulmonary effects from occupational exposures. This chapter reviews the role of occupational exposure in the prevalence and nature of chronic respiratory diseases, possible pathophysiologic mechanisms involved and currently applied methods and alternatives for the detection of these diseases.

1.2. CONTRIBUTION OF OCCUPTIONAL EXPOSURE

The contribution of occupational exposures to the pathogenesis of airway disease is still underestimated (Becklake 1985; 1989-a,-b), but certainly underrecognized in the practice of clinical and occupational medicine (Morgan, 1986). Reasons include the previously mentioned smoking habits in general populations (Higgins, 1989; The Health Report, 1985) and until recently of the pneumoconioses (interstitial diseases) in many dusty industries (Becklake 1985; 1989-b). Other reasons involve the focus on workplace studies of which the power is often limited by the size of the work force. In addition many workplace studies have been cross-sectional in design and usually unable to take account of health selection either into the workplace (the "healthy worker effect") (Becklake 1989-a; 1990) or out of the workplace (the "survivor effect") (Becklake, 1989-a; Higgins, 1989), so that workers who quit for reasons of ill health are not examined.

The strength of the evidence that implicates occupational exposure in the pathogenesis of chronic airway disease is mostly epidemiologic, and lies in several features. First is its variety: morbidity data have been collected in health surveys, mortality statistics, pathologic studies, and case series. Second, its consistency: by far the bulk of the evidence reviewed shows positive associations between air flow limitation and occupational exposure, despite the inevitable imprecision in assessing exposure, which, however detailed, can only be an imperfect characterization of the exposure profile over a working life. Finally, the wide base of evidence is not limited by time, place, or person. Much of the evidence has been gathered in surveys, based either in the workforce or the community (Becklake 1985; 1989-a,b). Workforce based surveys, formed the traditional approach to the study of occupational diseases and have furnished the bulk of the information; owing to health selection and survivor effects, however, they are likely to underestimate the true relative risk. Community-based studies are less likely to be comprised for these reasons, but they inevitably lack precision in the assessment of exposure. Evidence from community-based studies on the role of occupational exposure in the genesis of chronic airways disease is reviewed in some detail because it is less accessible, and less readily accepted among occupational medicine professionals than evidence from workforce based studies. Nevertheless the data are surprisingly consistent. Follow-up of a cohort (n = 1266, response

rate = 74%) in the Netherlands revealed that 10 to 30% of chronic obstructive airway diseases could be subscribed to occupation related factors (Heederik, 1990). The incidence of airway complaints was the highest in subjects with dust exposure (paper, textile, construction industry) (Heederik, 1990). A number of recent population studies (Viegi, 1991; Bakke, 1991-a,b; Meredith, 1991) confirmed that the estimates derived from the "Zutphen-study" is a reasonable indication, for the total working population.

1.3. DESCRIPTION OF AGENTS AND RISK GROUPS

Commonly agents are divided according to their nature, i.e. inorganic dusts and organic dusts and chemicals since these entities stand for different adverse respiratory effects and pathogenetic mechanisms. Recent investigations on occupational asthma, however have shown that this division is not so clear cut with regard to the pathogenetic mechanisms involved. In a recent review agents were therefore discussed on basis of the (most common) pathogenetic mechanism involved, i.e. immunological (IgE dependent, immunological non IgE dependent) and nonimmunological (Chan-Yeung, 1994). Ergo, inorganic dusts and organic dusts and chemicals may feature the same pathogenetic mechanism(s).

In general, most information on pathophysiologic mechanisms is obtained in occupational asthma. Occupational asthma, a disease characterized by variable airflow limitation and/or nonspecific bronchial hyperresponsiveness (NSBHR) due to a particular occupational environment and not to stimuli encountered outside the workplace (Fabbri, 1993) may encompass both immunological and non immunological causes. Immunological agents are characterized: 1) a latent period between onset of exposure and the onset of respiratory symptoms 2) in sensitized subjects, re-exposure to a small amount of the causative agent leads to the occurrence of asthma. Immunological causes can be further divided into those inducing asthma through an immunoglobulin E (IgE) dependent mechanism, and those inducing asthma through a non IgE dependent mechanism. It has been demostrated for many years that agents inducing asthma through an IgE-dependent mechanism are high molecular weight proteins or polysaccharides (organic dust). Recently, several low molecular weight compounds (chemicals and inorganic dusts) have been shown to produce specific IgE antibodies, by combining with a protein from a hapten-protein conjugate. To the high molecular weight compounds with an IgE dependent mechanism products of organic origin are reckoned such as animal products, plant proteins of polysaccharides, gums, enzymes and fish and seafood proteins. The most well known compounds in the group of low molecular weight chemical compounds with an IgE dependent mechanism are acid anhydrides (chemicals) and metals (inorganic dust) such as nickel, chromium, platinum and zinc. The majority of low molecular weight com-

pounds induce asthma by immunological mechanisms as yet unidentified because IgE antibodies to the offending agent were not found or only in a small proportion of patients with the disease. Well known examples of this category are diisocyanates and western red ceddar. Nonimmunological occupational asthma may or may not occur after a latency period. The main distinction between this type of occupational asthma and immunologically-dependent ones is that in the majority of cases re-exposure does not reproduce the symptoms. Examples of nonimmunolgic respiratory disease are Reactive Airways Dysfunction Syndrome (RADS) and potroom asthma (Chan-Yeung, 1994).

Agents that may cause asthma, either of occupational or non-occupational origin, may be classified as inducers (i.e., agents that cause the development of reversible airway bronchoconstriction to nonspecific and/or specific agents) and inciters (i.e., agents that trigger asthma attacks) (Dolovich, 1981; Kay, 1991).

Apart from occupational asthma, occupational exposure can be related to different other effects on the respiratory system depending on the type of exposure. Inorganic dust exposure is associated with long-term or chronic deleterious effects (pneumoconiosis, chronic bronchitis and emphysema) (Oxman, 1993), whereas organic dust exposure is associated with both acute (organic dust toxic syndrome, hypersensitivity pneumonitis) (Zejda, 1993) and chronic effects (chronic bronchitis and emphysema); chemical exposure is predominantly related to sensitization (occupational asthma) and irritant/acute effects on the mucous membranes (Venables, 1989). The extent of the adverse respiratory effects is determined by factors as (chemical) type, reactivity, sources and concentration of the agent(s) involved. Also the nature of exposure is related to the airway disease evoked although Becklake (1985) postulated that the concept of "nuisance" dusts should probably be discarded and replaced by the view (Becklake, 1989-a), that exposure to any dust if sufficiently intense, fine and for an adequate period, is capable of evoking lung tissue reactions. This concept has been confirmed for lung cancer and fibrosis in which particle overload is the basis for the penetration of fine particles in the interstitium (Morrow, 1992). In case of the airways, chronic inflammation of smaller ones, if unchecked, has the potency to set in motion the pathophysiologic processes that may eventually lead to airflow obstruction of a progressively irreversible nature (Becklake, 1989-a). In the following paragraphs airway diseases related to inorganic and organic dust, and chemicals will be reviewed.

1.3.1. Inorganic dust

In this paragraph the description of respiratory effects of inorganic dust is restricted to chronic airway effects. Asthmalike and other respiratory effects of metals will not be discussed.

Pleuropulmonary diseases caused by inhalation of inorganic dust are frequently restricted to pneumoconiosis, defined as "the accumulation of dust in the lungs and the tissue reactions to its presence" (Fraser, 1990). The accumulation of inorganic dust generally results in following pathologic reactions: focal and nodular or diffuse fibrosis or aggregates of particle-laden macrophages with minimal or no accompanying fibrosis (Fraser, 1990). Small nodular or irregular opacities as well as progressive massive fibrosis are described as roentgenographic manifestations of coal workers' pneumoconiosis (Fraser, 1990). "Spurious melanosis" (Watson, 1848), the first used terminology for coal-workers' pneumoconiosis, was considered as a relatively harmless pathologic condition in the early 20th century (Seaton, 1990). By the time of the second world war the harmful effects of coal dust inhalation had become apparent (Fletcher, 1948). However, in that period, an exaggerated importance was placed on radiologic detection of pneumoconiosis. Radiography of the chest was considered as the "only simple means of discovering the extent of the pathological changes in the lung during life" (Fletcher, 1948). Disablement due to coal working was since then related to radiologic criteria. An international accepted classification system and recommended terminology of radiographs of the pneumoconiosis was developed by the International Labour Office (ILO) (Jacobsen, 1970). Knowledge of the harmful effects of coal dust in the respiratory system expanded considerably in the past two decades as a result of epidemiological studies in coal workers. Now growing evidence exists on the role of respirable inorganic dust in the development of airflow obstruction, impaired diffusion capacity, chronic bronchitis and emphysema, and increase in mortality irrespective of the extent of radiographic abnormalities. This paragraph focuses on these non-pneumoconiotic effects associated with dust exposure in miners, but please bear in mind that similar effects have also been reported in gold miners (Sluis-Cremer, 1967) and other kinds of inorganic dust exposure (Bar-Ziv, 1974; Malmberg, 1993).

1.3.1.1 Coal dust exposure and airflow obstruction

In the last two decades, different reports of the pneumoconiosis Field Research (PFR of the Medical Coal Board in Great Britain) and of the National Coal Study in the United States have focused on the possible relationship between dust exposure and decline in ventilating capacity. Standards for airborne dust were introduced in 1970 in British coal mines to ensure that longterm average dust concentrations at coal faces would not exceed about 4 mg/m^3 (Jacobsen, 1970). A 35-year working life at the face, equivalent to approximately 60 900 working hours, would result in a total exposure of 144 gh/m^3 (Rogan, 1973). Rogan *et al.* (1973) reported a significant decrease in FEV_1 with increasing dust exposure in all the age/smoking-habit subgroups. They reported a loss of FEV_1 of 150 ml at a cumulative dust exposure of 240

gh/m^3. This loss of FEV_1 was considered to be of no clinical importance. Kibelstis *et al.* (1973) measured the ratio of FEV_1 to forced vital capacity (FVC) in a large group of American bituminous coal miners: the FEV_1/FVC ratio was less than predicted in 37.6% of non-smokers, 50.5% of ex-smokers and 58.8% of smoking miners.

The previous studies were based on men still at work in coal mining and a healthy worker effect can therefore contribute to underestimation of the quantitative dust/disease relationship. Men who had left industry could have suffered from a greater response to dust exposure than had been observed in working miners. Soutar and Hurley (1986) reported on relationship between lung function and individual cumulative exposure to respirable dust in a large group of miners and ex-miners. They found a significant inverse relationship between levels of FEV_1, FVC and FEV_1/FVC-ratio and exposure to respirable dust. The size of the coefficient for FEV_1 was -0.76 ml FEV_1/ gh/m^3 of exposure; that is equivalent to a loss of 228 ml FEV_1 in relation to a moderately high lifetime dust exposure of 300 gh/m^3 (Table 1.1).

TABLE 1.1: Estimated effect of coaldust on FEV_1 in miners and ex-miners (adapted from Soutar, 1986).

		Regression coefficient ml FEV_1/gh/m^3
Smoking habits	non-smokers	−0.90
	current smokers	−0.65
Occupational history	miners	−0.68
	ex-miners < 65 y	−0.89
Chronic bronchitis (c.b.)	without	−0.54
	with	−0.55
	ex-miners < 65 y with c.b.	−1.08
	ex-miners - other job	−2.00

Remarkably, the estimated effect of dust was higher in non-smokers (-0.90 ml/gh/m^3 dust) than in smokers (-0.65 ml/gh/m^3 dust). These effects of dust exposure were independent of the presence of pneumoconiosis. Ex-miners had on average lower levels of FEV_1 and FEV_1/FVC ratio than working miners despite the absence of a significant greater response to dust exposure than other men. Severe impairment in lung function was found in the group of ex-miners under 65 with chronic bronchitis. In a group of men who left voluntarily and took other jobs the estimated effect of dust exposure on FEV_1 was -2 ml FEV_1/gh/m^3 of exposure (Hurley, 1986). In this study, exposure to dust was

related to a parallel reduction of FEV_1 and FVC while smoking was mainly related to a reduction in FEV_1.

Marine *et al.* (1988) supported the findings that exposure to coal mine dust causes reductions in FEV_1, irrespective of smoking habits, radiologic state or concomitant bronchitic symptoms. They found between 90 and 100 ml FEV_1 decrements for every 100 gh/m^3 exposure to coal mine dust in smokers and non-smokers. When FEV_1 was expressed as a percentage of internally derived prediction equations, the proportion of men with a $FEV_1 < 80\%$ or a $FEV_1 < 65\%$ increased with increasing exposure to dust for both smokers (Figure 1a) and non-smokers (Figure 1b).

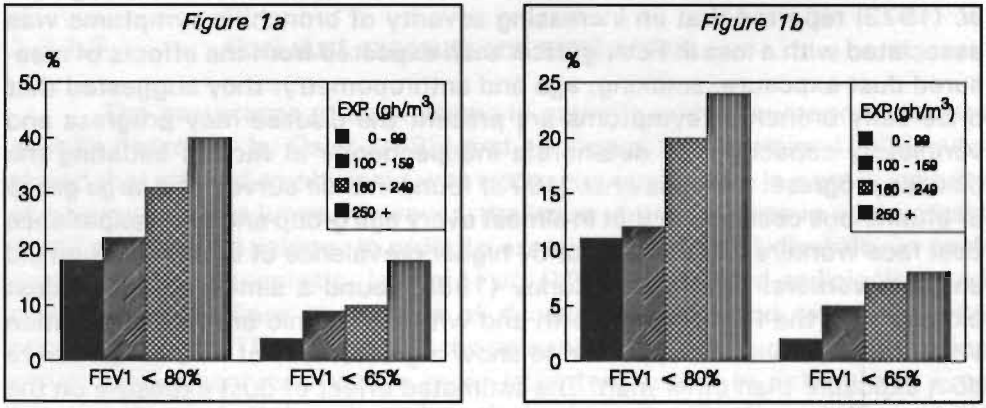

FIGURE 1: Decrement in FEV_1 expressed as a percentage of internally derived prediction equations showed increasing proportion of men with a $FEV_1 < 80\%$ or a $FEV_1 < 65\%$ with increasing exposure to dust for both smokers (Figure 1a) and non-smokers (Figure 1b). Data adapted from Marine (1988).

The equations were derived from a group of non-smoking miners without symptoms of chronic bronchitis and with no progressive massive fibrosis. The exposure-response relationship between various pulmonary function parameters and estimated cumulative dust exposure was recently confirmed in a large cohort of US coal miners (Attfield, 1992).

The relationship between FEV_1 and dust exposure was also demonstrated in longitudinal epidemiological surveys. Love and Miller (1982) found that a loss of FEV_1 was related to age, height, smoking habits and to the cumulative lifetime exposure to mixed coal-mine dust occurring before the study period. The longitudinal loss of lung function increased with exposure to coal mine dust and the magnitude of the predicted effect of the average exposure to dust appeared to be about one-third of the average loss attributable to smoking. The relationship between work in coal mines and loss in

ventilatory function was confirmed by data of American miners (Attfield, 1985). Again, these data were obtained in men who remained at work in the collieries.

1.3.1.2 Chronic bronchitis and respirable coal dust

In the early 20th century, sputum production in a collier was considered to be a healthy sign, indicating a rapid elimination of dust from the lungs (Haldane, 1918). In 1971, Rae *et al.* (1970) reported for the first time an increase in the prevalence of bronchitis among young and middle-aged men as their cumulative dust exposures increased. Two years after that Rogan *et al.* (1973) reported that an increasing severity of bronchitic symptoms was associated with a loss in FEV_1 greater than expected from the effects of measured dust exposure, smoking, age and anthropometry: they suggested that once early bronchitic symptoms are present the disease may progress and ventilatory capacity may deteriorate independently of factors initiating the disease progress. Kibelstis *et al.* (1973) found in their survey of a large group of bituminous coal miners that in almost every age group and work experience coal face workers had a significantly higher prevalence of bronchitis than did surface workers. Soutar and Hurley (1986) found a similar effect of dust exposure on the FEV_1 in men with and without chronic bronchitis, but men with chronic bronchitis appeared to show a greater loss of FVC in relation to dust exposure than other men. The estimated effect of dust exposure on the FVC was greatest in ex-miners under 65 with chronic bronchitis.

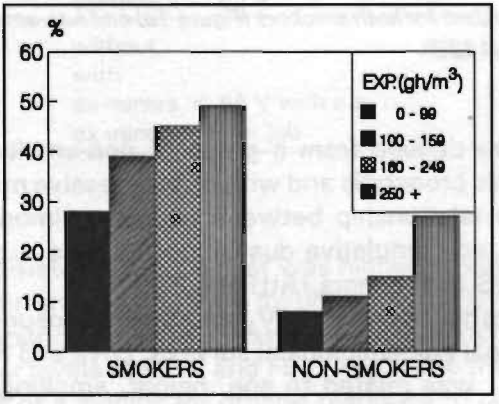

FIGURE 1.2: Increase in the percentage distibutions of chronic bronchitis with increasing exposure to dust for both smokers and non-smokers. Data adapted from Marine (1988).

Marine *et al.* (1988) clearly demonstrated a marked increase in the percentage distibutions of chronic bronchitis with increasing exposure to dust for both smokers and non-smokers (Figure 1.2). Estimates of prevalence of chronic bronchitis by smoking status at selected cumulative respirable dust exposure levels showed a larger increase in non-smokers versus smokers: prevalence of chronic bronchitis in non-smokers was twice the prevalence in smokers. The prevalence of respiratory dysfunction attributable to high dust exposures in non-smokers was similar in magnitude to the prevalence in smokers with zero dust exposure. The reported studies confirm the pathological and clinical observations of Osler (1895) that pneumoconiosis is associated with chronic bronchitis, and that it is the latter that really causes the chief symptoms.

1.3.1.3 Coal dust exposure and emphysema

The occurrence of emphysema in patients with pneumoconiosis was already described by Osler (1895) and confirmed by Cummins (1936) who stated that marked emphysema was a conspicuous feature in a great majority of pneumoconiotic lungs. However, the issue of emphysema in coalworkers is still a matter of debate. In order to explain the cause of disability in coalworkers' pneumoconiosis, Lyons *et al.* (1972) correlated radiological and physiological findings in a group of deceased miners and ex-miners with pneumoconiosis. They found that the presence of emphysema accompanying simple pneumoconiosis was a more important factor in determining lung function impairment than the radiological category or the degree of bronchitis as reflected by the Reid index. Furthermore, Lyons *et al.* (1981) compared the severity of emphysema in a group of non-smoking or ex-smoking deceased coalminers who suffered from pneumoconiosis during life with a comparable group of deceased miners who were cigarette smokers during life and who did not suffer from pneumoconiosis. They found little difference in severity of emphysema between the smokers and the non- and ex-smoking miners: centrilobular emphysema was the commonest type in both smokers and non-smokers. A post-mortem survey of emphysema in coalworkers and non-coalworkers dying of ischaemic heart disease demonstrated that predominantly centrilobular emphysema was significantly more frequent in coalworkers than in non-coalworkers even after adjustment for age and smoking habits. The severity of emphysema in coalworkers was related to the amount of dust in simple foci in the lungs (Cockcroft, 1982b).

Ruckley *et al.* (1984) studied the lungs of 450 deceased coal miners. They found that besides an increase in the relative frequency of emphysema with age at death and smoking habits, emphysema was associated with increasing evidence of pneumoconiotic disease: the proportion of subjects with any emphysema was 47% in the group men with no palpable dust lesions, 65% in miners with small, simple pneumoconiotic lesions and 83% in miners

with massive fibrosis. The centriacinar type of emphysema was slightly more frequent than the panacinar type: the presence of centriacinar emphysema was related to increasing exposure to coal dust in life. In simple pneumoconiosis but significantly in massive fibrosis, increasing amounts of ash with a given exposure to coal reduced the probability of finding centriacinar emphysema. The occurrence of centriacinar emphysema but not the extent of emphysema was associated with increasing amounts of dust retained in the lungs. The authors concluded that the association between exposure to respirable coal dust and emphysema in coal miners indicated a causal relationship, but that the presence of emphysema as a consequence of dust inhalation is mediated by both the extent and the nature of associated lung disease caused by that dust.

1.3.1.4 Respirable coal dust and diffusing capacity

Follow-up data of respiratory function in coal workers' pneumoconiosis mainly concern spirometric indices like FEV_1, FVC, and the FEV_1/FVC ratio. Few information is available on the effect of coal dust inhalation on diffusing capacity of the lung. Furthermore, reported data concern small patient groups. Catterall and Hunter (1965) reported data of single breath diffusing capacity and exercise testing in a group of 35 miners. According to the ventilatory function and the efficiency of gas exchange, four groups of patients were discerned: impaired ventilatory function was not always associated with a reduction in diffusing capacity. However, desaturation of arterial blood during exercise testing was related to a decrease in diffusing capacity. Rasmussen and Nelson (1971) performed extensive pulmonary function studies in a group of 368 coal miners. Their data confirmed that standard tests of ventilatory capacity can remain normal even in the presence of severe respiratory disability by impairment of oxygen transfer. In this study, impaired oxygen transfer was encountered considerably more frequently than was ventilatory insufficiency. Ventilatory function and oxygen transfer was not related to roentgenographic findings. Others have suggested that a gas transfer defect of the lungs is stronger related to the punctate or pinhead type of radiological opacities than to the micronodular type (Lyons, 1967). The conclusion can be drawn from these studies that evaluation of the functional status of coal miners must include assessment of gas exchange in addition to roentgenographic examination and measurement of ventilatory function.

1.3.2. Organic dust

There is a growing consensus on the adverse effects of organic dust on respiratory function in industrial workers. Organic dust generally entails

a wide range of possible respiratory effectors. The constituents of for example grain dust include grain particles, weeds and their disintegration products, insects, mites, bacteria, fungi and their metabolites, chemical residues, silica, and other substances. The physical, chemical, and biologic characteristics of grain dust are variable, depending not only on grain handling technologies for example, country and transfer elevators, but also on the predominant type of crop, climate, type of weather, and geographic region (Zejda, 1993).

This complex mixture is rich of high molecular weight plant and/or animal proteins, which can evoke an IgE dependent immunological reaction in the lungs. One would therefore expect acute respiratory reactions and asthma in exposed workers. Several authors have reported acute respiratory effects, however, workers exposed to grain dust such as grain elevator terminal workers and farmers as a rule do not, become sensitized to cereal flours but to other components in the grain dust. For example, occupational asthma in grain farmers is usually related to sensitization to storage mites such as Glycyphagus destructor (Davies, 1976). However, some studies indicate that inhalation of organic aerosols causes the development of chronic respiratory symptoms and lung function changes. Organic aerosols which have been studied include amongst others coffee (Jones, 1982), tea (Castellan, 1981), soy (Bush, 1977), fur (Gross, 1980), cotton dust (Jacobs, 1993) and animal food (Brooks, 1981; Smid, 1992).

Respiratory effects of grain dust will be discussed as an example of this form of exposure. Since organic dust exposure is far more complex than inorganic dust exposure this paragraph is restricted to respiratory effects other than hypersensitivity pneumonitis or farmer's lung associated with dust exposure in grain workers and fodder dust workers. These two occupations have different production processes but similar exposures.

1.3.2.1 Grain dust exposure and airflow obstruction

Cross sectional studies in grain workers have shown that spirometric variables are slightly but significantly lower than in control populations and that changes involve both lung volumes and airflow including changes in peripheral airways function (Dosman, 1980; Cotton, 1983). According to the exposure level 17% of the currently employed grain workers have an obstructive or restrictive profile of respiratory impairment, stable over 3 years of observation (Mc Duffie, 1989). Short-term and long-term observations suggest a characteristic pattern of spirometric response to continuous exposure to grain dust, composed of two distinct phases. The first phase comprises an acute reversible decline of FEV_1. The second phase is associated with a slow decrease in lung function, developing gradually over years of exposure, with modest fluctuation occurring across work-shift and across work-week.

In populations of workers exposed to grain dust, a rapid decline of average FEV_1 and FVC is particularly evident in those without recent exposure. In seasonal workers there is an average reduction of 300 ml at the end of the first and second week of employment that resolves within 5 weeks of employment commencement (James, 1990). In comparison with the pre-employment status, no significant declines of airflow variables are observed after approximately 10 to 20 weeks since the commencement of exposure (Dales, 1988; Broder, 1984) (Figure 1.3).

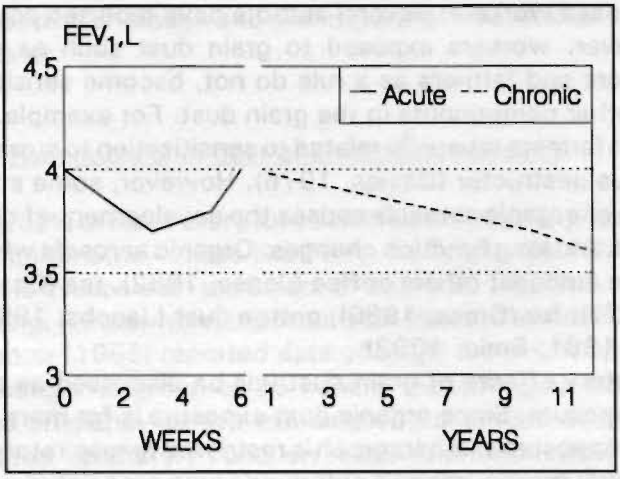

FIGURE 1.3: Acute and chronic decline in FEV_1 due to grain dust exposure. Data adapted from Zejda (1993).

This acute, reversible response may be accompanied by increased bronchial responsiveness and is related to mean airborne dust level measurements, but not to atopic status (James, 1990). When dust concentrations are extremely high, the decline may be immediate and profound (up to 800 ml), as documented in grain workers reexposed to barley dust after several days. Barley dust was described to trigger dramatic acute declines of FEV_1 in never exposed healthy men (Cockcroft, 1982).

Workers who remain exposed to grain dust may demonstrate small across-shift declines of FEV_1 and FVC, although in a small proportion of subjects the FEV_1 fall may exceed 10% (Chan-Yeung, 1980; doPico, 1983). Functional deterioration occurs over the work week (Chan-Yeung, 1980; Yach, 1985; Broder, 1980) and after rehire (Chan Yeung, 1989). In general, the across shift change does not depend on duration of work in the grain industry, smoking or atopic status and is larger in workers exposed to higher concentrations of grain dust (doPico, 1983; Corey, 1982). Longitudinal observations

show that grain workers also experience long-term deterioration in spirometric tests. The rate of decline is modest but larger than in civic workers (Dosman, 1991; Kaufman, 1982) and larger than in workers employed in other industries (Dales, 1987). The degree of annual decline depends on dust concentration, age and perhaps duration of exposure, smoking, across-shift changes, initial lung function and airways calibre (Dosman 1991; Dales, 1985). These findings are substantiated by studies based on observations covering at least 6 years (Dales, 1987). The data on determinants of respiratory impairment, however were obtained in only 38% of the initial cohort and only in those workers who did not change their smoking status. Both limitations favor a substantial underestimation of work-related effect, because of the "healthy worker effect", demonstrated in the grain industry (Broder, 1985), and the probability that newly symptomatic smokers might have changed their smoking habits. On the other hand, the lack of accelerated decline in lung function could be related to improvements in dust level management. In conclusion the evidence shows that exposure to grain dust is associated with short-term and long-term changes in lung function, which are correlated.

1.3.2.2 Chronic bronchitis and respirable grain dust

Cross-sectional studies document increased prevalence of chronic phlegm and chronic bronchitis in grain workers. The hypersecretory syndrome is associated with duration of exposure and may affect up to 23% of nonsmoking workers (Dosman, 1980). A survey of 5020 Canadian grain workers showed that chronic bronchitis was present in 15% of employees (Zejda, 1993). In nonsmokers prevalence ranged from 5.2 up to 14%, depending on the duration of exposure (exposure below 10 years and above 20 years respectively), whereas in smokers chronic bronchitis was present in 18.1% of workers in the shortest and in 34.8% of workers in the longest exposure groups (Zejda, 1993). Respiratory symptoms increase within the first 2 to 4 months of exposure and show the same trend in those employed for many years (Broder, 1984). The prevalence of sputum production increased significantly over 3 years in more than 3,000 workers studied longitudinally, despite a decrease in the proportion of current smokers in this population.

1.3.2.3 Grain dust exposure and emphysema

Prolonged exposure of laboratory animals to grain dust is associated with increased numbers of macrophages in the alveoli and may result in emphysema like damage of the lung tissue (Cotton, 1989). These results in animal models were not confirmed in humans. The predominant pattern of pulmonary function response involves simultaneous changes of FEV_1 and FVC,

often larger for FVC, suggesting the possibility of alveolar duct constriction, but not necessarily of emphysema. The accumulated observations support a recent view that not only obstructive but also restrictive type of impairment is specific for grain-related lung lesion (Zejda, 1993).

1.3.3. Industrial chemicals

Compared to animal and plant derived proteins, most industrial chemicals are low molecular weight compounds. These compounds are capable of evoking a whole spectrum of pathogenetic mechanisms, amongst which IgE dependent immunological reactions (e.g. acid anhydrides), IgE independent immunological reactions (e.g. diisocyanates) and nonimmunological reactions (e.g. potroom asthma) (Chan-Yeung, 1994). Besides sensitization (the potency of very low concentrations to evoke an allergic reaction), toxic effects of industrial chemicals include amongst others, irritation and asphyxiation (Table 1.2).

TABLE 1.2: Respiratory effects of industrial chemical exposure (adapted from Kurt, 1993).

Sensitizers / Asthmogens	Irritants	Simple asphyxiants	Toxic asphyxiants	Others
Colophony	Ammonia	Nitrogen	Carbon monoxide	Arsine
Diisocyanates	Chlorine	Methane	Hydrogen cyanide	Stibine
Piperazene	Fluorine	Carbon dioxide	Hydrogen sulfide	
Phenol	Nitrogen dioxide			
Phtalic anhydrides	Phosgene			
Trimellitic anhydrides	Ozone			
	Sulfur dioxide			

Differences between the pathogenesis of irritation and sensitization are however not so clear cut as they seemed to be. It has been suggested that prolonged irritation is a risk factor for sensitization (Wardlaw, 1988). But also the other way round, *i.e.* sensitization increases the susceptibility for irritation, has been suggested. Asphyxiation will not be discussed in this paragraph. Numerous industrial chemicals have been described as capable to induce irritation of mucous membranes and hypersensitivity in particular occupational asthma and the list of asthmogenic agents keeps growing steadily (Chan Yeung, 1986; Meredith, 1991).

1.3.3.1 Diisocyanates and airflow obstruction

Low molecular weight chemicals are of special interest since a large population is potentially exposed and they are potent sensitizers (Agius, 1994). In this paragraph respiratory effects of toluene diisocyanate, one of the most complex chemical sensitizers and an irritant as well are put forward as an example. Occupational asthma due to diisocyanates has recently been reviewed extensively by Vandenplas *et al.* (Vandenplas, 1993). Diisocyanates all have a -N=C=O group attached to a radical and react with compounds containing active hydrogen atoms to form a polymeric mass, polyurethane. The four most common diisocyanates are toluene diisocyanate (TDI), methylene diphenyl diisocyanate (MDI), naphtalene diisocyanate (NDI), and hexamethylene diisocyanate (HDI) (Figure 1.5.). These chemicals have been used in industry for over 40 years and have applications in the manufacture of plastics, foam surface coating, elastomers, adhesives, fibers, and in the production of polyurethane foam. Spray painting is a particularly dangerous form of exposure, since vapours (HDI, TDI) and particulates (MDI) are airborne and may be present in high concentrations.TDI induced asthma has been the most common cause of occupational asthma in many parts of the world for many years. However in recent years it has been replaced by HDI and MDI (Vandenplas, 1994). The prevalence of TDI-induced asthma varies from 5 to 10%, dependent upon the type of industry, the type of diisocyanate, and the level of exposure. In the Netherlands 10,000 workers are incidentally exposed, of whom 200 continuous > 20 h per week (Werkterreinanalyse TAUW, 1990). In 1974 approximately 500,000 workers in the USA were incidentally exposed to diisocyanates (NIOSH, 1978).

The dose and duration of exposure to which TDI will induce asthma are not known. Recommendations are that humans should not be exposed to concentrations above 20 ppb (WGD, 1991), but many cases of TDI-induced asthma occurred after a high level of exposure, e.g. a spill (Chan-Yeung, 1994). In those situations, a RADS-type of occupational asthma could not be excluded. In these cases, the lung's histologic appearance is not specific for isocyanate injury and the clinical outcome may not be predictable. Over the long term, some develop the histologic features of bronchiolitis obliterans and have a persistent respiratory impairment. Others may develop sensitization to isocyanates in association with this single overwhelming exposure (Vandenplas, 1993).

Wheezing, shortness of breath, chest tightness, cough, and the other clinical consequences of airway constriction may occur in workers with specific and non-specific bronchial hyperresponsiveness following exposure to isocyanates in the workplace. Clinically, once a subject has been sensitized to for example TDI, very low levels of TDI (as low as 1 ppb) may trigger an attack of asthma. Specific challenges with TDI induced isolated early, isolated late, dual or a continuous reaction in sensitive subjects (Figure 1.4). Once

asthma has developed, the majority had persistence of symptoms even after they were removed from exposure (Mapp, 1988). Favourable prognostic factors include early diagnosis and early removal from exposure (Chan-Yeung, 1994). The mechanism of TDI-induced asthma is not known. Specific IgE antibodies to a protein conjugate of TDI or a monoisocyanate were present in only a small proportion (10-20%) of patients who were shown to have specific asthmatic reactions on inhalation challenge test (Karol, 1994). Irrespective of the mechanism, airway inflammation was found in patients with TDI induced asthma both by broncheoalveolar lavage (BAL) and bronchial biopsy studies (Fabbri, 1988).

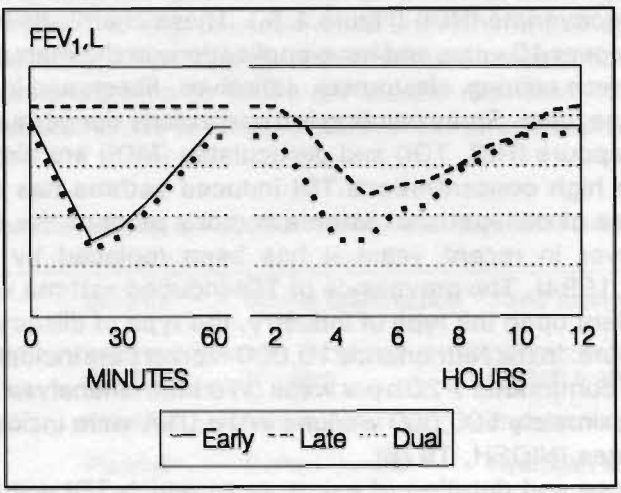

FIGURE 1.4: Possible positive reaction patterns after diisocyanate challenge.

1.3.3.2 Respiratory effects of low-level toluene diisocyanate (TDI) exposure

Besides occupational asthma, chronic effects of long-term low-level TDI exposure have been assessed by several groups of investigators. Adams (1975) assessed respiratory health of 180 workers employed from 1 to 11 years in two TDI manufacturing plants. The mean annual decline in FEV_1 and the forced vital capacity in these workers was not different from the values measured in a local population not exposed to TDI. In 63 former workers with TDI sensitivity and persistent symptoms followed from 2 to 11 years after leaving the workplace, the mean annual FEV_1 decline was only slighty excessive. A series of longitudinal surveys describing the respiratory health of employees at two polyurethane foam manufacturers using TDI disputed these

conclusions. In these reports, accelerated declines of both mean and annual and across shift FEV_1 were recorded (Peters, 1968, 1969, 1970). Initial work suggested that workplace TDI exposure caused alarmingly large decrements in FEV_1 when measured at the start and at the end of a Monday work shift (mean decline 220 ml), which persisted during the work week although to a lesser degree. Workers with chest symptoms had greater decline than those without. After 2 years the rate of FEV_1 decline was approximately twice what would be predicted. Those with excessive across shift declines also had a greater FEV_1 decline over time. This annual decline in lung function occurred in the presence of low TDI levels (< 20 ppb) and was independent of cigarette smoking.

Another longitudinal survey of the ventilatory function of workers engaged in the manufacture of TDI was reported in 1982 (Diem, 1982). This study began at the opening of the isocyanate producing-workplace, and pulmonary function was assessed before TDI manufacture started up. Environmental measurements and physiologic data were obtained over a 5-year period from a study population of nearly 300 workers. Exposure to TDI vapor was determined by personal monitors. Over 2000 personal samples were collected over 42 job titles. All employees had measurable TDI exposure, depending on both job and location. On average, TDI vapor concentrations exceeded the treshhold limit value of 20 ppb approximately 3 percent of the time. The average annual decline in FEV_1 among all study subjects was 24 ml, a value comparable to that expected in cross-sectional studies of "normal" populations. The average declines in $MEF_{75\text{-}25}$; MEF_{50} were 93 and 110 ml/L, respectively, larger than what could be expected on the basis of cross-sectional data. After controlling for smoking and atopic status, the annual declines in FEV_1, FEV_1% predicted and $MEF_{75\text{-}25}$ appeared to depend on TDI exposure and were recognized as excessive in "never smokers. Among never smokers, there was a 38-ml per year difference in the FEV_1 decline between the low- and high cumulative exposure categories ($P = 0.01$). In "current smokers" there was no observed difference in the amount of FEV_1 decline, regardless of the cumulative exposure level. For the low exposure category, there was a 27 ml per year ($P = 0.004$) excess decline in FEV_1 in current smokers compared to never smokers. TDI exposure correlated well with the annual change in lung function, whether it was determined by cumulative or peak exposure duration of exposure to more than 20 ppb.

1.4. PATHOPHYSIOLOGIC MECHANISMS OF AIRWAY OBSTRUCTION

Inflammation is a common feature in chronic airway disease. In general, inflammation is the response of vascularized tissue to injury and is usually beneficial since it serves to resolve and repair damage. Airway inflammation can be considered as an activation of a multicellular effector system in which the relative predominance of the effector cell type will be determined by the allergic or non-allergic type of response.

Cells migrating from the blood vessels that have been clearely identified in airway inflammation in asthmatic reactions include mast cells, involved in the initial phase of an allergic reaction (Holgate, 1985); polymorphnuclear leucocytes, important effector cells in host defence mechanisms (Atkins, 1977); eosinophils, first regarded as beneficial cells in asthma because of its mast cell mediator inhibiting properties (Archer, 1968; Goetzl, 1975) and now considered as the pivotal cell in allergic asthma; lymphocytes, prominent in asthmatic airways and of considerable importance for the pathogenesis of asthma (Azzawi, 1990). B cells produce IgE and its production is regulated by T-cells (Ishizaka, 1985); alveolar macrophages, the majority of inflammatory cells present in airway tissue, which are differentiated mononuclear phagocytic (Kay, 1989) (Figure 1.5).

In non-allergic airway inflammation mononuclear phagocytes play a central role in the host defence due their capacity to regulate the immune response by both the secretion of a variety of biologically active substances and the presentation of antigens to T-lymphocytes.

Both structural and effector cells produce a wide range of inflammatory mediators that include leukotrienes, enzymes, cytokines, reactive oxygen intermediates, eicosanoids and complement components. These agents contribute not only to the development of the inflammatory response but also to its modulation by feedback mechanisms. Among cytokines 4 types can be discerned: proximal, like TNF-α involved in the immediate reaction; secondary, chemotactic involved like IL-5, IL-8; secondary, growth-repair factors like TGF; inhibitory, amongst which the regulatory cytokines IL-4 and IL-8 (Figure 1.5).

This large group of cell-derived mediators have farranging effects on most organ systems in the body. Three general types of lymphokine mediated effects have been recognized: 1) costimulant effects or redundancy - more than one lymphokine is necessary to produce an effect; 2) quantitative or dose-dependent functionality effects of lymphokines - a signal from one lymphokine enhances the effect or secretion of another; and 3) a cascading type of activation specificity mediated by a single lymphokine released from an activated cell, which can in turn have a further enhancing effect on the same cell and on other potentially responsive cells.

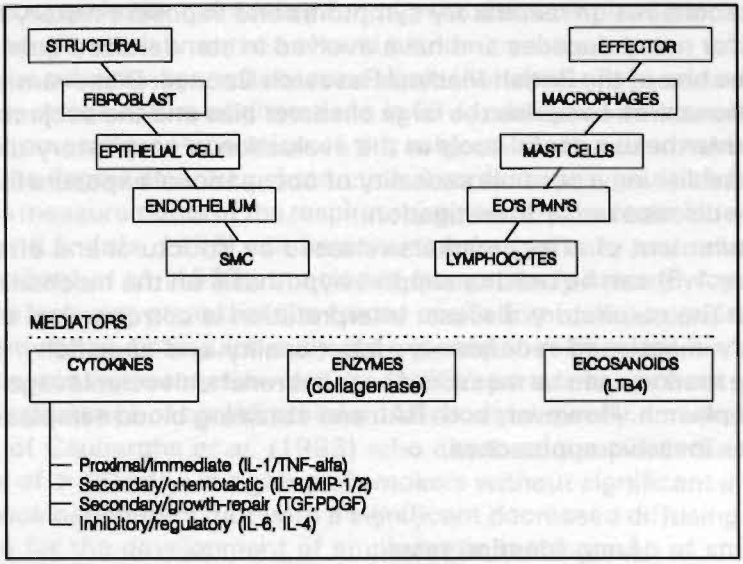

FIGURE 1.5: The inflammatory response of the lung depicted as an interplay between structural and effector cells mediated by cytokines, enzymes and eicosanoids. Nowadays it is recognized that also structural cells fibroblast, epithelium can activate effector cells. The net end effect is the complex sum of autocrine and paracrine pathways activated by the different acting cytokines. SMC: structural mesenchymal cell; EO: eosinophil; PMN: polymorphnuclear cells.

1.5. DETECTION OF OCCUPATIONAL CHRONIC AIRWAYS DISEASES

The usual approach to detect chronic airways disease is documenting changes by medical examination, spirometry, or peak flow-measurements, or screening. Screening is the process of finding subjects at risk of respiratory disease in the working population (Kreis, 1988). Because screening methods are used to identify asymptomatic employees who are not seeking medical assistance, the methods used should meet more precise criteria than diagnostic methods in a clinical setting. Reasonable criteria to judge screening methods include simplicity, acceptibility to subjects, precision or reproducibility, specificity, sensitivity and validity.

Several approaches are feasible in the detection of airway dysfunction:

1) The most direct approach is measurement of the lung function. In occupational medicine commonly flow volume curves and peak flow measurements are applied. In the clinical situation several techniques are available to detect airway obstruction i.e. flow volume curves, impedance measurements, bodyplethysmography.

2) Questionnaires on respiratory symptoms and exposure history have been used for many decades and have evolved in standardized questionnaires like the one of the British Medical Research Council. Disadvantages of the questionnaires comprise the large observer bias and the subjectvity. They are nevertheless useful tools in the evaluation of respiratory disease and for establishing a possible causality of occupational exposure in the respiratory disease under investigation.
3) Measurement of effect markers released by structural and effector cells (Figure 1.5) can be used to support hypothesis on the mechanisms involved in the respiratory disease. Interpretation is corroborated by the previously mentioned redundancy, functionality and specificity (par. 1.4). These markers can be measured both in bronchoalveolar lavage (BAL) and blood plasma. However, both BAL and obtaining blood samples are regarded as invasive approaches.

1.5.1. Lung function tests

During the last three decades lung function tests have evolved to clinical tools widely used in assessing respiratory status. In addition to their use in clinical case management, they have become a part of routine health examinations in respiratory, occupational and sports medicine and in public health screening. To maximize the clinical value of lung function tests and to assist those managing clinical lung function testing, the American Thoracic Society (ATS, 1987), has published guidelines, focussing primarily on spirometry, as the most widely used lung function test. The 1987 ATS statement on spirometry (ATS, 1987) outlined the steps necessary to achieve standardization: 1) equipment performance, validation, and quality control; 2) subject performance; 3) measurement procedures to determine acceptibility and reproducibility; 4) reference values and interpretation.

Currently surveillance of the lung function in occupational settings heavily relies on indices of the maximal forced expiration. The main advantages of flow volume curves are that they have been applied uniformly all over the world and that references values for the flow volume parameters exist that are adjusted for confounders like gender, age and height (Quanjer, 1983). The main disadvantage is that these measurements depend upon ***forced*** expiratory flow manoeuvers and therefore on patient compliance. To achieve maximal patient compliance the test needs to be performed by experienced personnel, not always present in occupational settings.

Furthermore, the question arises if current techniques are adequate since the advantages may be vitiated by lack of sensitivity, specificity, or validity. Although a decline in FEV_1 is generally considered as a measure of progressive airflow obstruction, different mechanical alterations of the respiratory system can influence this co-operation dependent parameter. Alternative

techniques to estimate pulmonary function at the workplace are not widely available. Ideally such a method should be non-invasive, cheap, easy to perform, require no patient compliance and easy to interpret. To provide additional information on the nature of alterations in FEV_1 additional measurements not dependent on active co-operation of the subjects can be used which reflect indirectly the degree of airway obstruction. In order to provide this information impedance measurements of the respiratory system by the technique of forced oscillations (Làndsér, 1976) can be considered as an easy and reliable technique. Brochard *et al.* (1987), concluded from a study among workers from a gas manufacturing plant that the forced oscillation technique affords sufficient sensitivity to distinguish subjects exposed to respiratory irrritants at a stage when mid expiratory flow volume (MEFV) parameters are not yet modified. The importance of combined information was recently demonstrated by the study of Cauberghs *et al.* (1993) who observed a rapid decline in FEV_1 (an excess of > 20 ml/y) in a group of smokers without significant alterations in impedance parameters, but with a significant decreased diffusing capacity suggestive for the development of emphysema in this group of smokers.

1.5.1.1 Impedance measurements using the forced oscillation technique (FOT)

In 1956 Dubois *et al.* described a method employing forced sinusoidal pressure oscillations to determine the mean resistance and reactance of the respiratory system. Initially, the measurements were performed during breath-holding at a single frequency at a time. Mead (1969) demonstrated that impedance can also be measured when forced oscillations are superimposed on spontaneous breathing. To assess the variations of impedance with frequency, various frequencies had to be applied in succession. Michaelson *et al.* (1975) introduced the random noise oscillation technique: oscillations of different frequencies are applied simultaneously, and a fast Fourier system is used to analyse the pressure and flow signals.

A further development of the technique is based on a forced pseudo-random noise signal (Làndsér, 1976), each frequency starting at a different randomly selected moment. This results in a small peak-to-peak size of the overall signal, improving the signal to noise ratio. The experimental setup is similar to the description given by Grimby *et al.* (1968) (Figure 1.6). The apparatus consists of a loudspeaker, which amplifies and transmits the signal generated by an oscillator, a high impedance side tube which enables the subject to breath spontaneously and a screen type pneumotachograph. Mouth pressure relative to atmospheric pressure and air flow, transduced to a pressure signal are recorded by two identical transducers and fed into an analyzing system, dividing pressure and flow.

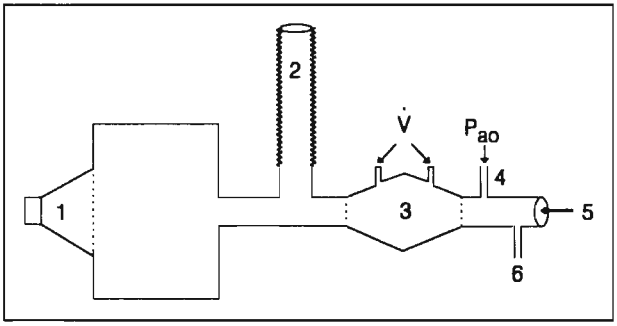

FIGURE 1.6: Schematic presentation of the forced oscillation technique.
1: Loudspeaker; 2: impedance tube; 3: pneumotachograph; 4: bias flow; 5: mouth piece; 6: pressure transducer.

The instantaneous relationship between pressure and flow is called the impedance (Z), reflecting the total mechanical characteristics of the respiratory system. This impedance can be divided into two components. One is the in phase component, sometimes referred to as the "real" part of impedance, or resistance (R). This component is due to overall flow-resistive properties of the total respiratory system. The second, out of phase component, or "imaginary" part, is the reactance (X). The reactance (X) is determined by the inertial and elastic properties of the system. A negative reactance is found at lower frequencies since at lower frequencies the reactance is based mainly on the capacity of the system. At higher frequencies the reactance is influenced predominantly by the inertial qualities of the system. The reactance then becomes positive. The frequency at which the reactance becomes zero is the resonant frequency (f_0). Input impedance measurements by means of forced oscillations have been used by many authors to evaluate the mechanical characteristics of the respiratory system during spontaneous quiet breathing, in healthy subjects and in patients with varied pulmonary disorders, and also to assess the response of the respiratory system to broncho-active agents.

Determination of the impedance of the respiratory system using forced oscillations enables the assessment of its mechanical characteristics during spontaneous quiet breathing at several frequencies simultaneously. In normal adults a slight increase in oscillatory resistance is generally observed in the frequency range of 4 to 30-52 Hz (Figure 1.7). In these subjects reactance is usually slightly negative at lower frequencies and changes from negative to positive at frequencies between 5 and 10 Hz. When signals consisting of frequencies below 4 Hz are applied it is observed that at the lowest frequencies, between 0.25 and 2 Hz, R is sharply increased when compared to higher frequencies.

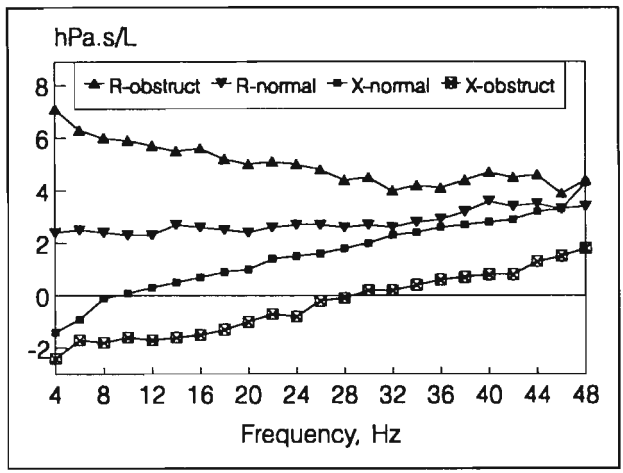

FIGURE 1.7: Impedance patterns for normal and obstructive adults.

Several studies have provided values for respiratory impedance in the presence of airflow obstruction, which are also reflected in Figure 1.7. Michaelson *et al.* (1975) studied 1 subject with chronic obstructive pulmonary disease. R was found to be markedly higher at lower frequencies than at higher frequencies and thus, a negative frequency dependence of resistance was observed. Reactance was highly negative at lower frequencies and X remained negative up to a frequency of about 20 Hz. Similar characteristics were reported by Làndsér *et al.* (1976) in 10 patients with chronic obstrucive pulmonary disease (COPD). Clément *et al.* presented data on a group of 43 subjects with obstructive airways disease with a moderate reduction in FEV_1 (50-77% of predicted) and a second group, comprising 26 subjects with severe COPD (FEV_1 less than 50% of predicted). In both groups a markedly negative frequency dependence of resistance was observed. In the group with moderately severe airflow obstruction resonant frequency was increased to around 20 Hz, in the group with severe airflow obstruction X was still negative at the highest evaluated frequency (24 Hz). Using forced pseudo-random noise oscillations with an extended frequency spectrum (up to 52 Hz), Wouters *et al.* (1989) found a similar negative frequency dependence of resistance in 20 patients with severe COPD, and a resonant frequency of around 28 Hz.

1.5.1.2 Influence of toxic inhalants on total respiratory impedance

Exposure of the respiratory system to toxic inhalants forms a continuous threat to the integrity of the respiratory system. Characterization of the mechanical impedance of the respiratory system by means of the forced

oscillation technique may provide a suitable tool in the assessment of abnormalities resulting from exposure to smoke, pollution, or occupational hazards.

Kjeldgaard *et al.* (1976) demonstrated that frequency dependence of oscillatory resistance is a sensitive index for differentiating smokers from non-smokers. A similar finding was reported by Hayes *et al.* (1984). These investigations observed significant differences in frequency dependence of oscillatory resistance, resonant frequency and conductance normalized by height between asymptomatic smokers and a control group. Jiemspirong *et al.* (1976) observed that, in contast to men, asymptomatic current female smokers showed elevated $\underline{R}$ values when compared to non smoking women. Quaedvlieg (1990) observed increased respiratory resistance at almost all frequencies, but most markedly at lower frequencies in the range from 8 to 28 Hz; respiratory resistance decreased with increasing frequency compared to non-smokers. Làndsér *et al.* (1976) on the other found no significant correlation of impedance parameters to smoking after standardization for age, weight, height, FEV_1 and vital capacity.

Brochard *et al.* (1987) studied the effects of occupational exposure to inhaled irritants in subjects working in a gas manufacturing plant. In workers exposed to respiratory irritants significant differences in forced oscillation parameters were observed compared with non-exposed subjects. They found that the slope of the resistance versus frequency curve appeared to be sensitive in detecting occupational chronic bronchitis at an early stage. Furthermore these investigations demonstrated that occupational exposure to inhaled respiratory irritants results in differences in the changes in frequency dependence of $\underline{R}$ and resonant frequency between air and HeO_2 breathing and concluded that the forced oscillation technique "affords sufficient sensitivity to distinguish subjects exposed to respiratory irritants at a stage when MEFV parameters are not yet modified".

Peslin *et al.* (1987) compared the findings of input impedance obtained with a head generator and transfer impedance by applying pressure variations around the chest in 39 healthy males and in 140 iron miners. Maximum expiratory flow indices were significantly lower in iron miners. Among input impedance indices, resistance was increased by almost 30% in miners and exhibited a slightly positive frequency dependence, while it hardly varied at all with frequency in control subjects. No differences in the imaginary part of input impedance were observed. For transfer impedance, most indices of $\underline{R}$ and $\underline{X}$ differed significantly. Smoking further enhanced the differences between control subjects and iron miners. Sepulveda *et al.* (1984) applied impedance measurements to detect cotton dust induced bronchoconstriction. Subjects showing across shift responses in MEFV curves showed limitation of flow predominantly located in the peripheral airways while subjects with no significant alterations in MEFV curves showed a central airways effect. In 54 workers with different degrees of occupation related diffuse interstitial lung disease (extrinsic allergic alveolitis, bleomycin-induced pneumonitis, silicosis,

asbestosis) van Noord *et al.* (1989) observed an increase of resistance at low frequencies causing a negative frequency dependence of the resistance and simultaneously a decrease in reactance at low frequencies. They stated that the observed change in resistance and reactance are not specific for restrictive lung disorders; similar changes are met also in moderately advanced obstructive lung disease (van Noord, 1989).

1.6. AIMS AND OUTLINE OF THIS DISSERTATION

From the introduction it has become clear that although we are aware of the adverse influence of occupational exposure on lung function early detection of these potentially reversible lung diseases might be optimized.

The aim of this thesis was to evaluate a multifactorial approach in order to characterize occupationally related respiratory impairment. We analyzed respiratory impairment by integration of lung function measurements, questionnaires and biological markers. Different populations of workers exposed to inorganic and/or organic dust and/or chemicals are examined. Besides generally used questionnaires and flow volume measurements, impedance measurements are applied in the different studies in order to obtain insights in the effects of exposure on the mechanical characteristics of the respiratory system. In order to support the determination pathophysiological effects of occupational exposure, biological markers are monitored.

Inorganic dust

As outlined previously, cells and mediators involved in mechanisms of both interstitial and airway diseases are largely the same. To assess a possible link between markers of interstitial lung disease and airway disease, a group of retired coal miners with and without coal worker's pneumconiosis (CWP) was studied. In animal models, TNF-α was demonstrated to be a crucial mediator in pulmonary fibrosis (Piguet, 1990); the same mediator was proven to be involved in the bronchial hyperreactivity induced by endotoxin (Kips, 1992). In the present study, we investigated if ex-vivo TNF-α release by peripheral blood monocytes is related to airflow obstruction (Chapter 2).

In addition to coal miners, a population of potato sorters mainly exposed to diatomaceous earth was studied. Diatomaceous earth is of interest since it was suggested that this inorganic dust, with a low content of crystalline silica, does not evoke interstitial lung disease like coal mine dust does. Besides flow volume and impedance measurements, serum procollagen type III was measured as a marker of possible changes in collagen type/content in this group of workers (Chapter 3).

Organic dust

Organic dust may evoke both acute and chronic respiratory impairment, which may be located in the peripheral airways (Sepulveda, 1984). We studied animal feed workers exposed to grain dust to measure the airway reponse to organic dust with flow volume and impedance measurements. Special emphasis was put to the exposure measurements in which the endotoxin fraction of the gathered organic dust was measured. Endotoxin has been put forward as a constituent of grain dust responsible for a part of the respiratory effects of this kind of organic exposure (Chapter 4) and - interestingly - is also a potent inducer of TNF-α release from various airway effector cells.

Chemical exposure

Since pathological findings were reported in both the central and peripheral airways after exposure to TDI (Fabbri, 1988) it was suggested that the airway response to isocyanate challenge may occur all along the tracheobronchial tree. In an attempt to localize the site of airway obstruction in early and late asthmatic reactions to isocyanates, impedance and flow volume measurements were applied during inhalation challenge with toluene diisocyanate (TDI) or methylene diphenyl diisocyanate (MDI) (Chapter 5).

Chapter 6 describes a two-year follow-up of flow volume and impedance measurements among workers in the chemical industry. The study was conducted to investigate if the mechanical characteristics of the respiratory system are related to respiratory symptoms and how possible alterations are reflected in spirometric values.

Finally, the present studies are discussed with regard to results and feasibility of a multidisciplinairy approach to detect and tackle occupational airway diseases in an early stage (Chapter 7).

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CHAPTER 2

Airflow obstruction and monocyte TNF-release in coal workers

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ABSTRACT

Respiratory health of 102 retired coal miners was assessed by chest radiographs, lung function measurements and questionnaires and related to tumor necrosis factor alfa (TNF-α) production by blood monocytes upon priming with different stimuli. The objective was to assess a possible relationship between airflow obstruction and TNF-α production in retired coal workers. No significant differences in lung function were observed between cases of coal workers' pneumoconiosis (CWP) (n = 27; > 0/0)) and references (n = 75; 0/0) and the effect of cumulative exposure on flow volume or impedance parameters was also not significant. TNF-α release upon stimulation of blood monocytes with coal mine dust was significantly increased in cases with International Labour Organization (ILO) score 0/1 (doubtful cases) compared to references and cases with a higher ILO score. Airflow obstruction either defined as a FEV_1 < 80% (n = 10; 5 cases of CWP) or as a resonance frequency > 15 Hz accompanied by a negative frequency dependence of resistance (n = 9; 4 cases of CWP) was significantly related to high levels of TNF-α release upon stimulation with endotoxin and silica, silica showing the strongest relation. These data suggest that in this group airflow obstruction is associated with an increased expression of inflammatory mediators indifferent of the presence of pneumoconiosis.

INTRODUCTION

Coal miners are at risk to a variety of respiratory diseases as a result of dust exposure (Attfield, 1992). While coal worker's pneumoconiosis (CWP) has received most of the attention, bronchitis and emphysema resulting from

exposure, clinically indistinguishable from their nonoccupational analogues, are more prevalent and are associated with significant morbidity among coal miners (Attfield, 1992; Soutar, 1986; Hurley, 1987). Research into the mechanisms of coal dust induced respiratory disease has mainly concentrated on mechanisms in the development of mineral dust-induced inflammation, leading to fibrosis in the interstitium. These mechanisms involve, amongst other, reactive oxygen species (Janssen, 1992) and alveolar macrophage (AM) or blood monocyte (BM) derived cytokines (Kelley, 1990). Previously, we (Borm, 1988) and others (Lasalle, 1990; Gosset, 1991) reported on the significance of AM derived cytokines such as, interleukin-1 (IL-1), interleukin-6 (IL-6) and TNF-α in coal dust induced pulmonary fibrosis. Ex vivo production of TNF-α by blood monocytes (BM) to various stimuli such as coal mine dust (CMD) or lipopolysacharide (LPS) was found to be increased in coal miners with CWP compared to references (Borm, 1988). Recently, several studies have demonstrated the (AM-derived) cytokines IL-6,8 and TNF-α are also important in the mechanism(s) of non-interstitial inflammation that may lead to airflow obstruction (Kips, 1992; Siracusa, 1992; Gosset, 1991). Kips *et al.* (1992) showed in a rat model that airway inflammation and bronchial hyperresponsiveness was caused by TNF and several clinical studies (Siracusa, 1992; Gosset, 1991) have shown that BM and AM from asthmatics have an enhanced capacity to produce TNF. We, therefore, hypothesized that TNF-α might also be involved in the airway obstruction frequently observed in coal miners. It was the purpose of this study to investigate *ex-vivo* TNF-α production by BM and the presence of airway obstruction in retired coal miners.

MATERIALS AND METHODS

Subjects and protocol

In a cross-sectional study retired, male coal workers (n = 106) from the Belgium coal mining industry Kempense Steenkoolmijnen (KS) were investigated of whom 102 completed all tests. Written informed consent was obtained from each worker and after evaluation of a validated questionnaire on respiratory symptoms, smoking and exposure, workers were recruited to complete three valid measurements of both impedance measurement using the forced oscillation technique (FOT) and flow volume curves. The FOT was always performed before registration of flow volume curves to avoid the influence of forced inspiratory manoeuvres on the bronchial tonus (Orehek, 1981). Chest radiograph (46 x 46 cm) were recorded at 70 kV (source at 1.5 m distance) and read by a panel of three occupational physicians experienced in reading and classifying pneumoconiosis according to UICC/ILO criteria (Parmeggiani, 1983) for compensation purposes. Classification of ILO scores into profusion score was made as following: profusion 0 is only 0/0 (n = 75);

profusion 1 is 0/1 (n = 7); profusion 2 is 1/0, 1/1, 1/2 (n = 4); profusion 3 is 2/1, 2/2, 2/3 (n = 8); profusion 4 is 3/2, 3/3 (n = 6); and profusion 5 is progressive massive fibrosis (n = 2). Subjects with profusion score 0 were classified as references and subjects with a profusion score greater than zero were classified as cases. From each worker a blood sample of 40 ml was obtained, stored at 4°C and processed at the same day for TNF assays. After completing the protocol all workers were informed personally by the occupational physician (LL) about the individual outcome of the chest radiograph and the lung function tests.

Exposure estimation

Occupational history data were obtained from (i) the medical file and (ii) a personal interview during follow-up, and applied to calculate exposure for the period(s) worked at the coalfaces and outside the coalfaces. The cumulative dust exposure at the coalface was calculated by multiplying the sum of the yearly mean dust concentrations for the colliery where the miner worked by the average time worked underground during one year. The resulting individual units are expressed as gram-hours per cubic meter of sampled air (gh/m^3). The total dust concentrations have been converted into "respirable" dust concentrations using the formula:

$$\text{Respirable dust concentration} = 1.1\ (\text{total dust concentration})^{1/2}$$

Furthermore, we estimated exposure underground at 1000 h per year: 6 h/shift (allowance for travelling time) * 220 shifts/year * 75% (25% taken as absenteeism rate). Estimation of the exposure outside the coalface was based on respirable dust levels per job (i.e. non-mechanized and mechanized headings, stone drivages). Quartz exposure was estimated by an average quartz content of 5-10% of Belgian coalface and stone drivage dust.

Lung function measurements

Forced expiratory flow volume curves were recorded according to European Community of Coal and Steel (ECCS) criteria using a Vitalograph P5 dry spirometer. Three recordings within 5% or a 100 ml range (FEV_1) were obtained from each subject. Parameters derived from the flow volume curves were: FVC, FEV_1, FEV_1/FVC ratio, PEF (peak expiratory flow), MEF (mid maximal expiratory flow), MEF_{75}, MEF_{50}, and MEF_{25}. MEF_{25} being the flow after 75% expiration. All flow volume values were related to the reference values of the ECCS (Quanjer, 1983) for individual diagnosis. The spirometer was calibrated at regular intervals and all subjects were measured on the same

spirometer. Two spirometric measurements failed due to lack of cooperation. Airflow obstruction was defined as a FEV_1 less than 80% predicted.

Impedance (Z_{rs}) of the respiratory system was measured using the technique described by Làndsér *et al.* (1976). A pseudo-random noise pressure signal containing all harmonics of 2Hz from 4 to 48 Hz is applied at the mouth by means of a loudspeaker during quiet breathing. Mean values of three successive measurements, each lasting 8 seconds were used in the analysis. Of the obtained impedance data, the resistance at 8Hz (R8), at 28Hz (R28), the difference between R28 and R8 (frequency dependence (FD), as a measure for the course of the resistance versus frequency curve, the reactance at 8Hz (X8) and the resonant frequency (f_0) were used for analysis. R8 was chosen because at this frequency usually coherence functions greater than 0.95 are obtained, which are not always obtained at lower frequencies during airflow obstruction. Airflow obstruction is deflected by a negative FD and an increased f_0 (usually > 15 Hz). The equipment was calibrated daily and all measurements were performed using the same apparatus, by the same investigator. Two measurements failed due to technical problems.

TNF-α assay

Monocytes were isolated from 30 ml blood as previously described (Borm, 1988). The cells attached to cluster well plates were tested for TNF-α production into the culture medium either spontaneously or in response to different stimulants. The (sterile) stimulants were used in the following final concentrations: endotoxin LPS *E. Coli* 0111B4, 3 or 1,000 ng/ml; coal mine dust, 5 mg/ml (contains 0.5 mg/ml silica); and silica, 0.5 mg/ml. After incubation of the cells (18 h), the plates were centrifuged and the cell-free supernatant frozen at −30°C until analysis for TNF-α. TNF concentrations in the cell-free supernatant of the monocyte incubations were determined with a TNF-specific ELISA as previously described (Borm, 1988). A standard titration curve was obtained by making serial dilutions of a known sample of rTNF-α. The lower detection limit of this ELISA is 10 pg TNF/ml.

Statistical analysis

The amount of cigarettes smoked was calculated from the questionnaire as the total number of cigarettes smoked multiplied by the average daily consumption times the number of days smoked (Table 1). Average lung function data for cases versus references and profusion scores were tested for significance using Student's t-test. Subjects using inhalation drugs were excluded from statistical analyses, because these drugs do influence lung function. Differences between the profusion score groups were tested by analysis of

variance (ANOVA). Confounders (age, height, weigth, smoking) in lung function measurements were adjusted by means of multiple linear regression. In this linear regression, lung function parameters were taken as dependent variables and the confounders and (cumulative) exposure as the independent variables. First the confounders and subsequently the cumulative exposure were put into the model of linear regression. Analysis of the lung function in relation to TNF levels and comparison of the questionnaire data to lung function outcome was done with a multiple logistic regression analysis (BMDP, Berkeley, CA, USA). All procedures if not stated otherwise were computed with SPSS-X program (SPSS-X Inc., Ill, USA).

TABLE 2.1: Demographic characteristics of the study population.

Parameter	Unit	References (n = 75)		Cases (n = 27)	
		$\bar{X}$	SD	$\bar{X}$	SD
GENERAL					
Age	y	47.9	5.1	50.3	5.6
Height	cm	170.4	6.5	169.8	5.7
Weight	kg	77.3	10.1	76.2	12.3
Smoking	n*10,000	113.7	105.8	152.4	128.6
EXPOSURE					
Underground	y	20.3	5.2	24.2	4.8
Mine dust	gh/m^3	89.5	57.6	133.5	46.7*
Quartz	gh/m^3	4.7	2.7	6.8	2.3*
PROFUSION SCORE					
0 (0/0)	n	75		-	
1 (0/1)	n	-		7	
2 (1/0, 1/1, 1/2)	n	-		4	
3 (2/1, 2/2, 2/3)	n	-		8	
4 (3/2, 3/3, 3/+)	n	-		6	
5 (PMF #)	n	-		2	

$\bar{X}$ = Mean; SD = Standard deviation; * = $P < 0.05$ tested with Student's t-test; # = Progressive massive fibrosis

RESULTS

The retired coal miners were relatively young (48.5 years) and on average 3.8 years out of function. In this population 27 cases of CWP (26.5%) were classified in five profusion score categories. Cases were relatively longer exposed than references resulting in a significantly higher cumulative exposure to coal mine dust (113.5 gh/m^3) and quartz (6.8 gh/m^3) compared to respec-

tively 89.5 and 4.7 gh/m^3 in references. Cases smoked more than references, but this did not reach the level of statistical significance. Prevalence of respiratory symptoms was high for the total group. Shortness of breath and wheezing were significantly more frequent among cases (78 respectively 62%) compared to references (54 respectively 43%). Comparisons of respiratory symptoms with TNF-α levels did not reveal significant correlations.

No statistical significant differences were observed between references and cases (Table 2.2) with respect to TNF-release or any lung function parameter measured.

TABLE 2.2: Results of lung function measurements and the TNF release.

Parameter	Unit	References (n = 75)		Cases (n = 27)	
		$\bar{X}$	SD	$\bar{X}$	SD
LUNG FUNCTION					
FVC	L	3.7	0.7	3.3	0.7
FEV1	L	4.7	0.8	4.4	0.7
R8	hPa.s/L	2.7	1.0	2.6	1.1
X8	hPa.s/L	−0.1	0.5	−0.3	0.4
FD	hPa.s/L	−0.1	0.5	−0.2	0.6
f_0	Hz	10.3	3.9	11.6	4.0
TNF-α RELEASE	pg/ml				
No stimulation		0.2	0.2	0.2	0.2
LPS 1000		4.4	4.4	5.0	5.4
LPS 3		2.1	2.0	2.3	2.5
Silica		0.7	0.7	0.8	1.1
Coal mine dust		1.9	1.4	2.1	1.3

For explanation of the parameters see methods.
* = $P < 0.05$ tested with Student's t-test

Coal dust induced TNF-α release of BM was increased ($P < 0.08$, ANOVA) in subjects with profusion score 1 (0/1; doubtful cases) versus references and miners with CWP at higher (> 1) profusion scores (Figure 2.1). Interestingly, also spontaneous TNF release was not significantly different among profusion categories.

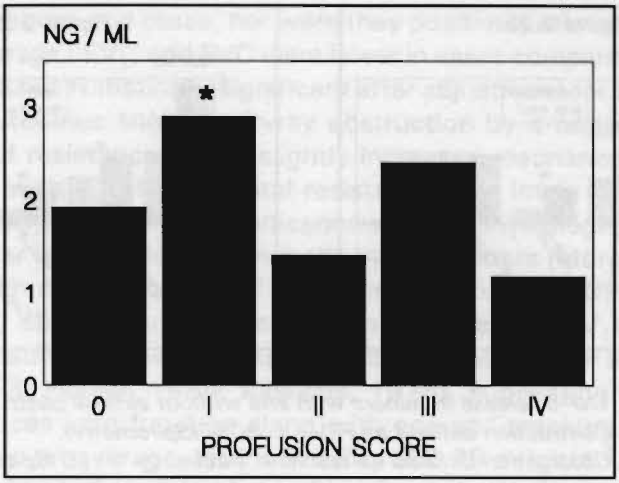
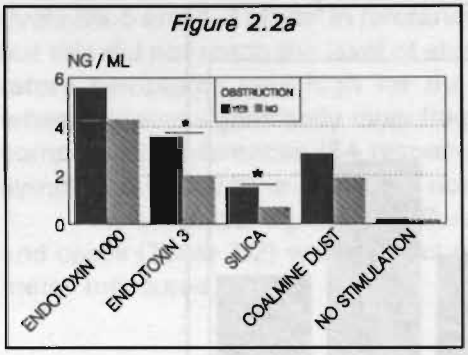


FIGURE 2.1: TNF-α release after stimulation of blood monocytes with coal dust per profusion score ($P < 0.05$).*

No significant correlation between cumulative dust exposure and lung function were found in the multiple linear regression. Although FVC tended to increase FEV_1 tended to decrease with increasing cumulative exposure (resulting in a decrease of FEV_1/FVC ratio), none of these relations was significant. The decrease in FEV_1 was estimated at 0.39 ml/gh/m^3. Also the impedance parameters $\underline{R}8$ and $\underline{X}8$, tended to decline with increasing exposure but did not reach the required level of significance. Correlation of quartz exposure to lung function parameters showed similar results, because quartz content was directly derived from dust exposure either as a 5 or 10% fraction.

No lung function parameter under study was found to be different among subjects from the various profusion categories. Airflow obstruction, however, was detected more frequently in miners with CWP as compared to references. Flow volume revealed 10 subjects with airflow obstruction (5 cases, 5 references), while impedance parameters indicated 9 subjects (4 cases, 5 references). In terms of relative risk this means a 2.2 to 2.7 risk for miners with CWP to have airflow obstruction (data unadjusted for smoking).

Interestingly, when comparing the TNF-α release from miners with or without airflow obstruction, BM from subjects with airflow obstruction have significantly higher TNF release upon stimulation with the low dose LPS or silica as compared to miners without airflow obstruction. In figures 2 and 3 the TNF-release upon different stimulations is illustrated when obstruction was detected by spirometry (Figure 2.2a) or impedance measurements (Figure 2.2b). Respiratory symptoms, more prevalent in miners with CWP, were not related to TNF levels.



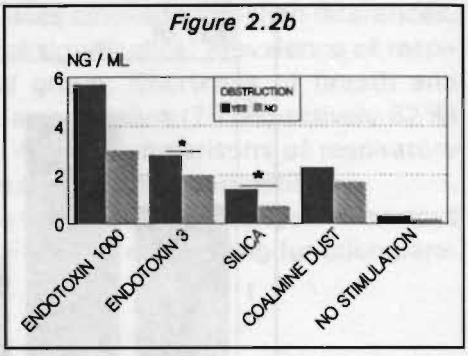


FIGURE 2.2 : TNF-α release in miners with and without airflow obstruction.
2.2a : Obstruction defined as FEV_1 < 80% (Spirometry).
2.2b : Obstruction defined as resonant frequency > 15 Hz concurrent with a negative frequency dependence of resistance (Impedance).
** Statistical significance P < 0.05 (Mann-Whitney-U test)*

DISCUSSION

This study demonstrates that airflow obstruction, irrespective of the presence of CWP, is positively related to TNF-α release from peripheral blood monocytes upon stimulation. With respect to restrictive effects of dust exposure our observations confirm previous reports. The observed larger cumulative exposure for the cases is in line with several British studies reporting an increased prevalence of CWP with increasing cumulative exposure (Hurley, 1982). Neither flow volume nor impedance parameters were found to be significantly different between references and retired cases of CWP (n = 27) and multiple linear regression did not reveal a significant dose dependent relationship of exposure with lung function. Impedance outcomes suggest airway obstruction extending to the peripheral airways. Similar observations were made in coal miners (Morgan, 1974) and in patients with diffuse interstitial lung disease (Noord, 1989).

The pattern of TNF-α release versus profusion score was similar to previous studies by our laboratory (Borm, 1988): coal dust induced release of TNF-α were significantly increased in the group with ILO score 0/1 cases compared to controls and to cases with higher profusion scores. These data and other reports on coal workers (Lasalle, 1990; Gosset, 1991) combined to work in animal models (Piguet, 1990) support the established crucial role of TNF in the pathogenesis of pneumoconiosis.

The observed larger cumulative exposure for the cases is in line with several British studies reporting an increased prevalence of CWP with increasing cumulative exposure (Hurley, 1982; Morgan, 1974). However, both flow volume and impedance parameters failed to show significant differences

between references and cases, nor were they positively related to cumulative exposure. Average FEV_1, and FVC were lower in cases compared to references (Table 2.1) but not statistically significant after adjustment for age and height. Impedance outcomes suggest airway obstruction by a negative frequency dependence of resistance, and a slightly increased resonance frequency. In contradiction with this finding total resistance was lower in cases than in controls. Although the clinical significance of these physiologic changes is not yet clear similar observations were made by coal miners (Morgan, 1974) and in patients with diffuse interstitial lung disease (Noord, 1989).

Airway obstruction (illustrated by a decrease in FEV_1) as a result of coal dust exposure has been reported both in cases with CWP as in references (Morgan, 1973; Hurley, 1986; Kibelstis, 1978), suggesting that coal dust exposure reduces lung function along with age and smoking habits. In this study we found an average decline in FEV_1 of 0.39 ml/gh/m^3. The value did not reach the level of statistical significance, probably because of the small number of subjects studied. The figure, however, is consistent to British (Morgan, 1973; Hurley, 1986) and US coal miner (Kibelstis, 1978) studies reporting decreases in FEV_1 varying between 0.49 and 0.76 ml/gh/m^3 total dust.

Our results demonstrate a significantly increased TNF-α secretion by BM's upon *ex-vivo* stimulation with silica and low dose endotoxin in retired coal miners with airflow obstruction, independent of the presence of CWP. These findings fit with recent studies demonstrating the involvement of TNF-α in the pathogenesis of non-interstitial inflammation (Siracusa, 1992; Gosset, 1991). Siracusa *et al.* (1992), showed that immediate, late and dual respiratory reactions to occupational agents were associated with an increase in the spontaneous production of TNF released by PBM. Gosset *et al.* (1991) demonstrated that IgE dependent activation enhances the production of TNF and IL-6 production by AM and BM in late asthmatic reactions. Moreover, the AM from asthmatics had an enhanced capacity to produce TNF and IL-6. Under controlled conditions (a rat model), Kips (1992), showed that TNF causes bronchial hyperresponsiveness and airway inflammation. Pretreatment with anti-TNF antibody diminished this effect.

Association of TNF-α with restrictive lung function was recently investigated by Schwartz and coworkers (Schwartz, 1993) in subjects with asbestosis and reported that concentations of TNF-α released by BM were not significantly related to restrictive lung function.

In conclusion, this study demonstrates that TNF-α may be involved in both interstitial and non-interstitial effects of coal mine dust since levels of TNF-α were increased in early stadia of CWP and TNF-alfa secretion by BM's upon *ex-vivo* stimulation and TNF-α was significantly increased in retired coal miners with airflow obstruction independent of the presence of CWP.

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CHAPTER 3

Respiratory effects and serum type III procollagen in potato sorters exposed to diatomaceous earth

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ABSTRACT

Exposure to diatomaceous earth with low non-crystalline silica contents (< 1%) is rarely reported to cause pneumoconiotic disease, whereas airway obstruction and bronchitis are more frequently reported. We investigated the occurrence of pneumoconiosis and airflow obstruction in 172 male workers from 5 potato sorting plants (55 controls, 29 salesmen, 72 currently exposed and 16 retired exposed) exposed to non-crystalline silica containing dust from former sea terra's (7.7 - 15.4 mg/m^3). The presence of pneumoconiosis was evaluated by chest radiographs (exposed only) and serum levels of type III procollagen (P-III-P) were measured as an estimate of fibrogenetic activity. Lung function was assessed by flow volume curves and impedance measurements. A validated questionnaire was used to record respiratory symptoms. No pneumoconiotic abnormalities were demonstrated by chest radiographs. In line with this finding serum P-III-P levels were not elevated in exposed workers as compared to controls, suggesting no differences in fibrogenetic activity. In fact, serum P-III-P levels decreased significantly ($P < 0.03$) with increasing cumulative exposure. Flow volume parameters indicated airflow obstruction, related to (cumulative) dust exposure; the annual decline in forced expiratory flow volume in 1 second (FEV_1) was estimated at 10.5 ml/y ($P < 0.05$). Airway obstruction was confirmed by impedance analysis: in the retired group impedance changes were compatible with airway obstruction extending into the peripheral airways.

We conclude therefore that this group of potato sorters is not at an increased risk for pneumoconiosis, but that (prolonged) surveillance in this group is desirable in order to detect early indications of airflow obstruction.

INTRODUCTION

Diatomaceous earth is an amorphous nonfibrous silicate derived from skeletal remains of diatoms that are deposited on marine and lake floors. It consists, in its native form, of 1% crystalline silica (crystobalite). Calcination at high temperatures (600-1000°C), however, may lead to the formation of up to 60% crystobalite (Murata, 1974). This highly inflammatory crystalline silica and potential carcinogen (IARC, 1987) is used for filtration, insulation, and as a filler and polisher (Rom, 1992). A recent mortality study showed increased standard mortality ratios due to crystalline silica exposure in diatomite industry (Checkoway, 1993). The increased ratios could be explained by increased rates of lung cancer and non malignant respiratory disease (excluding infectious diseases and pneumonia). Diatomite pneumoconiosis from crystalline silica is rare and has mainly been described in the diatomite- processing industry and in several brewery workers exposed to diatomaceous earth used for filtering purposes (Davis, 1988).

Few data are available about the deleterious respiratory effects of noncalcined diatomaceous earth with low crystalline content. Industrial hygiene evaluations in potato sorting in the Netherlands have consistenly found elevated dust exposures caused by the diatomaceous earth on the potatoes, grown in former sea terraces.

The main purpose of this cross-sectional study was to determine whether respiratory hazards were present due to exposure to the exposure mix, high in diatomaceous dust in potato sorters. To assess pneumoconiotic effects, both chest X-rays as well as serum type III procollagen (P-III-P) levels, previously suggested to be a sensitive marker of active fibrosis (Janssen, 1992), were applied. Flow volume curves and impedance measurements were performed to detect airflow obstruction and respiratory symptoms were recorded by means of a questionnaire.

SUBJECTS AND METHODS

Study design and protocol

Male Caucasian workers (n = 174) from 5 potato sorting plants of the same agricultural cooperative in the Netherlands were studied (Table 3.1) and 172 complete data sets were obtained. The study was conducted in the month of October, just after the potato harvest at peak activity of these plants. Written informed consent was obtained prior to measurements (response rate 88%). Salesmen (n = 29) who experienced short-term exposure during the taxation of the crop (1 h/day on average) were regarded as a separate group. Questionnaires were obtained from 55 office staff (I = controls), 29 salesmen (II = salesmen), 72 blue collar workers (III = currently exposed), and 16 reti-

red blue collar workers (IV = retired exposed). After evaluation of the questionnaire, workers completed three valid measurements of both flow volume curves and impedance using the forced oscillation technique (FOT). The FOT was always performed before registration of flow volume curves to avoid the influence of forced inspiratory manoeuvres on the bronchial tonus (Orehek, 1981). A 10-ml blood sample was taken and the serum frozen and stored for determination of serum P-III-P, as previously described (Janssen, 1992). P-III-P was measured as a marker of active fibrosis. A chest radiograph (± 100 kV, 125 mA) was made in exposed and retired workers only. All radiographs were classified by a panel of three occupational physicians experienced in reading and classifying coal workers' pneumoconiosis according to ILO (International Labour Organization) criteria (ILO, 1980). Within 2 months after the study all workers were informed individually by the occupational physician (KDK) about the personal outcome of measurements.

TABLE 3.1: Demographic characteristics of the four groups studied.

Parameters (Unit)	I # n = 55		II n = 29		III n = 72		IV n = 16	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Age (y)	38.3	9.4	44.5	10.9	46.4*	10.9	63.8**	6.2
Length (cm)	184.4	6.6	181.2	5.9	178.2	7.0	175.2*	6.2
Weight (kg)	83.6	9.3	86.9*	11.4	82.7	8.7	81.0	7.7
Employment (y)	12.4	9.7	16.2*	11.8	11.8	8.4	22.4**	7.8
Smokers								
current (n)	26	47%	14	48%	36	50%	5	31%
past (n)	18	33%	12	42%	25	35%	9	56%
never (n)	11	20%	3	10%	11	15%	2	13%
Packyears	9.6	7.9	12.2	9.6	13.2	8.9	16.5*	8.7
Cum. dose (g)	-	-	63.2	77.1	117.8	87.4	199.5	94.3

explanation of groups: I = controls; II = salesmen; III = current exposed; IV = retired exposed.
Significantly different from controls (I) at: * $P < 0.05$; ** $P < 0.01$ tested by analysis of variance.

Exposure assessment

Stationary monitoring using a continuous dust monitor (Hund TM Data ®) based on light diffraction was used to identify main sources and places of exposure. In 1988 job exposure was extensively measured using personal samplers (Casella ®, cyclone) with at least four (8h) samples per job. Five different job categories could be distinguished. Total dust and respirable dust

were determined gravimetrically, and quartz content of two respirable samples was determined by MT-TNO (Delft, The Netherlands) using a Fourier transform infrared spectrophotometer (Bruker Type 113V ®). Assuming homogeneous lognormal distribution of the data, geometric mean and geometric standard deviations were calculated (Table 3.2). In order to obtain the estimated exposures in units of gram-hours per cubic meter (gh/m^3), the total exposure time was determined as 1200 work hours/year, taking into account the seasonal character of this industrial activity. Job continuity of most workers allowed proper estimation of the individual (cumulative) dose of total dust and of silica exposure from each worker's job history. No data were available on exposure to herbicides, fungicides, or other pollutants.

TABLE 3.2: Geometric mean (GM) and geometric standard deviation (GSD) of total dust and respirable quartz exposure among different jobs.

Job	Number of measurements	Total dust exposure (mg/m^3)		Respirable quartz (mg/m^3)	
	n	GM	GSD	GM	GSD
Sorting	4	6.9	1.9	0.23	1.4
Transport	5	6.9	1.6	0.13	1.7
Loading	4	5.6	1.8	0.20	1.5
Pelleting/Package	4	10.8	2.0	0.32	1.9
Reading	4	13.3	1.7	0.35	1.8
Total group	21	8.2	1.9	0.23	1.8

Lung function measurements

The basis for the impedance measurement was described previously (Làndsér, 1976). Briefly, a pseudo-random noise pressure signal containing all harmonics of 4 up to 52 Hz was applied at the mouth by means of a loudspeaker. Recorded random pressure and flow signals were analyzed by spectral analysis techniques and impedance was partitioned into resistance (R) and reactance (X). Of the obtained impedance data, the resistance at 8Hz (R8), at 28Hz (R28), frequency dependence of resistance (FD, the difference between R28 and R8), the reactance at 8Hz (X8) and the resonant frequency (f_0) were used for analysis. Only measurements with a coherence function ≥ 0.95 were used. R8 was chosen because at this frequency usually coherence functions ≥ 0.95 were obtained, which were not always obtained at lower frequencies. Airway obstruction, based on impedance criteria, was considered in this study in the presence of a negative FD concurrent with an

increased f_0 (usually $\geq$ 15 Hz). Three successive measurements, each lasting 8 s were performed in each subject. No active cooperation of the subject was required during the measurement procedure. The apparatus was calibrated daily and all subjects were measured on the same apparatus by the same observer.

Forced expiratory flow volume curves were recorded according to European Community of Coal and Steel (ECCS) criteria using a dry spirometer (Vitalograph P2 ®). Three recordings within 5% or a 100 ml range were obtained from each subject. Parameters derived from the flow volume curves were: FVC, FEV_1, FEV_1/FVC ratio, peak expiratory flow (PEF) and maximal mid expiratory flow (MMEF). Flow volume values were expressed as a percentage of the reference values of the ECCS (Quanjer, 1983) for individual diagnosis. A FEV_1 value less than 80% predicted was considered as a measure of airflow obstruction. The spirometer was calibrated at regular intervals and all subjects were measured on the same spirometer. Two spirometric and two FOT measurements failed due to technical problems.

Blood assays

A 10-ml blood sample was obtained from each worker. The serum of the sample was stored at $-80°C$ until analysis of P-III-P. Serum P-III-P was assessed using a commercial assay (Behringwerke AG, Marburg, Germany) as described previously (Janssen, 1992). This assay used Fab antibody fragments that binded with equal affinity to the two known reactive collagen species, col 1 and col 1-3 (Risteli, 1986). This resulted in an inhibition curve whose slope was parallel to the standard, a result not always achieved by assays that employ the intact antibody.

Statistical analysis

Individual dust/silica exposure was expressed as a cumulative measure (g dust) and estimated by multiplying the average time worked in a job times the average exposure for that job category. When a subject had worked in different jobs estimated cumulative exposures were added. Smoking was expressed as packyears (Table 3.1). Average lung function data for the different groups were tested for significance using analysis of variance (ANOVA). Confounders (age, height, weight, smoking) in lung function measurements were controlled by means of multiple linear regression. In this linear regression, lung function parameters were taken as dependent variables and the confounders and (cumulative) exposure as the independent variables. First the confounders and subsequently the cumulative exposure were put into the model of linear regression. Comparison of the questionnaire data to lung function

outcome was done with a multiple logistic regression analysis (BMDP, Berkeley, CA, USA). All procedures, if not stated otherwise, were computed with SPSS-X program (SPSS Inc., Ill, USA).

RESULTS

Exposure

Mean exposure of the total group to total dust was 9.9 mg/m^3 (n = 21, range 2.4 - 21.6 mg/m^3) with highest exposure among readers and those involved in pelleting and package (Table 3.2). The mean exposure was just below the Dutch standard of 10 mg/m^3 for total nuisance dust. Mean respirable dust exposure was 2.21 mg/m^3 (n = 21, range: 0.5 - 6.7 mg/m^3), with a mean non-crystalline silica content of the respirable fraction of 1% (n = 3). The mean respirable quartz exposure was 0.27 mg/m^3 (range: 0.09 - 0.84 mg/m^3). The Dutch standard for respirable quartz is 0.15 mg/m^3. In Table 3.2 the geometric mean and standard deviations are shown for several jobs in these potato sorting plants.

Respiratory effects

On the chest radiographs no irregular densities could be observed and, in consequence, the radiographs were classified 0/0 according to the ILO criteria. In line with this finding of serum P-III-P levels were similar in the control and exposed groups (Table 3.3). In all exposed groups however, serum P-III-P levels tended to be lower. Cumulative exposure was significantly ($p \leq 0.03$) inversely related to serum P-III-P levels after adjustment for packyears smoking. Flow volume values adjusted for differences in age and length were expressed as percentage predicted (Table 3.3). All spirometric parameters, except FVC, were significantly lower in currently exposed workers (group III) than in controls. Differences were even more pronounced when parameters were compared to data from retired workers (group IV). The FOT outcome showed that $\underline{R}8$, FD, $\underline{X}8$ and f_0 were statisticaly significantly different in group III compared with the control group. Again, differences were more pronounced when controls were compared with group IV. The comparison of salesmen (group II) to controls did not reveal significant differences in lung function data.

Airflow obstruction ($FEV_1 \leq 80\%$) was observed in 23 subjects (Table 3.3), resulting in an incidence rate of 23/172 (13.4%). Impedance criteria indicative for airway obstruction (FD < 0 concommittent with an in crease in f_0) were found in 15 subjects or 8.7% of the studied population.

Cumulative dust exposure in the 16 workers with $FEV_1 \leq 80\%$ from group III and IV was significantly higher compared to workers without airflow obstruction (223.4 vs 112.5 gh/m^3; $P \leq 0.001$).

TABLE 3.3: Average results (mean and standard deviation) of flow volume curves, impedance measurements, serum P-III-P levels and differential cell counts in the four groups of workers.

Parameter (Unit)	I Controls (n = 55)		II Salesmen (n = 29)		III Currently exposed (n = 72)		IV Retired exposed (n = 16)	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
SPIROMETRY								
FVC (% predicted)[A]	109.5	10.9	110.4	11.4	107.3	11.5	109.0	16.4
FEV_1(% predicted)	99.0	11.8	100.2	12.5	93.4	15.6	93.4	22.7
FEV_1/FVC	74.2	7.2	74.0	7.5	70.4	9.3	66.4	8.2
PEF (% predicted)	98.8	13.2	102.2	15.5	93.2	17.6	88.9	23.1
MEF (% predicted)	78.5	21.1	79.2	22.2	67.6	24.4	59.5	33.1
IMPEDANCE								
R8 (hPa.s/L)	2.26	0.56	2.22	0.57	2.42	0.97	2.85	1.38
FD (hPa.s/L)	0.36	0.37	0.38	0.36	0.21	0.63	−0.28	0.87
X8 (hPa.s/L)	0.04	0.20	0.05	0.21	−0.11	0.51	−0.47	1.06
f_0 (Hz)	8.17	1.90	8.16	1.82	9.63	4.40	12.62	7.9
BLOOD								
Serum P-III-P (ng/ml)	50.9	9.39	47.34	8.01	46.92	8.52	46.04	9.14

[A] Percentage predicted of European Community of Coal and Steel reference values (Quanjer, 1983). Significantly different from controls (I) at: * $P < 0.05$; ** $P < 0.01$ tested by analysis of variance.

The effect of exposure on lung function was further analyzed by multiple linear regression analysis after adjustment for smoking, height and age (Table 3.4). Retired exposed workers were not included in this analysis, because of the large difference in age and the lack of proper controls for this group. Results of this analysis for the separate lung function parameters are listed in Table 3.4. These results show that only FEV_1 significantly differs between controls and currently exposed. The decrease in FEV_1 (in group III) due to dust exposure was found to be 124 ml/11.8 years of employment (10.5 ml/year). This is equivalent to 1.0 ml/gh per cubic meter. No interaction between smoking and cumulative exposure was found.

TABLE 3.4: Results of the multiple linear regression (MLR) analysis including smoking, height, age and cumulative exposure as independent variables for the lung function parameters. The MLR was carried out on all workers excluding retired exposed, because of the large differences in age.

Lung function		R^2%	β	P
Parameter	Unit			
FVC	L	57	−0.088	0.132
FEV_1	L	60	−0.124	0.032
FEV_1/FVC	%	24	−0.139	0.079
MEF	L/s	39	−0.117	0.100
FD	hPa.s/L	14	−0.089	0.293
$\underline{X}8$	hPa.s/L	16	−0.144	0.084
f_0	Hz	19	0.142	0.085

R^2 = percentage of variance explained by the model; β = Slope due to cumulative exposure to silica containing total dust; P = level of significance.

Complaints of cough, phlegm, chronic bronchitis (productive cough for 3 months in 2 subsequent years), shortness of breath, wheezing and asthmatic attacks were related to aberrations in each lung function parameter, dust exposure and personal characteristics (age, length, smoking) using multiple logistic regression (Table 3.5). Complaints of phleghm (19%) and chronic bronchitis (14%) in this population were positively related ($P < 0.05$) to cumulative dose. Asthmatic attacks were reported by eight subjects (4,6%) and positively related to age ($P < 0.10$). Cough and wheezing were positively related to smoking ($P < 0.10$). Most symptoms, even cough, were related to FOT parameters (f_0, $\underline{R}8$ and $\underline{X}8$), whereas FEV_1 was related to chronic bronchitis and asthmatic attacks.

TABLE 3.5: Results of the multiple logistic regression analysis studying the relation between the data of the questionnaire, lung function parameters and confounders.

Respiratory symptom	n	$\chi^2 < 0.10$	Confounder
Cough	34	$\underline{R}8$, f_0	packyears, age
Phleghm	32	-	cumulative dose
Chronic bronchitis	24	FEV_1	cumulative dose
Short of breath	20	$\underline{X}8$, f_0	-
Wheezing	34	f_0, FEV_1	packyears, age
Asthmatic attacks	8		age

χ^2 : Statistical significance was tested with the χ^2-test ($P < 0.1$)

* : Explanation of parameters: $\underline{R}8$ = resistance at 8 Hz, $\underline{X}8$ = reactance at 8 Hz, f_0 = resonant frequency

DISCUSSION

To the best of our knowledge, the present paper firstly describes deleterious respiratory effects in the potato sorting industry. Among workers in this industry, airflow obstruction manifested by significant changes in spirometric and impedance parameters was demonstrated. These changes occurred in the absence of radiological evidence of pneumoconiosis. Furthermore, FEV_1 was significantly related to cumulative dust exposure while impedance parameters were more markedly related to airway complaints. These results might be corroborated by "healthy worker" influences since exposed workers are seasonal laborers, i.e., in case of ill health they are less likely to be rehired in the next season.

Evidence for a relationship between inorganic dust exposure and airflow obstruction is reported in various cross-sectional and longitudinal studies in coal workers. Airflow obstruction in coal workers was related to cumulative exposure to respirable dust, irrespective of the presence of pneumoconiosis, smoking habits or concommittent bronchitic symptoms. Subjects with airflow obstruction had a significantly higher cumulative exposure than subjects without airflow obstruction. Decreases in FEV_1 were markedly consistent among different studies in British and US coal miners and varied between -0.49 and -0.76 ml/gh per cubic meter total coal dust (Begin, 1987; Manfreda, 1984; Marine, 1988). We have calculated a decrease in FEV_1 (in group III) of 1.0 ml/gh per cubic meter due to noncalcined diatomaceous earth exposure.

When FEV_1 was dependent on the active cooperation of the study subject, airflow obstruction was confirmed in the present study by significant changes in impedance parameters, assessed by the technique of forced oscillations. No active cooperation of the subjects is required for this procedure (Nagels, 1980). Resistance versus fequency and reactance versus frequency curves in the group of exposed workers showed frequency dependence of resistance and a decrease in reactance values, resulting in an increase in resonant frequency. These curves were explained by airway obstruction extending into the peripheral airways (Clément, 1983).

Although exposure to respirable free silica was above its treshold limit value (TLV) no irregular densities on the chest radiographs were observed. In the present study, procollagen type III- N terminal peptide (P-III-P) was used as a marker to detect fibrogenic activity possibly indicative for pneumoconiosis. P-III-P is released during synthesis of type III collagen as a result of N-terminal cleavage of type III procollagen (Kelley, 1989; Madri, 1980). Areas of active fibrosis show an increase in the proportion of type III to type I collagen (Kelley, 1989; Madri, 1980; Bateman, 1981). Thus serum P-III-P levels can be considered as a potentially important marker of active fibrosis. Measurement of P-III-P in serum and bronchoalveolar lavage has provided insight into several pulmonary interstitial diseases (Okazaki, 1983) including silicosis (Bateman, 1981) asbestosis (Cavallieri, 1988) and coal workers' pneu-

moconiosis (Janssen, 1992). But even in the absence of X-ray detectable fibrosis several investigators have reported increases in serum P-III-P levels in individuals exposed to inorganic dust like silica or asbestos. Serum levels did not differ between exposed groups and controls. To our surprise serum P-III-P levels showed a significant decrease with increasing exposure, indicating slightly lower fibrogenetic activity. The meaning of this finding is unclear, but two facors might be involved. Firstly, amorphous silica might protect against silica fibrosis. The data suggest an adaptation of serum P-III-P production with increasing exposure to diatomaceous dust in this population. Secondly, interruption of work during the summer might allow clearance of dust from the lungs.

The analysis of questionnaire data showed that diatomaceous earth dust exposure was significantly related to complaints of productive cough and chronic bronchitis. A marked increase in the percentage distributions of chronic bronchitis with increasing exposure to dust for both smokers and non smokers was reported earlier after coal dust exposure (Marine, 1988). Comparison of questionnaire data to lung function outcome, suggests that the FOT is a sensitive tool to detect and objectify airway complaints, such as cough, shortness of breath and wheezing.

In conclusion, this study shows that this semi agricultural working population is not at an increased risk to develop pneumoconiosis. The main respiratory effect of the dust, mainly generated from clay adhering to incoming potatoes, observed was airflow obstruction with or without chronic bronchitis. Unfortunately, our data do not allow discrimination, between the effects of total dust and respirable silica and the contribution of exposure to organic dust, growth inhibiting agents and herbicides, as possible contaminants of the harvested potatoes. Although our data do not allow a conclusion about a causal role of the specific agent(s), results derived from flow volume curves and complaints are well in line with data obtained after other forms of inorganic dust exposure. Therefore, prolonged surveillance to assess the harmful nonpneumoconiotic effects associated with this type of dust exposure is desirable.

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CHAPTER 4

Respiratory symptoms and lung function in animal feed workers

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ABSTRACT

In a study among 194 male workers exposed to endotoxin-containing organic dust in animal feed mills, lung function was measured by flow volume curves and impedance measurements and respiratory symptoms were recorded by means of a validated questionnaire. The aims were to detect and localize airway obstruction caused by fodder dust and endotoxin, and to relate respiratory symptoms to both types of lung function measurements. Flow volume and impedance parameters were significantly related to present exposure. All impedance parameters, but of the spirometric measures only MEF_{75}, were significantly related to cumulative dust or endotoxin exposure. The changes in impedance parameters were for overall increasing resistance at 8 Hz and decreasing reactance at 8 Hz, reflecting an increase in peripheral airflow obstruction, with increasing exposure. The changes in all lung function parameters were more strongly related to (cumulative) endotoxin exposure than to inspirable dust exposure. All impedance parameters and FEV_1 showed a good correlation with complaints of chronic bronchitis and breathlessness. Impedance measurement of the respiratory system proved to be a useful tool for objectively assessing (early) airflow obstruction in workers exposed to inspirable dust and endotoxin and in localizing airflow obstruction.

INTRODUCTION

Chronic exposure to organic dust is known to cause respiratory symptoms and airflow obstruction, mostly studied in grain workers (Chan-Yeung, 1980; Dosman, 1980; Cotton, 1982) and in cotton industry (Schilling, 1956). In workers exposed to grain dust significant dose-response relationships were reported by Huy *et al.* (1991) for chronic phleghm production, breathlessness on exertion, FEV_1, FVC, and total dust exposure in workers. Few studies in the animal feed industry have been reported. One study (Zuskin, 1989) demonstrated both acute, across-shift and pre-shift effects of feed dusts on lung function and respiratory symptoms. Another recent cross-sectional study among 315 workers in Dutch animal feed industry confirmed the chronic effects on lung function, but failed to demonstrate a relation between the level of exposure and symptom prevalence (Smid, 1992). Similar to other studies on organic dusts endotoxin was suggested to be an important factor in both acute and chronic respiratory impairment (Smid, 1992). In this paper results will be presented from a cross-sectional study among 194 workers involved in animal feed processing, with emphasis on respiratory symptoms and lung function measured by flow-volume curves and input impedance testing. Recent investigations showed that impedance measurement by the forced oscillation technique (FOT) is able to measure the distribution and magnitude of airway resistance along the respiratory system (Wouters, 1990). Moreover the technique was shown to be suitable in early diagnosis of airflow obstruction to irritants in an occupational setting (Brochard, 1987) and of airflow obstruction due to inorganic dust (Jorna, 1992). In this study impedance measurements were applied along with flow volume curves to (i) detect and localize airflow obstruction caused by animal feed dust/endotoxin, and (ii) to relate respiratory symptoms to the outcome of both types of lung function measurements.

METHODS

Subject selection

In a cross-sectional study male Caucasian workers (n = 205) from five grain elevators of the same agricultural cooperation in the Netherlands were studied. Characteristics of the study population are summarized in Table 4.1 Written informed consent was obtained from each worker (participation rate 88%). Subjects were asked to complete the British Medical Research Council questionnaire on respiratory complaints at home before lung function measurements. Additional questions were asked concerning job history and exposure. Exposure to organic dust and endotoxin was measured to estimate present and historic cumulative exposure.

TABLE 4.1: Characteristics of animal feed workers and controls (mean ± SEM[A]).

Parameter	Unit	Controls n = 54		Exposed n = 139	
		$\bar{X}$	SEM	$\bar{X}$	SEM
Age	y	38.9	1.5	42.3	0.8
Length	cm	178.2	0.9	176.4	0.6
Weight	kg	78.2	2.2	78.1	1.9
Years in industry	y	14.1	1.4	16.3	0.7
Years exposed	y	0.9	0.4	16.3	0.7
Years smoked	y	9.8	1.0	12.2	0.6
Packyears	y	6.5	1.3	9.5	0.7
Smoker current	n	16	26%	53	40%
past	n	18	30%	58	44%
never	n	27	44%	22	17%
FVC	L	5.63	0.12	5.37	0.11
FEV_1	L	4.51	0.11	4.16	0.11
FEV_1/FVC	%	80.6	1.00	77.3	0.70
PEF	L/s	12.34	0.25	11.14	0.25
MEF	L/s	4.45	0.18	3.67	0.12
MEF_{75}	L/s	9.54	0.34	8.41	0.26
$\underline{R8}$ [B]	hPa.s/L	2.23	0.08	2.73	0.10
FD [B]	hPa.s/L	0.29	0.05	0.03	0.06
$\underline{X8}$ [B]	hPa.s/L	−0.03	0.03	−0.19	0.05
f_0 [B]	Hz	8.46	0.18	10.41	0.42

[A] SEM = standard error to the mean
[B] For explanation of impedance parameters see methods

Production process

Briefly, the production process comprises the following steps. Raw materials arrive by ship or by truck. Mainly grains (especially corn, wheat and barley), other vegetable products such as cassava, tapioca and waste products from the food industry are used. The raw materials are cleaned, stored in silos, and mixed in a batch process. Fats, molasses, vitamins, and minerals are added in small quantities. The mixture is stored again in silos, usually after being pressed to pellets. The product is transported to the customer by bulk trucks. Only a part of the produced animal feed is packed and transported in bags. Production workers occupied eight job titles as previously described (Heederik, 1991; Smid, 1992). The job titles involved in production are unloaders, crane drivers, facility operators, press operators, expedition workers, truck drivers, maintainance personnel and "jacks of all trades". Fifty-five non-production workers, rarely exposed to dust in production processes

(laboratory personnel, gate keepers, consultants, office workers), were regarded as controls. Some of these non-production workers had previously held production jobs. Maintainance personnel (n = 5) were excluded from the study since they may have been exposed to other respiratory hazards, such as welding fumes.

Industrial Hygiene Sampling Methods

(Airborne) grain dust was monitored by gravimetric personal air sampling (n = 54) of all job titles. A large number of samples (n = 17) were taken in the group with "jacks of all trades", because of the large variability in exposure. DuPont (P-2500) airpumps with PAS-6 filters were used to draw air samples. The sampling rate was approximately 1.2 L/min and the minimum sampling duration was 7.30 hours. The filter was positioned near the worker's breathing zone. Pumps were calibrated immediately before and after each use and monitored throughout the sampling period. The dust sampled is characterized by a 50% cut-off diameter of 30 μm (Smid, 1992) and resembles the inspirable dust fraction that passes the mouth and nostrils when inhaled. Endotoxin was quantified spectrophotometrically using a modified kinetic-QCL assay (BioWhittaker Inc, 192 Test kit). The method is based upon the endotoxin catalyzed activation of a pro-enzyme in Limulus amoebocyte lysate (LAL) (Smid, 1992).

Lung function measurements

Impedance measurements were performed on equipment described by Làndsér *et al.* (1976). Briefly, the seated subject, wearing a noseclip, supports the cheeks with his hands and breathes quietly via a tube. A pseudo-random noise pressure signal containing all harmonics of 4 up to 52 Hz is applied at the mouth by means of a loudspeaker. Mouth pressure and flow signals are recorded by transducers with identical frequency characteristics (Validyne MP45 ®) and analyzed by spectral analysis techniques. The relationship between pressure and flow is called impedance. Impedance is partitioned into the real part or resistance (R) and the imaginary part or reactance (X). The accuracy of the computations is evaluated at each frequency by means of a coherence function. Only the values with coherence functions exceeding or equal to 0.95 are retained; a value of 1.00 would represent complete absence of noise or alinearities. Three successive measurements, each lasting eight seconds were performed in each subject. Of the obtained impedance data, analyzed parameters included: resistance at 8 Hz (R8) and at 28 Hz (R28), the difference between R28 and R8 (frequency dependence (FD); signifying the course of the resistance versus frequency curve) the reactance at 8 Hz

($\underline{X}8$) and the resonant frequency f_0. The apparatus was calibrated daily and all subjects were measured by the same investigator.

Flow volume curves were measured using a dry 'rolling seal' spirometer (Vicatest-V, Mijnhardt, Bunnik, The Netherlands), according to ATS criteria (ATS, 1987). The spirometer was calibrated daily and all subjects were measured on the same spirometer by the same investigator. Parameters analyzed were FVC, FEV_1, FEV_1/FVC, peak expiratory flow (PEF), and forced expiratory flow at 25% and 75% of the expired VC (MEF_{75}, MEF_{25}), and maximal mid-expiratory flow (MEF).

Impedance measurements were always performed prior to registration of flow volume curves to avoid the influence of forced expiratory manoeuvres on the bronchial tone (Orehek, 1981). All subjects were measured at least four hours after beginning their shift since acute respiratory effects of endotoxin exposure are measurable 4 h after exposure (Rylander, 1989). Lung function measurements were obtained from 205 male workers: 60 office staff (group I = not exposed), 145 blue collar workers (group II = exposed). Since three spirometric- and three impedance-measurements failed due to technical problems and the previously mentioned maintainance personnel (n = 5) was excluded, eventually 194 workers were considered in this study (Table 4.1).

Data handling and analysis

Occupational category means were used to estimate the present inspirable dust and endotoxin exposure (Table 4.2). Present exposure levels were ranked according to Huy *et al.* (1991) in four exposure categories: not exposed, 0 - 4 mg/m^3, 4 - 9 mg/m^3, > 9 mg/m^3, representing arithmetic means (AM). Effects of present exposure on lung function were analyzed by regression analysis with age, height, packyears and years after smoking cessation as predictor variables. Weight was included as predictor variable because overweight status is associated with increased total respiratory resistance and decreased total respiratory reactance (Michels, 1991). The results were expressed as adjusted mean values (SEM). Each subject's individual (cumulative) dose of total grain dust and of endotoxin exposure was derived from his personal job-history. The product of the occupational category mean (AM) and the number of years employed with that job title was computed for every job that was reported by the worker. Cumulative exposure was computed for each worker by adding the results for all jobs held. Chronic effects of grain dust and endotoxin on flow volume and impedance parameters were tested by multiple linear regression. In this linear regression, lung function parameters were taken as dependent variables and the confounders (age, height, weight, smoking) and (cumulative) exposure as the independent variables. First the confounders and subsequently the cumulative exposure were put into the model of linear regression. Correlation between respiratory symptoms and lung

function parameters was tested by multiple logistic regression (BMDP, Berkeley, CA, USA).

TABLE 4.2: Mean dust (mg/m^3) and endotoxin (ng/m^3) concentrations by job category[A] (n = 54 samples).

	Airborne dust (mg/m^3)			Endotoxin (ng/m^3)		
	AM[B]	GSD[C]	range	AM[B]	GSD[C]	range
Unloaders	23.8	3.9	2.8 - 58.2	14.2	6.5	0.3 - 48.9
Crane drivers	1.8	2.3	0.5 - 3.7	0.4	1.9	0.1 - 0.7
Facility operators	2.8	3.3	0.4 - 9.4	1.4	2.2	0.2 - 0.8
Press operators	9.7	2.4	2.5 - 25.5	21.7	6.1	0.9 - 93.1
Expedition workers	15.7	4.7	0.6 - 130.8	7.3	6.8	0.1 - 56.9
Truck drivers	3.3	2.8	0.6 - 14.3	1.8	3.9	0.2 - 7.2
"Jacks of all trades"	36.1	4.4	0.7 - 199.2	29.1	5.8	0.3 - 317.2

[A] maintainance personnel was excluded (see methods)
[B] AM = arithmetic mean
[C] GSD = geometric standard deviation

Symptoms (cough, phleghm, chronic bronchitis, breathlessness, wheezing, asthmatic attacks) were included if a subject had positively responded to a question in a category. Three separate logistic regression analyses were performed with the respiratory symptom as the dependent variable and the confounders (age, height, packyears) and spirometric or impedance or exposure parameters as the independent variables. Exposure was not regarded as a confounder in this relationship.

Subjects using inhalation drugs (4 in the exposed group only) were excluded from statistical analyses since this may lead to an underestimation of respiratory resistance. All procedures if not stated otherwise were computed with the SPSS-X mainframe software package (SPSS-X Inc., Ill, USA).

RESULTS

Estimation of exposure

In these facilities 54 measurements of dust and endotoxin exposure were gathered. For the seven job titles in the production group (maintainance personnel was exluded), mean exposures to inspirable dust and endotoxin are given in Table 4.2. Regression analysis revealed that average dust and endotoxin exposure differed significantly among different job titles ($P < 0.01$). Highest exposures were met with in unloaders, press operators, and "jacks of all trades".

Pulmonary function - present exposure

Mean pulmonary function values of controls and exposed workers are listed in Table 4.1. On average, exposed are older, shorter, have smoked more packyears and have similar weight compared to controls. All flow volume parameters, FD and $\underline{X}8$ are decreased, whereas $\underline{R}8$ and f_0 are increased in exposed compared to control workers. These findings indicate an increase in airflow obstruction in the exposed group. Mean FEV_1 and MEF-parameters decreased significantly with increasing exposure by category adjusted for age, height and smoking (Table 4.3). Differences between exposure groups were most pronounced for MEF_{75}. Interestingly, all impedance parameters were significantly different between exposure groups. $\underline{R}8$ and f_0 increased at higher exposure while $\underline{X}8$ decreased with higher exposure (Table 4.3).

TABLE 4.3: Mean values (SEM†) of lung function in control workers and production workers classified into exposure categories, with respect to airborne dust.

Lung function		Controls		0 - 4 mg/m³‡		4 - 9 mg/m³‡		> 9 mg/m³‡	
Parameter	Unit	n = 54		n = 97		n = 17		n = 25	
Spirometry									
FVC	L	5.63	0.12	5.34	0.08	5.37	0.20	5.28	0.21
FEV_1	L	4.51	0.11	4.20	0.08*	4.10	0.08*	3.99	0.20$
PEF	L/s	12.34	0.24	11.46	0.18*	10.69	0.35#	10.35	0.32$
MEF	L/s	4.45	0.21	3.80	0.13#	3.52	0.40#	3.30	0.29$
MEF_{75}	L/s	9.54	0.34	8.74	0.22#	8.03	0.61#	7.38	0.49$
Impedance									
$\underline{R}8$	hPa.s/L	2.23	0.09	2.52	0.09#	2.54	0.28#	3.50	0.34$
$\underline{X}8$	hPa.s/L	−0.03	0.03	−0.08	0.04#	−0.24	0.17#	−0.50	0.16$
FD	hPa.s/L	0.29	0.04	0.16	0.06*	0.07	0.13#	−0.43	0.20$
f_0	Hz	8.46	0.20	9.21	0.35*	10.88	1.47#	14.11	1.48$

† SEM = standard error to the mean; ‡ Exposure categorization by arithmetic mean (AM); Levels of significance: * $P < 0.05$, # $P < 0.01$, $ $P < 0.001$. Statistical testing by linear regression, adjusted for differences in age, height, weight and smoking (see methods).

Frequency dependence of oscillatory resistance became more negative with increasing exposure. Roughly, similar differences between exposure groups and lung function parameters were observed when workers were stratified into three groups with respect to endotoxin exposure. Difference in effects on lung function between present dust and endotoxin exposure was not observed, because few subjects changed between groups when classified to endotoxin exposure with group sizes equal to dust exposure (data not shown). No interaction was observed between exposure and smoking when including an interaction term.

Pulmonary function – cumulative exposure

Lung function was clearly inversely related to cumulative exposure after adjustment for age, height and smoking (Table 4.4). Among flow volume parameters only the MEF_{75}, however, reached statistical significance ($P < 0.05$). On the other hand, all impedance parameters were significantly related to cumulative inspirable organic dust and endotoxin exposure. The estimated cumulative exposure to endotoxin appeared to be more stronger related to lung function decrements than cumulative dust levels. This is reflected in consistently higher regression coefficients, although statistical significance is higher only for frequency dependence (Table 4.4). No interaction was observed between exposure and smoking.

TABLE 4.4: Relation between cumulative dust and endotoxin exposure and spirometric/impedance values, after adjustment for age, height, weight, packyears and years after quitting smoking (n = 135).

Lung function		Cumulative dust exposure	
Parameter	Unit	Dust (y* g/m^3)	Endotoxin (y* g/m^3)
FVC	L	−0.021	−0.036
FEV_1	L	−0.042	−0.114
MEF	L/s	−0.105	−0.270
PEF	L/s	−0.168	−0.270
MEF_{75}	L/s	−0.252	−0.630
MEF_{50}	L/s	−0.112	−0.360
MEF_{25}	L/s	−0.026	−0.072
R8	hPa.s/L	0.221	0.720
FD	hPa.s/L	−0.140	−0.451
X8	hPa.s/L	−0.130	−0.339
f_0	Hz	1.191	4.056

Levels of significance: $P < 0.05$, $P < 0.01$, $P < 0.001$

Respiratory symptoms

Most respiratory symptoms were related to age and smoking habits in a logistic regression analysis. Prevalence of chronic bronchitis and (ever) wheezing ranged from 7% in the control workers to 32% in the highest exposure group (> 9 mg/m^3) (Figure 4.1).

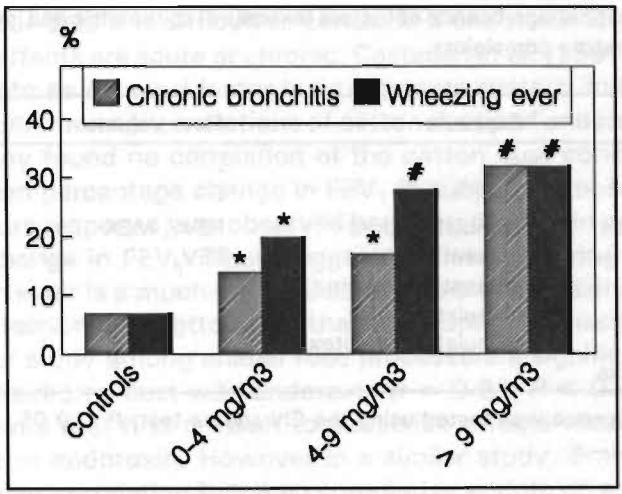

FIGURE 4.1: Prevalence of respiratory symptoms versus present exposure categories. Significance was tested by multiple logistic regression (see methods); $P < 0.05$, # $P < 0.01$.*

Smoking and age adjusted odds ratio's indicated significant differences in reported symptoms (chronic bronchitis, wheezing) between production and control workers. Moreover, a clear exposure-response relationship was present, since prevalence of respiratory symptoms increased with exposure. Endotoxin exposure revealed similar relationships (data not shown).

Respiratory symptoms and lung function

Chronic bronchitis was significantly related to cumulative dust exposure, shortness of breath was related to present dust and present endotoxin exposure (Table 4.5), whereas wheezing was related to cumulative dust and endotoxin measures. Since both respiratory symptoms and lung function were different among exposure categories with respect to present dust exposure, we investigated whether symptoms were related to lung function parameters using two logistic regression analyses (one with flow volume parameters, one with impedance parameters). These analyses revealed that chronic bronchitis is significantly associated with a decrease in FEV_1 and MEF. Shortness of breath was significantly related to changes in lung function (R8, FD and FEV_1). As a group, individuals with asthmatic episodes differed from others in terms of impedance parameters, but not in terms of exposures.

TABLE 4.5: Statistical significance of factors (exposure, spirometric and impedance) in relation to the respiratory complaints.

Symptom	Exposure	Flow volume	Impedance
Cough	-	-	-
Phlegm	-	-	-
Chronic bronchitis	cumulative dust	FEV_1, MEF	-
Breathlessness	present dust	FEV_1	R8, FD
	present endotoxin		
Wheezing (ever)	cumulative dust	-	-
	cumulative endotoxin	-	
Asthmatic attacks	-		R8, X8

Statistical significance was tested using the Chi square test; $P < 0.05$.

DISCUSSION

This study provides further evidence that organic dust exposure in animal feed industry may induce airflow obstruction and respiratory symptoms (Zuskin, 1989; Smid, 1992). Lung function in exposed workers was found to be inversely related to both present and cumulative exposure to inspirable dust. A significant exposure-response relationship was observed between present dust exposure and lung function parameters from both techniques applied. On the other hand, all impedance parameters correlated significantly with cumulative dust exposure, while only MEF_{75} was a significant descriptor of airflow obstruction due to cumulative dust exposure. Present and cumulative exposure to airborne endotoxin proved to be a better descriptor than total dust of changes in all lung function parameters.

Dust exposure in fodder dust industry in The Netherlands has been studied elaborately by Smid and coworkers (1992). Because we had no indication that exposure levels in our fodder dust facilities would differ significantly from those reported by Smid *et al.*, the characterization of dust exposure itself was conducted by measuring endotoxin levels in the dust. In general, endotoxin levels in the dust depend on a number of factors like: the nature of the dust, land of origin of the product, conditions during transport, the particle size during processing, temperature and humidity. For the workers particle size seems to be the most important factor involved in the endotoxin/dust ratio. In general large dust particles will contain relatively small ratios of endotoxin whereas small dust particles will contain a relatively large ratio of endotoxin.

No measurements of dust or endotoxin were performed in the office environment. However, a possible exposure of the office workers, to endotoxin, only tends to reduce the reported significance between our exposed and control group.

From our data it is difficult to conclude if endotoxin is a causal factor and whether effects are acute or chronic. Castellan *et al.* (1987), showed that endotoxin might be a causal factor in an exposure system. In the absence of a correlation between concentrations of cotton dust and endotoxin ($r = 0.07$; $P = 0.46$) they found no correlation of the cotton dust concentration with the group mean percentage change in FEV_1 in subjects exposed. In contrast a clear exposure response was observed between endotoxin and group mean percentage change in FEV_1. They suggested that measuring the endotoxin concentration in air is a much more reliable means of assessing the risk of an acute airway response to cotton dust than measuring the mass concentration of dust. In our study among animal feed processors a significant correlation coefficient of airborne dust with endotoxin ($r = 0.81$; $P < 0.01$) was observed. This means that it is difficult to subscribe effects measured to either airborne dust or endotoxin. However in a similar study, Smid *et al.* (1992) showed a higher correlation between cumulative endotoxin exposure and all flow volume parameters studied than between dust and lung function decrements (Smid, 1992). Our linear regression revealed similar ß-values for flow volume parameters with cumulative exposure in the same order of magnitude but probably due to a smaller population the level of significance was not always reached. Kennedy *et al.* (1987) showed that lung function (FEV_1, FEV_1%) and respiratory symptoms (byssinosis and chronic bronchitis) were related to present exposure in cotton workers; we also observed a concentration dependent decrease in lung function with increasing present exposure. Correlation of lung function parameters to both present and cumulative exposure indicate that it is difficult to separate both effects. We are therefore uncertain if the observed effects are chronic or acute but presume that both influenced the results.

In previous studies of animal feed workers, flow-volume parameters have generally been measured to evaluate acute and chronic airflow obstruction. A more sensitive analysis of the response to exposure may be possible with other lung function techniques. Rylander *et al.* (1989) applied carbon monoxide diffusing capacity measurement (DL_{CO}) and found a decrease 4 hours after acute inhalation of nebulized endotoxin, suggesting an inflammatory effect of endotoxin exposure.

Sepulveda *et al.* (1984) applied impedance measurements to detect cotton dust induced bronchoconstriction. Subjects with across shift responses in MEFV curves showed limitation of flow predominantly located in the peripheral airways and subjects with no significant alterations in MEFV curves showed a central airways effect. In this cross-sectional study among animal food processors the observed changes in impedance parameters with increasing present dust levels suggest an airflow obstruction extending to the peripheral airways. Evidence for a causal role of endotoxin in the mechanism of the observed airflow obstruction was recently provided by Kips *et al.* (1992). They showed changes in airway responsiveness and airway inflammation in

rats after challenge to an aerosol of endotoxin. Furthermore a significant increase in secondary release of Tumor Necrosis Factor (TNF), a pro inflammatory marker, after endotoxin challenge was observed. This indicates that the airway inflammation caused by endotoxin exposure might be mediated through TNF release.

A significantly higher prevalence of most (chronic) respiratory symptoms was found in the exposed workers compared to control workers. Separate analysis of exposure-categories revealed a concentration (present dust and endotoxin) dependent increase of shortness of breath prevalence. Cumulative dust exposure was a significant predictor of ever wheezing. Prevalence values were similar to those reported by Huy *et al.* (1991) in grain workers and to animal dust exposed non-smokers (Zuskin, 1989). Different symptoms correlated well with (individual) outcome of lung function measurements. Chronic bronchitis was associated with a general decrease in all spirometric parameters whereas impedance parameters did not change significantly. Recently, Wesseling *et al.* (1992) reported normal impedance data in patients fulfilling the criteria of chronic bronchitis.

Possibilities for primary prevention of the observed effects include personal protection (mouthpieces) and an improvement of the ventilation. Secondary prevention might be improved by creating a health-hazard model that implements the dust-endotoxin ratio. Currently one facility is renovated with a new ventilation system. In future we plan to evaluate the respiratory consequences for the workers of this effort.

From the present study, it can be concluded that: 1) there is an apparent dose-related adverse respiratory effect of working in dust exposed areas of animal feed facilities; 2) the dust in these facilities is contaminated with endotoxin and dose-response relationships indicate that the effect of endotoxin is more substantial than the effect of "dust"; 3) impedance testing appeared to be more sensitive than spirometric testing with respect to detecting adverse respiratory effects of animal feed dust exposure.

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CHAPTER 5

Respiratory impedance in isocyanate induced asthmatic reactions

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ABSTRACT

In an attempt to localize the site of airway obstruction in early and late asthmatic reactions to isocyanates, impedance and flow volume measurements were applied to eleven subjects during inhalation challenge with toluene diisocyanate (TDI) or methylene diphenyl diisocyanate (MDI). Six subjects were found to be responsive to isocyanate challenge (a decrease in $FEV_1 \geq 20\%$ from baseline). Average changes in FEV_1, PEF and MEF_{75-25} values differed significantly between sham and challenge in isocyanate reactors. Changes in resistance at 8Hz (R8) and reactance at 8Hz (X8) showed response vs. time profiles similar to spirometric parameters in all subjects, reflecting a sensitivity equal to flow volume parameters. However, two distinctly different reaction patterns could be discerned with the impedance measurements. Both the 2 early and 2 out of 4 late reactions were characterized by impedance changes that suggest peripheral airway obstruction. In two late asthmatic reactions however, obstruction appeared to be localized in the central airways. This finding suggests that the asthmatic response to isocyanates may occur all along the bronchial tree. Interestingly, both subjects with a centrally localized late asthmatic reaction showed absence of bronchial hyperresponsiveness both before and after challenge. Larger groups have to be studied to confirm the two response patterns and to investigate possible differences in underlying mechanisms.

INTRODUCTION

Asthmatic reactions induced by laboratory exposure to isocyanates in sensitized subjects are generally defined as early, late or dual, depending upon the time of onset after challenge (Banks, 1989). Late asthmatic reactions induced by TDI (toluene diisocyanate) are associated with a transient increase in non specific bronchial hyperresponsiveness (NSBHR) (Mapp, 1985) and a cellular influx into the airways which can be demonstrated in broncheoalveolar lavage fluid (Fabbri, 1987). These responses can be influenced by anti-inflammatory drugs (Fabbri, 1985). Pathological findings were reported in both the central and peripheral airways in a subject who died at work after exposure to TDI (Fabbri, 1988). These data indicate that the airway response to isocyanate challenge may occur all along the tracheobronchial tree. To study response localization within a short time course such as inhalation challenge repetitive measurements are necessary. Alternatives like studies on deposition of radioactive aerosol may give indirect evaluation on the site of obstruction (Santolicandro, 1986), but are also invasive. Lung function measurements used in the surveillance of isocyanate challenges usually include forced expiratory flow volume curves. However, maximal inspiratory maneuvers can cause transient bronchodilatation or bronchoconstriction that can affect the subsequent maximal flow volume curve (Orehek, 1981). Furthermore parameters used, i.e. FEV_1 and FVC do not allow a clear distinction between central and peripheral airway obstruction. Partial flow volume curves are able to localize airway obstruction (Sterk, 1986), but demand high patient compliance and trained subjects. Measurement of the impedance of the respiratory system with the technique of forced oscillations in bronchial challenge tests enables localization of response to pharmacological agents (Wouters, 1989). Furthermore this technique is readily applicable as a non-invasive lung function measurement in occupational medicine (Wouters, 1990).

The objectives of this study were 1) to test if impedance measurements are suited for the assessment of the response in isocyanate challenges, i.e. if impedance measurements show the same time dependence of airflow obstruction as flow volume parameters and 2) to investigate possible response patterns of impedance measurements, indicative for different localization of airflow obstruction along the tracheobronchial tree.

METHODS

Subjects

Eleven subjects (10 male, 1 female) suffering from work related complaints of shortness of breath during exposure to isocyanates were challenged with TDI or MDI (methylene diphenyl diisocyanate) to confirm occupational asthma. Characteristics of the study population are listed in Table 5.1.

TABLE 5.1: Characteristics of the study population.

Subject No.	Sex M/F	Age y	Reaction type	Isocyanate	Exposed y	Atopy (+/−)	Baseline FEV_1 % predicted	$PD_{20}FEV_1$ (mg methach.) before challenge	$PD_{20}FEV_1$ (mg methach.) after challenge
Positive									
1	M	27	early	MDI	11	−	96.9	0.09	nm*
2	M	34	early	MDI	3	+	112.2	> 1.40	> 1.40
3	M	43	late	TDI	25	+	94.9	0.73	0.36
4	F	30	late	TDI	11	+	108.4	0.11	0.09
5	M	43	late	TDI	29	−	122.3	> 1.40	> 1.40
6	M	29	late	MDI	8	+	120.3	> 1.40	> 1.40
Negative									
7	M	41	-	TDI	26	+	76.6	> 1.40	> 1.40
8	M	54	-	TDI/MDI	22	+	94.8	0.34	0.18
9	M	49	-	TDI/MDI	2	+	100.8	0.58	0.42
10	M	28	-	TDI	1	+	98.4	0.23	0.08
11	M	51	-	TDI	30	−	104.5	1.03	0.62

* nm = not measured, because medical treatment was required due to the severity of reaction

In all patients a complete medical history was obtained together with chest radiography, ECG, routine blood tests, skin tests with common aero-allergen extracts, lung function measurements and methacholine challenge on the first day. Respiratory viral infection in the last month was excluded. No subject used systemic or inhaled steroids in the month before the study. Sympathomimetics, antihistamines, theophylline, anticholinergics or cromolyn were stopped at least 24 h before the challenge procedure. On the second day subjects were sham exposed to obtain baseline impedance and flow volume values. At the end of the day a methacholine challenge was performed. This procedure was repeated on the third day when the subjects were challenged with a specific isocyanate, i.e. TDI and/or MDI according to occupational exposure. Two subjects were challenged with both TDI and MDI on separate days one week apart. Control lung function measurements were performed at 9.00 a.m. on the fourth and fifth day. The study was approved by the local ethics committee, and informed written consent was obtained from each subject.

Challenge

Airway responsiveness to methacholine and inhalation challenge with TDI or MDI were performed as described previously (Mapp, 1988). Briefly, subjects were exposed to TDI or MDI (5 -10 ppb) in a 9 m^3 exposure chamber. Exposure was monitored using a MDA 7005 monitor. Impedance measurements and flow volume curves were obtained before challenge and 15 min, 30 min, and hourly for 7 h after challenge. Lung function was monitored as soon as a subject complained of shortness of breath during the whole proce-

dure. The test was considered positive when FEV_1 decreased by at least 20% from baseline after exposure to TDI or MDI. An early response was defined as a positive response within 1 h and a late response was defined as a positive response at least 2 h after start of the challenge. The maximal duration of challenge was 30 min, or the challenge procedure was stopped if a subject showed a positive reaction within 30 minutes.

Lung function

Spirometry was performed according to the recommendations of the American Thoracic Society (ATS, 1987) with a Sensormedics 2130/922 Spirometry System (Sensormedics, Anaheim CA). At least 3 acceptable forced expiratory maneuvers were performed each time. FVC or FEV_1 values had to be in the range of 5%. FVC, FEV_1, peak expiratory flow (PEF), and maximal expiratory flow 75-25% of FVC (MEF_{75-25}), were obtained from the curve with the largest sum of FVC and FEV_1.

Impedance (Z_{rs}) of the respiratory system was measured using the technique described by Làndsér *et al.* (1976). A pseudo-random noise pressure signal containing all harmonics of 2 Hz from 4 to 48 Hz is applied at the mouth by means of a loudspeaker during quiet breathing. Measurements were performed in the seating position while the subject, wearing a noseclip supported the cheeks with his hands. Recorded pressure and flow signals were analyzed by spectral analysis techniques. The relationship between flow and pressure is called impedance. Impedance is partitioned into a real part or resistance ($\underline{R}$) and an imaginary part or reactance ($\underline{X}$). The reactance depends on the compliant and inertial properties of the system: a negative reactance is found at lower frequencies since at lower frequencies the reactance is mainly determined by the capacitance of the system. At higher frequencies the reactance is influenced predominantly by the inertial qualities of the air in the airways (Wouters, 1989). The reactance then becomes positive. The influences of capacitance and inductance on $\underline{X}$ cancel out at the resonant frequency (f_0). A coherence function is calculated at each frequency: only values greater than 0.95 were retained; a value of 1.00 represents complete absence of noise or alinearities. Mean values of three successive measurements, each lasting 8 seconds were used in the analysis. Of the obtained impedance data, the resistance at 8Hz ($\underline{R}8$), at 28Hz ($\underline{R}28$), the difference between $\underline{R}28$ and $\underline{R}8$ (frequency dependence (FD), as a measure for the course of the resistance versus frequency curve) the reactance at 8Hz ($\underline{X}8$) and the resonant frequency (f_0) were used for analysis. $\underline{R}8$ was chosen because at this frequency usually coherence functions greater than 0.95 are obtained, which are not always obtained at lower frequencies during airflow limitation. Impedance measurements were performed before spirometry because forced inspirations may influence bronchial smooth muscle tone (Orehek, 1981). The equipment was

calibrated daily and all measurements were performed using the same apparatus, by the same investigator.

Data analysis

Group data were expressed as means ± standard error of the mean. Asthmatic responses to isocyanates were classified (early, late or dual) and quantified based on graphical analysis of impedance or flow volume parameters vs time plots (e.g. Figure 5.1). Maximum fall of a parameter was defined as the largest difference in values measured. Variation coëfficients of the lung function parameters were calculated from sham exposure data as:

$$\frac{1}{n}\sum_{i=1}^{i=n}\frac{(X_i - X)^2}{X} \times 100$$

RESULTS

Clinical findings

Six out of eleven subjects with suspected isocyanate asthma showed a positive response to challenge; two persons had an early reaction and four had a late reaction (Table 5.1). Subjects in the group with a negative response to isocyanate challenge were slightly older, but the difference in age was not significant. The duration of occupational exposure to TDI or MDI ranged from 1 to 30 y. Four persons with a positive response and four with a negative response were atopic (positive skin reaction to one or more common aeroallergen extracts). Three subjects with a late response and one subject with a negative response to isocyanate challenge were not reactive to methacholine ($PD_{20}FEV_1$ > 1.4 mg) before and after isocyanate challenge. Of the other six subjects 5 showed a decrease in $PD_{20}FEV_1$ after isocyanate challenge. In one subject $PD_{20}FEV_1$ was not measured due to the severity of the response after TDI challenge. This subject had to be treated medically in order to return to baseline spirometric values, the other responders returned spontaneously to baseline spirometric values.

Sham and Challenge Findings

Table 5.2 shows maximum changes in FEV_1%, $\underline{R}8$ and $\underline{X}8$ during sham exposure and isocyanate challenge. Within day variability derived from shams

only ranged from 2-5% for FEV_1, from 2-8% for R8 and from 8-10% for f_0. During sham exposure the average maximum fall in FEV_1 was 7% (SE 1.50) compared to baseline in isocyanate responders. R8 increased 0.49 hPa.s/L while X8 decreased 0.31 hPa.s/L compared to baseline values. During challenge, the average maximum fall in FEV_1 was 39% compared to baseline; increase in R8 was 2.21 hPa.s/L, and the decrease in X8 was 1.51 hPa.s/L. Changes in lung function after challenge were approximately five times larger than after sham exposure (FEV_1 7/39, R8 0.49/2.21, and X8 0.31/1.51).

TABLE 5.2: Characteristics of the study population

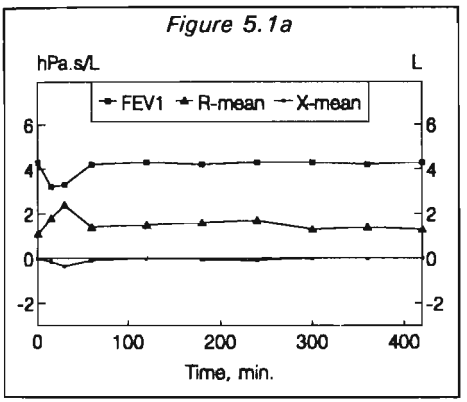
Subject No.	Reaction type	Max. fall FEV_1 sham	Max. fall FEV_1 challenge	Max. fall PEF% challenge	Max. fall MEF% challenge	Max. incr. R8 sham	Max. incr. R8 challenge	Max. incr. X8 sham	Max. incr. R8 challenge
Positive									
1	early	10%	88%	71%	81%	0.43	4.09	0.32	4.47
2	early	1%	31%	39%	64%	0.11	1.37	0.16	0.56
3	late	9%	26%	24%	44%	0.47	1.76	0.24	1.05
4	late	12%	31%	28%	40%	1.16	3.36	0.46	2.46
5	late	5%	40%	41%	61%	0.32	1.06	0.22	0.29
6	late	5%	21%	20%	31%	0.48	1.63	0.48	0.24
Average		7%	39%	37%	52%	0.49	2.21	0.31	1.51
SD		(1.50)	(9.21)	(7.35)	(6.38)	(0.14)	(0.90)	(0.05)	(0.68)
Negative									
7	-	5%	6%	3%	6%	0.63	0.47	0.12	0.19
8	-	10%	10%	8%	10%	0.84	0.49	0.34	0.33
9	-	13%	12%	17%	20%	0.44	0.55	0.43	0.27
10	-	9%	16%	15%	24%	0.61	0.42	0.01	0.21
11	-	9%	9%	7%	9%	0.42	0.11	0.11	0.02
Average		9%	11%	10%	14%	0.59	0.41	0.20	0.20
SD		(1.17)	(1.49)	(2.33)	(3.01)	(0.07)	(0.07)	(0.07)	(0.05)

* nm = not measured, because medical treatment was required due to the severity of reaction.

In subjects with a negative response to isocyanate challenge differences observed between sham and challenge data were not significant: average maximum fall in FEV_1 was 9% during sham and changes to 11% after challenge, average maximum increase in R8 decreased slightly whereas average maximum decrease in X8 did not alter after challenge. Average changes in PEF and MEF_{75-25} were in the same order of magnitude as the changes observed in FEV_1 both during sham and challenge.

Since maximum average changes in one single parameter do not provide details about the response measured by both techniques, response versus time curves were plotted for FEV_1, R8 and X8. An immediate type response pattern is illustrated in figure 5.1a: FEV_1 falls 31% within 30 min, at the same time R8 increased and X8 decreased. A late type response pattern is illustrated

in figure 5.1b: a maximal fall of FEV_1 of 27% was found six hours after challenge. At the same time R8 increased and X8 decreased. In all responses consistent profiles of impedance parameters (R8, X8) compared to FEV_1 were obtained. Changes in impedance indices occurred at equal time-points in the same individuals as decreases in FEV_1. Recovery from bronchoconstriction also revealed no differences. In the subject that was treated with ß2-sympaticomimetics for severe airway obstruction impedance values returned to baseline within 10 minutes, whereas flow volume parameters returned to baseline within 2 hours.



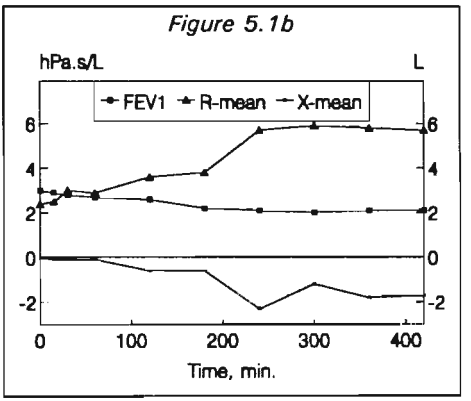
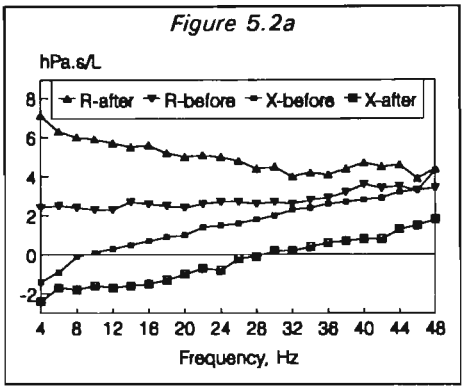


FIGURE 5.1: Response versus time profiles in spirometric (FEV_1) and impedance (R8 and X8) parameters in an early (Figure 5.1a) and a late (Figure 5.1b) airway response to isocyanate challenge.

Impedance patterns

In late responders two distinctly different reaction patterns to isocyanate provocation were observed by impedance measurements. Figure 5.2a shows the first reaction pattern observed in late reactors no. 3 and 4. Six hours after challenge R was increased at all frequencies but especially at lower frequencies. R8 increased from 2.26 hPa.s/L to 5.98 hPa.s/L and R became frequency dependent, i.e. the slope of R between R8 and R28 had a negative value. At the same time X was decreased at all frequencies. This resulted in an increase in f_0 from 8.76 Hz to 27.67 Hz. This pattern was also observed in both early responders (no. 1 and 2) and in positive methacholine challenges. Figure 5.2b shows the second reaction pattern observed in late reactors (no. 5 and 6). A parallel increase in R was observed at all frequencies. The average resistance increased from 1.87 to 3.21 hPa.s/L in the absence of frequency dependence. No clear changes in X were observed and thus f_0 remained almost unchanged (8.07 Hz before and 8.32 Hz after challenge).



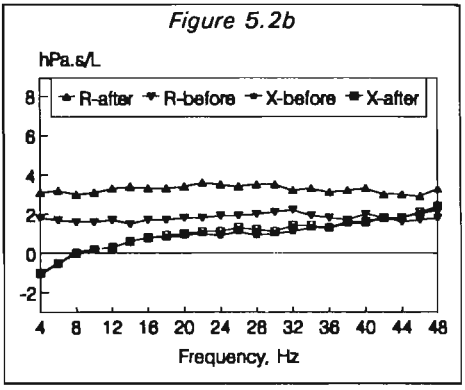


FIGURE 5.2: Mean resistance (R) and reactance (X) values (hPa.s/L) before and 5 h after TDI challenge in a subject with peripheral airways obstruction during a late asthmatic response (Figure 5.2a) and, before and 6 h after TDI challenge in a subject with central airways obstruction during a late asthmatic response to TDI (Figure 5.2b).

DISCUSSION

In this study we have confirmed distinctly different responses to isocyanate challenge. Both early and late responses were observed and changes in FEV_1 and impedance parameters R8 and X8 were consistent. A dual response was not observed. Furthermore similar response versus time patterns were observed for these three parameters. Analysis of the impedance findings in subjects with a positive isocyanate challenge test revealed two different response patterns. The first response pattern, characterized by an increase in the oscillatory resistance especially at lower frequencies, and a decrease in resistance with increasing frequency was observed in two early and two late reactions. In these subjects more negative values of reactance at lower frequencies and an increase in resonant frequency were found. The second response pattern consisted of a large fall in FEV_1 which was neither accompanied by frequency dependence, nor by significant changes in the reactance of the respiratory system; however a parallel increase in R at all frequencies was observed. The latter pattern was observed in two late reactions.

The factors determine the bronchial response to isocyanates in sensitizised subjects are largely unknown. Recently, Vandenplas *et al.* concluded that the main determinant of bronchial hyperresponsiveness is not concentration nor duration of exposure per se but the product of both factors that is, total dose (1993). They showed that the pattern of reaction may change depending upon concentration, although cumulative dose remained constant. Another important component of bronchial asthma is nonspecific bronchial hyperresponsiveness to methacholine aerosol. A number of investigations have

indicated that nonspecific bronchial hyperresponsiveness is also present among TDI reactors (Butcher, 1977; Chester, 1979). Burge found that there was a correlation between the dose of toluene diisocyanate (TDI) required to induce an asthmatic reaction in 30 subjects and the level of histamine reactivity (Burge, 1982). A major question is whether nonspecific bronchial hyperresponsiveness is a predisposing factor or a result of the asthma. Bronchial hyperresponsiveness may wane when the patient has been away from exposure for a period of time and may never become abnormal in those with immediate asthmatic reactions only (Lam, 1979). This is confirmed by case reports of patients with an immediate response upon TDI challenge without bronchial hyperresponsiveness (Smith, 1980; Mapp, 1986-a). In our study three subjects with a positive response to isocyanates did not respond to methacholine challenge (maximal dose 1,440 μg) before nor 8 h after isocyanate challenge. In subject no 2 an immediate reaction was elicited in the absence of bronchial hyperresponsiveness. In late but not early asthmatic reactions induced by TDI, Mapp *et al.* (1986-b) reported increased hyperresponsiveness to methacholine. This finding was confirmed in subjects no 3 and 4, but absent in subjects no 5 and 6 showing late asthmatic responses upon isocyanate challenge without bronchial hyperresponsiveness to methacholine. One might discuss the maximal level of methacholine challenge as being low since Vandenplas *et al.* observed a late reaction to TDI accompanied by a drop in PC_{20} from 5.6 to 2.3 mg/ml (Vandenplas, 1993), but interestingly, these findings were accompanied by a different pattern of impedance parameters as compared to late responders with bronchial hyperresponsiveness both in a reactor to TDI and a reactor to MDI.

In bronchial challenge tests impedance measurements have been used by several investigators (Noord, 1989; Wouters, 1988). Some of these studies have provided comparisons of absolute changes in spirometric values and impedance data in induced bronchoconstriction (Noord, 1989; Wouters, 1988). The sensitivity of this technique in detecting induced bronchoconctriction has been evaluated by several investigators. Van Noord *et al.* (1989) concluded that the forced oscillation technique (FOT) is a sensitive indicator of induced changes in airway calibre: the sensitivity of impedance derived parameters was between that of the specific airway conductance (sGaw) using a bodyplethysmograph and FEV_1. Yet, response evaluation measuring sGaw or FEV_1, allows only a quantitative assessment of the response, whereas the FOT may enable analysis of the response to bronchoactive agents with respect to localization of response along the tracheobronchial tree.

Frequency dependence of resistance, indicating a decrease of resistance with increasing frequency is described as a common finding in patients with COPD (Grimby, 1968; Hyatt 1970; Michaelson; 1975, Hayes, 1979; Clément, 1983) and in asthmatics after induced bronchoconstriction (Wouters, 1989; Noord, 1989). Separate analysis of the frequency dependence of pulmonary resistance, chest wall resistance and resistance of the total respiratory system

has demonstrated that frequency dependence of the total respiratory system can be explained by the frequency dependence of the pulmonary resistance (Kjeldgaard, 1976; Nagels, 1980). The frequency dependence of resistance over an extended frequency range was explained by different models of the respiratory system (Cutillo, 1983), characterized by partitioning of the airway resistance and shunt capacitances. In these models frequency dependence of resistance is a reflection of an increase in peripheral airway resistance, and the capacitance of the airways act as compliant structures mechanically in parallel with the airspaces which they supply (Mead, 1969; Cauberghs, 1983; Ying, 1990).

The parallel increase of resistance at all frequencies without frequency dependence can be explained by the response localization to isocyanates in the central airways. Evidence for this explanation can be derived from studies in anaesthesized tracheostomized dogs (Harf, 1985, Bates, 1986) demonstrating that a two- to threefold increase in central airway resistance by narrowing the trachea did not result in a negative frequency dependence of resistance, and from a study demonstrating a parallel increase of resistance during normal breathing after narrowing the upper airway by external application of pressure to the trachea (Coe, 1988).

It has to be noted that the results of the impedance measurements are not corrected for possible changes of lung volume: frequency dependence of resistance decreased at higher lung volumes (Ying, 1990). Therefore, air trapping as reported during acute broncho-obstructive responses can lead to an underestimation of resistance and frequency dependence and an overestimation of reactance, mainly at lower frequencies (Mead, 1969).

In conclusion, our impedance data support previous findings that the response after isocyanate challenge may be localized in the peripheral as well as in the central airways. The small population size and the different response patterns observed however, do not allow speculations on the mechanism(s) involved. Although impedance measurements seemed to have the same sensitivity as flow volume curves in the response assessment in isocyaate challenges, i.e. impedance measurements showed the same response vs time patterns, two different response patterns were observed, indicating that impedance measurements provide information on airway disease not supplied by history and free floating spirometry. Larger groups have to be studied to confirm the two response patterns observed and to investigate possible differences in underlying mechanisms.

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CHAPTER 6

A two year follow-up of lung function in chemical workers

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ABSTRACT

A two year follow-up of 145 workers by flow volume and impedance measurements was conducted to study the influence of chemical exposure on lung function and to relate rapid declines in FEV_1 to changes in impedance parameters in order to evaluate the contribution of airway obstruction in a possible decline in FEV_1.

The average decline in FEV_1 over the two years was 80 ml which was not markedly different from the predicted decline of 64 ml, i.e. an excessive decline of 8 ml/year. The prevalence of airflow obstruction (FEV_1 < 80% predicted) was about 8% in both measurement periods. The prevalence of airflow obstruction was significantly increased among workers of the acids producing plant (Odd's ratio 3.00, confidence interval 1.19 - 7.59, $P < 0.05$). A rapid decline in FEV_1 was related to a similar significant decrease in FVC (280 ml/2 years; $P < 0.05$) and to minimal changes in impedance parameters.

It is concluded that this study population is not at increased risk to obstructive lung function deterioration due to chemical exposure, and that impedance parameters might provide a simple tool to provide additional information on mechanical characteristics of the respiratory system in order to explain an observed decline in FEV_1.

INTRODUCTION

A progressive decline in forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) is related to occupational exposure as recently described in several population-based studies (Heederik, 1992; Oxman, 1993; Bakke, 1991; Kauffmann, 1982; Viegi, 1991). Both inorganic and organic dust (Becklake, 1985; Cotes, 1987) as well as chemical exposure (Clayton, 1981) are described as causal factors. Other factors related to a progressive decline in FEV_1 include aging (Cauberghs, 1993), smoking (Peat, 1990), fat mass (Higgins, 1982), airway obstruction (Clément, 1982), bronchial hyperresponsiveness (Parker, 1990; Lim, 1988) and environmental exposure (Kaufmann, 1982). Although a decline in FEV_1 is generally considered as a measure of progressive airflow obstrucion, different mechanical alterations of the respiratory system can in/fluence this co-operation dependent parameter. To provide information on the nature of alterations in FEV_1 additional measurements not dependent of active co-operation of the subjects can be used which reflect indirectly the degree of airway obstruction. In order to provide this information impedance measurements of the respiratory system by the technique of forced oscillations (Làndsér, 1976) can be considered as an easy and reliable technique to assess the mechanical characteristics of the respiratory system. Brochard *et al.* (1987) concluded from a study among workers from a gas manufacturing plant that the forced oscillation technique affords sufficient sensitivity to distinguish subjects exposed to respiratory irritants at a stage when mid expiratory flow volume (MEFV) parameters are not yet modified. Several cross-sectional investigations (Brochard, 1987; Jorna, 1992-a,b, 1993) have shown that impedance measurements offer a simple non-invasive instrument to detect airway obstuction in different forms of exposure. The importance of combined information was recently demonstrated by the study of Cauberghs *et al.* (1993) who found no significant changes between groups of smokers and non-smokers with normal and rapid decline in FEV_1 (decline of FEV_1 > 20 ml/y); the rapid decline in FEV_1 was related to a significant decrease in diffusing capacity, suggestive for the development of emphysema in the rapidly declining smokers.

The present study was performed to assess the prevalence of airflow obstruction by combined measurement of flow volume curves and impedance in a group of workers from a chemical plant in relation to chemical exposure. Furthermore, follow-up data on changes of flow volume and impedance data were obtained after a 2 year follow-up period.

MATERIAL AND METHODS

Study population and protocol

Male workers (n = 160) from a chemical plant were investigated during routine yearly medical control in the months of April and May 1990. Prior to investigation written informed consent was obtained. All subjects completed three flow volume curves and three impedance measurements. Impedance measurements were always performed before flow volume curves to avoid the influence of forced inspiratory manoeuvers on the bronchial tonus (Orehek, 1981). In the months of April and May 1992 the same procedure was repeated after evaluation of a questionnaire on respiratory symptoms, smoking and working history in the same group of workers (n = 150). Finally, 145 complete data-sets were obtained and analyzed. Chemical agents with possible respiratory hazards related to the most important plants and production processes were retrieved from the company's chemical safety data-base. Eight production groups were distinguished: chemicals, acids, polymers, polyurethanes, fibers, bulk/transport and maintainance personnel and a control (not exposed to hazardous chemicals). Individual exposure was qualitatively retrieved from medical files and through monitoring records.

Lung function measurements

Forced expiratory flow volume curves were recorded according to European Community of Coal and Steel (ECCS) criteria using a dry spirometer (Vitalograph Ltd, Buckingham, England). Three recordings within 5% or a 100 ml range were obtained from each subject. Parameters derived from the flow volume curves were: FVC, FEV_1, FEV_1/FVC ratio, peak expiratory flow (PEF), maximal mid expiratory flow (MEF). The first 4 parameters describe the effort-dependent part of the flow volume curve and the last one is generally considered as a reflection of the effort-independent part of the flow volume curve. All flow volume values were related to the reference values of the ECCS (Quanjer, 1983) for individual diagnosis. The spirometer was calibrated at regular intervals and all subjects were measured on the same spirometer.

Impedance (Z) of the respiratory system was measured using the technique descibed by Làndsér *et al.* (1976). The method was previously described by Wouters *et al.* (1989). Mean values of three successive measurements, each lasting 8 seconds were used in the analysis. Of the obtained impedance data, the resistance at 8Hz ($\underline{R}8$), at 28Hz ($\underline{R}28$), the difference between $\underline{R}28$ and $\underline{R}8$ (frequency dependence (FD), signifying the course of the resistance versus frequency curve), the reactance at 8Hz ($\underline{X}8$) and the resonant frequency (f_0) were used for analysis. Criterium for acceptation of an impedance value was a coherence function ≥ 0.95. $\underline{R}8$ was chosen because at

this frequency usually values of coherence function ≥ 0.95 were obtained, which was not always the case at lower frequencies. A negative FD and an increase in f_0 were considered as criteria of airway obstruction. The equipment was calibrated daily and all measurements were performed using the same apparatus, by the same investigator in both studies.

Statistical analysis

Packyears smoking was calculated from the questionnaire (Table 6.1). Non-smokers were life time abstainers, ex-smokers had quit smoking before entry in 1990, and smokers did generally not alter smoking habits during the period of follow-up. One subject had quit smoking during the observation period.

TABLE 6.1: Average results (mean ($\bar{X}$) and standard deviation (SD)) of flow volume and impedance parameters of the same group of chemical workers (n = 145) in 1990 and 1992.

Parameter	Unit	1990		1992		Correlation Coefficient[$]
		$\bar{X}$	SD	$\bar{X}$	SD	
GENERAL						
Age	y	38.8	9.10	40.8	9.10	
Height	cm	176.5	6.40	177.0	6.50	
Weight	kg	78.6	9.40	79.4	8.90	
Smokers current	n	48	-	47	-	
past	n	47	-	48	-	
never	n	50	-	50	-	
Cigarettes x 1,000	n	66.4	80.0	69.8	83.3	
FLOW VOLUME						
FVC	L	5.23	0.76	5.16	0.73	0.93**
FEV_1	L	3.95	0.68	3.87	0.69	0.92**
FEV_1/FVC	%	75.22	7.60	73.98	7.51	0.69*
PEF	L/s	8.63	1.71	8.83	1.63	0.59
MEF	L/s	4.31	1.24	4.07	1.24	0.82**
IMPEDANCE						
R8	hPa.s/L	2.41	0.77	2.36	0.74	0.62
FD	hPa.s/L	0.46	0.54	0.34	0.48	0.40*
X8	hPa.s/L	0.13	0.33	0.11	0.26	0.48
f_0	Hz	7.92	3.07	7.97	2.38	0.56

[$] correlation between (individual) 1990 and 1992 values.
Significance was tested by Student's t-test: * $P < 0.05$; ** $P < 0.01$.

Average lung function data for the different groups were tested for significance using the Student's t-test. Relationships between alterations in FEV_1 and changes in impedance parameters were tested by a Chi-square test. Differences between groups were tested by analysis of variance (ANOVA). Airflow obstruction was defined as a $FEV_1 < 80\%$ predicted for flow volume curves or as a negative FD concurrent with an $f_0 > 15$ Hz for impedance measurements. Chronic bronchitis was defined as persistent cough for 3 three months in two subsequent years. Odd's ratios (OR) and confidence intervals (CI) for airflow obstruction were calculated per production process. For the calculation of the OR's, workers not occupied in this process were regarded as controls. All procedures if not stated otherwise were computed with SPSS-X program (SPSS Inc., Ill, USA).

RESULTS

General data

Although small differences in lung function parameters were observed (Table 6.1) between the two years, none reached the level of statistical significance. Only one subject changed smoking habits during the observation period. Correlation coefficients for FVC, FEV_1, FEV_1/FVC ratio, MEF and FD between 1990 and 1992 data were significant ($P < 0.05$; Table 6.1), and higher for spirometric parameters compared to impedance parameters. The decrement in FEV_1 of 80 ml in two years did not differ significantly from the predicted decline of 64 ml based on a 2 year increase of age at the same height. This resulted in an average excessive decline of 16 ml/2 y compared to reference equations derived from healthy non-smoking controls. Average values of impedance parameters could be considered as normal in both years: a resonance frequency of approximately 8 Hz and a positive frequency dependence of resistance. Air flow limitation defined as a $FEV_1 < 80\%$ predicted was present in 8% of the subjects (n = 12) in 1990 and in 7% of the subjects (n = 10) in 1992. Eight subjects had air flow limitation both in 1990 and 1992, six subjects were included in one year only. Airway obstruction according to impedance criteria was present in 9% (n = 14) and 8% (n = 12) of the subjects in 1990 and 1992 respectively.

Impedance parameters accompanying a rapid decline in FEV_1

Correlation coefficients between flow volume and impedance parameters were calculated to compare the outcome of both measurements. All impedance parameters $\underline{R}8$, $\underline{X}8$, FD, and f_0 were significantly correlated to FEV_1% predicted and to almost all the other flow volume parameters (FVC%

predicted, FEV_1/FVC, and MEF% predicted). Predicted values for spirometric parameters were used to exclude the influence of age and height. Correlation coefficients between FEV_1 and impedance parameters were similar in both years, therefore only correlation coefficients of 1992 (Table 6.2) are shown. In general these coefficients were low.

TABLE 6.2: Correlation coefficients of flow volume and impedance parameters in measurements performed at follow-up in 145 subjects.

Parameter	R8 (hPa.s/L)	X8 (hPa.s/L)	FD (hPa.s/L)	f_0 (Hz)
FEV_1 (%)	−0.26**	0.35**	0.14	−0.38**
FVC (%)	−0.21*	0.28**	0.06	−0.30**
FEV_1/FVC (%)	−0.20*	0.26**	0.17	−0.26**
MEF (%)	−0.14	0.26**	0.18	−0.25**

Levels of significance, see footnote Table 6.1.

Since no criteria for a rapid decline in FEV_1 are available we sought for a surrogate subdivision. Alterations in FEV_1 were arranged in order of size. The total group was then divided into 4 groups with an approximately equal number of subjects; $\blacktriangle FEV_1 < -210$ ml (n = 38), $\blacktriangle FEV_1 < -90$ ml (n = 34), $\blacktriangle FEV_1 < 0$ ml (n = 37) and $\blacktriangle FEV_1 > 0$ ml (n = 36). These groups were compared with regard to changes in flow volume and impedance parameters. The decline in FEV_1 was accompanied by a decline in FVC, FEV_1/FVC ratio and MEF (Table 6.3). With increasing decline in FEV_1 over two years R8 and f_0 tended to increase, whereas X8 tended to decrease and FD remained the same. These trends were only significant between the group with the largest decline in FEV_1 (> 210 ml decline) compared to the group with an increase in FEV_1 (ANOVA; $P < 0.05$). Reported smoking and respiratory complaints (chronic bronchitis, wheezing and shortness of breath) in the 4 groups of FEV_1 alterations showed that subjects with the most rapid decline in FEV_1 smoked less ($P < 0.05$) and had slightly more complaints of shortness of breath without higher prevalence of chronic bronchitis than their collagues with more stable FEV_1 values.

TABLE 6.3: Relationship between alterations in FEV_1 (▲), alterations in other lung function parameters (FVC, FEV_1/FVC, MEF, R8, X8, FD, f_0), packyears smoking and respiratory complaints (1992).

Parameter	Unit	▲ < −0.21L n = 38	▲ < −0.09L n = 34	▲ < 0.0L n = 37	▲ > 0.0L n = 36
FVC	L	−0.28*	−0.10*	−0.02	0.05
FEV_1/FVC	%	−1.41**	−1.33**	−0.34*	1.32
MEF	L/s	−0.58**	−0.50**	−0.19*	0.22
R8	hPa.s/L	0.11*	−0.03	−0.03	−0.19
FD	hPa.s/L	−0.11*	−0.06	−0.06	0.03
X8	hPa.s/L	−0.10	−0.16*	−0.16	−0.08
f_0	Hz	1.10**	−0.09	−0.09	−0.49
Packyears	y	5.4	7.5	7.5	8.1
Chronic bronchitis	%	10	17	17	32
Shortness of breath	%	24	20	20	17
Wheezing	%	14	17	17	20

Levels of significance, Chi square test: * $P < 0.05$, ** $P < 0.01$
Explanation of parameters see Table 6.1.

Odd's ratios (Table 6.4) relating airflow obstruction to different plants revealed an increased prevalence of risk for airflow obstruction in all plants except the polyurethane plant. However, the Odd's ratio for the acid producing plant (3.00, confidence interval 1.19 - 7.59; $P < 0.05$) was the only reaching statistical significance. These are crude Odd's ratios because no correction for smoking was made. Workers from the acid producing plant smoked on average significantly more than others (23 vs 16 cigarettes/day).

TABLE 6.4: Odds' ratios indicating the presence of airflow obstruction in the different plants. Data not adjusted for smoking.

Plant	Odds Ratio	Confidence interval (90%)
Chemicals	1.2	0.16 - 7.30
Acids	3.0*	1.19 - 7.59
Polymers	2.4	0.56 - 10.70
Polyurethanes	0.5	0.16 - 1.40
Fibers	2.1	0.64 - 7.06
Bulk/transport	1.5	0.81 - 2.83
Maintainance	2.3	0.90 - 8.98

DISCUSSION

In this study an average small decline in FEV_1 of 16 ml/2 years was attributed to occupational chemical exposure. In addition, minor changes in impedance parameters were observed over this period. These data indicate that the total group is not at an excessive risk for lung function decline due to occupational exposure. This is also reflected in a low prevalence of air flow limitation, i.e. 10% in 1990 and 8% in 1992, based on flow volume criteria. Based on impedance data, similar prevalence rated were observed. The fact that not necessarily the same subjects were obstructive in both years indicates that the obstruction was reversible in some subjects. Odd's ratios however, revealed that workers from the acid producing plant are at increased risk to airflow obstruction, probably due to the strong irritating effects of the compounds used like phosphoric-, nitric-, sulphuric acid, or to the smoking habits.

The rate of decline of FEV_1 is generally used to detect changes in lung function after different forms of exposure. Frequently, no further information is obtained on the nature of these alterations in FEV_1. To provide this information, impedance measurements were performed in the present study. Impedance measurements are sensitive in detecting air flow limitation as was shown by measurements in patients with COPD and asthma (Wouters, 1989), and in positive challenge tests to non-specific stimuli like histamine and methacholine (Wouters, 1990) and specific chemical stimuli like toluene diisocyanate (Jorna, 1993). In these tests air flow limitation is defined as a decrease of FEV_1 (> 20% compared to baseline) is usually accompanied by an increase in resistance at lower frequencies resulting in a negative frequency dependence concurrent with a decrease of reactance at all frequencies.

A rapid decline in FEV_1 in this study was associated with minimal changes in impedance parameters. The rapid decline in FEV_1 was mainly accompanied by changes in FVC. Similar findings were recently reported in smokers and non-smokers where a rapid decline in FEV_1 was associated with a significant decrease in VC, without significant changes in impedance parameters (Cauberghs, 1993). In that study subjects with a rapid decline in FEV_1 tended to demonstrate lower values of DL_{CO}, weight and FEV_1/VC, suggestive for the development of emphysema in this group of smokers. The authors (Cauberghs, 1993) concluded that the fact that carbon monoxyde diffusing capacity turned out to discriminate between people with a rapid and a normal demographic decline pointed out the possibility of using this measurement, in combination with FEV_1/VC and weight as a predictor of rapid decline of lung function in middle-aged healthy male smokers.

Reduction in simple breath diffusing capacity is an early finding after smoke inhalation. Measurement of diffusing capacity can not only be used to predict the development of chronic pulmonary disease, but also as a predictor of arterial oxygen desaturation during exercise in this patient group (Owens, 1984) or to evaluate exertional dyspnea (Mohsenifar, 1992).

Remarkably smoking habits, expressed in packyears, was significantly lower in the group of workers with rapid decline in FEV_1. An adaptive response possibly related to a higher intensity of breathlessness or an increased sensitivity for the harmful effects of smoke inhalation can be hypothesized. Otherwise, the group of workers with no decline in FEV_1 has a relatively higher prevalence of symptoms of chronic bronchitis compared to the rapid declinig group. These data are in accordance with previous reports on the effects of smoke inhalation on large and small airways (Burns, 1991).

During the first years of smoking a rapid decline in FEV_1 may be observed which gradually leads to a relatively small decline or steady state. This might explain why the group with no decline in FEV_1 smoked a higher number of cigarettes. In line with this finding is the higher prevalence of chronic bronchitis and wheezing. Shortness of breath was experienced most prevalent in the group with the most rapid decline in FEV_1.

The influence of exposure on the prevalence of air flow obstruction is largely influenced by the smoking habits. Workers form the acid producing plant did smoke significantly more than other workers. On the other hand the possible influence of exposure to the acid aerosols H_2SO_4 and HNO_3 and their precursors should not be neglected since they are strong irritants which are more potent in subjects with asthma and chhronic bronchitis (Folinsbee, 1992). Short periods of inhalation of H_2SO_4 have a variable effect on airway function with some studies showing eveidence of airflow obstruction and others showing no change even at high concentrations (1,000 $\mu g/m^3$) for up to 2 hr in normal subjects (Hackney, 1989). Asthmatics appear to be more susceptible with changes occurring at concentrations of 350 $\mu g/m^3$ in adults (Koenig, 1983). In the presence of low concentrations of ammonia in the mouth acid aerosols may be much more potent bronchoconstrictors than has hitherto appreciated. Studies of exposure to HNO_3 vapor have been performed only recently. HNO_3 vapor is taken up almost entirely in the upper airways. Latter study suggests possible pulmonary function responses in asthmatics and some alteration in macrophage function (Koenig, 1989). The extent of the effect of occupational acid aerosol exposure on lung function of subjects with air flow limitation has to be resolved.

It is concluded that this study population is not at increased risk to lung function deterioration due to chemical exposure, and that impedance parameters might provide a simple tool to provide information on mechanical characteristics of the respiratory system in order to explain an observed decline in FEV_1.

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CHAPTER 7

General discussion

The need for a proper appreciation and quantification of causes in occupational lung disease is underscored by the dimension of the problem described by several recent reviews (Cullen, 1990-a,b; Heederik, 1992; Oxman, 1993), the results described in this thesis and the awareness that a significant proportion of occupational lung diseases remains unrecognized. Lung diseases are rarely as specific as they used to be, no longer take epidemic proportions, but arise in more susceptible individuals. This is one reason why research on occupational lung disease nowadays is a cross road for diverse scientific disciplines, where the skills and viewpoints of physicians and biologists must be combined with those of epidemiologists, inhalation toxicologists, engineers and many other specialists. It is only through this multidisciplinary approach that we can achieve understanding and then practice control of the factors that induce occupational lung disease. We applied such a multidisciplinary approach integrating exposure measurements, lung function measurements, questionnaires and biological (effect) markers to several workplace populations and observed several features that forward further discussion.

When summarizing data on airflow obstruction (Table 7.1) in the different surveys (all male workers) the total average prevalence of a $FEV_1 < 80\%$ is 8.1% (n = 613). Age seemed to be no major descriptor of the observed differences between groups and, interestingly, total group prevalence is the same in exposed and non-exposed workers, suggesting no effect at all of exposure. The prevalence observed in the surveys among potato sorters (13.4%), chemical workers (8.3%) and - notably - grain workers (3.1%) are lower than data from open population studies. These figures are likely to be underestimations due to the "healthy worker effect", the exclusion of people

ill at the time of study and a (expected) lower response among people with some illness. Roughly, the total average prevalence of airflow obstruction assessed by FOT criteria (f_0 > 15 concurrent with frequency dependence of resistance) was in the same order (10.4%) to that found by spirometry (8.1%). Similar conclusion could be drawn from the study among workers exposed to inorganic diatomaceous earth, where airway obstruction is not different among exposed and controls and not dependent on the method used. Clear differences in prevalence of airway obstruction however, were measured by FOT between controls and exposed grain dust workers (5.5% vs 18.7%), whereas these differences were not observed by spirometry (3.7% vs 2.8%). Unfortunately, in the studies among coal miners and chemical workers no control group was available for such a comparison.

TABLE 7.1: Prevalence of airflow obstruction in the populations of male workers studied in cross-sectional studies described this thesis.

Population		% Obstruction[A]			% Obstruction[A]				% Obstruction[A]			
		n	FEV_1	FOT	n	Age	FEV_1	FOT	n	Age	FEV_1	FOT
Coal miners	b	102	8.8	9.8	102	49.4	8.8	9.8	-	-	-	-
Potato sorters	c	172	13.4	8.7	117	46.4	13.4	8.5	55	38.3	12.7	9.1
Grain workers	c	194	3.1	14.9	139	42.3	***2.8***	***18.7***	54	38.9	***3.7***	***5.5***
Chemical workers 1990	d	145	8.3	6.9	145	38.8	8.3	6.3	-	-	-	-
Total		613	8.1	10.4	504	43.6	8.1	11.1	109	38.6	8.2	8.2

[A] Criteria for airway obstruction (yes/no) were FEV_1 < 80% for spirometry and f_0 > 15 and FD < 0 for FOT

b Exposed only and originally selected for a case-control (1:2) study

c Studies have internal, not-exposed control group

d Exposed only

Differences in impedance patterns were demonstrated after inorganic or organic dust exposure. In workers exposed to inorganic diatomaceous dust a parallel increase of resistance over all frequencies was observed compared to controls, suggesting an obstruction in the upper airways (van Noord, 1989). On the other hand, in grain dust workers an asthma-like pattern of impedance was observed, i.e. a frequency dependence of resistance in the range of 8 - 28 Hz and concurrently a decrease of reactance. The latter pattern suggests an airway obstruction extending to the peripheral airways (Wouters, 1990).

Explanations for the incompatible impedance patterns in potato sorters and grain dust workers could be sought in variables determining biologically active dose during and after exposure such as exposure levels and duration, deposition, durability and defence. Average exposure levels were similar in potato sorters (8.2 mg/m^3) and grain dust workers (9.4 mg/m^3) and workers had spent equal time in the industry (13 respectively 14 years), suggesting a similar dose of respirable dust in both studies. Although the cumulative exposure seems equivalent, the pattern of deposition -and, therefore, the internal dose- is highly dependent on particle size distribution. Unfortunately, we have no data on this variable but there are no reasons that indicate deposition as a determining factor in the impedance patterns. In fact, changes in both impedance patterns are directly related to external exposure.

Defense mechanisms, including mucociliary clearance and (non) immunological tools, constitute another source of variables that might explain for differences in impedance patterns between inorganic (diatomaceous) and organic (grain) dust. Although all defense lines are used after inhalation of both types of dust, it can be hypothesized that the inflammatory axis plays a role only at high (chronic) dose of inorganic dust, while immunologic pathways are readily triggered in the short-time exposure to grain dust (Zejda, 1992). Among the various mechanisms and mediators that are involved, TNF was shown to play an essential role in the non-humoral response to inhalation of both types of dusts. Previously, it was demonstrated by us (Borm, 1988) and others (Lassalle, 1991) that monocytes or AM of coal miners release more TNF upon mild stimulation and especially at early stages of CWP and during PMF. Recent studies (Gosset, 1991-a; Dubar, 1993) have demonstrated that in addition to silica, other factors are also involved. On the other hand, monocyte or AM TNF-α release has also been shown to be affected in the (IgE-mediated) asthmatic response to occupational agents (Siracusa, 1992; Gosset, 1991-b). Moreover, in a rat model Kips (1992) showed that TNF is a crucial mediator in bronchial hyperresponsiveness and airway inflammation caused by endotoxin.

Interestingly, we demonstrated that monocyte TNF-α secretion was increased in retired coal miners with airflow obstruction, independent of the presence of CWP. If the increased monocyte TNF-α release is a consequence of the airflow obstruction, this could only be mediated by a secondary, priming factor (Mohr, 1992) that could be tested in subjects before and on several time-points after a nonspecific or a specific challenge. This finding does however, confirm that the TNF-α is not only involved in IgE driven responses (Gosset, 1991-b) but also in non-specific airway obstruction as caused by inorganic dust exposure. Organic dust on the other hand contains animal and plant derived proteins and bacterial LPS. Proteins are thought to trigger IgE dependent asthma-like reactions in the airways, which could explain the

asthma-like impedance pattern observed in exposed workers. Bronchial responses to inhaled endotoxin are probably due to nonspecific proinflammatory effects (Kips, 1992). Both cellular and humoral response response were shown to be involved in the airflow obstruction induced by inhalation challenge with LPS or grain-dust extracts in normals and asthmatics (Rylander, 1989; Kennedy, 1987). These findings are supported by our study among fodder workers, where a dose-response relationship was observed between lung function and exposure to grain-dust, but dust/endotoxin ratio's were a better predictor of lung function decline then inspirable dust levels. Moreover, in a recent follow-up study in a subpopulation (n = 12) of the original cohort of grain workers (Borm, 1994) levels of monocyte TNF-α release were measured before exposure (monday morning, before the shift) and at the end of the week (friday afternoon, after the shift). Results showed elevated levels of TNF-α release by PBM's upon stimulation at the end of the week compared to the beginning of the week. The observed elevation of release was positively related to exposure (inspirable dust) (Borm, 1994).

In conlusion, the multidisciplinary approach applied in the studies among potato sorters, coal miners and grain workers showed that apparently uniform prevalence data based on changes in FEV_1 can be based upon different airway mechanics and different pathophysiologic mechanisms. Without the (more specific) information obtained by biological markers or impedance patterns the link between airflow obstruction and TNF would have been missed and an underestimation of airway disease in grain workers would have been the result. Our observations in isocyanate asthmatics further support for the extra value of impedance measurements when applied with flow-volume measurements in clinical studies. Among four late asthmatic reactions to diisocyanate-challenge two different impedance patterns were observed. From these findings we hypothesized that different compartments of the tracheobronchial system contribute to the response to this sensitizing agent. Early as well as late reactions were characterized by impedance changes that suggest peripheral airway obstruction. In two late asthmatic reactions however, obstruction appeared to be localized in the central airways, based on a parallel increase in resistance and no alteration in resonance frequency. These findings are somewhat contradictionary to the general concept in occupational asthma, starting from the principle that all inflammatory processes in occupational asthma may be studied in the central airways (Saetta, 1992). Since up to now, it is impossible to obtain cells (and mediators) from the peripheral airways it is difficult to corroborate this concept. Perhaps neither concept will fully cover the ungoing processes during an asthmatic reaction and it is more likely that these reactions might be localized all along the tracheobronchial tree. Proof for this could be obtained from experiments investigating the relation between different impedance patterns found in late asthmatic

reactions and different mechanism(s).

Opposite observations with regard to airway obstruction were observed in the follow-up study among workers in chemical industry where a decrease in FEV_1 was not reflected in changes of impedance. A two year follow-up of a group of chemical workers showed that a progressive decline in FEV_1 is not necessarily associated with changes in impedance parameters. The implication of this finding, recently confirmed by Cauberghs *et al.* (Cauberghs, 1993), is that one does not know a priori which functional index or indices will be altered first and thus will turn out to be a useful predictor of decline. It would be of interest to be able to detect those individuals before their pulmonary function is markedly altered. Detection by means of the direct determination of the rate of decline of FEV_1 is impractical, because this necessitates several measurements over a span of 6 to 8 y (Cauberghs, 1993). To this end, the physiologic changes accompanying or preceding a rapid decline in FEV_1 and VC should be known. Cauberghs concluded that the FEV_1 is a global parameter not only related to airway obstruction but also to restrictive impairment. Although cross-shift decline of FEV_1 (Heederik, 1990; Weill, 1993), non specific bronchial hyperreactivity (Enarson, 1987) and atopy (Cockcroft, 1981) are risk factors for the development of airflow obstruction, their predictive value is rather low or unknown.

One alternative could be the use of validated biological (susceptibility) markers derived from immunological or biochemical mechanisms shown to play a role in airway obstruction. Our findings in coal miners suggest that the ability to release mediators (such as TNF) upon ex-vivo stimulation of peripheral blood monocytes might be used as a source to develop markers of airway disease. In retired miners airflow obstruction was clearly related to ex-vivo TNF-α release from peripheral blood monocytes. The investigated group however was to small to discern differences between the relationship of impedance with TNF levels and spirometry and TNF levels. Similar studies in other populations might resolve the question if this finding is of any clinical relevance, or perhaps useful as tool in the screening for subjects susceptible to airway disease. Although we have investigated TNF-alfa, other mediators/cytokines should not be excluded as candidate markers. In fact a candidate biomarker can be derived from any (critical) signal in the continuum of events between causal exposure and resultant disease (Schulte, 1989; Borm, 1994).

Another alternative to detect individuals susceptible to excessive deterioration of airway function might be provided by respiratory symptoms. In our follow-up study among chemical workers, the highest incidence of shortness of breath was observed in the group with the most progressive decline in FEV_1, and was also correlated to the largest increase in resonance frequency. Since the other studies had a cross-sectional design, respiratory symptoms can only be related to type and level(s) of exposure. The importance of

chronic bronchitis due to inorganic dust exposure, indicated in the first chapter, was underscored in our study among (retired) coal miners and potatoe sorters. Shortness of breath and wheezing were reported frequently (> 60%) and cough and phleghm were reported by 80% of the miners. However, it should be noted that this was a selected population and therefore prevalences are not representative for all coal miners. In potato sorters most prominent symptoms were cough, phleghm, wheezing. Symptoms of cough and phleghms were significantly related to lower flow volume parameters. Chronic bronchitis in chemical workers, on the other hand, was most frequent among workers without a decline in FEV_1, and might be due to the fact that these subjects are beyond the progressive decline in FEV_1 and have reached a 'steady state'. This demonstrates that a relationship between chronic respiratory symptoms and lung function is not always present. On the other hand exposure to grain dust was predominantly related to complaints of breathlessness and wheezing. Interestingly, these symptoms among grain dust exposed workers were best related to changes in impedance parameters, whereas symptoms of chronic bronchitis due to inorganic dust were strongly associated to flow-volume parameters. Obviously, the pattern of respiratory symptoms due to organic dust exposure is distinctly different from those caused by inorganic dust exposure.

In addition to its discriminative power with regard to the site of obstruction and its value towards respiratory symptoms, we have shown that impedance measurements are sensitive in detecting airflow obstruction in humans. Up to now, studies were performed predominantly in small groups, according to well defined protocols, e.g. methacholine provocation tests (Hayes, 1984; Quaedvlieg 1990). Larger groups were not frequently studied, since data analysis was complex and time consuming. Computerized analysis overcame these difficulties, and made the FOT readily applicable to workplace and epidemiological surveys outside the clinical setting. We applied the FOT in occupational settings, thereby studying its performance, advantages over spirometry and the effect of different kinds of exposure. The main reasons for failure of an impedance measurement are of technical nature. In our hands, the technique was very suitable for repetitive measurements, and could be applied in the determination of hyperreactivity in occupational settings, or as a substitute for determination of peak flow. It is worth mentioning that impedance measurements can be done in workers near the source of exposure, which allows an estimation of the acute respiratory effects of exposure. Moreover, it can also be used in workers with respiratory failure, or subconscious due to inhalation accidents. Among its advantages the technique is noninvasive, user friendly, has a low observer bias and is not time consuming, especially when compared to flow-volume measurements. A major disadvantage concerns the interpretation of data of different research groups, as there is

currently no uniform equipment used for impedance measurements. Furthermore, the absence of well-defined diagnostic criteria and reference values complicates interpretation of the data. We conclude that, the FOT is a good complementary tool in screening and research in occupational medicine, but its strongest feature is that it can help to detect site and type of defence to a known occupational agent.

In conclusion, it is clear that future investigation of occupationally induced lung disorders will largely focus on the contribution of the workplace to non-specific respiratory disorders, such as chronic airway obstruction and lung cancer, diseases that continue to increase in incidence in the general population (Weill, 1993). Classic tools and strategies have serious shortcomings for early detection of respiratory impairment and discerning individuals susceptible to occupational obstructive lung diseases become more complex. The flaws in the use of FEV_1 measurements and questionnaires only in the (early) detection of occupational airflow obstruction favor the consideration of supplementary measurements. As was demonstrated in this thesis measurements of impedance and biological markers should be listed as alternatives. Particularly challenging remains the prospect of developing biomarkers that are minimally invasive or non-invasive, to detect or quantify exposure, identify susceptibility to an untoward response, and uncover early indicators of the disease process.

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CHAPTER 8

Summary

This thesis reviews the role of occupational exposure in the prevalence and nature of chronic respiratory diseases, the pathophysiologic mechanisms involved and routine methods and alternatives for the detection of these diseases. The dimension of lung disease in the Netherlands is indicated by a number of prevalence studies showing that 10 - 20% of adult men and 5 - 10% of adult women have chronic non specific lung disease (CNSLD, requiring medication). The presented data show the need for a proper appreciation and quantification of occupational causes. This is underscored by indications that a proportion of occupational lung diseases remains unrecognized, because they are rarely as specific as they used to be, no longer take epidemic proportions and arise in more susceptible individuals. On the other hand the incidence of these non-specific respiratory disorders, such as chronic airway obstruction and lung cancer, continues to increase in incidence in the general population. Classic tools (FEV_1 measurements and questionnaires) have serious shortcomings for early detection of respiratory impairment and discerning susceptible individuals. These arguments favor the consideration of supplementary measurements or alternative strategies in the (early) detection of occupational airflow obstruction.

The aim of this thesis was to evaluate a multifactorial approach in order to characterize occupation related respiratory impairment by integration of (new) lung function measurements, questionnaires and levels of biological markers. This aim was operationalized in different populations of workers exposed to inorganic dust, organic dust and chemicals.

In Chapter 2, the respiratory health of 102 retired coal miners (exposed to inorganic dust) is evaluated based on chest radiographs, lung function mea-

surements, questionnaires and release of tumor necrosis factor alfa (TNF-α) by blood monocytes. No significant differences in lung function were observed between subjects with coal workers' pneumoconiosis (CWP) (n = 27) and references (n = 75) nor was there an effect of cumulative dust exposure on flow volume or impedance parameters. TNF-α release upon stimulation of blood monocytes with coal mine dust was significantly increased in doubtful cases (ILO score 0/1) compared to references and cases with a higher ILO score. A relationship between airflow limitation and TNF-α release was demonstrated by increased TNF-α release in individuals with FEV_1 < 80% or impedance characteristics of obstruction. Although these data suggest that airflow obstruction is associated with an increased expression of inflammatory mediators in this selected population, further studies in other populations are needed to validate the significance of this observation to monitor (early) respiratory impairment.

In Chapter 3, the prevalence of pneumoconiosis and airflow obstruction in another group of workers exposed to inorganic dust is described. In 172 male workers from 5 potato sorting plants (55 controls, 29 salesmen, 72 currently exposed and 16 retired exposed), exposed to diatomaceous earth dust from former sea terra's, the presence of pneumoconiosis was evaluated by chest radiographs. Levels of serum type III procollagen (P-III-P) were used as a marker of fibrogenetic activity. Pneumoconiotic abnormalities were not demonstrated by chest radiographs nor were serum P-III-P levels increased in exposed workers compared to controls. In fact serum P-III-P decreased significantly (P < 0.03) with increasing cumulative exposure. Both flow volume parameters and impedance analysis indicated airflow obstruction, related to (cumulative) dust exposure. The annual decline in FEV_1 was estimated at 10.5 ml/year while impedance changes in the retired group revealed that this airway obstruction extended into the peripheral airways. Apart from the conclusion that this group of workers is not at an increased risk for pneumoconiosis, our integral strategy showed that (prolonged) surveillance in this group is desirable in order to detect early indications of airflow obstruction. Flow volume measurements are, in this case, a valid tool to do so.

Airway obstruction caused by fodder dust (and endotoxin), was related to the outcome of respiratory symptoms and impedance or spirometry data in a study among 194 male workers and is described in Chapter 4. Flow volume and impedance parameters were significantly related to ***present*** exposure. All impedance parameters, but of the flow volume parameters only MEF_{75}, were significantly related to ***cumulative*** dust or endotoxin exposure. The changes in impedance parameters (increasing resistance at 8 Hz and decreasing reactance at 8 Hz) reflected an increase in peripheral airflow obstruction, with increasing exposure. All lung function parameters were more strongly related to (cumulative) endotoxin exposure than to inspirable dust

exposure, but only impedance parameters and FEV_1 showed a good correlation with complaints of chronic bronchitis and breathlessness. Therefore, in this population, impedance measurement of the respiratory system proved to be the best tool to assess and localize airflow obstruction and objectify respiratory symptoms.

In Chapter 5, impedance and flow volume measurements were applied in a clinical setting to eleven subjects with (suspected) occupational asthma. Six subjects were found to be responsive (a decrease in $FEV_1 \geq 20\%$ from baseline) upon challenge with toluene diisocyanate (TDI) or methylene diphenyldiisocyanate (MDI). Average changes in both flow volume and impedance values differed significantly between sham and challenge in isocyanate reactors. Moreover, resistance at 8Hz ($\underline{R}8$) and reactance at 8Hz ($\underline{X}8$) vs. time profiles were similar to spirometric findings in all subjects. However, two different reaction patterns could be distinguished with the impedance measurements. Both early and 2 out of 4 late reactions were characterized by impedance changes that suggest peripheral airway obstruction. In two late asthmatic reactions obstruction appeared to be localized in the central airways. Interestingly, both subjects with a centrally localized late asthmatic reaction showed absence of bronchial hyperresponsiveness both before and after challenge. These findings suggest that the asthmatic response to isocyanates may occur all along the bronchial tree, but larger groups are needed to confirm our observations. Furthermore, possible differences in underlying mechanisms should be investigated.

A two-year follow-up of 145 workers by flow volume and impedance measurements was conducted to study the effect of chemical exposure on lung function and to relate (rapid) declines in FEV_1 to changes in impedance parameters (Chapter 6). The average decline in FEV_1 over two years was 80 ml which is not markedly different from the predicted decline of 64 ml, i.e. an excessive decline of 8 ml/year. The overall prevalence of airflow obstruction ($FEV_1 < 80\%$ predicted) was about 8% at both measurement moments, but was significantly increased among workers of the acids producing plant (Odd's ratio 3.00, confidence interval (1.19 - 7.59)). Interestingly, a rapid decline in FEV_1 (> 210 ml/2 years) was associated with a similar decrease in FVC. Combined to changes in impedance parameters predominantly a restrictive loss of lung function in suggested and it is concluded that this study population is not at increased risk to lung function deterioration due to chemical exposure. In this case, however impedance parameters provided the necessary information on mechanical alterations of the respiratory system in order to explain the observed decline in FEV_1.

In conclusion first, uniform changes in flow volume parameters by occupational causes can be based on different mechanics and pathophysiologic

mechanisms. Apparently uniform prevalence data on airway obstruction were found among exposed and non-exposed workers when considering all workers screened. When considering workers exposed to inorganic dust (Chapter 2 and 3) the above overall conclusion still holds and is irrespective of the method used. A clear difference in prevalence of airway obstruction, however was observed using impedance analysis in grain dust workers (18.7%) versus controls (5.5%); in contrast, evaluation of airway obstruction by conventional flow volume curves revealed no differences between exposed (3.7%) and controls (2.8%). The follow-up study among chemical workers, on the other hand, showed that a prospective decrease in FEV_1 (and FVC) was not accompanied by analogous changes in impedance. This demonstrated that impedance analysis can differenciate between contributing factors in subjects with a progressive decline in FEV_1.

Secondly, the benefits of a strategy combining different lung function techniques, respiratory symptoms and biological markers arre illustrated by the outcome of our studies among asthmatics (Chapter 5) and coal miners (Chapter 2) with airway obstruction. At a 20% decrease of FEV_1 two different impedance patterns were observed in isocyanate induced late asthmatic reactions with the same FEV_1 versus time curve. Hypotheses on the localization of airway obstruction in occupational asthma and the involvement of inflammatory mediators in this response, are the most prominent outcomes from the applied strategy. Linking of lung function data to the outcome of ex-vivo TNF release in coal miners, led to the observation that TNF is related to airway obstruction and forwards future research on its application as a biological (susceptibility) marker.

It is concluded that not the extent of exposure, but the site of injury and nature of pathogenetic mechanism that determine the tools to be used in the detection of respiratory impairment due to exposure. Despite the additional information obtained by our approach no uniform strategy for the (early) detection of airflow obstruction could be distilled.

CHAPTER 9

Samenvatting

Dit proefschrift beschrijft de rol van beroepsmatige blootstelling in de prevalentie en aard van chronische luchtwegaandoeningen, onderliggende pathofysiologische mechanismen en routinematige en alternatieve methoden voor de opsporing van deze aandoeningen. Recente onderzoeken tonen aan dat 10 - 20% van de volwassen mannen en 5 - 10% van de volwassen vrouwen lijden aan chronische a-specifieke aandoeningen van de luchtwegen (CARA). Deze mensen zouden eigenlijk medicatie moeten gebruiken. De rol van beroepsmatige oorzaken en de noodzaak van een adequate quantificatie in deze worden onderstreept in een aantal onderzoeken. Het is echter aannemelijk dat een deel van de beroepsgebonden longziekten niet herkend worden daar deze aandoeningen niet meer zo specifiek zijn als vroeger, geen epidemische proporties meer aannemen, maar voorkomen in meer gevoelige personen. Aan de andere kant blijft de incidentie van deze a-specifieke luchtwegaandoeningen, zoals chronische luchtwegobstructie en longkanker, toenemen binnen de algemene bevolking. Klassieke middelen zoals FEV_1 metingen en vragenlijsten hebben ernstige tekortkomingen in de vroege detectie van de achteruitgang van de luchtwegen en om gevoelige personen te onderscheiden. Deze argumenten leidden tot het overwegen van aanvullende metingen of alternatieve strategieën in de vroege opsporing van beroepsmatige exposities.

Het doel van dit proefschrift is de evaluatie van een multifactoriële aanpak om beroepsgebonden luchtwegaandoeningen te karakteriseren. Dit bestond uit het toepassen van (nieuwe) longfunctie metingen, vragenlijsten en het bepalen van biologische markers. Dit doel werd geoperationaliseerd in verschillende cohorten werknemers blootgesteld aan anorganisch stof, organisch stof of chemicaliën.

In Hoofdstuk 2, werd de gezondheid van de luchtwegen van 102 gepensioneerde mijnwerkers (blootgesteld aan anorganisch stof) geëvalueerd aan de hand van röntgenfoto's van de thorax, longfunctiemetingen, vragenlijsten en afgifte van tumor necrosis factor alfa (TNF-α) door bloed monocyten. Significante verschillen tussen longfuncties van personen met coal workers' pneumoconiosis (CWP) (n = 27; 0/0)) en de referentie groep (n = 75; > 0/0) werden niet waargenomen, noch was er een effect van cumulatieve blootstelling op flow volume of impedantie parameters. TNF-α afgifte na stimulatie van bloed monocyten met kolenstof was significant toegenomen in personen met een International Labour Organization (ILO) score 0/1 (twijfelachtig of pneumoconiose aanwezig is) vergeleken met de referentiepopulatie en zieken met een hogere ILO score. Een relatie tussen luchtwegobstructie en TNF-α afgifte werd aangetoond door toegenomen TNF-α afgifte in personen met een FEV_1 < 80% of impedantie karakteristieken van obstructie. Alhoewel deze gegevens suggereren dat luchtwegobstructie is geassocieerd met een toegenomen expressie van inflammatoire mediatoren in deze geselecteerde populatie, is verder onderzoek in andere populaties nodig om de significantie van deze observatie te staven.

In Hoofdstuk 3, werd de prevalentie van pneumoconiose en luchtwegobstructie beschreven in een ander beroepsgroep die is blootgesteld aan anorganisch stof beschreven. In 172 mannelijke werknemers van 5 aardappelsorterende bedrijven (55 controles, 29 vertegenwoordigers, 72 huidig blootgestelden en 16 gepensioneerde blootgestelden) blootgesteld aan stof van voormalige zeebodems dat niet-kristallijne silica bevat (7.7 - 15.4 mg/m^3), werd de aanwezigheid van pneumoconiose geëvalueerd met behulp van röntgenfoto's. Serum type III procollageen (P-III-P) werd gemeten als een marker voor de fibrogene activiteit. Pneumoconiotische afwijkingen werden niet geconstateerd op de thoraxfoto's noch waren serum P-III-P niveaus verhoogd. Serum P-III-P niveaus daalden juist significant ($P < 0.03$) met toenemende cumulatieve blootstelling. Zowel flow volume parameters als de impedantie analyse wezen op luchtwegobstructie die gerelateerd was aan de cumulatieve blootstelling. De jaarlijkse afname van de FEV_1 werd geschat op 10,5 ml/j, terwijl veranderingen in impedantie parameters in de gepensioneerde groep luchtwegobstructie in de perifere luchtwegen suggereerde. Naast de conclusie dat deze groep aardappelsorteerders niet een verhoogd risico heeft op het ontwikkelen van pneumoconiose, toonde onze integrale strategie dat een verlenging van de bewaking van deze groep wenselijk is om vroege indicaties van luchtwegobstructie op te sporen. Flow volume curves zijn, in dit geval, een valide middel om dat te doen.

Luchtwegobstructie veroorzaakt door veevoeder (en endotoxine), werd gerelateerd aan de rapportage van luchtwegklachten en impedantie en spirometrie data in een studie bij 194 mannelijke werknemers en staat beschreven in Hoofdstuk 4. Flow volume en impedantie parameters waren significant gerelateerd aan de huidige blootstelling. Alle impedantie parameters, maar van de

spirometrische maten alleen MEF_{75}, waren significant gerelateerd aan de cumulatieve stof of endotoxine blootstelling. De veranderingen in impedantie parameters (toenemende weerstand bij 8 Hz en afnemende reactantie bij 8 Hz) gaven een toenemende perifere luchtwegobstructie te zien met toenemende blootstelling. Alle longfunctie parameters waren sterker gerelateerd aan (cumulatieve) endotoxine blootstelling dan aan inspirabel stof blootstelling, maar enkel impedantie parameters en FEV_1 toonden een goede correlatie met klachten van chronische bronchitis en kortademigheid. Daarom is, in deze populatie, impedantiemeting van het respiratoire systeem het beste middel om luchtwegobstructie vast te stellen en luchtwegklachten te objectiveren.

In Hoofdstuk 5, werden impedantie en flow volume metingen toegepast bij elf personen met een verdacht op beroepsasthma in een klinische setting. Zes personen toonden een positieve reactie (FEV_1 daling $\geq$ 20% ten opzichte van de uitgangssituatie) na provocatie met tolueen diisocyanaat (TDI) of methyleen diphenyl diisocyanate (MDI). Gemiddelde veranderingen in zowel flow volume en impedantie waarden verschilden significant tussen de uitgangssituatie en provocatie in personen met een positieve reactie op de challenge. Profielen van de weerstand bij 8 Hz ($\underline{R}8$) en de reactantie bij 8 Hz ($\underline{X}8$) in de tijd waren gelijkwaardig aan de profielen van spirometrische parameters in de tijd in alle personen. Echter, met behulp van de impedantiemetingen konden twee verschillende reactiepatronen worden waargenomen. Zowel de vroege als 2 late reacties werden gekarakteriseerd door impedantieveranderingen die perifere luchtwegobstructie suggereren. In twee late astmatische reacties bleek de luchtwegobstructie in de centrale luchtwegen te zijn gelokaliseerd. Opmerkelijk was dat beide personen met een centraal gelokaliseerde positieve reactie geen detecteerbare bronchiale hyperreactiviteit vertoonden, noch voor noch na de provocatie met isocyanaten. Deze resultaten suggereren dat de astmatische respons op isocyanaten in principe overal langs de bronchiaalboom gelokaliseerd kan zijn. Grotere groepen dienen onderzocht te worden om deze bevindingen te bevestigen. Bovendien zouden mogelijke verschillen in het onderliggende pathogenetisch mechanisme van beide geobserveerde impedantiepatronen onderzocht kunnen worden.

Een follow-up van twee jaar van 145 werknemers met flow volume en impedantie metingen werd uitgevoerd om de invloed van chemische exposite op de longfunctie te bestuderen en om snelle afnames van de FEV_1 aan veranderingen in impedantie parameters te relateren (Hoofdstuk 6). De gemiddelde afname van de FEV_1 gedurende twee jaar was 80 ml wat niet duidelijk verschilt van de voorspelde afname van 64 ml, i.e. er was een overmatige afname van 8 ml/jaar. De algemene prevalentie van luchtwegobstructie (FEV_1 < 80% voorspeld) was ongeveer 8% op beide meetmomenten, maar was significant verhoogd in werknemers van de zuren producerende fabriek (Odd's ratio 3.00, confidence interval (1.19 - 7.59)). Interessant is dat een snelle daling in FEV_1 > 210 ml/2 jaar) gerelateerd is aan gelijkwaardig significante veranderingen in FVC. Gecombineerd met veranderingen in impedantie para-

meters werd voornamelijk een restrictief verlies van de longfunctie gesuggereerd en we concludeerden dat deze groep werknemers niet een verhoogd risico lopen op longfunctie afname ten gevolge van chemische blootstelling. In dit geval, bezorgden impedantie parameters de benodigde informatie over mechanische veranderingen van het respiratoire systeem om de waargenomen veranderingen in FEV_1 te verklaren.

Concluderend ten eerste, uniforme veranderingen in flow volume parameters door beroepsmatige oorzaken kunnen gebaseerd zijn op verschillende mechanieken en pathophysiologische mechanismen. Ogenschijnlijk uniforme prevalentie data van luchtwegobstructie werden waargenomen in blootgestelde en niet-blootgestelde werknemers als werknemers uit alle studies in ogenschouw worden genomen. Deze conclusie gaat ook op voor studies de bij werknemers blootgesteld aan anorganisch stof en is onafhankelijk van de longfunctiemethode die werd toegepast. Een duidelijk verschil in de prevalentie van luchtwegobstructie werd echter waargenomen tussen veevoeder werknemers (18.7%) en controles (5.5%); In tegenstelling, evaluatie van luchtwegobstructie met behulp van conventionele flow volume curves leverde geen verschillen op tussen blootgestelden (3.7%) en controles (2.8%). De follow-up studie bij chemische werknemers, aan de andere kant gaf te zien aan dat een prospectieve afname van FEV_1 (en FVC) niet werd vergezeld van analoge veranderingen in impedantie parameters. Dit toonde aan dat impedantie analyse kan differentiëren tussen factoren die een bijdrage leveren aan een progressieve afname van de FEV_1.

Ten tweede, de voordelen van een strategie die verschillende longfunctietechnieken, luchtwegklachten en biologische markers combineert wordt geïllustreerd door de resultaten van onze studies bij astmatici (Hoofdstuk 5) en mijnwerkers (Hoofdstuk 2) met luchtwegobstructie. Bij een daling in de FEV_1 van ten minste 20% werden twee verschillende impedantiepatronen waargenomen in isocyanaat geïnduceerde late astmatische reacties terwijl het FEV_1 versus tijd profiel hetzelfde was. Hypothese over de lokalisatie van de luchtwegobstructie in beroepsastma en dat ontstekingsmediatoren bij deze reactie betrokken zijn, zijn de belangrijkste bevindingen van de toegepaste strategie. Het koppelen van longfunctie gegevens aan de uitkomst van *ex-vivo* TNF afgifte in mijnwerkers leidde tot de observatie dat TNF is gerelateerd aan luchtwegobstructie en is een ondersteuning voor toekomstig onderzoek naar haar toepassing als een biologische gevoeligheid marker.

We concluderen dat niet de intensiteit van de blootstelling, maar de plaats waar de blootstelling aangrijpt en de aard van het pathogenetische mechanisme bepalen welke middelen toegepast dienen te worden in de opsporing van luchtwegaandoeningen ten gevolge van blootstelling. Ondanks de toegevoegde waarde van onze aanpak kon geen uniforme strategie voor de opsporing van luchtwegobstructie worden gedestilleerd.

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CURRICULUM VITAE

The author of this thesis was born on April 29, 1963 in Arnhem. He graduated from high school St. Dominicus College, Nijmegen in 1982 and went to the agricultural university Wageningen, were he degreed in Human Nutrititon in 1988. He became Assistant Trainee at the department of Occupational medicine, environmetal health and toxicology, University of Limburg, The Netherlands. During the reorganization his appointment was transferred to the Department of Respiratory Diseases, University of Limburg.

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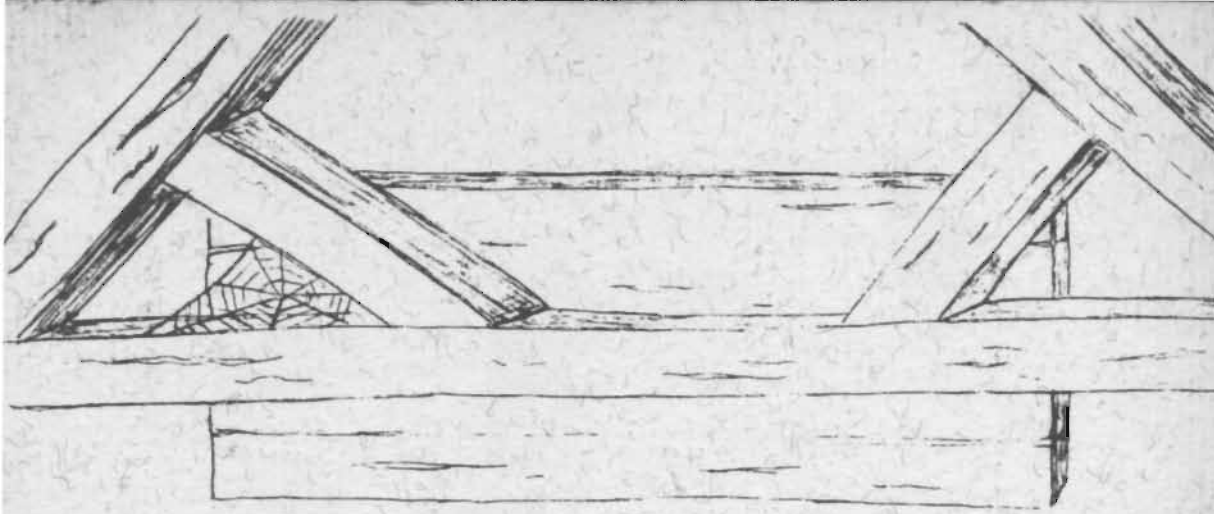

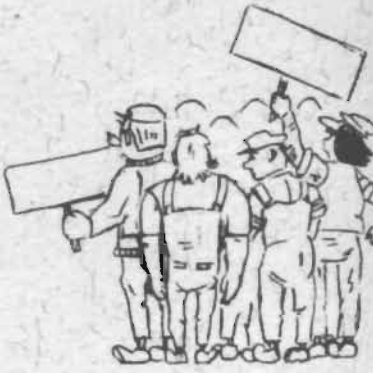

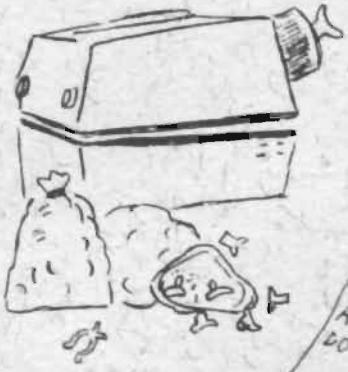
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