Exposure to organic dust and respiratory disorders

An epidemiological study in the animal feed industry

Tjabe Smid

STELLINGEN

1. Blootstelling aan stof in de mengvoederindustrie leidt bij werknemers tot chronische en acute effecten op het ademhalingsstelsel.
(dit proefschrift)

2. In de mengvoederindustrie zijn respiratoire effecten sterker gerelateerd aan endotoxineblootstelling dan aan stofexpositie.
(dit proefschrift)

3. Het invoeren van een MAC waarde voor graanstof is van weinig meer dan rituele betekenis zolang in de mengvoederindustrie overschrijdingen van de MAC waarde voor hinderlijk stof aan de orde van de dag zijn.

4. Bij onderzoek van relaties tussen omgevingsfactoren en longfunctie heeft het gebruik van continue effectvariabelen de voorkeur boven dichotomisering daarvan.
(J Vestbo, KM Knudsen, FV Rasmussen. Forced expiratory volume: why dichotomise? Br J Ind Med 49 (1992) 445-447).

5. Het gebruik van geometrisch gemiddelde expositieniveaus voor de berekening van cumulatieve blootstelling van graanwerkers, zoals beschreven door Huy en coauteurs, is onjuist.
(T Huy et al. Grain dust and lung function. Am Rev Respir Dis 144 (1991) 1314-1321).

6. De rol van een mogelijk healthy worker effect wordt in rapportages over observationeel epidemiologisch onderzoek doorgaans onderbelicht.

7. Het begrip mens- en milieuvriendelijk ondernemen ('memo') is een geromantiseerde en onjuiste betiteling voor het werk in ambachtelijke graanwindmolens.

8. Als MAC waarden op basis van beschikbare kennis geoptimaliseerd zouden worden vastgesteld, zouden wijzigingen even vaak verlagingen als verhogingen betreffen. Dat in praktijk uitsluitend verlagingen voorkomen geeft aan dat gezondheidkundige argumenten slechts een beperkte rol spelen.

9. De kritiek van stralingshygiënisten op het overheidsmilieubeleid met betrekking tot ioniserende straling komt voort uit de onjuiste premisse dat risico's van ioniserende straling en van andere milieuinvloeden niet vergelijkbaar zouden zijn.
(AS Keverling Buisman. Een jaar uit het leven van Nico Nors. NVS nieuws 1991, nr 4, 25-26).

10. De uitspraak van Ulenbelt 'Dat vormings- en jongerenwerk(st)er het zwaarste beroep blijkt te zijn (...)' is onjuist. Scores op enkele schriftelijke vragen over geestelijke belasting zijn immers een volstrekt ontoereikende operationalisatie van het begrip zwaarte van het beroep.
(P Ulenbelt. Stellingen bij het proefschrift: Omgaan met blootstelling aan chemische stoffen. Amsterdam 1991).

11. De overheidseis dat erkende veiligheidskundigen een technische of natuurwetenschappelijke vooropleiding dienen te hebben gevolgd beperkt in belangrijke mate de ontwikkeling van de veiligheidszorg.

12. Dankzij de ontwikkeling van de epidemiologie komt roken steeds meer in een kwaad daglicht te staan. Het is echter voor de epidemiologie een gemis dat berekeningen op de achterkant van een sigarendoosje steeds minder worden gemaakt.

13. Energiebesparing leidt tot negatieve invloeden op de levensverwachting.
(B.L. Cohen. Catalog of risks extended and updated. Health Physics 61 (1991) 317-335).

14. Toevoeging van het woord 'humane' in de naam van de vakgroep epidemiologie van de landbouwuniversiteit heeft voor vakgenoten in de epidemiologie een effect dat tegengesteld is aan het beoogde.

15. Dankzij de toegenomen veiligheid in de burgerluchtvaart is het verstrekken van zwemvesten aan vliegtuigpassagiers nauwelijks zinvoller dan het uitreiken van parachutes aan passagiers van veerboten.

Stellingen behorend bij het proefschrift:
Exposure to organic dust and respiratory disorders. An epidemiological study in the animal feed industry.
Tjabe Smid, 30 juni 1993.

EXPOSURE TO ORGANIC DUST AND RESPIRATORY DISORDERS

an epidemiological study in the animal feed industry

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EXPOSURE TO ORGANIC DUST AND RESPIRATORY DISORDERS

an epidemiological study in the animal feed industry

PROEFSCHRIFT
ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op woensdag 30 juni 1993
des namiddags te half twee in de aula
van de Landbouwuniversiteit te Wageningen

Dit onderzoek werd gefinancierd door
het Directoraat Generaal van de Arbeid
van het Ministerie van Sociale Zaken en
Werkgelegenheid

Omslag illustraties:
- A. Gärtner. Précis d'hygiëne public et privée. Liège, 1895
- De Nederlandse Mengvoederindustrie, ± 1955

CIP gegevens Koninklijke Bibliotheek Den Haag
ISBN 90-5485-136-8

Voor mijn ouders
Voor Els en Jord

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Voorwoord

Dit proefschrift had nooit geschreven kunnen worden zonder de hulp en steun van een groot aantal mensen. In de allereerste plaats geldt mijn dank daarvoor Dick Heederik, die zowel hulp als steun was vanaf het moment dat wij beiden onze eerste beroepsmatige schreden op het pad van de wetenschap zetten. Ook nadat ik van dat pad afweek en Wageningen verliet was het altijd prettig en leerzaam met Dick te bomen over het onderzoek, dat ik toch altijd ook als ons onderzoek heb beschouwd. Ik ben dan ook blij dat Dick als copromotor kan optreden. Mijn promotor Jan Boleij was een grote steun tijdens het onderzoek en tijdens het schrijfwerk dat daarna moest gebeuren. Ook van professor Quanjer heb ik in verschillende fasen van het onderzoek veel mogen leren.

Het onderzoek was natuurlijk niet mogelijk geweest zonder de deelname van alle werknemers die voor ons vaak bijna letterlijk de longen uit hun lijf wilden blazen. Ook degenen in bedrijven en bedrijfsgezondheidsdiensten die zorgden dat het onderzoek kon worden uitgevoerd ben ik veel dank verschuldigd.

In verschillende fasen van het onderzoek heb ik veel geleerd van de wijsheid van Klaas Biersteker, Taeke Pal, Han Willems en Wim de Kort en van alle andere leden van de begeleidingscommissie. De bijeenkomsten van de commissie en de andere contacten waren altijd erg plezierig en inspirerend.

Verder bedank ik alle mensen die in Wageningen en in het veld meegewerkt hebben aan de uitvoering: Hans Marquart, Gert Mensink, Rudolf van der Haar, Mieke Lumens, Anita Groenendijk, Tonnie Zweers, Jan Burema, Wim Braun, Reini Pater en Pieter Versloot. Zonder hen had dit onderzoek niet kunnen worden uitgevoerd. Een speciale vermelding verdient hier Remko Houba die een belangrijk deel van de analyses uitvoerde en op wonderbaarlijke wijze orde wist te scheppen in de chaos van verzamelde gegevens.

Jos van Hutten en Miquette Lulofs hebben een belangrijke rol gespeeld bij de totstandkoming van de schriftelijke weerslag van het onderzoek. Ontelbare veranderingen, onleesbare krabbeltjes en steenkolen Engels hebben zij weten om te vormen tot een heus proefschrift.

Ook de collega's bij de (toenmalige) vakgroepen Gezondheidsleer en Luchthygiëne en -verontreiniging waren een grote steun, door de prettige werksfeer en door de hulp, adviezen en de vele hand- en spandiensten die onmisbaar waren.

Ik bedank ook familie, vrienden en collega's van de KLM die vooral de laatste twee jaren tegen beter weten in toch altijd weer belangstellend waren naar de voortgang, en steeds weer allerlei subtiele stapjes op het continuüm van 'grotendeels klaar' naar 'helemaal klaar' naar waarde wisten te schatten.

Vooral ook Els ben ik veel dank verschuldigd. Ik dreigde soms in deze paragraaf de mededeling op te nemen dat het ondanks haar toch gelukt was. 'Dankzij haar' is natuurlijk veel meer van toepassing; ik heb altijd veel steun gehad, ook al hebben Els en Jord zich te vaak zonder mij moeten vermaken. Ik zal het jullie niet weer aandoen.

1. Introduction and study definition

Respiratory disorders account for a substantial part of public health problems in western countries. In the Netherlands 19% of diagnosed sick leave cases in 1988 were caused by respiratory diseases (CBS 1990b). In 1989 26,600 workers had a disability pension due to respiratory disorders, 3.4% of all disabled workers (CBS 1991b). 8700 people died from primary respiratory causes in 1988, 7% of all deaths (CBS 1991a). These figures comprise ICD 9 codes 460 to 519 (CBS 1979), and therefore exclude respiratory cancer.

In the Netherlands, as well as in other western countries, mortality rates for chronic obstructive pulmonary diseases (COPD) and related causes have shown an increase during the last 10-20 years (Weber et al. 1990). Figure 1.1 shows relative mortality from COPD as published by Wever et al. (1990), updated with information of 1988 and 1989 (CBS 1990a, 1991a). Rates per 1,000,000 are given for the total of ICD-9 (CBS 1979) codes 490 (bronchitis not specified), 491 (chronic bronchitis), 492 (emphysema) and 496 (other chronic airflow obstruction). Mortality from asthma (ICD code 493) is not taken into account. As well as the other causes in ICD group 490-496 (chronic obstructive pulmonary diseases and related causes), however, asthma mortality is relatively low, and trends can not yet be identified (Fig 1.2). In both figures rates are given for primary causes which are not adjusted for age.

Although smoking is undoubtedly a major external cause for this increasing mortality and morbidity (Wever et al. 1990), other environmental factors also play an important role in the etiology of respiratory diseases. Both indoor and outdoor pollution, as well as occupational exposure are reported as contributing factors to respiratory morbidity and mortality. The universality of smoking habits and the widely accepted evidence of their role in the development of airway disease have atendency to overshadow the role of other environmental pollutants, such as occupational exposures (Becklake 1985). Heederik & Pal (1993) mention several reasons why research on chronic non-specific lung diseases (CNSLD) has resulted in limited attention for occupational exposure. Most of these reasons relate to methodological problems resulting in insufficient sensitivity of research methods and statistical models.

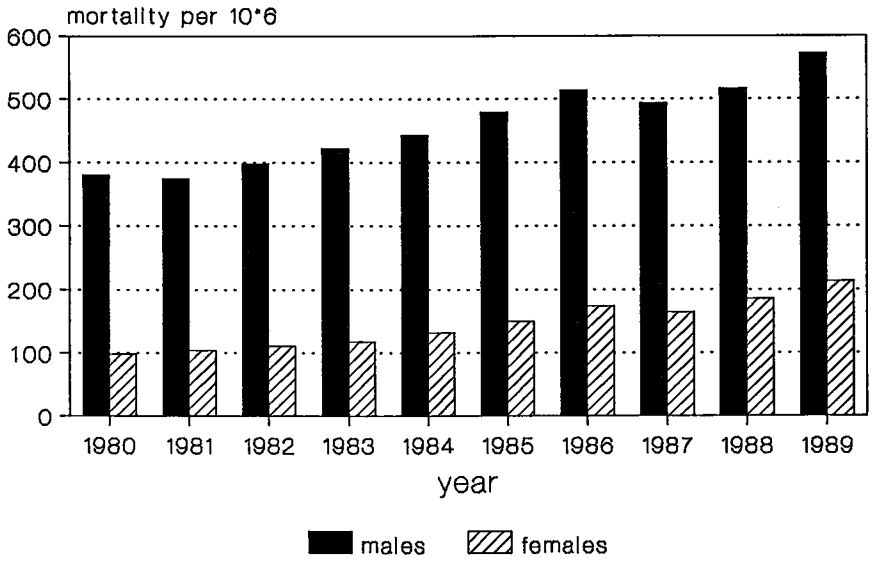

Figure 1.1 Total mortality rates per 1,000,000 for COPD (ICD 490 + 491 + 492 + 496) by year and sex, in the Netherlands 1980-1989

Nevertheless, it has been known for ages that occupational dust exposure is related to respiratory diseases. An almost obligatory citation in research in this field is related to Ramazzini, the 17th century father of occupational medicine (Ramazzini 1700). However, before that, in 1566 Agricola, in his classical work on metalliferous mining 'De Re Metallica', wrote on lung diseases in miners: '(...) the dust, which is stirred and beaten up by digging, penetrates into the windpipe and lungs, and produces difficulty in breathing and the disease which the Greeks called asthma.' (Agricola, cited in Hunter (1975)). Ramazzini, however, was the first author who gave extensive descriptions of lung diseases caused by organic dust, in millers, bakers, grain threshers, and in flax and hemp workers (Ramazzini 1700). It took until the nineteenth century before Patissier (1822) in France, Thackrah (1832) in Britain and somewhat later Coronel (1860, 1861) in the Netherlands wrote on lung diseases caused by vegetable dusts. As

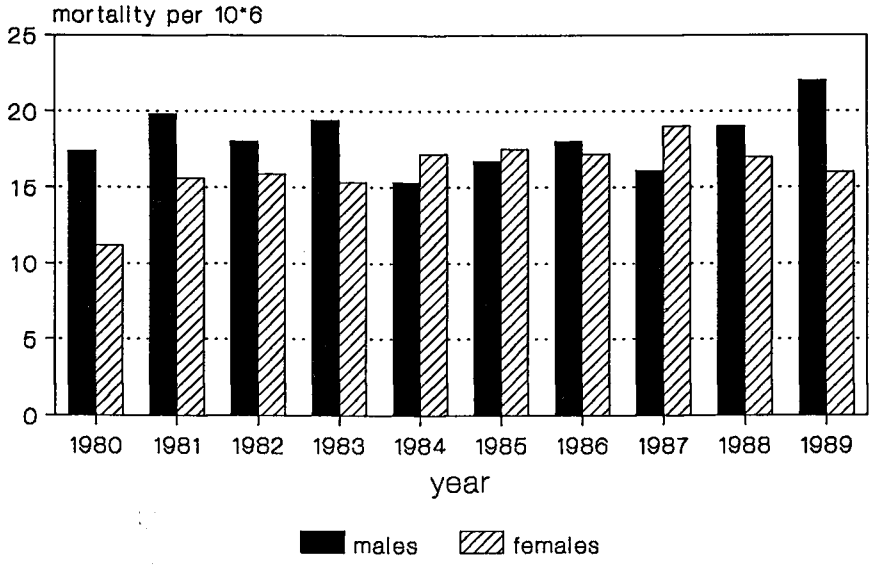

Figure 1.2 Total mortality rates per 1,000,000 for asthma (ICD 493) by year and sex, in the Netherlands 1980-1989

far as it is known, the first time attention was called in writing in the Netherlands, to the health risks of grain and flour dust, was in 1880. In a survey by Domela Nieuwenhuis a porter in a steam flour mill, whose job it was to fill and transport sacks with bran, reported frequent cough as the only occupational disease in his work ('niet dan veel hoesten en kugen'). The working conditions are described as stale and dusty ('bedompen en veel stof'), and the working week lasted 90 hours. A bread baking mate trade union reported to Domela Nieuwenhuis on many chest diseases caused by inhalation of bad agents ('vele borstkwalen door de slechte inademing'). Working conditions in private bakeries were described as damp with inhalation of flour dust and carbon monoxide fumes, and bad for health 'Vele Partuculieren werkplaatsen zijn vochtig, tevens veel inademing van meelstof en kolendamp, schadelijk voor de gezondheid (...)' (Welcker 1978).

During the nineteenth century, the first publications that relate respiratory problems to grain dust exposure using statistics were published. Thackrah (1832) mentions mean chest circumference and capacity of the lung as measured by the pulmometer (202 cubic inches = 3.3 l) in a group of corn millers. Thackrahs hypothesis, however, was that diminishment in lung capacity and chest circumference in general is caused by frequently leaning forward.

At the end of the nineteenth century several tables on mortality by occupation were published, i.e. by Arlidge in 1892 and Heijermans in 1905. The results, however, were inconclusive for exposure to grain dust (grain millers and bakers).

In the 20th century a vast amount of research was published on grain dust and other organic dust, and lung disorders. The complex nature of the dust, however, as well as the methodological issues mentioned earlier (Heederik & Smid 1993) have hampered the development of knowledge on exposure response relationships, mechanisms and active components that is needed for the occupational risk assessment. A major contributing factor to this lack of knowledge is the absence of detailed exposure assessment. Most of the research was performed from a health-oriented point of view; thus, much effort was spent in identifying and further characterising of effects, but exposure was poorly quantified. Only during the last two decades this field of attention has started to develop for organic dust related working conditions.

Although the adverse effect of organic dust is known for several ages, scientists (and legislative authorities) have hardly been able to establish guidelines or limits for occupational exposure.

The subject of this thesis is an epidemiological study into the relationship between exposure to grain dust and some of its components, and spirometric lung function changes and respiratory symptoms. Grain dust is broadly defined as the dust that is present in occupational environments in which grains and related products (pulses, oil seeds) are handled and processed. The study was performed in the animal feed sector, a major branch of agricultural industry in the Netherlands, in which approximately 6000 workers are exposed to grain dust.

The relevance of this study, however, relates to a considerably larger number of workers in the Netherlands. In this report, it is estimated that outside the agricultural sector, 20,000 to 35,000 workers are exposed to grain dust. The total number of workers that are exposed to organic dust is approximately 200,000 (Heederik & Smid 1988).

The scientific relevance of our study, apart from adding epidemiological information on grain dust and airway disorders to the existing vast body of knowledge, is that it is one of the first field studies in which extensive and reliable measurements of personal exposure to grain dust and one of the most discussed active components (endotoxin) are made. Because of this detailed information on personal exposure to dust and endotoxin, the sensitivity and accuracy for detecting exposure response relationships is considerably larger than in most of the existing field studies.

First, respiratory disorders in general are discussed first with special attention to terminology and methodological issues in the research of exposure related airway diseases (chapter 2).

The Dutch animal feed industry is described in the next chapter (3), with focus on its history and its present characteristics, as well as the production process.

A definition of grain dust, a review of the literature on grain dust and health, as well as estimates of the population at risk in the Netherlands are given in chapter 4, which was translated and updated from a Dutch publication in 1989.

The results of the study are presented in three consecutive parts. The first comprises an overview of the exposure measurements and their quality characteristics, as well as results, generalised to the animal feed industry and to exposure limits (chapter 5).

Then the results are given of cross-sectional and retrospective analyses of the relationship between lung function levels and respiratory symptoms, and exposure. Exposure characterisation varies from a dichotomy of an exposed and a control group, to estimates of present and historical personal exposure to inspirable dust and endotoxin (chapter 6).

Acute respiratory effects, indicated by work related respiratory symptoms and cross-shift and cross week lung function changes are related to dust and endotoxin exposure in chapter 7.

Finally, in the last chapter (8) a general disussion and conclusions are presented.

2. Respiratory disorders - epidemiology, terminology and methodological issues

2.1 Epidemiology and relevance for occupational health

In occupational health, two major fields for attention related to respiratory health can be distinguished; malignant respiratory diseases and chronic non-specific lung diseases.
In the Netherlands, in 1988, malignant diseases of the airways accounted for 11.6% of all deaths in males, and 1.9% in females. Allthough smoking is by far the most important cause of bronchial carcinomas, it is estimated that occupational exposure may account for an excess mortality for bronchial carcinoma of up to 10% (Seaton 1984b). Agents which may contribute to this phenomenon are asbestos, radon, chromium, nickel, coal combustion products, etc. For the current situation in the Netherlands, however, this figure may be an overestimation, since there are no underground mining activities, the use of asbestos has practically ceased, and the standards of industrial hygiene are (relatively) high.

As mentioned in chapter 1, nonmalignant respiratory disorders account for a significant part of sick leave, disability pension and mortality in the Netherlands and in other western countries. Nonmalignant respiratory diseases, as defined by the International Classification of Diseases revision 9 (CBS 1979), comprise several categories that are mentioned in table 2.1. In 1988, mortality accounted for 8.2% of all deaths in males, and 5.6% of all deaths in females. By far the two most common categories were chronic non-specific lung diseases (ICD 491-494, 496) and pneumonia (ICD 480-486) (CBS 1991).

With respect to occupational health, infectious respiratory diseases may be considered a relatively small problem, allthough for some occupations (medical and medical related professions and farmers) the risk is clearly present (Seaton 1984c).

Chronic non-specific lung diseases, however, have a clear relationship with occupational exposure. Heederik & Pal (1993) give an overview of nine general population studies showing relationships between occupational exposure to dust, fumes or gases, and respiratory symptoms and/or lung function decreases. Odds ratios for the studies with respiratory symptoms as endpoint varied from 1.3 to

2.1. In his cross-sectional analysis of a Dutch general population ('Zutphen') study, Heederik et al. (1989b) estimate odds ratios for physician-diagnosed CNSLD and exposure to dust, fumes or gases between 1.4 and 2.6, depending on confounding analysis. A longitudinal analysis with incidence data showed a significant incidence density ratio of 1.4 for CNSLD and occupational exposure. The exposed fraction of the population was estimated to be approximately 0.3. The etiological fraction for CNSLD, using this incidence density ratio, is 11% (Heederik 1990). Since other studies have indicated comparable odds ratios and exposed fractions, it may be estimated that at least 10% of CNSLD morbidity is related to occupational exposure.

Table 2.1 ICD-9 classification for nonmalignant airway diseases (CBS 1979)

ICD code (group)	Description
460-466	acute airway infections
470-478	other diseases of the upper airways
480-487	pneumonia and influenza
490-496	chronic obstructive lung diseases and related disorders
	490 bronchitis not specified as acute or chronic
	491 chronic bronchitis
	492 emphysema
	493 asthma
	494 bronchiectasis
	495 extrinsic allergic alveolitis
	496 other chronic airway obstruction
500-508	pneumoconioses and other lung diseases caused by external influences
510-519	other airway diseases

2.2 Chronic non-specific lung diseases: terminology

The concept of chronic non-specific lung diseases (CNSLD) is used for a group of related respiratory disorders, comprising chronic bronchitis, asthma and emphysema. It is considered to be present in case of chronic or periodic shortness of breath, wheezing (periodic or exercise-induced), or chronic or episodic cough and/or expectoriation, in the absence of other diseases such as tuberculosis, lung cancer, pneumoconiosis and fibrotic lung diseases (Post et al. 1992, Heederik 1990). Airflow limitation is not a prerequisite for CNSLD, although it is an accompaniment in many cases. In general, this concept is used predominantly in the Netherlands, or by those who accept the so-called Dutch hypothesis (chapter 2.5).

A related and more generally (especially in the USA) used concept is COPD or COLD, chronic obstructive pulmonary/lung disease, where obstruction of airflow is required for the diagnosis. It comprises emphysema, chronic bronchitis, and peripheral airways disease. Asthma is separated from this group, since it is characterized by variability of airway obstruction, while in chronic obstructive lung diseases the variability is limited (Post et al. 1992, Quanjer 1990).

A third categorisation is used in Great Britain, where again a distinction is made between asthma, and bronchitis and emphysema. Chronic bronchitis is divided between a syndrome characterised by hypersecretion (simple chronic bronchitis) and obstructive chronic bronchitis (Post et al. 1992).

Table 2.2 is adapted from Post et al. (1992) and gives a general overview of different categorisation and terminology in the three countries mentioned. It must, however, be kept in mind that often the separate syndromes are difficult to distinguish, and that explicit criteria for this are scarce. In epidemiological research on these subjects, the validation of CNSLD or COPD related syndromes is therefore usually performed by an operational definition. The end-point of those studies may thus be defined as positive answers to one or more standardised questions relating to respiratory symptoms, or as lung function changes.

Table 2.2 Classification of CNSLD syndromes in the Netherlands, Great Britain and the USA (adapted from Post et al. 1992).

Netherlands	Great Britain	USA
ASTHMA	**ASTHMA**	**ASTHMA**
- shortness of breath	- shortness of breath	- shortness of breath
- periodic	- periodic	- periodic
- obstructive	- obstructive	- obstructive
- decrease of lung function	- decrease of lung function	- decrease of lung function
* peak flow	* peak flow	* peak flow
* bronchial hyperreactivity	* bronchial hyperreactivity	* bronchial hyperreactivity
- reversible	- reversible	- reversible
- sometimes allergic	- sometimes allergic	- sometimes allergic
	simple chronic	
BRONCHITIS	**BRONCHITIS**	
- (productive) cough	- (productive) cough	
- decreased lung function	- persistent	
- persistent/chronic		
	OBSTRUCTIVE CHRONIC BRONCHITIS	
	- shortness of breath	
	- obstructive	
	- decreased lung function	
	- persistent	
		COPD/COLD/CAO
		- (productive) cough, shortness of breath
		- with/without obstruction
		- decreased lung function
		- persistent/chronic irritative reaction
EMPHYSEMA	**EMPHYSEMA**	
- shortness of breath	- shortness of breath	
- decreased lung function	- decreased lung function	
- persistent/chronic	- persistent/chronic	
- obstruction	- obstruction	

2.3 Lung function

As can be seen from table 2.2, lung function decreases are among the criteria for the majority of syndromes mentioned as elements of CNSLD. The usual lung function test in this respect is the spirometrically (or pneumatically) measured forced expiratory manoeuvre. Other lung function measurements may also be used, but fail to meet some of the essential criteria for use in epidemiological studies. According to Morgan (1984c), these criteria are acceptability, simplicity, objectivity, reproducibility and validity (sensitivity, specificity and predictive value). Most of the 'alternative' tests do not meet the criteria of simplicity or acceptability. Variables that can be measured by forced expiratory manoeuvres are:

- FVC; forced vital capacity;
- FEV_1; forced expiratory volume at one second;
- MMEF (or FEF_{25-75}); maximum mid expiratory flow: forced expiratory flow between 25% and 75% of forced vital capacity;
- MEF_{75} (or FEF_{25}), MEF_{50} (or FEF_{50}) and MEF_{25} (or FEF_{75}); Maximum expiratory flow when (respectively) 75%, 50% and 25% of forced vital capacity remains to be exhaled;
- peak expiratory flow (PEF).

Morgan (1984c) gives an overview of these variables according to his criteria for use in epidemiological studies. Table 2.3 is adapted from his publication. The validity score is left out, since the concept of validity suggests agreement between a lung function variable and 'the disease', or 'what the researcher wants to know'. As mentioned before, the common use of lung function variables in epidemiological studies is related to operational definitions; in other words 'the disease' or 'what the researcher wants to know' is defined in terms of lung function values.

Table 2.3 Rating of various spirometric variables for epidemiologic research according to four criteria (Morgan 1984c)

criteria	FVC	FEV_1	MMEF	peak flow	MEF_{50}	MEF_{25}
acceptability	+++	+++	+++	+++	+++	+++
simplicity	+++	+++	+++	++	−	−
objectivity	++	++	++	−	++	++
reproducibility	+++	+++	−−	++	−	−−

Acceptability is described as the absence of discomfort for the subject. Therefore, a test should neither cause pain nor be associated with risk.

The test should likewise be simple, so that the subject can carry out the instructions. Special attention must be paid to a relationship between inability to perform the test and respiratory disorders. If subjects are excluded from epidemiological studies because they cannot meet the criteria for acceptable forced expiratory manoeuvres, (partly) because of respiratory disorders, then the results of the study may be influenced.

Objectivity is related to the absence of observer bias and dependency of the results on the subject's full cooperation. A predominant problem in this field, in spirometry, is effort dependency of the results. Both the instructions of the observer and the cooperation of the subject may influence effort, and therefore the value of measured variables. A peculiar problem is the negative effort dependency of FEV_1, as described by several authors (Miller 1987, Krowka et al. 1987).

The fourth criterion, reproducibility, is probably most important for epidemiological analysis. The criteria of simplicity and objectivity are partly reflected in this criterion: if a test is not simple to perform, or if the subject's effort or observer's skills are not optimal, then the reproducibility will be influenced in a negative way. The reproducibility will be directly reflected in

large confidence intervals after epidemiological modelling with lung function variables as dependent variables.

Both the American Thoracic Society and the European Community for Coal and Steel (ECCS) (Quanjer 1983) have published guidelines for the standardisation of lung function testing. The ECCS guidelines which are used in this study, contain requirements for equipment, calibration, the test procedure, and the selection and acceptance of variables from, at least three, manoeuvres. The guidelines are aimed at maximizing reproducibility of test results.

As can be seen from table 2.3, the reproducibility and thus the statistical value for epidemiological analyses varies between lung function variables. The most reproducible variables are FVC and FEV_1. Willems and Heederik (1984) give coefficients of variation (CV; EPA 1987) for repeated measurements over the day for both variables of 2-5%. The other variables have CV values varying from 5 to 20%. It must, however, be kept in mind that the use of CV values assumes a linear relationship between the value of a given lung function variable, and its standard deviation. In practice, however, it appears that for smaller lung function values, the CV values are somewhat larger (Quanjer 1992, personal communication).

Most epidemiological studies on lung function direct prevailing attention to FVC and FEV_1. Apart from the statistical virtues because of small CV values, this is caused by the fact that FVC and FEV_1 are relatively easy to measure, whereas the other variables require sophisticated equipment and data processing facilities. Furthermore, for diagnostic purposes, it is doubted whether the other variables provide useful information not contained in the FEV_1 and FVC (American Thoracic Society 1987).

2.4 Questionnaires on respiratory symptoms

The first standardised questionnaire on respiratory symptoms was developed by the British Medical Research Council (BMRC 1960). It was widely accepted, and in several countries translated and/or slightly adapted versions were published (Heederik 1990a). In the Netherlands, a shortened, self administered

version is often used (Biersteker et al. 1974). The questions relate to cough, productive cough, shortness of breath, wheezing, frequent wheezing and asthma, and are given in table 2.4 (in Dutch). This questionnaire was used in the animal feed study.

According to the criteria mentioned by Morgan (2.3., Morgan 1984c) of measurements for epidemiologic studies, the acceptability and simplicity are maximal. In the self administered version, objectivity in terms of absence of observer bias is also excellent, but there is a clear dependency on the subject's cooperation. Reproducibility of questionnaires has been much studied, but studies are hampered by the natural variability of symptoms.

Nevertheless, Heederik characterises the reproducibility as unsatisfactorily low, as indicated by percentage of agreement ranging from 65 to 90% in different studies. Self administered versions may improve this score since observer bias is excluded (Post et al. 1992, Heederik 1990).

The validity of the questionnaire, as indicated by sensitivity and specificity, is highly dependent on the definition of the 'objective' symptom or disease. In general, it may be stated that the sensitivity is better than the specificity, in other words the percentage of false positives outnumbers the percentage of false negatives (Post et al. 1992, Heederik 1990).

Table 2.4 Questionnaire for respiratory symptoms (Biersteker et al. 1974)

1. Heeft u gedurende de laatste twee jaren wel drie maanden achtereen vrijwel dagelijks gehoest?
2. Heeft u gedurende de laatste twee jaren wel drie maanden achtereen slijm opgehoest?
3. Heeft u wel eens last van kortademigheid als u met leeftijdgenoten in normaal tempo op vlak terrein wandelt?
4. Heeft u ooit last van piepen op de borst gehad?
5. Heeft u dit de laatste twee jaren wel eens meer dan één week achtereen gehad?
6. Heeft u wel eens aanvallen van benauwdheid (astma) gehad?

In general, it can be concluded that questionnaires may be of use in epidemiologic studies, when questions on validity are excluded through

operational definitions and when study group sizes are adequately large to increase statistical power and thus cope with statistical variation caused by low reproducibility.

2.5 Etiology of CNSLD

The interrelationship between exogenous and endogenous factors in the etiology of CNSLD has often been a topic of discussion during the last decades. As already mentioned, smoking is clearly related to the development of CNSLD, but it is also obvious that not all smokers develop CNSLD, and that not all CNSLD patients have a smoking history. In terms of analogies it can be used for occupational exposure.

It is probable that the phenomenon can partly be explained by competition of risks; smokers for example run an increased risk of coronary heart diseases (Weiss & Speizer 1984).

Two hypotheses have been stated for the etiology of CNSLD. The first is referred to as the Dutch hypothesis. It states that all constituents of CNSLD (asthma, bronchitis and emphysema) are clearly interrelated, and that in susceptible individuals CNSLD develops as a result of allergic and non-specific stimuli. This susceptibility is genetically predisposed, and is characterised by increased bronchial reactivity or atopy. Because of this increased susceptibility, subjects react heavily to exogenous stimuli through airflow obstruction and mucus secretion, and hence to decreased clearance capacity of the lung and imflammation. In the long term, these reactions may lead to a more rapid lung function decline and development of chronic impairment of lung function. The phenomenon that not all smokers develop CNSLD might therefore be explained by the absence of predisposition.

The alternative explanation is the British hypothesis, which states that exogenous stimuli, and especially smoking, lead to hypersecretion and airway inflammation, and subsequently to increased infections. The infections destroy lung tissue and thus may lead to accelerated lung function decline and the development of clinical disorders (Quanjer 1990, Weiss & Speizer 1984).

In a recent symposium on these matters with several eminent experts it was concluded that the increased knowledge on immunology, (patho)physiology and the natural history of CNSLD may lead to the recognition of a distinction between asthma and COPD. Asthma may be characterised by malfunctioning of the immunological system, whereas chronic bronchitis appears to be associated with smoking. The central question why so many smokers do not develop COPD or why not all COPD patients are smokers, however, remains to be answered (Quanjer 1990).

3. The animal feed industry

3.1 History

At the end of the nineteenth century the expansion of animal breeding activities started as a product of rationalization of agricultural life. In the 1890's, before the implementation of import duties for grains, large amounts of foreign grain products were imported and transformed into animal products that could be exported to areas of industrial development in neighbouring countries. The development of the industrial vegetable oil industry also produced large amounts of high quality waste products that could be used to feed animals. The availability of cheap animal feed and the increase of live stock mutually influenced each other, leading to a gradual growth of rational animal breeding that lasted until the current times (de Nederlandse mengvoederindustrie 1955, Bläsing 1986).

Although nutritional standards for animal feed were already published in 1864 in Germany, the use of rationalized and optimized mixtures of raw materials became common during the 1930's. Before that time most farmers used single component feeds or simple mixtures of available raw materials. In one of the first animal feed quality surveys in the Netherlands, in 1904 in Goes, 298 samples were studied. The vast majority of the samples were (indigenous or foreign) single component meals, 9 were simple mixtures of two or three meals, and 46 were more heterogenous mixtures of mainly grain flour, meal from pulses and waste products from oil industries. This latter group can be considered as precursors of modern animal feeds. Both the quality and the price, however, were doubted by the conductor of the survey, the governmental agricultural research station; 'These products are sold, using all kinds of fine sounding names, without declaration of contents, sometimes much too expensive' (Hissink 1905).

The widespread acceptance of modern industrially produced and rationally composed animal feed was only seen in the 1930's. In 1939, in an article on modern mixtures, an author in an agricultural newspaper states that 'If one thing has increased during the last five to ten years, it is the use of mixed animal feeds! (...) We can speak of a true revolution in the field of animal feed that has

taken place in relatively short time!' (van der Meulen 1939). The use of 'additives', such as chalk, charcoal and cod-liver oil was also common at that time (Bläsing 1986, p 76). Until 1930, meal and cattle cakes were used. After that time, with the rapid increase of industrial production, other types such as pellets and crumbles became available. The foundation of large scale industrial animal feed production facilities took place in the 1930's and 40's, and some of the facilities that were founded at that time are still in use. After these decades of industrial founding, increased use of foreign raw materials such as soy and cassava, increased use of additives, and upscaling led to the present animal feed production.

3.2 Production of animal feed

The production of animal feed is a relatively simple mechanical dry bulk process; raw materials are mixed and ground, addition of additives takes place, and the mixture is (usually) pressed into pellets that can be shipped in sacks or bulk trucks. The production process is summarised in figure 5.1. The process is usually highly mechanised, and the processed quantities are large. Approximately 80% of the animal feed production is produced in facilities with production capacities ranging from 50,000 to several hundreds of thousands of tons per year (Produktschap voor Veevoeder 1991).

The raw materials that can be used in animal feeds are numerous. Depending on the use of the feed, specifications vary. The two main criteria are protein and energy content. Since raw materials account for 80% of the price of animal feed, it is very profitable to optimize the composition using information on prices using sophisticated computer programs.

Raw materials can be grouped into four main categories:

- grains; mainly wheat, corn and barley;
- other vegetable products, such as cassava and pulses;
- waste products from the food industry, such as citrus pulp, extracted soya-meal, corn gluten, beet pulp, and extracted coprameal;

- others, such as vitamins, minerals, fats, molasse and additives (i.e. anti-oxydants).

Figure 3.1 gives the relative use of raw materials in the European Community in 1987. It must be taken into account that the use of raw materials may vary by country. The proportion of grains for example varies from 12% (Netherlands) to 65% (Spain) (Roelfsema 1991).

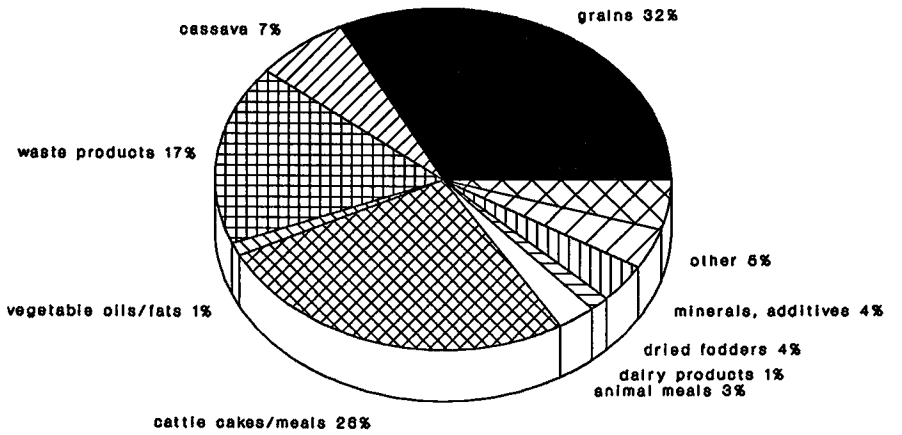

Figure 3.1 Industrial use of raw materials for animal feed in the European Community, excluding Greece and France, in 1987 (Roelfsema 1991)

Most of the raw materials arrive by ship or by truck. Ships are unloaded by a crane or by a mechanical or pneumatic unloading pipe. Trucks are unloaded pneumatically (bulk trucks) or the materials are dumped into a pit. After weighing and possible pretreatments such as cleaning, the raw materials are transported to silos. Transport in animal feed facilities is almost always mechanical, mainly with screw- and belt-conveyors and elevators. Raw materials may also be stored in tanks (fat, molasses) or in sacks (additives). Premixes are often used: mixtures of additives, vitamins, minerals etcetera, and sometimes a

carrier. Storage of the raw materials is a delicate process, since humidity, temperature and oxygen concentration may influence microbiological activity and germination.

After storage, the actual animal feed production takes place in a bulk process, in which the raw materials are weighed, ground and mixed, and additives, minerals, fats etc. are added. The technology used and the order of processing may vary between facilities, and between different products. The process is usually operated from a central control room, but separate steps in the process may be operated manually.

After the production of the meal, the product is almost always pelletised. The first step is conditioning: injection of steam and addition of molasses into the meal. The actual pelletising takes place in a centrifugal press. After cooling down, the product is stored into silos again.

The animal feed may than be packed in sacks, or can be transported in bulk trucks to the customers (Bläsing 1986, v.d. Poel 1988).

The process is highly mechanised, and in modern facilities most of it is operated from a central control room. The number of workers operating an animal feed facility is usually no more than 20 to 30 in one shift. Their jobs can be grouped into 8 categories, which are described in detail in 5.3.3.

3.3 Present characteristics

After the beginning of large scale industrial production of animal feed in the 1940's, the production has risen steadily until approximately 1985, when production limiting measures for milk production were taken by the European Community. Other reasons for the stabilization are legislation on manure production and an increase in efficiency of feed utilization. Figure 3.2 shows the total production of animal feed between 1964 and 1989 in the Netherlands. As can be seen the stabilized production volume in the second half of the eighties is around 16 million tons per year, approximately three times the production of 1964 (Vermeer 1983, Produktschap voor Veevoeder 1991, Roelfsema 1991).

In the European Community, the total production of animal feed is approximately 100 million tons per year. Germany, France and the Netherlands are the main producing countries. Each of these countries accounts for one sixth of the total production (Roelfsema 1991).

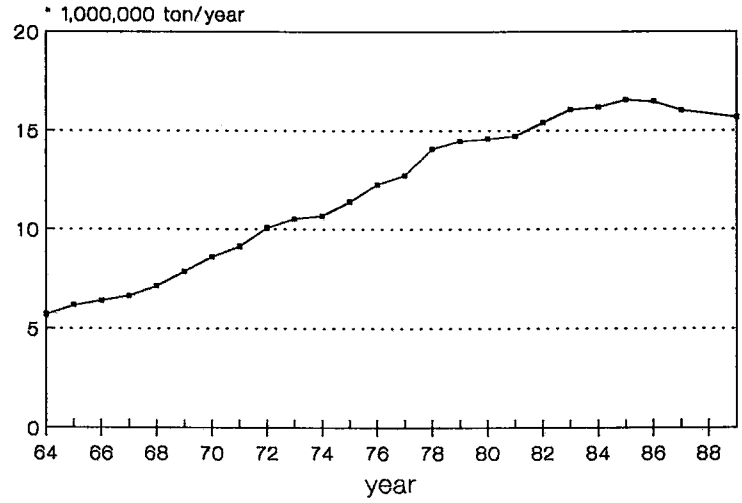

Figure 3.2 Animal feed production in million tons per year in the Netherlands 1964-1989 (Vermeer 1983, Produktschap voor Veevoeder 1991)

In the Netherlands, in 1989, animal feed was produced in 354 facilities with 10,770 employees. 7342 Workers (including administrative staff) took part in the actual production (Produktschap voor Veevoeder 1991). The number of animal feed facilities has decreased dramatically in the last decades. Figure 3.3 shows the number of facilities between 1972 and 1987 by their size, and indicates clearly that the decrease is caused predominantly by decreasing numbers of small facilities. There is, however, a significant increase in large facilities (> 100,000 tons production volume per year), from 12 in 1972 to 37 in 1989 (Produktschap voor Veevoeder 1991). Their importance for the total yearly production volume is also rapidly increasing, as can be seen from figure 3.4 which shows the relative amount of production between 1972 and 1987, by facility size.

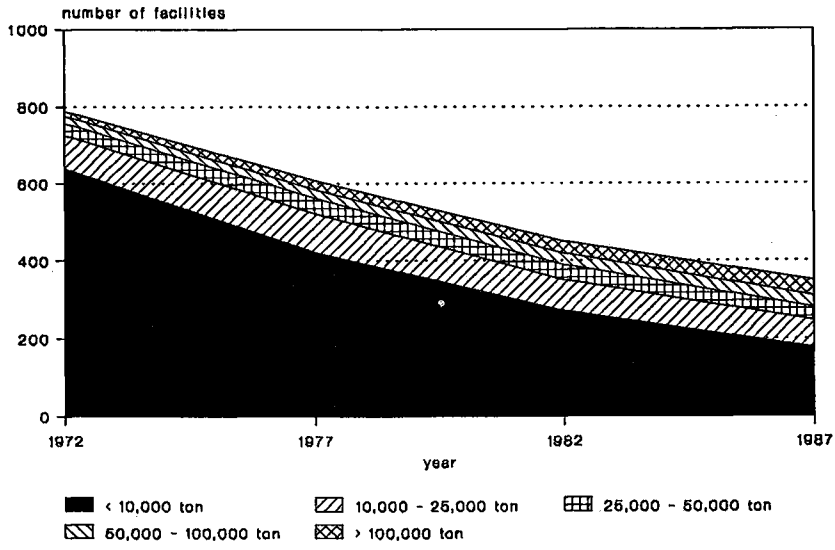

Figure 3.3 Number of facilities in the Netherlands by production volume 1972-1987 (Vermeer 1983, Produktschap voor Veevoeder 1987a)

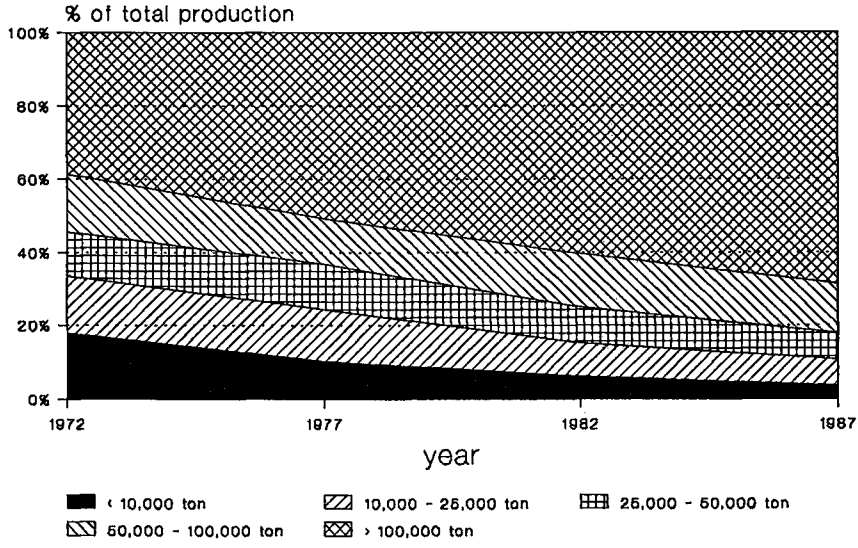

Figure 3.4 Relative production volume in the Netherlands by production volume 1972-1987 (Vermeer 1983, Produktschap voor Veevoeder 1987b)

The majority of workforce is employed by the largest facilities; in 1989 54% of the workers operated 37 (10% of all) facilities with a production volume of over 100,000 tons per year. The productivity of workers is larger than in the smaller facilities; the 37 largest facilities produce 74% of all animal feed. The relationship between workforce and production volume is shown in figure 3.5, which shows a relative (to all Dutch workers) number of workers and a relative (to national) production volume, by facility size (Produktschap voor Veevoeder 1991).

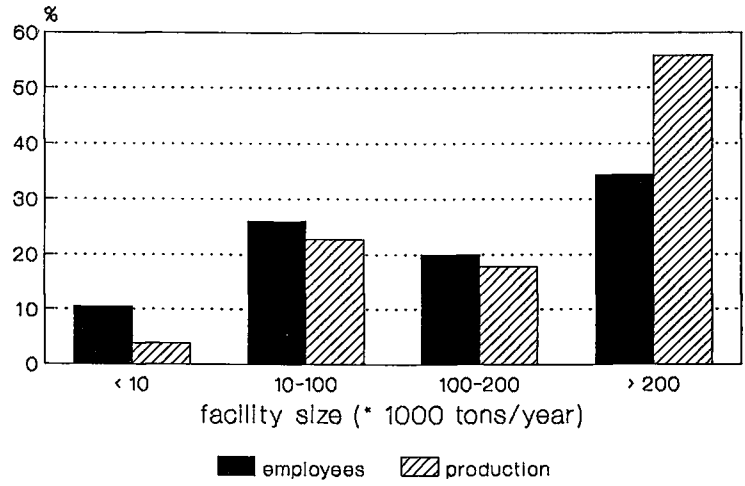

Figure 3.5 Relative number of workers and production volume, to total national animal feed production workforce (7342 workers) and production volume (15.659 tons/year), in 1989. By facility size as indicated by annual production volume (Produktschap voor Veevoeder 1991)

Taking into account the stabilized production during the last years, the upscaling of production facilities and the positive relationship between facility size and productivity, it is expected that the total number of workers in the animal feed industry will continue to decrease in the coming years.

4. Grain dust and health - a review of the literature[1]

Occupational exposure to dust of biological origin is increasingly related to respiratory disorders during the last years. A classical example is byssinosis, a syndrome mainly characterized by respiratory symptoms that can be caused by exposure to cotton dust (WHO 1983, Rylander 1990). Other types of biological dusts can originate from paper (pulp) (Heederik et al. 1987b), coffee (Jones et al. 1982), tobacco (Gleich et al. 1980), tea (Zuskin et al. 1985), hemp (Zuskin et al. 1990) and agricultural production farms (Bringhurst et al. 1959, Madsen et al. 1976).

This chapter shows the result of a literature study of occupational effects of exposure to grain and flour dust. Some attention is paid to the scarce literature on non-occupational exposure. Most of the literature cited is foreign. In the following chapters the results of the Dutch studies will be published.

4.1 Definitions and population at risk

A large number of - mainly epidemiological - publications has been published in the last 15 years on the subject of (respiratory) impairment and exposure to grain dust. In most studies 'grain dust' has a broader meaning than dust deriving from cereal grains. Grain dust may originate from the processing of several grains, such as wheat, oats, barley, rye, sorghum, maize and rice, but also from pulses (soy beans, peas, mustard) and oil seeds (rape seed, flax and sunflower) (Patel et al. 1981, Becklake 1980, Donham 1986). The reason for this is that most of the research was performed in American and Canadian grain elevators. In these facilities the products are stored and transferred, and sometimes dried and cleaned. In the Netherlands similar mixed exposures will be found in the animal feed industry and in grain elevators where raw materials for the animal feed industry are transferred. In this article the term grain dust will refer to the

[1] Translated and updated from Smid & Heederik: Graanstof en gezondheid. T Soc Gezondheidsz 67 (1989) 53-60. Part of this chapter was published in Heederik & Smid: Mortality and morbidity of organic dust exposed populations. In: Rylander & Jacobs (eds.) Handbook of organic dust. 1993 (in press)

broad meaning that is generally used in the literature. The effects of flour dust that are mentioned in the literature will be discussed as well.
Exposure to grain dust is common in farm environments, during the production of grain and when animal feed is used (Darke et al. 1976, Manfreda et al. 1986, Rylander 1986, Warren et al. 1984, Watson et al. 1986, von Essen et al. 1987). This review, however, will be focused on health risk in the grain industry. The literature that deals with farm environments will not be discussed extensively.

The Central Bureau of Statistics (CBS) publications show that in the Netherlands in 1985 166,000 people were employed in the food and confectionary industry. Approximately 50% were employed in non clerical or commercial jobs. Assuming that this percentage is also applicable to the animal feed industry (12,000 employees), the flour mills, and rice and barley husk facilities (2000), the exposed population will count at least 7000 workers. Other industries in which exposure to grain dust may take place, such as the pasta industry, are not mentioned separately in the CBS statistics. Assuming that several thousand workers are exposed to grain dust in other industries, the total number of exposed people is estimated to be 10,000.

Not included in this number are the self employed bakers and workers in the bakery trade. In 1985 this group counted 35,000 people. Since most of the bakeries are relatively small, the percentage of people in production work is larger than 50%. Not all production workers on the other hand will be exposed to grain or flour dust. Due to these uncertainties only a rough estimate of the exposed fraction can be made; the number of exposed workers in the bakery trade is estimated between 10,000 and 25,000.

The total number of workers exposed to grain and flour dust outside the agricultural industry is thus estimated to be between 20,000 and 35,000 (CBS 1984a, 1987a and b).

4.2 Exposure

Like other organic dusts, grain dust is a heterogeneous mixture. The main part is the grain itself, but a variety of other components may be found;

- non-grain plant matter (Becklake 1980, Donham 1986, Whidden et al. 1979);
- fungi, moulds and spores, mainly Aspergillus sp. and Cladosporium sp., and in humid grain thermophylic Actinomycetes (Darke et al. 1976, Farant & Moore 1978, Palmgren et al. 1983a and b, Gemeinhardt & Bergman 1980, Gemeinhardt 1981, Donham 1986, Whidden et al. 1979);
- mycotoxins, such as aflatoxins, zearalenone, vomitoxin, ochratoxin and toxin T2 (Palmgren et al. 1983a, 1986, Silas et al. 1987, Sorenson et al. 1984, 1986, de Jong 1987a, Tanaka et al. 1990);
- bacteria and their (bio)chemical components and excreta, such as endotoxins and proteolytic enzymes (deLucca et al. 1984, 1987, Palmgren et al. 1983b, Dutkiewicz 1986, Popendorf 1986);
- mites, such as Glycyphagus Destructor en Tyroglyphus Farinae, and other insects, such as the grain weevil (Becklake 1980, Yoshida 1982, Davies et al. 1976);
- other animal matter, including parts of insects, rodents and birds and their excreta (Becklake 1980, Yoshida 1982);
- pesticides (HCB, HCH, lindane, aldrin, DDT, malathion), fumigating agents (phosphine and carbon tetrachloride), and fertilizers (Palmgren & Lee, 1984, Becklake 1980, Produktschap voor Veevoeder 1987a, de Jong 1987b, Deer et al. 1987);
- inorganic matter such as soil, silica etc, including quartz (Farant & Moore 1978, Yoshida 1982).

Reports on ambient air concentrations and personal exposure are usually reported as gravimetric concentrations of dust. In general personally monitored concentrations of total or inspirable dust vary from a few to several tens of milligrams per cubic meter. The respirable fraction usually accounts for 10 to 50 gravimetric% of the total dust. Apart from the animal feed industry data from research in the Netherlands are available from a flour mill (Houba & Zweers 1988), two seaport grain elevators (Wieling et al. 1987, Heederik et al. 1989a), a wheat starch producing facility (Oostendorp & Kromhout 1985) and two traditional wind grain mills (unpublished).

The results of personal measurements showed exposure similar to those found in foreign research. In the wheat starch mill the geometric mean of 43 exposure measurements was 7.4 mg/m^3 and a geometric standard deviation of 4.2, indicating a large variability in exposure. In the grain mill the geometric mean was 6.8 mg/m^3 with a geometric standard deviation of 4.5 (n=98). In the seaport elevators, exposures of up to hundreds of mg/m^3 were found, and in the traditional wind mills all 16 measurements exceeded the Dutch limit value for nuisance dust (10 mg/m^3). Except for one seaport elevator all measurements were conducted with the PAS-6 air sampling head (ter Kuile 1984). In practice the PAS-6 is reported to sample a slightly smaller particle fraction than total dust. The sampled dust resembles the inhalable fraction (ISO 1981, 1983, Boleij et al. 1987). The measurement technique that was used in the grain elevator also led to an underestimation of the total dust fraction, by tens of percents (Wieling et al. 1987).

The authorities in the Netherlands have not established limit values for exposure to grain dust. The national MAC (maximum allowed concentration) list gives a value of 10 mg/m^3 total nuisance dust and 5 mg/m^3 respirable nuisance dust. Nuisance dust is defined as '..dust particles (...) that are known not to cause adverse effects at non excessive exposure for years'. The rest of this chapter will show that this definition cannot be applied to grain dust. The American ACGIH has established a TLV (threshold limit value for 8 hours time weighted average) of 4 mg/m^3 total grain dust (wheat, oats, barley) (ACGIH 1992). A criteria document for occupational standards from the Swedish NIOH and the United States NIOSH states that 'it is presently impossible to recommend an exposure concentration at which all workers would be protected from adverse health effects' (Brown 1988).

4.3 Non-respiratory effects

Most research on effects of grain dust that has been reported in the literature considers respiratory effects. A second field of attention is formed by mainly clinical studies on skin disorders. An increasing number of studies indicate

relationships between mortality and cancer incidence with occupational grain dust exposure.

4.3.1 Skin disorders

Considerable attention has been paid to dermatitis in bakers for several decades. So-called bakers eczema was a well known phenomenon until the use of baking aids was forbidden around 1960 in several countries (Baird 1945, Heyl & Reinert-Dilthey 1968).

Nowadays, although the number of skin disorders has decreased, up to 4% of bakers apprentices with a job history of 1-5 years reported eczema in a German study (Herxheimer 1973). Skin disorders are also reported in clinical studies (Heyl & Reinert-Dilthey 1968, Heyl et al. 1970, Nethercott & Holness 1989). Both irritant and allergic contact dermatitis are found, with several causal agents, such as rye and wheat flour, additives, flavoring agents, or food mites (Nethercott & Holness 1989). The relationship with respiratory disorders, however, appears to be weak, although an inhalation allergy has been hypothesised as one of the causal mechanisms (Heyl et al. 1970).

An epidemiological study in Canadian grain elevators showed prevalences of pruritus ('grain itch') of over 50% in two cohorts. Exposure to barley and oats was reported to provoke the greatest number of complaints (Hogan et al. 1986).

In an Italian study of 204 workers in 15 animal feed mills, the prevalence of (clinically verified) occupational contact dermatitis was 13.7%. Duration of employment was positively associated with the contact dermatitis rate. 7.8% was an irritant contact dermatitis, while 5.8% had an allergic base (Mancuso et al. 1990). A large variety of clinical reports support the authors' conclusion that additives, rather than the feed itself, are a cause of sensitisation in animal feed workers and farmers. Among the many additives mentioned in the literature were antioxidants (ethoxiquin (Mehlhorn & Beetz 1971, van Hecke 1977, Burrows 1975)), antibiotics (tylosin (Neldner 1972), virginiamycine (Tennstedt et al. 1978)), growth promoters (furazolidone (de Groot & Conemans 1990), quinoxaline dioxide (Dawson & Scott 1972)), other pharmaceuticals (ethylenediamine dihydroiodide (Fisher 1975), and trace elements (cobalt

(Mancuso et al. 1990)). Although there is a consensus on the dominant role of additives as a cause of allergic contact dermatosis (Burrows 1975, Mancuso et al. 1990), some grains such as barley are also reported as a sensitizing agent (Cronin 1979).

4.3.2 Mortality and cancer incidence studies

An increasing number of mortality and incidence studies is aimed at identying risks of occupational exposure in the grain and flour industry. Two incidence studies, from grain mills in Sweden (Alavanja et al. 1987b, McLaughlin et al. 1987) and from the animal feed industry in Denmark (Olsen et al. 1988) indicate an increased incidence of (primary) liver cancer. In the reports from both studies, exposure to aflatoxin was hypothesised as the cause of liver cancer. In a Dutch mortality study in a vegetable oil pressing facility with exposure to aflatoxin (van Nieuwenhuize et al. 1973, Hayes et al. 1984) an increased mortality of non- malignant liver disease and (respiratory) cancer was found. The study cohort (71 workers and 67 controls) was too small to detect differences in mortality from liver cancer. The increased mortality of respiratory cancer was not confirmed in the Scandinavian incidence studies. Since no adjustment for smoking habits was made, the smoking behavior of the oil press workers may have been a confounding factor. In a ten year cohort study in Danish bakers (Tüchsen & Nordholm 1986), however, an increased mortality from lung cancer was also detected. The authors cited several mortality studies confirming their findings, but also some negative studies. Among others, carcinogenic mycotoxins such as aflatoxin, sterigmatocystine, and zearalenone are hypothesised as causes.

Lymphatic and hemopoietic malignancies were reported in two proportionate mortality studies in the USA, in the corn wet milling industry (Thomas et al. 1985), and in the grain industry (Alavanja et al. 1987a).

An elevated risk of bladder cancer was found in the US corn wet milling industry (Thomas et al. 1985), and an Ohio case control mortality study also identified working in grain mills as a risk factor for bladder cancer (Steenland et al. 1987).

Alavanja (1987b) noted that in the USA chemicals, such as pesticides and fumigants, are probably used to a larger extent than in Europe. On the other hand, the use of raw materials containing aflatoxins is relatively rare in the USA. His hypothesis that the apparent difference between cancer sites may be caused by different characteristics of the industry appears to be plausible, and is confirmed by the available research. The comparison between the available study reports is clearly hampered by considerable differences in study design. Case-control studies and cohort studies are reported, and in the latter risk estimates are expressed as PMR, SMR, SIR etc. Therefore risks are expressed as relative to both external controls and to total mortality or incidence. Because of this, and because of the lack of detailed exposure data, qualitative and quantitative comparison of risks is not possible.

A list of mortality and incidence risks that are not found consistently in the cited studies is extensive. In a NIOSH study (Milham 1976), an increased proportionate mortality rate of emphysema among millers of grain, flour and feed was found in the state of Washington in 1950-1971. In the grain industry study by Alavanja and co-workers (1987a) pancreas cancers were also elevated. In the study among union members in the corn wet milling industry in the United States (Thomas et al. 1985), mortality for pancreatic cancer was also elevated in blacks, as was mortality of diabetes, and chronic nephritis in the total study group. In the Danish animal feed study, the incidence of salivary gland tumours and multiple myeloma was also increased.

In a small Danish study in a seaport grain elevator indications were found for a decrease of variables of sperm quality of 16 workers, compared to a control group (Foldspang et al. 1987). The (present) uniqueness of this study, as well as some methodological shortcomings hamper the interpretation of the results, but the intriguing results suggest the need for further study.

4.4. Respiratory effects

4.4.1 Introduction

A wide variety of respiratory effects has been reported from numerous studies. The heterogeneity of reported mechanisms and effects is undoubtedly caused by the heterogenous and complex nature of the exposure. A further complication is the occurrence of a healthy worker effect in the observational epidemiological research. In the study of the corn wet milling industry mentioned before (Thomas et al. 1985) a decreased mortality for non malignant respiratory symptoms was reported among black workers. In a study in Canadian west coast grain elevators a lower prevalence of atopy compared to controls was found in grain workers (Chan-Yeung 1980). The follow-up study after three years revealed that workers who had left the industry had lower metacholin sensitivity in bronchial provocation tests than those who stayed in the industry (Enarson et al. 1986). In another Canadian study grain workers were found to show less serum precipitin reactions against grain dust extract than a control group (Broder & Mc Avoy 1981). In the follow-up study after three years it was found that workers who stayed in the industry had less respiratory symptoms at the baseline study than those who left (Broder et al. 1985).

An analysis of published research shows several more or less clearly defined syndromes; extrinsic allergic alveolitis, grain or flour fever, and flour or bakers asthma. Furthermore a large number of mainly epidemiological studies indicate acute and chronic lung function changes and respiratory symptoms, although no clear syndrome can be detected. Symptoms indicate a chronic bronchitis, but allergic reactions and asthmatic components are also detected (Dosman & Cotton 1980, Seaton 1984a, DoPico et al. 1984a and 1986, DoPico 1986).

4.4.2 Extrinsic allergic alveolitis

Extrinsic allergic alveolitis (EAA) is also called hypersensitivity pneumonitis. Some hours after exposure, respiratory and systemic symptoms appear. Chills, fever, cough and dyspnoea are most frequently reported (Levy & Fink 1985, Molina 1984). In contrast with grain fever mentioned further, the mechanism of

EAA is immunologic. Precipitating antibodies (IgG) are present, but the mechanism has not yet been revealed in details (Levy & Fink 1985). EAA has been found among others in farmers who work with mouldy hay, straw, and grain. This syndrome is called farmers lung. The causal factors are usually thermophylic actinomycetes, but may also be bacteria (Erwinia Herbicola) (Dutkiewicz 1985). In grain workers EAA is probably rare (Flaherty 1982, Molina 1984, Levy & Fink 1985). In malt workers the so called malt workers lung has been reported. This EAA is caused by the fungi A. fumigates and A. clavatus (Pepys 1980).

4.4.3 Grain fever

Grain or flour fever has been referred to as organic dust toxic syndrome. Symptoms indicate a systemic reaction: malaise, chills, fever, dyspnoea, and leucocytosis that appear during or up to 4-8 hours after exposure to high concentrations of grain dust, or during exposure after prolonged absence from exposure (DoPico et al. 1982 b, Flaherty 1982, Morgan 1984b, DoPico 1986).

Some authors have mentioned a possible causal role of endotoxins in the development of grain fever (Pernis et al. 1961, Morgan 1984a, Flaherty 1982, DoPico et al. 1982b, DoPico 1986). Endotoxins have often been detected in grain dust (Olenchock et al. 1980, DeLucca 1984, Heederik et al. 1987a). Exposure to moulds may also play a role in the development of the grain fever syndrome (DoPico 1986, Pratt et al. 1986).

The mechanism that causes grain fever is different from the cause of extrinsic allergic alveolitis that is characterized by comparable symptoms. In grain fever no precipitating antibodies are found, symptoms develop only at very high exposures, and in a large majority of the exposed people. No chronic characteristics are found after repeated occurrence of grain fever. (Morgan 1984a, Flaherty 1982, DoPico et al. 1982b, DoPico 1986).

The number of workers that reported to have experienced symptoms of grain fever ranged from none to 33% in foreign studies. No data on the situation in the Netherlands are available.

4.4.4 Bakers asthma

Flour or bakers asthma is a well known and frequently found occupational disease among millers and bakers. The classical syndrome is an IgE mediated bronchial asthma with a latency time of many years, and is usually preceded by allergic rhinitis (Thiel & Kallweit 1984, Herxheimer 1973, Bergmann 1979). As causal agents proteins in rye and wheat flour are usually mentioned (Björksten et al. 1977, Blands et al. 1976, Sutton et al. 1984). Other causal agents can be the baking agent α-amylase (Baur et al. 1986), Alternaria and Aspergillus fungi (Klaustermeyer et al. 1977), and the grain weevil (Frankland & Lunn 1965). Mites are mentioned in connection with allergic reactions in bakers (Jimenez Diaz 1947) and in grain workers (Warren et al. 1983, Revsbech & Andersen 1987). A dual reaction, characterized by an immediate and late reaction, also was reported (Hendrick et al. 1976).

Allergic reactions after exposure to soy bean dust were also reported in the literature. Kuissu et al. (1980) described an allergic conjunctivitis and Bush & Cohen (1977) a direct and late allergic reaction.

The prevalence of allergic diseases was estimated between 10 and 20% in West Germany (Herxheimer 1967, 1973, Thiel & Kallweit 1984).

4.5 Epidemiological research on lung function and respiratory symptoms

Epidemiological research conducted in recent years has mainly been focused on the effects of long term exposure on symptoms and (mainly spirometric) lung function, and on acute effects.

4.5.1 Chronic effects

Many cross-sectional and a few longitudinal studies were conducted to detect chronic effects. An overview of longitudinal and of the main cross-sectional research is given.

In a twelve year follow-up study among Paris area workers, exposure to grain dust was identified as related to accelerated FEV_1 decreases (Kauffmann et al.

1982). Chan-Yeung et al. (1980) found a lower FVC and FEV_1 in 600 grain elevator workers on the Canadian west coast compared to a control group of office and saw mill workers. The prevalence of respiratory symptoms and eye and nose irritation was elevated. In a follow-up after 2.5 years the results were confirmed (Chan-Yeung et al. 1981). The largest decreases in FEV_1 and MMEF were found in older workers, compared to a control group. In a second follow-up study after six years it was found that the 10% of the workers who showed the largest decreases in FEV_1, compared to the 10% who had the best trend were exposed to significantly higher doses during the base line study (Enarson & Chan-Yeung 1984).

Corey et al. (1982) found an inverse relationship between three day mean personal respirable dust exposure and the level of FEV_1, MEF_{50} and MEF_{25} in 17 grain workers who did not use respiratory protection.

In a longitudinal study among 500 grain workers in Saskatchewan a difference in three year change of FVC and FEV_1 was found between exposed workers and controls (Dosman et al. 1985).

In another longitudinal Canadian study among 441 grain workers in Thunder Bay no differences to a control group were found (Broder et al. 1985).
Research was also conducted outside the North American continent.

In a flour mill in Sudan differences in FVC and FEV_1 level, and respiratory symptoms were found between 100 workers and 30 controls (Awad al Karim et al. 1986).

In South Africa differences in respiratory symptoms were detected between 582 non white grain workers and 153 controls. No differences in the lung function level were found. However, cross-week changes were found to be different between groups for FVC, FEV_1 and MMEF (Yach et al. 1985).

A Chinese study among 655 grain elevator workers in Chenyang revealed 'highly significant differences of respiratory symptoms (Anonymous 1990).
In a study in Scotland lung function levels did not differ between 75 grain workers and 48 controls (Patel et al. 1981).

In an Amsterdam grain elevator, significant differences in FEV_1, MMEF, MEF_{50}, MEF_{25} and respiratory symptoms were found between workers and

controls in a total of 71 subjects (Wieling et al. 1987). Exposure levels were to a large extent in excess of 10 mg/m^3.

In a Dutch flour mill differences in lung function were found between two groups of 18 workers with high and low exposure levels. Differences were detected for all studied flow variables, but not for FVC (Houba & Zweers 1987).

A study among 318 British bakery employees revealed that FEV_1/FVC ratio was related to exposure levels. 35% of the workers reported one or more chest symptoms (Musk et al. 1989).

The prevalence of respiratory symptoms in the studies mentioned are very different. In the Dutch studies the prevalence of CNSLD symptoms ranged from a few percents to 33% (chronic phlegm in the flour mill (Houba & Zweers 1988)). In foreign studies the prevalences in exposed workers also ranged from one to several tens of percents.

An East German study was conducted among 324 workers that were partly exposed to fertilizer dust and partly to pesticides. Both groups showed a lowered vital capacity and FEV_1 compared to a control group (Lehnigk et al. 1985).

4.5.2 Acute effects

Acute effects of grain dust exposure have been well known for ages (Ramazzini 1700, Patissier 1822). Symptoms can be eye and nose irritation, cough, phlegm, wheezing and shortness of breath (DoPico et al. 1983, Broder et al. 1980, Chan-Yeung et al. 1980).

Research for acute reversible lung function changes has been conducted as observational studies and as experiments. The first field studies were conducted about ten years ago. Broder et al. (1980) studied 77 grain workers in a period of little activity in a grain elevator. Half of the workers were laid off for several months in this period. In this group respiratory symptoms decreased during the layoff, and increased after rehiring. All workers showed an increase in flow variables during the period of low activity, and a decrease after restoration of the full elevator activity. The results of this study indicate that lung function abnormalities due to exposure to grain dust can be at least partly reversible.

In a second study by Broder et al. (1984) among newly hired grain workers a small decrease in FVC was found, as well as an increase in respiratory symptoms, in the initial months of employment. In both studies exposure levels were not assessed.

In Australia 119 seasonal grain workers showed a decrease in FEV_1 after 18 days of employment (James et al. 1986).

Corey et al. (1982) studied 47 grain workers during one week. Mean exposure levels were approximately 1 mg/m^3 respirable dust and 6 mg/m^3 semi total dust. Compared to a control group FVC and FEV_1 decreased during the week (Monday morning - Friday morning). A correlation between exposure and cross-shift decreases of MEF_{50} and MEF_{25} was also found.

Another observational study was conducted by DoPico et al. (1983) in over 200 grain workers who were exposed to a mean personal total dust concentration of 3.3 mg/m^3. Significant relationships between dust exposure and cross-shift lung function changes were found for FVC, MEF_{50} and MEF_{25}.

Cockcroft et al. (1983) found considerable cross-shift decreases of FVC and FEV_1 in six grain workers who were exposed to high concentrations of dust, despite the use of air stream helmets.

In the study from Sudan, mentioned above, decreases of FVC and FEV_1 were found in a group of 100 flour mill workers. The average total dust concentration ranged from 1.3 to 3.6 mg/m^3, dependent on the (stationary) measurement spot (Awad el Karim et al. 1986).

Chan-Yeung and co-workers (1980) studied 485 grain workers in the port of Vancouver and found significant cross-shift decreases on Mondays, and decreases during the week, for both FVC and FEV_1. A group of 65 sawmill workers that was considered a control group showed significant increases for the variables mentioned.

After 2.5 years a follow-up study was conducted among the same workers (n=396). The mean yearly decrease of lung function variables was computed by comparing the Friday afternoon measurements. The authors reported a significant correlation between 'lung function' and the cross-shift and cross-week changes in the base line study ($p=0.037$). It is, however, not clear which lung function variables are concerned, nor what the p-value relates to (Chan- Yeung

et al. 1981). In the second follow-up, that was conducted with 267 workers after six years, significant correlations between annual decline and cross-week changes in the base line study were found for FVC, FEV_1 and MMEF (Tabona et al. 1984).

A Danish study among 132 grain elevators was aimed at identifying diurnal variation in peak flow expiratory rate. The difference between the highest and lowest PEFR was related to respiratory symptoms, and weakly related to grain dust exposure (Revsbech & Andersen 1989).

Apart from the observational studies mentioned, experiments with grain dust were also conducted.

Cockcroft and co-workers found considerable changes in FEV_1 and FVC in six people without previous exposure who spent two hours in a barley silo. The dust concentration was extremely high; the environmental total dust concentration was 583 mg/m^3 and the respirable dust level was 31 mg/m^3 (Cockcroft et al. 1983, McCarthy et al. 1985).

DoPico et al. (1982a) found a more then 20% decrease in FEV_1 in 5 out of 11 grain workers after bronchial provocation with durum wheat extract. In four of the five reactors the reactions did not appear immediately, but only after a few hours. The fifth person reacted immediately and was the only subject that reacted to the durum wheat dust itself. No reactions were found after provocation with extracts of mites and insects. This study is one of the few observational studies that investigated the effects on lung function of components of grain dust or dusts of different types of grain. Cross-shift lung function changes (FVC, FEV_1, MEF_{50}, MEF_{75}) were found during exposure to soy bean dust (Zuskin et al. 1988). Effects of barley dust are described in the report of Cockcroft et al. (1983) mentioned earlier. Barley dust is repeatedly reported to be perceived to produce symptoms (Manfreda et al. 1986, Patel et al. 1981). Endotoxin was reported to be related to acute changes in FEV_1 in swine confinement buildings (Donham et al. 1989).

4.5.3 Discussion and conclusions on epidemiological research

From the available research it can be concluded that both chronic and acute effects are found very frequently. The nature of respiratory effects is not unequivocal, although the obstructive effects appear to be most pronounced. The symptoms which point towards chronic bronchitis appear to prevail (chronic phlegm and wheezing). Allergical mechanisms that may play a role in the development of respiratory effects have been studied intensively. Apart from the grain dust itself, several components were the object of research.

As previously mentioned in the discussion of bakers asthma components that were related to allergic symptoms may be flour proteins, moulds and insects. Results of research for immunologic mechanisms are contradictory. Chan-Yeung and co-workers (1980) for example found no positive skin test on grain dust extract in grain workers, while DoPico et al. (1977) found a significant relationship between positive skin tests and work related symptoms.

The relationship between atopy and lung function changes is not a clear one either. Some authors (DoPico et al. 1977, Yach et al. 1985) found lung function levels to be related to reactions to common allergens. Others, however ((Chan-Yeung et al. 1980, Grzybowski et al. 1980, Broder et al. 1983), found no relationship. According to an Australian study (Cookson et al. 1986) an allergic constitution was a risk factor for the development of respiratory symptoms in seasonal workers, but in the longitudinal Vancouver study no relationship between atopy and the development of symptoms or lung function decreases was detected (Chan-Yeung et al. 1981, Tabona et al. 1984).

The role of immunologic mechanisms in the development of respiratory symptoms and lung function changes is not sufficiently clarified. Nevertheless it is clear from the research that other than immunological mechanisms must be important as well.

A mechanism that may include complement activation is still hypothetical. Olenchock and Major (1979) showed that grain dust activated the complement system in vitro. The endotoxin that was already mentioned as an active component may play a role in complement activation (Roitt 1984, Burrell 1984). DoPico et al. (1981, 1983), however, did not find support for this hypothesis in vivo.

A mechanism that may play a role in the development of lung function changes is bronchial hyperreactivity. Tabona et al. (1984) measured methacholine responsiveness in the second follow-up of the longitudinal study by Chan-Yeung and co-workers (1980). Increased airway responsiveness in grain workers was found to be positively correlated with longitudinal lung function decline. DoPico et al. (1982a) found a relationship between reactions on a durum wheat provocation and increased metacholin responsiveness in a group of 11 grain workers. To what extent the hyperreactivity is caused by exposure to grain dust is not clear. Results of several studies suggest a direct effect on bronchial hyperreactivity; a group of 52 non smoking grain workers showed increased bronchial reactivity when compared to a control group of teachers (Gerrard et al. 1979). A comparison between 29 lifetime non smoking grain workers and 29 controls also revealed increased bronchial reactivity in the exposed subjects (Mink et al. 1980). In 160 bakers an increased prevalence of bronchial hyperreactivity was found after comparison with a control group (Langhorst et al. 1985). Two studies in seasonal grain workers also indicate a (seasonal) exposure related trend in airway responsiveness, in 29 wheat workers with a history of wheat associated asthma (Hensley et al. 1988), and in 41 grain handlers (James et al. 1990). Two other studies, however, failed to detect this phenomenon. James and co-workers (1986) found no changes in bronchial reactivity in 119 seasonal grain workers, and Dales and co-workers (1988) found a decrease in airway responsiveness during employment in 45 river port grain handlers. Although the results of the presented studies are inconclusive, the indications for an exposure related increase airway responsiveness are strong.

A subject that has received much attention in the literature is the interrelationship of smoking habits and grain dust exposure as causal factors for the development of lung function declines and chronic bronchitis. Studies with lifetime non-smokers show that lung function changes and symptoms of chronic bronchitis can be related to grain dust exposure (Dosman et al. 1980). Some authors found indications for a synergistic effect in their studies (Cotton et al. 1982, Herbert et al. 1981, Dosman et al. 1985). Other studies, however, showed a mere additive effect (Cotton et al. 1983, DoPico et al. 1984b, 1986, Wieling et al. 1987).

4.6 Studies of non-occupational exposure

As far as we know three studies are published that relate morbidity or mortality to the proximity of grain handling facilities. A cluster of Hodgkin's disease was found in a small town in Michigan. The incidence in a twenty year period was 10 cases in the 1250 inhabitants town, while 0.74 cases were expected. The only industrial facility in the town was a grain elevator, and most of the cases resided near the elevator. The authors suggest chronic immune stimulation by mitogenic substances (especially phytohemagglutinin in navy beans) as a predisposing factor for the development of Hodgkin's disease (Schwartz et al. 1978). Although the increased incidence rate is clearly significant, the causal link to dust exposure is highly speculative. In a recent discussion on cluster analysis, Rothman mentions five reasons why the study of clusters may be handicapped (Rothman 1990). The Michigan study does not meet two of Rothmans criteria. The first is a phenomenon called 'Texas sharp shooting'; the sharpshooter first fires at the barn and then paints the bulls-eye around the bullet hole. Thus the space-time cluster is defined after the cases were identified. In the Michigan cluster the authors defined the study group as the population of the town, but also took into account malignancies that appeared in people who left the town. The second criterion that was not met is the characterisation of exposure. Proximity to the grain elevator was not quantified, prevailing winds were not taken into account, and measurements of the actual dust exposure were not conducted. A further handicap of the analysis may be that the extensive investigations for lymphatic malignancies may have resulted in higher incidence rates than could be detected in the general population studies that were used as reference.

Furthermore, the findings in the Michigan study were not supported by the results of studies in occupationally exposed groups (4.3.2). Two studies identified a relationship between lymphatic malignancies and occupation. Alavanja and co-workers (1987a) found elevated proportionate mortality ratios in grain industry workers. However, no increased mortality rates for Hodgkin's disease were found. In the corn wet milling industry elevated PMR's were also

found, but an increase of Hogdkin's disease was not reported (Thomas et al. 1987).

Another study on the relationship between proximity to industry and health problems was aimed at lung cancer mortality in Louisiana. In a case control study, slightly elevated relative risk estimates were found for over ten years of residence near grain industry. The authors hypothesise particulates as causal factors, but also state that, since the numbers were small, no conclusions were warranted (Gottlieb et al. 1982).

A third study was very carefully conducted in Barcelona, after frequent outbreaks of asthma. All 13 epidemics that were registered in a two year period, coincided with the unloading of soybeans in the harbour, as well as with meteorological conditions that favoured transportation of dust from the harbour to the city. The likelyhood of a causal relationship was increased by a serological case control study in which the IgE levels against extracts of soybean from Barcelona harbour were found to be highly significantly raised in patients (Antó et al. 1989, Sunyer et al. 1989).

In general it can be concluded that data on effects of grain dust during non-occupational exposure are scarce and, as far as malignant diseases concern, not very convincing. The asthma epidemics study by Antó et al., however, was carefully performed, and gives very strong indications for the relationship between soy bean dust exposure and asthma.

4.7 Conclusions

A vast amount of research on non-respiratory effects of grain dust exposure is available. The main focus is on skin disorders, and on mortality and cancer incidence.

The prevalence of bakers eczema has decreased rapidly after the use of some baking aids was forbidden. Nevertheless there is still a vast amount of literature on both irritant and allergic contact dermatitis in bakers and grain mill workers, caused by several agents, such as grain, additives, and mites. The animal feed

industry is, due to the extensive use of additives, frequently mentioned as a risk for allergic skin disorders.

The reports on mortality and cancer incidence indicate an increased risk for several malignancies in branches of industry with regular exposure to grain dust. Most consistent are liver cancer in European studies, and lymphatic and haemopoietic malignancies as well as bladder cancer in North America. Exposure to aflatoxin and other mycotoxins may be related to the liver cancer incidence, whereas the other diseases may be related to the use of pesticides and fumigants.

Several effects of grain and flour dust on the respiratory system have been reported from a wide variety of studies. Extrinsic allergic alveolitis is rarely reported in connection with grain dust, but (bakers) asthma and to a lesser extent grain fever are reported frequently. Symptoms and lung function changes that indicate chronic bronchitis are also frequently reported, although the mechanism, and especially the role of immunological factors and bronchial reactivity, need more clarification.

A large number of components in grain dust have been related to respiratory disorders; moulds, endotoxins, mites, pesticides and of course the grain dust itself. Apart from studies on mechanisms, research into the role of active components in the dust appears to be relevant for the development of knowledge in this field. Epidemiologic research needs a reliable characterization of exposure. The use of indirect exposure estimates such as time weighted averaged total dust concentrations or dichotomous variables indicating exposure/non exposure may easily lead to veiled or underestimated exposure-response relationships.

When these two conditions are met, a clear identification of mechanisms and active components, it will be possible to establish clear guidelines for exposure to grain dust. There is, however, massive evidence that the (operational) MAC values of 10 mg/m^3 total nuisance dust and 5 mg/m^3 respirable dust are clearly to high to prevent respiratory disorders.

5. Exposure to dust, endotoxin and fungi in the animal feed industry[1]

Tjabe Smid, Dick Heederik, Gert Mensink, Remko Houba and Jan S.M. Boleij

5.1 Abstract

In the Dutch animal feed industry approximately 6000 workers are exposed to organic dust, originating mainly from raw materials such as grain, pulses (peas and beans) and waste products from the vegetable oil and starch industries.

In this study, 79 stationary dust samples and 530 personal dust samples from eight animal feed production facilities were analyzed. The stationary total dust samples showed gravimetric concentrations ranging from 0.2 to 25 mg/m^3 (Geometric Mean 1.3 mg/m^3). Concentrations of smaller particle fractions (respirable, thoracic, and inspirable dust) were considerably lower. Personal inspirable dust concentrations were considerably higher than stationary concentrations, and were strongly related to job titles. Pooled personal inspirable dust concentrations ranged from 0.2 to 450 mg/m^3 (GM = 2.4 mg/m^3). After adjusting for differences between inspirable and total dust, 25% of the measurements exceeded the Dutch MAC (Maximum Allowed Concentration) for total nuisance dust (10 mg/m^3), and 42% exceeded the ACGIH Threshold Limit Value for grain dust (4 mg/m^3). Endotoxin concentrations ranged from 0.2 to 1870 ng/m^3 inspirable dust (GM = 5.1 ng/m^3). Endotoxin appeared to be less prevalent in respirable dust than in larger dust fractions. Concentrations in dust appeared to be related to stages in the production process. Colony forming units of fungi ranged from 130 to 15300 CFU/m^3 (GM = 2300 CFU/m^3), and were in parallel measurements more strongly related to endotoxin concentrations than to dust concentrations.

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[1] Am. Ind. Hyg. Assoc. J. 53 (1992) 362-368

It is clear that workers in the Dutch animal feed industry are frequently exposed to dust levels above recommended Dutch and American levels. Exposure levels to endotoxin and to fungi are quantified. This information provides a basis for a preliminary health risk analysis and for the design of effect oriented studies.

5.2 Introduction

Production of animal feed is a important activity of the European agro-industry. In the Netherlands, approximately 500 facilities employ 10,000 workers, among whom 6,000 are exposed to organic dust. Some organic dusts, and particularly grain dust, have been described to be related to increased respiratory symptoms and lung function changes, to allergic disorders such as extrinsic allergic alveolitis, and to the organic dust toxic syndrome (Chan-Yeung et al. 1981, Zuskin et al. 1989, Levy & Flink 1985, Morgan 1984a, DoPico et al. 1984b).

The occupational exposures were studied in eight storage and production facilities that were considered typical for the Dutch animal feed industry. The exposure measurements were part of a larger epidemiologic study aimed at identifying the respiratory effects associated with exposure to dust and major components of it in the animal feed industry.

The production process of animal feed is simple (figure 5.1). The raw materials arrive by ship or by truck, are cleaned, and stored in silos. In a batch process, the raw materials are ground and mixed with fats, molasses and additives, such as vitamins and minerals. The mixture is then usually pressed into pellets and stored in silos again. The animal feed is either packed and shipped in sacks or shipped in bulk trucks. The process is highly mechanized, and most of it is operated from a central control room. Exposure of workers

therefore mainly takes place during unloading, cleaning, maintenance and similar tasks.

Raw materials used in animal feed production are:

- Grains; mainly corn, wheat and barley;
- Other vegetable products; such as cassava and pulses (soy beans, peas and horse beans);
- Waste products from the food industry; such as citrus pulp, extracted soy meal, corn gluten, beet pulp, and extracted copra meal.

Although grains were traditionally the major ingredients in animal feed, their importance has gradually diminished in favor of other vegetable and waste products. By 1986, grains constituted 13% of the raw materials, where it had been 70% in the fifties. The European Community policy, however, is to increase the use of grains again.

The dust in animal feed mills is a heterogeneous mixture of all kinds of organic dusts. The prevailing part is the raw material itself, but other components may be fungi and bacteria and their toxins, insects and parts of animals and their excreta, agricultural chemicals such as pesticides and fertilizer and inorganic matter such as silica dust (Yoshida 1982, Farant & Moore 1978).

In this study, gravimetric dust concentrations of four different particle size fractions were measured. In the dust samples, determinations were made of endotoxin, a toxin produced by gram negative bacteria that may cause systemic and pulmonary pathology (Jacobs 1989). Airborne fungi concentrations were also determined.

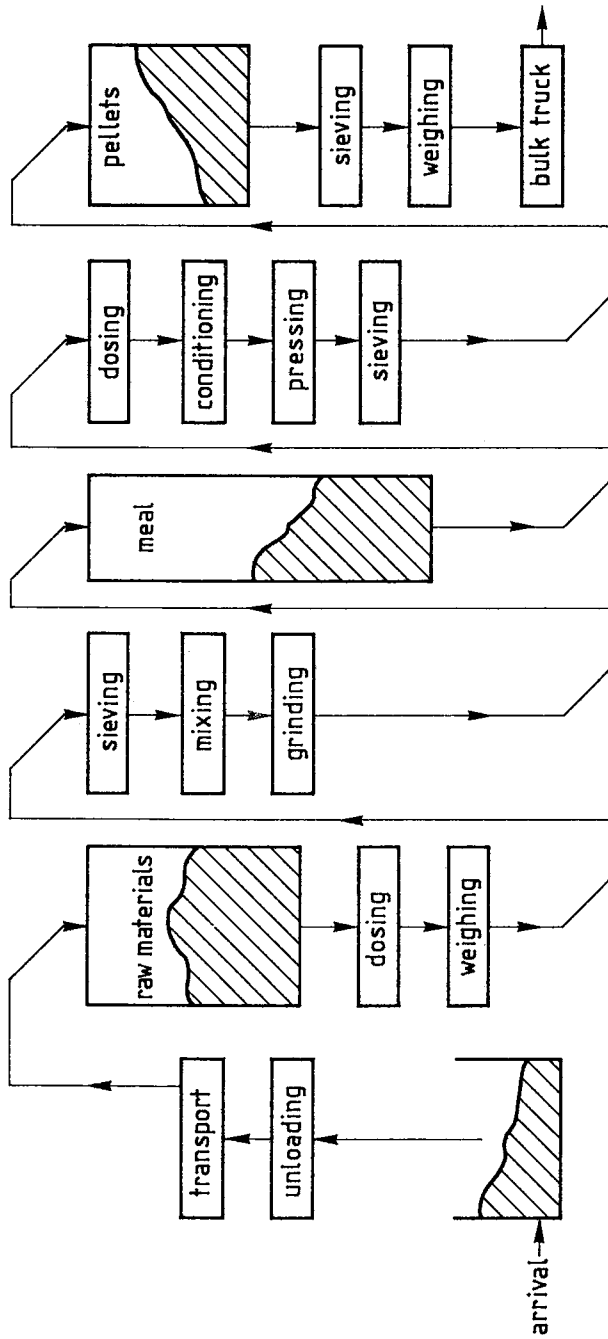

Figure 5.1 Production process in an animal feed mill (adopted with permission from Beumer 1984)

The purpose of the study was to reveal characteristics of exposure that are relevant for the evaluation of potential health risks and for the design of studies on exposure response relationships.

General characteristics are described first, such as detection limits, reproducibility, and frequency characteristics.

Extensive stationary measurements were aimed at identifying particle size distribution and mutual relationships of dust and endotoxin levels in the various dust fractions, and airborne fungi concentrations.

Personal measurements were aimed to establish absolute exposure levels of dust and endotoxin in the animal feed industry. By comparing exposure levels to limit values and concentrations that may cause pathology in other industry, preliminary information on potential health impact of the exposure may be assessed. Relating exposure to job categories and facilities was performed in order to identify parameters that may determine exposure levels, both for endotoxin and dust. This information can be used in the development of a preliminary health risk evaluation and in the design of effect oriented studies.

5.3 Study design

5.3.1 Production facilities

Eight production and storage facilities were studied during 1 to 24 days, in most cases in two periods (spring and autumn). The facilities differed in a number of characteristics, such as age, number of employees and technology level. Table 5.1 shows some of these characteristics of the eight facilities. The facilities were selected with the aim of establishing a representative sample, through the cooperation of regional health services and the main animal feed producing companies. With the exception of the very small facilities (less then 10 workers which account for less than 10% of the workforce) the sample is considered to be representative on the characteristics mentioned.

Stationary sampling was conducted in seven mills, and personal samples were taken in all eight.

Table 5.1 Characteristics of the facilities

nr.	number of employees	age of facility (year)	arrival of raw materials	type of facility	main products	automation[1]	dust control[2]	days measured
1	< 25	> 25	ship	elevator	–	0	0	6
2	< 25	< 10	ship	elevator	–	++	+	6
3	25-50	10-25	ship	elevator/ production	bulk/sacks	+	0	2
4	25-50	> 25	truck	production	sacks	+	0	2
5	25-50	> 25	ship	production	bulk/sacks	0	0	12
6	25-50	10-25	ship	production	bulk	++	+	1
7	> 50	10-25	ship	production	bulk/sacks	+	+	24
8	25-50	< 10	ship/ train	production	bulk	++	+	12

[1] automation of production 0 = low, + = fair, ++ = high
[2] dust control (i.e. separated production rooms, exhaust systems, enclosed production process, control rooms, regular cleaning) 0 = low, + = fair

5.3.2 Stationary sampling

Stationary sampling was conducted in locations where worker activities were concentrated. The main purpose of the stationary sampling was to describe characteristics of theairborne dust that could not be determined by personal sampling. These characteristics included the relationship between several particle size fractions and the concentrations of airborne fungi.

All stationary measurements consisted of four parallel measurements of different particle size fractions, over six to eight hours. At a moment during this time, fungal concentrations were measured. Measurements were taken at 1.5 m above the floor.

The following dust fractions were determined:

- **total dust**

  Total dust was determined using a modified Schleicher and Schüll PL050/1 sampling head with a face velocity of 1.25 m/s in the inlet opening and a flow rate of 23.5 l/min (Staubforschungsinstitüt 1973). Whatman GF/A glass fiber filters were used in a modified filter cassette.

  The method is considered by the Dutch legal requirements to measure total dust. The dust samples were used to determine the proportion of measurements exceeding the total dust MAC (Maximum Allowed Concentration) of 10 mg/m^3 and the ACGIH threshold limit value of 4 mg/m^3.

- **inspirable dust**

  Inspirable dust was determined using a portable pump at a flow rate of 2.0 l/min, and a filter cassette with a 6 mm diameter inlet opening (ter Kuile 1984), on a 2.4 cm diameter glass fiber (Whatman GF/A) filter. Although this technique meets the requirements for total dust sampling, the captured dust fraction is considerably smaller than the one described earlier under total dust, and resembles the inspirable dust fraction that is characterized by a 50% cut-off diameter (D_{50}) of 30 μm (ISO 1981).

- **thoracic dust**

  The thoracic dust fraction, with a D_{50} of approximately 10 μm (ISO 1981), was determined using cyclones as described by Vrins and Hofschreuder (1983), with Whatman GF/A glass fiber filters, at a flow rate of 15 l/min.

- **respirable dust**

  The respirable (or alveolar) dust fraction, characterized by a D_{50} of 5 μm (ISO 1981), was determined with the same cyclones that were used for measuring the thoracic fraction, at a flow rate of 50 l/min.

All samples were analysed gravimetrically, and endotoxin in the samples was analysed using the Limulus Amoebocyte Lysate test as described by Attwood et al. (1987)

The concentration of airborne fungal particles was determined using the N-6 modification of the Andersen sampler (Jones et al. 1985) on malt extract agar plates during one minute at a flow rate of 28.3 l/min. Results were expressed as

colony forming units (CFU) per m^3. Details of this method are described in appendix (Smid et al. 1989).

5.3.3 Personal sampling

In all facilities, personal inspirable dust sampling was performed on all production workers during one or more days. Technical maintenance workers were excluded from the study population since their dust samples might contain considerable amounts of non-organic dust, such as metal dust originating from welding fumes. Inspirable dust was sampled in the workers' breathing zone during fullshift periods of approximately eight hours. The inspirable dust fraction was sampled according to the method described earlier. As in the stationary samples endotoxin analyses were performed.

All workers were grouped into categories by job title. The job categories consisted of one or more job titles with similar tasks and working environments. The following eight categories were used:

Unloaders work mainly in barges where raw materials are piled up using shovels or brooms to be unloaded by a crane or a suction pipe.

Crane drivers operate the crane that is used to unload the raw materials from barges.

Facility operators and bulk loaders operate the production process or the loading of bulk trucks from a central control room.

Press operators operate the machines used to press the milled materials into pellets. They spend most of their time in the production rooms, and partly in local (press) control rooms.

Production managers and their deputies are usually not directly engaged in production work, and spend considerable time in production rooms.

Transport workers are usually lift truck operators, but may have other transport tasks, mainly in receiving deliveries or in the storage of sacks and the preparation of deliveries.

Packers operate the packing machines where the animal feed is packed into sacks.

Other production jobs are characterized by a variety of tasks, such as operating and checking several machines, checking silos and cleaning.

5.4 Results

5.4.1 Detection limits, reproducibility and frequency characteristics

Detection limits were computed by adding the mean plus three times the standard deviation of approximately 10 blanks, and dividing it by the mean sampled volume (Smid et al. 1989). For all dust fractions, detection limits varied from 0.1 to 0.3 mg/m^3, and for endotoxin from 0.1 to 0.3 ng/m^3, depending on the particle size fraction. In the statistical analyses, values below the detection limits were considered as being 2/3 of this limit.

In order to quantify the reproducibility, CV-values (coefficients of variation) (EPA 1987) were computed for a sample of simultaneous duplicate measurements for all dust and endotoxin fractions. For stationary samples, CV-values varied from 11% to 23% for dust and from 31% to 49% for endotoxin. In the personal (inspirable) dust fraction, CV values, were 38% for dust and 52% for endotoxin computed from a sample of (n=8) workers who carried two measurement sets. Since CV values are the result of measurement and analytical errors, the differences between dust and endotoxin can be explained by a larger analytical error for endotoxin analysis. Differences in CV values for stationary and personal measurements are caused by differences in sampling errors.

For fungi, every CFU value was corrected for a simultaneous blank on the measurement spot, and all samples were taken in duplicate.

For all 11 exposure indices (dust and endotoxin in four stationary and one personal fractions, and CFU concentrations), the distribution was skewed to the right when all data were pooled. For all indices, a log transformation lead to a distribution that resembled a normal distribution, as was indicated by the Kolmogorov goodness of fit test. Although in a few cases the deviation from normality was still significant, log normality was assumed in the statistical analyses as is indicated by the computation of geometric means and standard deviations.

5.4.2 *Stationary measurements*

A total of 79 complete sets out of 92 sets (86%) of stationary measurements were available for statistical analysis, i.e. sets with no missing values for dust, endotoxin or fungi CFU concentrations. Missing values were caused by technical failures such as pump breakdown, filter damage etc. and were therefore non-systematic. The measurements were conducted in seven mills; in facility 6 no stationary samples were taken. Although small differences (related to the total variance) between the studied facilities existed, all measurements were pooled.

Table 5.2 shows geometric means and standard deviations for all 11 exposure indices. Both endotoxin- and dust concentrations were related to the particle-size fractions; the smaller fractions accounted for a relatively small part of the total concentrations. For all size fractions, the variance in endotoxin concentration was considerably larger than in dust concentrations. The GSD's for all four dust fractions were about 3 and for endotoxin they ranged from 4 to 6.7. Endotoxin concentrations were usually low with some extremes that accounted for the large GSD's.

Table 5.2 Characteristics of stationary measurements for various dust fractions, and endotoxin concentrations in these fractions, and CFU concentrations (n=79)

	GM	GSD	range
dust fraction:			
total (mg/m^3)	1.3	2.9	0.2-25
inspirable (mg/m^3)	0.8	3.4	0.2-20
thoracic (mg/m^3)	0.4	2.9	0.7-4.9
respirable (mg/m^3)	0.1	2.6	0.1-1.8
endotoxin fraction:			
total (ng/m^3)	2.6	6.7	0.1-1850
inspirable (ng/m^3)	1.9	4.9	0.2- 160
thoracic (ng/m^3)	0.8	5.0	0.2- 610
respirable (ng/m^3)	0.1	4.0	0.1- 17
fungi (CFU/m^3 * 1000)	2.3	2.6	0.13-15.3

GM = geometric mean
GSD = geometric standard deviation

The - for stationary samples - relatively large variance in dust concentrations can be explained by the heterogeneity of the 79 measurements (various places in seven facilities).

Concentrations of viable fungi ranged from 130 to 15300 CFU's per m^3, with a variance that was slightly smaller than for dust concentrations (GSD = 2.6).

Table 5.3 shows the correlation between log transformed CFU-concentrations and the other eight log transformed stationary sample exposure indices. CFU concentrations appear to be clearly related to endotoxin concentrations, as indicated by Pearson correlation coefficients between 0.32 and 0.38 ($p < 0.05$ with inspirable endotoxin and $p < 0.01$ for the other three size fractions). The relation of CFU levels with dust is less clear. Mutual correlations between the endotoxin concentrations in the stationary samples were stronger; all but one were between 0.37 and 0.83 ($p < 0.01$).

Table 5.3 Correlation coefficients (Pearson's R) of log transformed CFU concentrations with log transformed dust- and endotoxin concentration (n=79)

dust fraction	CFU-dust	CFU-endotoxin
total	0.11	0.38**
inspirable	0.23	0.32*
thoracic	0.29 *	0.38**
respirable	0.21	0.37**

* = $p < 0.05$
** = $p < 0.01$

The endotoxin content of dust in the various stationary sampled size fractions is given in table 5.4. Since the distribution of these four indices resembled a log-normal distribution, characteristics are given as geometric means and standard deviations. The variance of the endotoxin content of dust appear to be clearly smaller than in air (compare table 5.2).

Where the geometric mean concentrations of endotoxin in dust in the three larger fractions were comparable, it was clearly lower in the respirable fraction, indicating that endotoxin levels in the smaller particles were relatively low.

Table 5.4 Endotoxin content of dust (ng/mg) in stationary particle size fractions (n=79)

dust fraction (ng/mg)	GM	GSD	range
total	2.0	4.6	0.1-270
inspirable	2.3	2.7	0.2- 32
thoracic	1.9	3.3	0.2-120
respirable	1.1	3.4	0 - 35

GM =geometric mean
GSD=geometric standard deviation

Table 5.5 shows mutual relations between the dust fractions; the three smallest fractions are given as a proportion of the total dust concentration. The distributions of all three proportions were skewed to the right, but a log transformation did not improve normality. Therefore frequency characteristics are given as a median proportion and range. From the table it is clear that although the variance in proportions is considerable, the median proportions are clearly related to the size fraction. The median proportion of 0.65 for inspirable to total dust, can be used to compute from the stationary inspirable dust measurements to what extent the personal total dust MAC was exceeded.

Table 5.5 Median and range of proportions of 3 stationary dust fractions in total dust (n=79)

dust fraction	median	range
inspirable	0.65	0.11- 7.7
thoracic	0.37	0.05- 2.4
respirable	0.10	0.01- 0.57

5.4.3 Personal measurements

A total of 530 personal measurements taken from 187 workers in all eight facilities were available. A histogram of all pooled data is given in figure 5.2, illustrating the log-normal distribution. In order to investigate if dust exposures were determined by job characteristics or by facility characteristics, analyses of variance were performed on dust and endotoxin levels. As factors, job category and facility were used simultaneously to explain for (log transformed) exposure levels. Table 5.6 shows the results, indicating that job category was more important in explaining both dust and endotoxin levels, than the facility in which the workers were employed. When the two factors were analysed separately, or when a correction was made for the unequal distribution of workers over the facilities by weighted analyses of variance, the results remained similar.

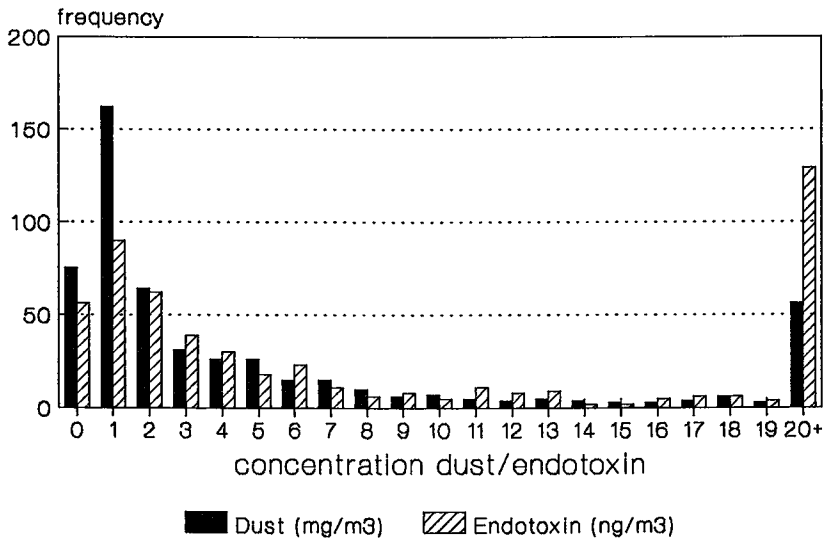

Figure 5.2 Histogram of pooled personal inspirable dust (mg/m^3) and endotoxin (ng/m^3) measurements, taken from 131 workers in eight facilities (n = 570).
The last columns ("20+") represent exposures of 20 mg/m^3 dust and above, and 20 ng/m^3 endotoxin and above.

Table 5.6 Analysis of variance of log-transformed personal dust and endotoxin concentrations (n=530)

factor		dust		endotoxin	
	Df	MS	F	MS	F
job category	7	28,2	15,8*	37.2	14.3*
facility	7	11,6	6,5*	18.8	7.2*
rest	515	1,8		2.6	

Df =degrees of freedom
MS =mean squares
* =p<0.001

Table 5.7 shows frequency characteristics of personal dust exposures for all eight job categories. Geometric means generally ranged from 0.8 to 3.4 mg/m^3, with the exception of the unloaders who's exposure had a geometric mean of 9.8 mg/m^3. The variance in all categories was relatively large. Due to these high variances, inter group differences in body (lung) burden can not be derived from geometric means. The arithmetic mean is a more appropriate variable to represent differences in total burden. Figure 5.3 shows these arithmetic mean exposures for all job categories. Arithmetic standard deviations are not given because of the skewness of the distribution. The 'other production jobs' category clearly deviates from the other categories (except from the unloaders), where the comparison of geometric means did not show elevated exposure levels. Overall the average (AM) dust exposure is almost 10 mg/m^3, almost all of which is attributable to the exposure of unloaders.

The number of measurements in excess of the Dutch MAC for total nuisance dust (10 mg/m^3) was computed after dividing the personal (inspirable) dust exposures by the median ratio of inspirable to total dust based on the static measurements, thus adjusting for the smaller particle size fraction. In total, 25% of the measurements exceeded the MAC. Only the facility operators (category 3) showed less than 5% of measurements in excess of the TLV (2%). For all other job categories the number of measurement above the MAC was more than 5%,

with a maximum of 57% for the unloaders. When the ACGIH grain dust TLV was used (4 mg/m^3 total dust, ACGIH 1992), 42% of all measurements was in excess. In four of eight job categories the excess percentage was higher than 50%, again with a maximum for the unloaders (80%).

Table 5.7 Characteristics of personal dust concentrations by job category

job category (n)	GM (mg/m^3)	GSD	range (mg/m^3)
unloaders (69)	9.8	4.6	0.2-450
crane drivers (23)	2.5	4.7	0.2- 27
facility operators (63)	0.8	2.7	0.2-9.4
press operators (54)	1.3	3.2	0.2-100
production managers (42)	1.6	3.5	0.2- 49
transport workers (61)	1.2	2.8	0.2- 14
sackers (20)	3.4	2.9	1.1- 45
other (198)	3.2	4.9	0.2-250
total (530)	2.4	4.7	0.2-450

GM = geometric mean
GSD = geometric standard deviation

Table 5.8 shows the results of the endotoxin measurements. The differences between job categories correspond with those found for the dust concentrations; the unloaders (category 1) clearly deviated from the other job categories. Geometric standard deviations, however, were considerably higher then for dust concentrations, and ranged up to an extreme value of 8.7 for the crane drivers. The reason for this is probably that in some facilities the crane drivers occasionally perform high-exposure unloading tasks as well as the relatively low exposure crane driving. The high GSD of 6.0 in the 'other production jobs' category can be explained by the divergent tasks that are part of this category. The highest variances were found in the job categories that showed high GSD's for dust as well.

Table 5.8 Characteristics of personal endotoxin measurements by job category

job category (n)	GM (mg/m^3)	GSD (mg/m^3)	range
unloaders (69)	28.5	5.4	0.2-1150
crane drivers (23)	10.6	8.7	0.2- 420
facility operators (63)	1.2	4.3	0.2- 50
press operators (54)	2.3	4.2	0.2- 160
production managers (42)	2.5	5.0	0.3- 160
transport workers (61)	2.4	5.2	0.2- 880
sackers (20)	3.7	3.8	0.5- 75
other (198)	7.0	6.0	0.2-1870
total (530)	5.1	6.7	0.2-1870

GM = geometric mean
GSD = geometric standard deviation

Analogous to the dust exposure differences in body burden can be illustrated by arithmetic means, as shown in figure 5.3. As for dust, the unloaders' category clearly deviated from the rest.

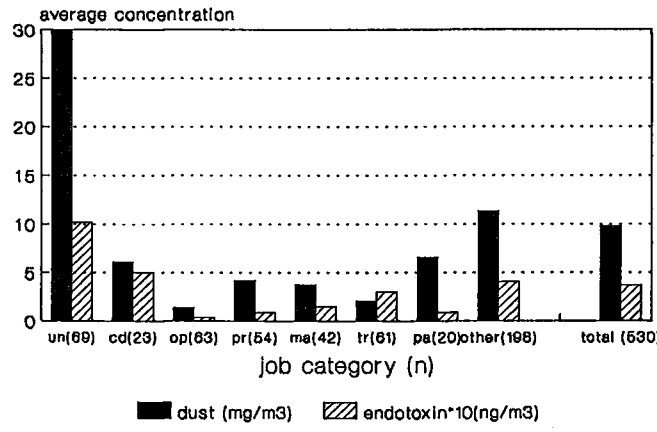

Figure 5.3 Arithmetic mean personal dust and endotoxin concentration per job category, taken from 131 workers in eight facilities (n=530).
un = unloaders; cd = crane drivers; op = facility operators; pr = press operators; ma = production managers; tr = transport workers; pa = packers; other = other production jobs

No standards are available for endotoxin, but two values (100 ng/m^3 and 10 ng/m^3) can be considered. 100 to 200 ng/m^3 is suggested to be a threshold for overshift FEV_1 decline (20), and in sensitive persons Castellan et al. (1987) estimated 9 ng/m^3. In our study, depending on job category, the percentages of values in excess of 100 ng/m^3 ranged from 0 to 22%. For 10 ng/m^3 these figures were 11 to 71%. In total, 35% of the personal measurements were in excess of 10 ng/m^3.

Table 5.9 shows geometric mean concentrations of the endotoxin content in the sampled dust. The two categories that are almost exclusively in contact with the raw materials (unloaders and crane drivers) clearly showed the highest of endotoxin content of dust. The only category in which contact with dust is limited to the end product (packers) showed the lowest endotoxin content. All other job categories are characterized by possible exposure to raw materials as well as the end products. These findings indicate that the endotoxin levels in dust decrease with the continuation of the production proces.

Table 5.9 Endotoxin content of personal dust samples

job category (n)	GM (ng/mg)	GSD (ng/mg)	range
unloaders (69)	2.9	4.3	0.1 - 94
crane drivers (23)	4.3	3.5	0.3 - 130
facility operators (63)	1.5	2.4	0.2 - 12
press operators (54)	1.8	2.5	0.2 - 66
production managers (42)	2.2	2.3	0.5 - 17
transport workers (61)	2.0	4.0	0.02 -1620
sackers (20)	1.1	2.8	0.07 - 7
other (198)	2.2	3.2	0.01 - 740
total (530)	2.1	3.2	0.01 -1620

GM = geometric mean
GSD = geometric standard deviation

5.5 Discussion and conclusions

The facilities in which the study was performed can be considered as a representative sample from the Dutch animal feed industry, with the exception of the very small ones. Generalizing from the personal exposure of the studied workers to all animal feed workers is facilitated by the finding that personal exposures seem to be more determined by job category than by facility.

The exclusion of technical maintenance personnel from the study may have led to an underestimation of the percentage of MAC exceedence, since maintenance workers may be exposed to large concentrations. The relative small number of maintenance workers, however, makes this a minor problem.

A very large number of personal dust samples (25%) exceeded the equivalent Dutch MAC for nuisance dust. Dust exposures were in the range of levels that were reported in US and Canadian grain elevators to be related to respiratory effects (Chan-Yeung et al. 1981, DoPico et al. 1983). Although some job titles contributed considerably to the high degree of non compliance, no category could be excluded from the risk of MAC exceedence.

Dust concentrations were in the same range as results reported from a Yugoslavian animal feed mill, although a precise comparison can not be made due to different measurement methods (Zuskin et al. 1989). Apart from these and a former GDR study indicating larger concentrations (Gemeinhardt et al. 1981) no other studies on animal feed mills were reported.

The stationary measurements showed that dust levels mainly consisted of larger particles. The median proportion of respirable in total dust was only 10%. An evaluation for health risks based on total dust mass would imply that the larger particle size fractions are more relevant than the respirable dust.

The larger particles were the main source of endotoxin exposure. This finding was not surprising, since the endotoxin content of the dust was similar for all fractions, with the exception of the respirable dust concentration that was approximately 50% lower. There is no plausible explanation for this finding. Endotoxin levels were in the same range as the concentrations that were found in grain elevators (DeLucca & Palmgren 1987).

Fungi were measured as colony forming units. Because a particle may contain more than one spore, this methods underestimates the total number of viable spores. Fungal CFU concentrations were approximately 10 to 1.000 times lower than levels that were reported in grain elevators (Farant & Moore 1978, Palmgren et al. 1983a) and in (former) GDR animal feed mills (Gemeinhardt et al. 1981). Differences in measurement methods, however, prevent a detailed comparison.

Because endotoxin levels were more strongly related to CFU's then to dust levels, it appears that there is a microbiological parameter that is only partly related to dust concentrations. The independence from dust levels of this microbiological parameter is supported by the finding that endotoxin concentrations in dust appeared to be related to the stages of the production process. This finding may be of major importance for studies in which effects are linked with exposure variables. Although dust and endotoxin exposure can be separated to some extent, endotoxin-related effects that may be found may well be caused by fungi or perhaps even by other microbiological parameters.

Personal dust concentrations were considerably higher than could be expected from stationary samples. A clear cause is probably that for many 'dust producing' tasks (repair work, cleaning), the worker is much closer to the source than the stationary sampling equipment. Another reason is that for technical reasons no stationary samples could be taken in some very dusty locations, for example in a ship with raw materials. Nevertheless it is obvious that stationary sampling can not be used to estimate personal dust exposures.

Differences in geometric mean personal exposure levels were found to be only partly related to arithmetic mean levels, suggesting that geometric means may not adequately classify job categories into levels of body burden. The main reason for this was the large difference in geometric standard deviations between the job categories.

An important result from this study is that personal exposure was more clearly determined by job category than by facility, indicating that the large differences between facilities in level of mechanization or implementation of dust control measures did not result in a decrease of personal dust exposures. A probable cause is the difference in tasks between modern and traditional

facilities within the same job categories; because routine tasks are mechanized, dusty tasks such as cleaning and repair jobs have an increased contribution to the exposure. A beneficial effect of dust control can thus be compensated by a changing set of tasks that constitute a job. Another cause may be that preventive behavior is more prominent in highly dusty environments, as was suggested in a Canadian study in which an effect of increased ventilation was seen on stationary samples but not on personal samples (Farant & Moore 1978). The suggested reason was that before the improvements were implemented, workers tended to leave dusty environments as soon as they had performed their assigned tasks.

Measures that will result in decreasing personal exposure will therefore be difficult to implement. Regarding the exposure levels, however, they will be essential to prevent health risks.

5.6 Acknowledgements

The authors thank the workers for their cooperation, and Rudolf van der Haar, Hans Marquart, Anita Groenendijk, Mieke Lumens, Tonnie Zweers, Reini Pater, Pieter Versloot and Jos van Hutten for their help.

6. Dust- and endotoxin related respiratory effects in the animal feed industry[1]

Tjabe Smid, Dick Heederik, Remko Houba, Philip H. Quanjer

6.1 Abstract

A cross-sectional study of 315 animal feed mill workers was undertaken in 14 animal feed mills in the Netherlands. Primary aims were to explore relationships between exposure to organic dust and respiratory symptoms and chronic lung function changes. The study comprised monitoring dust and endotoxin exposure, spirometric lung function measurements (FVC, FEV_1, maximal mid-expiratory flow and flow-volume parameters) and a questionnaire for respiratory symptoms. The exposure was measured during spring and autumn. The average 8-hour personal inspirable dust exposure was 9 mg/m^3 grain dust (range 0.2-150 mg/m^3) and 25 ng/m^3 endotoxin (range 0.2-470 ng/m^3), based on 530 personal dust measurements. On the basis of these measurements and an occupational history of the workers, the number of years 'worked in dust' and the cumulative dust and endotoxin exposure were calculated. The prevalence of most chronic respiratory symptoms tended to decrease with increasing years of exposure. It is argued that probably the "healthy worker effect" is responsible for this finding. In general a strong negative association between most of the exposure variables and lung function was found. The endotoxin exposure was more strongly related to decreases in lung function than the dust exposure. These results suggest that endotoxin exposure is an important factor in the development of respiratory diseases. The lung function changes occur at endotoxin levels lower than previously reported in field studies.

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[1] Am Rev Respir Dis 146 (1992) 1474-1479 (published in revised form)

This study was supported by a grant form the Directorate General of Labor of the Netherlands Ministry of Social Affairs and Employment.

6.2 Introduction

Respiratory effects of organic dust have been reported from several studies. The main exposures that attracted scientific attention were cotton and grain dust and their constituents (Chan-Yeung et al. 1980, 1981, Dosman et al. 1980, 1985, Cotton et al. 1982, 1983, WHO 1983). Other types of organic dust have also been studied, deriving from a variety of products such as wood, flax, hemp, jute, tobacco, tea, etc. (WHO 1983, Chan-Yeung et al. 1984, Bouhuys et al. 1961, El Ghawabi 1978, Gleich et al. 1980, Zuskin et al. 1985). There is limited evidence of respiratory effects of dust in animal feed mills. An increased prevalence of respiratory symptoms and decreased lung function values were found in a cross-sectional epidemiological study (Zuskin et al. 1989).

In several experimental studies endotoxins have been hypothesised as agents playing an important role in the etiology of cross-shift lung function changes (Rylander 1985). Endotoxin exposure was suggested to be related to chronic lung impairment in an epidemiological study among cotton workers (Kennedy et al. 1987).

In this paper results will be presented of a cross-sectional study of lung function and respiratory symptoms and their relationship to dust and endotoxin exposure in the Dutch animal feed industry. It is part of a larger study aimed at the assessment of exposure levels and acute as well as chronic lung disorders. A detailed description of the exposure data has been published elsewhere (chapter 5). Data analysis of acute lung function changes and work-related symptoms is in progress.

6.3 Materials and methods

6.3.1 Population

A total of 315 Caucasian male workers from 2 animal feed elevators and 12 production facilities took part in the study. Briefly, the production process comprises the following steps. Raw materials arrive by ship or by truck such as grains (mainly maize, wheat and barley), other vegetable products (cassava and

pulses) and waste products from the food industry. The raw materials are cleaned and stored in silos and than ground and mixed in a batch process. Fats, molasses, vitamins, and minerals are added in small quantities. The mixture is then stored again in silos, usually after pressing to pellets. Transport to the customer is mainly done by bulk trucks, but part of the animal feed is packed and transported in sacks. In the elevators only storage and shipment of raw materials takes place.

Job titles can be divided into two large categories: production and non-production workers. Production workers (n=265) were divided over eight job titles. The job titles involved in production are unloaders, crane drivers, facility operators, press operators, production managers, transport workers, packers, and other production workers. A detailed description of the job titles can be found in chapter 5.3. Fifty non-production workers were employed in the animal feed industry, but were rarely exposed to dust in the production process (laboratory personnel, truck drivers, gate keepers, etc.). Maintenance personnel was excluded from the study since they may have been exposed to other respiratory hazards, such as welding fumes. Apart from the 315 animal feed workers, an external control group (n=125) was recruited from two other companies: a milk processing plant and a wholesale company. These controls had the same socio-economic status and performed comparable (manual) work, without exposure to agents that may effect the respiratory system. Data on age, height, working history and smoking habits are given in table 6.1. The work history is given as the number of years the workers were employed in the industry and the number of years they actually held production jobs in which they were exposed to dust.

6.3.2 Exposure

In eight facilities eight-hour personal inspirable dust samples were taken from the production workers category. The dust sampled is characterized by a 50% cut-off diameter of 30 μm, and resembles the inspirable dust fraction that passes the mouth and nostrils when inhaled (ISO 1981). In the samples gravimetric dust concentrations and endotoxin levels were determined using the Limulus

Amoebocyte Lysate (LAL)-test. Details of sampling methods and analyses are given elsewhere (Kateman et al. 1990).

Table 6.1 Characteristics of animal feed workers and external controls: means and standard deviations

	animal feed workers	controls
n	315	125
age (year)	39.4 (11.0)	34.7 (10.0)
length (cm)	178 (7)	176.9 (7.6)
years smoked	15.9 (12.7)	12.8 (11.4)
pack-years	11.0 (10.8)	8.7 (9.2)
smokers	55%	59%
ex-smokers	24%	16%
non-smokers	21%	25%
appointment in industry (year)	13.7 (9.6)	8.4 (6.3)
production job animal feed (year)	11.2 (9.7)	-

6.3.3 Questionnaire

A short self-administered questionnaire with items derived from the British Medical Research Council questionnaire was used to collect information on respiratory symptoms (Biersteker et al. 1974). The questions included chronic cough and chronic phlegm (daily at least three months during the last two years), shortness of breath, ever wheezing, frequent wheezing (at least one week during the last two years), and chest tightness.

6.3.4 Lung function

Forced expiratory lung function measurements were conducted after a period of at least 48 hours without exposure to organic dust, between 11 am and 3 pm. During this period the circadian rhythm is at its maximum and in a stable phase (Dimich & Sterling 1981). Vicatest-V dry 'rolling seal' spirometers (Mijnhardt,

Bunnik, The Netherlands) which were calibrated twice a day were used by trained assistants. Measurements and procedures including BTPS adjustments and procedures of data selection were in accordance with the standards of the European Community for Coal and Steel (Quanjer 1983). Variables recorded were the following: Forced Vital Capacity (FVC); Forced Expiratory Volume in 1 second (FEV_1); Maximum Mid-Expiratory Flow (MMEF); Peak Expiratory Flow (PEF); and Maximum Expiratory Flow rates at 75, 50 and 25% of the vital capacity (MEF_{75}, MEF_{50} and MEF_{25} respectively).

6.3.5 Statistical analyses

Relationships between exposure and respiratory disorders were analysed using SAS software. For lung function analyses linear regression models were used to relate lung function variables to exposure characteristics, allowing for age, height and smoking habits. Symptoms were related to exposure using maximum likelihood logistic regression models (SAS procedure CATMOD) to compute odds ratios after adjustment for potential confounders. Relationships between exposure and respiratory disorders were studied in four ways:

- a comparison between grain production workers and controls;
- a comparison of categories with different current exposure levels within the group of grain workers;
- an analysis of the relationship between respiratory disorders and number of years the workers held production jobs in the animal feed industry;
- an analysis of the relationship between respiratory disorders and estimated cumulative exposure.

6.4 Results

6.4.1 Exposure

From eight facilities a total of 555 measurements of dust exposure were available for 206 workers. In 530 samples the endotoxin concentration was determined (187 workers). Since separate analyses for the 530 samples showed that

dust as well as endotoxin exposure was more strongly determined by job category than by facility (chapter 5, appendix 2), overall job title mean exposures were calculated and differences between facilities were omitted. For workers who took part in the measurements for more than one day the individual exposure was the mean of the repeated measurements. Arithmetic mean exposures were used in the analysis. For the eight job titles in the production group, mean exposures to inspirable dust and endotoxin are given in table 6.2. Personal mean inspirable dust exposures ranged from 0.2 mg/m^3 to 150 mg/m^3. Mean exposures for job titles ranged from 1.7 mg/m^3 (facility operators) to 29.7 mg/m^3 (unloaders). Personal mean endotoxin exposures were between 0.2 ng/m^3 and 470 ng/m^3, with job title means ranging from 6 ng/m^3 (facility and press operators and packers) to 68 ng/m^3 (unloaders).

Table 6.2 Characteristics of personal mean dust and endotoxin concentrations by job category

job category (n)	dust			endotoxin		
	AM (mg/m^3)	GSD	range (mg/m^3)	AM (ng/m^3)	GSD (ng/m^3)	range
unloaders (69)	29.7	3.9	0.6-150	68	3.8	2 -420
crane drivers (23)	5.0	4.0	0.2- 14.4	36	7.9	0.2-144
facility operators (63)	1.7	2.3	0.5- 5.9	6	3.9	0.2- 50
press operators (54)	3.0	2.7	0.2- 26.2	7	3.7	0.2- 58
production managers (42)	3.8	3.2	0.3- 19.4	12	3.7	0.5- 59
transport workers (61)	2.8	2.5	0.3- 14.2	30	3.0	0.3-320
sackers (20)	4.8	2.0	1.2- 10.3	6	2.2	0.5- 19
other (198)	11.5	4.0	0.2-105	37	4.2	0.2-470

AM =arithmetic mean
GSD =geometric standard deviation

6.4.2 Respiratory symptoms and lung function

As expected, most symptoms were related to age and smoking habits in a logistic regression analysis. The number of pack-years was more clearly related

to symptoms than other variables quantifying smoking habits, and was subsequently used to adjust odds ratios for smoking habits.

Analysis of lung function with linear regression analysis was performed with age, height and smoking habits as possible confounders. Of several variables representing smoking habits the number of pack-years was most clearly related to lung function and was therefore used in analyses to adjust relationships between exposure and lung function variables.

6.4.3 Comparison of grain production workers and (external) controls

Table 6.3 shows prevalences and odds ratios of respiratory symptoms for animal feed production workers compared to all controls. For all workers prevalences of respiratory symptoms ranged from 4% (chest tightness) to 16% (ever wheezing). Odds ratios were adjusted for smoking habits and age. The odds ratios indicate no significant differences in reported symptoms, except for frequent cough that is more frequently reported in the exposed group (OR = 2.04; $p < 0.05$).

Table 6.3 Prevalence of respiratory symptoms of animal feed production workers (n=265) vs external controls and non-production animal feed workers (n=175). Odds ratio corrected for age and pack years smoked.

	production workers n (%)	external controls & non-production n (%)	odds ratio (adjusted)
chronic cough	26 (10)	8 (5)	2.04*
chronic phlegm	11 (4)	8 (5)	0.81
shortness of breath	13 (5)	9 (5)	0.80
ever wheezing	42 (16)	28 (16)	0.90
frequent wheezing	15 (6)	4 (2)	2.20
chest tightness	10 (4)	11 (6)	0.63

* = $p < 0.05$

Differences in lung function (and the standard errors of the differences) between nonproduction and production workers after adjustment for age, height, and smoking habits are shown in table 6.4. Most lung function variables were lower for the external control subjects, with a statistically significant difference in FVC, FEV_1, and MMEF. Only the peak flow was lower in the exposed group; however, this difference was small and did nog reach statistical significance. The other variables were also lower among external controls subjects, but the differences did not reach statistical significance. In all these analyses we found a poor fit of regression models with unstable coefficients for age and height for most lung function variables. Exclusion of external control subjects resulted in a much better fit for all lung function variables. A comparison with the age- and height-specific reference values recommended by the European Committee for Coal and Steel (Quanjer 1983) revealed that animal feed production workers and internal control subjects had a average FVC that was significantly above the reference level (respectively, 108 and 112% of predicted; t-test: $p < 0.05$), whereas the external control subjects had FVC values that did not differ from the reference level (100,5%; t-test: $p > 0.10$).

Table 6.4 Differences (Δ) and standard errors of Δ (SE) in lung function between 265 animal feed production workers and 125 external control workers, and 50 internal control workers after adjustment for age, height and pack-years of tobacco smokes in a linear regression analysis*

	External Control Workers		Internal Control Workers	
	Δ	SE	Δ	SE
FVC, L	-0.357	0.077	0.168	0.107
FEV_1, L	-0.254	0.068	0.214	0.095
MMEF, L/s	-0.251	0.310	0.309	0.183
PEF, L/s	0.099	0.260	1.300	0.360
MEF_{75}, L/s	-0.137	0.229	1.022	0.318
MEF_{50}, L/s	-0.270	0.167	0.505	0.231
MEF_{25}, L/s	-0.115	0.074	0.115	0.102

* = $p < 0.05$
** = $p < 0.01$
SE = standard error

The FEV_1 and flow-volume variables for animal feed production workers and internal control subjects were equal to or slightly above the reference level, whereas the external control subjects had significantly higher values for most of these lung function variables. This suggested that the external control subjects differed with respect to variables other than the exposure.

Part of the non-production group was exposed to grain dust in the past, but when the non-production workers who had worked in production jobs and thus had been exposed in the past were excluded, the results of the analyses hardly changed. Further analyses were therefore performed with a control group of 50 non-production workers only.

6.4.4 Current exposure

Table 6.2 indicates that job title mean endotoxin exposures can be stratified into three groups: the unloaders group is the highest exposed category (job title mean = 67 ng/m^3, 25 workers participated in the respiratory disorders measurements). Intermediate exposure levels (30-40 ng/m^3, n=154) characterize the crane drivers, transport workers and the 'other production jobs' group. Low exposure levels are found for the other job titles, including the non-production workers (< 15 ng/m^3, n=136). Inspirable dust exposures show a distribution pattern among the job titles which is different from the endotoxin exposure. Two exposure groups of approximately equal sizes were created; a low exposure group with levels under 5 mg/m^3 (n=177) and a high exposure group with levels in excess of 5 mg/m^3 (unloaders and 'other production groups'; n=138). Table 6.5 shows adjusted odds ratios for reported respiratory symptoms for the high dust exposure category compared to the low dust exposure group. No clear differences could be seen, except for the prevalence of chronic phlegm, that was significantly lower in the highly exposed group (OR = 0.19, $p < 0.01$). Adjusted odds ratios for the intermediate and high endotoxin exposure groups, as related to the low exposed workers, are also given in table 6.5. Although no statistically significant odds ratios could be detected, five out of six symptoms showed increased odds ratios for the high endotoxin group, and all six were elevated compared to the intermediate exposure group.

Table 6.5 Adjusted odds ratios for respiratory symptoms for high dust exposure categories vs low dust exposure categories and for intermediate and high endotoxin exposure categories vs low endotoxin exposure categories (n=315)

symptom	dust exposure high[1]	endotoxin exposure intermediate[2]	endotoxin exposure high[2]
chronic cough	1.20	1.44	1.96
chronic phlegm	0.19*	0.44	0.59
shortness of breath	0.57	0.45	2.22
ever wheezing	0.88	1.04	1.11
frequent wheezing	1.14	1.70	2.60
chest tightness	0.65	0.42	1.22

* = $p < 0.05$
[1] compared to low dust exposure
[2] compared to low endotoxin exposure

Table 6.6 shows the differences in lung function for the dust and endotoxin categories, after adjustment for age, height and smoking habits by linear regression. All lung function variables showed decreased values with increasing exposure to both dust and endotoxin. The differences between the endotoxin categories appear to be somewhat greater than those between the dust categories. When (job title mean) dust and endotoxin exposures were related to adjusted lung function values statistically significant negative associations with 5 lung function variables were found. The estimated lung function losses for the overall mean dust level were 64 ml for the FVC ($p < 0.05$), 70 ml for the FEV_1, 568 ml/s for the PEF, 370 ml/s for the MEF_{75} and 138 ml/s for the MEF_{25} ($p < 0.05$). For the mean endotoxin level the losses were 112 ml (FVC), 122 ml (FEV_1), 910 ml/s (PEF), 650 ml/s (MEF_{75}) and 258 ml/s (MEF_{50}). All differences but the two with $p < 0.05$ were significant at the 1% level. As with the division into categories the lung function decreases seem to be more strongly related to endotoxin levels than to inspirable dust levels.

Table 6.6 Differences in lung function between low and high dust exposure categories, and between low endotoxin categories vs intermediate and high endotoxin. Adjusted for age, height and pack-years with linear regression (n=315)

	dust		endotoxin			
variable	high[1]	SE	intermediate[2]	SE	high[2]	SE
FVC (l)	-0.127*	0.075	-0.141*	0.077	-0.246*	0.142
FEV_1 (l)	-0.152*	0.076	-0.125	0.078	-0.370**	0.144
MMEF (l/s)	-0.128	0.137	-0.218	0.141	-0.296	0.260
PEF (l/s)	-1.091**	0.265	-1.099**	0.268	-2.373**	0.494
MEF_{75} (l/s)	-0.633**	0.239	-0.830**	0.241	-1.678**	0.445
MEF_{50} (l/s)	-0.262	0.172	-0.364*	0.177	-0.633*	0.326
MEF_{25} (l/s)	-0.011	0.076	-0.080	0.079	0.096	0.145

* = $p < 0.05$
** = $p < 0.01$
SE = standard error
[1] compared to low dust exposure
[2] compared to low endotoxin exposure

6.4.5 Number of years exposed to dust

The number of years employed in production of animal feed was related to current respiratory symptoms and lung function variables. Years worked in non-production jobs were not counted as production years, although the non-production workers were included in the analysis since part of them had held production jobs in the past. Figure 6.1 shows adjusted odds ratios for respiratory symptoms for four subgroups of production years history: 0-2 years (n=80); 2-10 years (n=83); 10-16 years (n=75); and 16-40 years (n=77). It indicates that for chronic cough, chronic phlegm, and for ever and frequent wheezing elevated prevalences were found in the two middle groups, while the group exposed longest reported less symptoms. Due to the small group sizes, however, none of the odds ratios was statistically significant. Odds ratios for shortness of breath and for chest tightness appeared to decrease with increasing production years. Logistic regression for these two symptoms was performed, in order to estimate odds ratios for production years after adjustment for age and pack-

years. For both symptoms the coefficient for production years was significant, with an odds ratio of 0.56 for shortness of breath and of 0.50 for chest tightness, for the mean number of production years (11.2). Although symptoms did not show a positive relationship and for some variables even a decrease with increasing production years, the lung function clearly decreased with increasing exposure, as can be seen from table 6.7. Only the FVC was not significantly related to the job history, but decreases of the FEV_1 and all flow-volume variables were highly significant, indicating respiratory disorders.

Table 6.7. Differences in lung function per 10 years production work, after adjustment for age, height and pack-years with linear regression models (n=315)

variable	difference per 10 years	SE
FVC (l)	-0.06	0.05
FEV_1 (l)	-0.14**	0.04
MMEF(l/s)	-0.27**	0.09
PEF (l/s)	-0.61**	0.18
MEF_{75} (l/s)	-0.59**	0.16
MEF_{50} (l/s)	-0.34**	0.11
MEF_{25} (l/s)	-0.12**	0.05

** = $p < 0.01$
SE = standard error

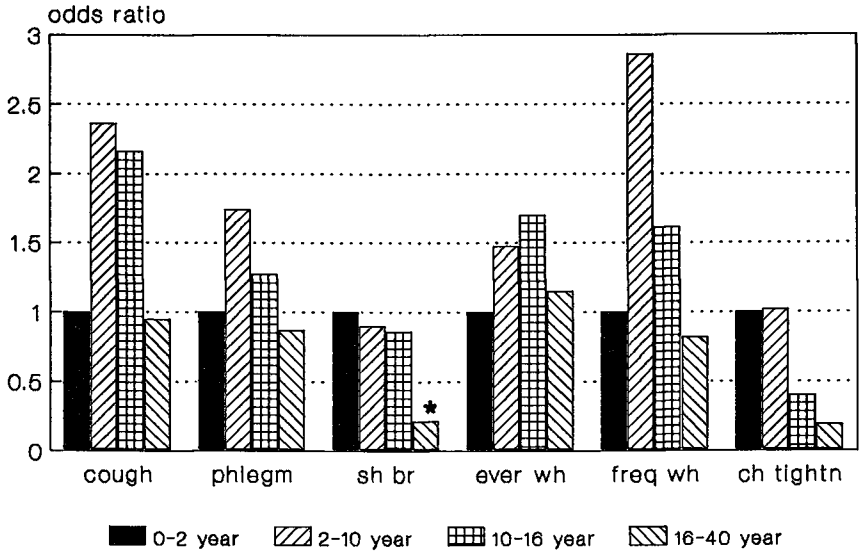

Figure 6.1 Odds ratios of reported respiratory symptoms*) for four job history categories related to the 0-2 year category. Adjusted for age and pack-years

*) chronic cough, chronic phlegm, shortness of breath, ever wheezing, frequent wheezing and chest tightness.
* = $p < 0.05$

6.4.6 Cumulative exposure

Cumulative exposures to dust and endotoxin were estimated for each animal feed worker. The product of the job title category mean exposure 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 every worker by adding the results for all jobs held. Of the 315 animal feed workers, 312 had reported sufficient information on job history to allow calculation of the cumulative exposure. Relating cumulative exposure levels with respiratory symptoms did not result in any relevant information that indicated consistency. Therefore no results of these analyses are presented. However, cumulative

exposure is clearly related to lung function, as can be seen from table 6.8 where estimated adjusted effects of cumulative dust and endotoxin exposure levels are presented. All flow variables except the MEF_{25} were significantly related to both exposure indices. The cumulative exposure to endotoxin appeared to be more strongly related to lung function variables than the cumulative dust levels. Current and historical exposure variables were analysed together in a variety of models in order to detect their interdependent effects on lung function variables. The effects of both types of exposures were found to be independent, indicating that present exposure and historical exposure were separate risk factors. Table 6.9 shows mean estimated effects of three retrospective and two current exposure variables. As already mentioned, both historical and current endotoxin exposure appeared to be more clearly related to lung function than dust exposures. The relationship of current exposure on FVC appears to be more pronounced than that of historical exposure. The current exposure, especially the endotoxin exposure, may be related to a restrictive effect as well.

Table 6.8. Relationship between cumulative dust and endotoxin exposure and lung function, after adjustment for age, height and pack-years (n=312)

variable	dust (year*gram/m^3)$^{-1}$ ß	SE	endotoxin (year*μgram/m^3)$^{-1}$ ß	SE
FVC (l)	-0.46	0.31	-0.19	0.12
FEV_1 (l)	-0.74**	0.29	-0.34**	0.12
MMEF(l/s)	-1.03*	0.58	-0.60**	0.24
PEF (l/s)	-6.02**	1.08	-2.42**	0.46
MEF_{75} (l/s)	-4.51**	0.98	-2.04**	0.41
MEF_{50} (l/s)	-1.94**	0.72	-0.96**	0.29
MEF_{25} (l/s)	-0.05	0.31	-0.14	0.12

* = $p < 0.05$
** = $p < 0.01$
SE = standard error

Table 6.9. Estimated effects for mean duration of production work (11.2 year), mean cumulative dust exposure (111 year*mg/m^3) and mean cumulative endotoxin exposure (311 year*ng/m^3); and for mean current exposure to dust (8.6 mg/m^3) and endotoxin (24.8 ng/m^3). Adjusted for age, height and smoking (pack-years)

variable	production years	cumulative exposure		current exposure	
		dust	endotoxin	dust	endotoxin
n	315	312	312	315	315
FVC (l)	-0.067	-0.051	-0.059	-0.064*	-0.112**
FEV_1 (l)	-0.156**	-0.082**	-0.106**	-0.070**	-0.122**
MMEF(l/s)	-0.302**	-0.114*	-0.187**	-0.063	-0.131
PEF (l/s)	-0.683**	-0.670**	-0.753**	-0.568**	-0.910**
MEF_{75} (l/s)	-0.660**	-0.502**	-0.635**	-0.370**	-0.650**
MEF_{50} (l/s)	-0.381**	-0.216**	-0.299**	-0.138*	-0.258**
MEF_{25} (l/s)	-0.134**	-0.005	-0.044	-0.020	-0.002

* = $p < 0.05$
** = $p < 0.01$

The inter-relationship between exposure variables and smoking habits in the explanation of respiratory disorders was tested in all statistical models by adding an interaction term to the model. In none of the models the interaction coefficient reached a significance level, indicating that the effects of smoking and exposure on lung function are additive. In order to verify if no important differences existed between the 8 facilities in which exposure measurements took place and the other 6 facilities, all analyses mentioned were also performed on the subgroup of workers from the 8 facilities. No major differences with the results presented were found.

6.5 Discussion

The complex nature of organic dust in occupational settings is a major handicap in studies aimed at identifying relationships between exposure characteristics and health effects. This hampers comparison of the results of studies that were conducted in different industries. Nevertheless the nature of the dust found in grain elevators in northern America and usually referred to as grain dust is to some extent comparable to the dust in the animal feed industry. Becklake (1980) described several components that may be identified in grain dusts: plant matter of grain (cereal grains, oil seeds and pulses); non grain plant matter; fungal matter and fungal spores; animal matter including insects; agricultural chemicals; and inorganic matter. Bacterial matter, including endotoxins, may be added to this list (DeLucca & Palmgren 1987). Except for studies in grain elevators, the results of this study can be compared to the results of the Yugoslavian study in an animal feed mill mentioned earlier (Zuskin et al. 1989).

For both animal feed workers and controls the prevalence of respiratory symptoms was low compared to those reported by Zuskin et al. (1989) and those found in grain elevator studies (Chan-Yeung et al. 1981). Other studies in different occupational environments with the same questionnaire (Kateman et al. 1990, Bongers et al. 1987, Heederik et al. 1987b) also resulted in low prevalences, indicating that the self administered questionnaire may have a low sensitivity compared to the usual oral interviews or medical examinations.

Several results of our study are indicative of the occurrence of selection bias, interacting with obvious exposure effects. Most lung function variables were lower in external controls than in animal feed workers, chronic phlegm was less prevalent in the high dust exposure category, and two reported symptoms (shortness of breath and chest tightness) were inversely related to the number of years worked in production jobs. Studies in grain elevators have also shown healthy worker effects. Others (Chan-Yeung et al. 1980, Gerrard et al. 1979) found less allergic respiratory diseases in grain workers than in controls in a cross-sectional study. In a longitudinal study workers who left the industry had increased respiratory symptoms at the baseline study (Broder et al. 1985), and in a retrospective study positive correlations between lung function and employment

duration were detected (Corey et al. 1982). The detection of a 'healthy worker effect' in our study and in several other cross-sectional and retrospective studies indicates that these observational studies may be seriously handicapped in their power to detect respiratory disorders and probably underestimate exposure-effect relationships.

Nevertheless the study clearly showed exposure related respiratory disorders. Respiratory symptoms appeared to be related to current endotoxin levels, but not to dust levels. Lung function levels were clearly related to current dust levels and more strongly to endotoxin levels. The use of retrospective exposure estimates resulted in ambiguous information on symptoms. A selective effect was indicated for dyspnoea and chest tightness, but other symptoms first increased and then decreased with increasing duration of employment in animal feed production. For the calculation of the cumulative exposure it is assumed that current dust and endotoxin levels are representative of historical exposure levels. This assumption is plausible, since our exposure study showed that differences between job titles more strongly determine the exposure levels than the facilities do. The facilities clearly differed with respect to age which ranged from one to forty years. Lung function variables were very clearly related to all retrospective exposure measures, indicating airflow limitation. Current exposure levels were also related to (FVC) changes.

The apparent discrepancy between effects of exposure on lung function and on symptom prevalences may have several causes. The relatively low prevalences may have resulted in limited power to establish reliable relationships with exposure characteristics. Other factors may have played a modifying role, such as job satisfaction and age (Dreyer-Wright et al. 1970). The inverse relationship between job history and dyspnea and chest tightness may be (partly) caused by such phenomenon. The decline of the other symptoms in the categories with the highest number of exposed years corroborates with this explanation. A third explanation for the discrepancy of effects on lung function and symptoms is that a healthy worker effect might be more pronounced for individuals with perceived symptoms than for individuals with (minor) lung function changes.

Comparing the results of our study to the Yugoslavian (cross-sectional) animal feed study (Zuskin et al. 1989) in detail is difficult, because of its preliminary

nature reflected in the small scale and the less extensive exposure characterization. A striking difference, however, is the apparent absence of a healthy worker effect in the Yugoslavian study, although the exposure levels appear to be in the same range. The increased respiratory symptoms and decreased lung function values corroborate with our study.

A number of studies that have been conducted in grain elevators indicated respiratory effects of grain dust exposure. Several studies showed relationships between current exposure and respiratory disorders (Dosman et al. 1980, Cotton et al. 1982, 1983, Corey et al. 1982). In two studies a relationship with the duration of employment was found (DoPico et al. 1977, Sheridan et al. 1980). In two longitudinal studies respiratory disorders were detected in grain elevator workers (Chan-Yeung et al. 1981, Dosman et al. 1985). The most commonly detected indicators of respiratory disorders were decreased FEV_1 and flow variables, and increased respiratory symptoms. Our results are in concordance with these findings, but due to different statistical techniques and the already mentioned healthy worker effect, a detailed comparison of the magnitude of the respiratory disorders is hardly possible.

A finding of major importance in our study is that both symptoms and lung function were more clearly related to (present and retrospective) endotoxin exposure than to inspirable dust exposure. To our knowledge only one study that indicated chronic effects of endotoxin has been published. Kennedy and co-authors (1987) reported an exposure-response relationship of current endotoxin exposure (that was indistinguishable from retrospective exposure), with FEV_1 and prevalence of chronic bronchitis and byssinosis in the cotton industry. Endotoxin concentrations in our study were considerably lower, in spite of personal monitoring which usually yields higher results. The authors estimated the effect of current endotoxin to be -0.242 to -0.778 L per $\mu g/m^3$ endotoxin, which is considerably lower than our estimate of -4.91 L per $\mu g/m^3$ (table 9: 0.122 L per 24.8 ng/m^3). Similar differences between the two studies can be found when retrospective endotoxin exposure estimates are compared. Several reasons for these differences might exist, such as differences in sampling techniques and laboratory analysis (Olenchock et al. 1989), non-linear dose response relationships and variations in endotoxin toxicity. Since the measure-

ments in our study were clearly more extensive the statistical phenomenon of attenuation may have played a role (Heederik et al. 1991). A large measurement error or (non-differential) misclassification of exposure may lead to underestimation of exposure-response relationship. It is, however, not clear whether these factors can exclusively explain the inconsistency between the results of our study and the study among cotton workers.

Several authors have suggested a possible role of endotoxin in the etiology of chronic bronchitis studies (Rylander 1985, Olenchock et al. 1989). It is, however, not clear whether the possible endotoxin effect is dependent on other toxins, such as gossopyl and tannins in cotton dust (Jacobs 1989). The differences in exposure-effect estimates between our study and the cotton workers' study may be explained partly by the role of these other factors. In our exposure study, levels of airborne fungi were more strongly related to endotoxin levels than to dust levels in stationary samples. This suggests an interrelationship of microbiological variables that is only partly dependent on dust concentrations. The existence of this interrelationship may point towards a role of other agents of a microbiological nature in the etiology of lung function impairment. Although this study adds evidence to the existence of endotoxin related chronic respiratory disorders, more information is needed to further elucidate the role of biologically active constituents in organic dust.

6.6 Acknowledgements

We thank the workers for their cooperation. Jan Boleij and Bert Brunekreef of the Wageningen University, Robert Castellan and Mike Attfield from the National Institute of Occupational Safety and Health (USA) gave helpful comments on the manuscript. We also thank Jos van Hutten, Rudolf van der Haar, Hans Marquart, Anita Groenendijk, Mieke Lumens and Tonnie Zweers for their assistance in different stages of the project.

7. Dust- and endotoxin related acute lung function changes and work related symptoms in the animal feed industry[1]

Tjabe Smid, Dick Heederik, Remko Houba

7.1 Abstract

This study is the result of an analysis respiratory symptoms during work and acute lung function changes in the animal feed industry. Reported respiratory and related symptoms during work were compared between 265 exposed animal feed workers and a control group consisting of 175 external controls and non exposed workers in the animal feed industry. Symptoms indicating respiratory and nasal irritation were significantly increased in the animal feed workers. Prevalences ranged from 9% (cough) to 21% (sneezing). Reported cough after work was also significantly increased. In 119 workers, a total of 457 cross shift spirometric lung function changes were measured. Almost all lung function variables showed a decrease during the work shift, as could be expected since the circadian rhythm is in a downward phase during the measurement period (2 PM - 10 PM). When the workers were grouped into dust and endotoxin exposure categories according to their job title, an exposure-response trend was seen for MMEF and MEF_{50}. The effect of endotoxin was stronger then that of dust, both in magnitude and significance. For the same lung function variables and for FEV_1 and MEF_{25} a significant cross week change was also detected. The results of this study are in concordance with other studies that indicate acute effects on lung function and elevated prevalences of respiratory symptoms during work caused by exposure to grain dust.

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[1] Am J Ind Med (submitted)

7.2 Introduction

Work related respiratory symptoms and cross-shift lung function changes have been reported from numerous studies in occupational environments with exposure to organic dust. Studies have been carried out in coffee workers (Zuskin et al. 1981), tea and wood workers (Al Zuhair et al. 1981), agricultural environments (Thelin et al. 1984, Donham 1989, Heederik et al. 1991), the wool industry (Zuskin et al. 1976), jute, hemp and flax workers (Zuskin & Valic 1973, El Ghawabi 1978, Mc Kerrow et al. 1965, Zuskin et al. 1990), spice grinders (Chan et al. 1990) and in a variety of other industries. Most attention, however, has been paid to cotton workers (Morgan 1984b, Rylander 1990) and, to a lesser extent, to grain and flour workers (DoPico et al. 1983, Chan-Yeung et al. 1980, Awad el Karim et al. 1986). The role of endotoxins as a cause of cross-shift lung function decreases in organic dust environments has been debated intensively in recent publications (Rylander 1987, Jacobs 1989, Palchack et al.1988, Milton et al.1989). Endotoxin related acute lung function changes have only been reported from several experimental studies (Castellan et al. 1987, Rylander et al. 1989). To our knowledge a relationship between cross-shift lung function changes and endotoxin exposure was reported in only one field study. Donham and co-workers (1989) found a relationship between endotoxin exposure and cross-shift decrement of FEV_1 and MEF_{25} in swine confinement workers.

Although extremely high dust exposures in animal feed mills were reported in East European studies (Jindrichova et al. 1971, Gemeinhardt et al. 1981), the evidence of cross-shift lung function changes is limited. To date the results of only one (Yugoslavian) study on cross-shift lung function changes and work related symptoms in this industry have been published (Zuskin et al. 1989).

In this paper results are presented from a study on work related respiratory symptoms and cross-shift and cross-week lung function changes in the Dutch animal feed industry. It is part of a larger study aimed at the quantification of exposure levels together with acute and chronic respiratory disorders. Reports on exposure data (chapter 5) and on chronic respiratory disorders (chapter 6) have been published recently.

7.3 Materials and methods

7.3.1 Production proces and subjects

The production process of an animal feed facility is simple and described in detail in chapter 5. The raw materials arrive by ship or by truck: grains (mainly maize, wheat and barley), 30-40 other vegetable products (cassava and pulses) and waste products from the food industry. The raw materials are cleaned and stored in silos. The materials are ground and mixed in a batch process, and fats, molasses, vitamins, and minerals are added in small quantities. The mixture is then usually pressed into pellets and stored in silos. The animal feed is either packed and shipped in sacks, or shipped in bulk trucks.

A total of 315 male Caucasian workers from 12 animal feed production facilities and 2 elevators took part in the study, and 125 male controls were selected from a milk processing plant and a wholesaler's. The controls had the same socio-economic status but were not occupationally exposed to organic dust or other known lung damaging agents. The 315 animal feed workers were divided into 265 production workers and 50 non production workers (laboratory personnel, gate keepers, truck drivers) who are only occasionally exposed to dust in small quantities. For the comparison of animal feed workers and controls, the non production workers were considered as controls. Data on age, standing height and smoking habits of 265 animal feed workers and 175 controls are given in table 7.1.

In three facilities forced expiratory lung function measurements were conducted in (dust exposed) production workers before and after the workshift on Mondays, Tuesdays and Fridays. A total of 119 workers took part in this study. In all three facilities the lung function measurements were conducted in two periods after a six month interval. 69 workers took part in both series.

7.3.2 Exposure

In eight facilities eight-hour personal inspirable dust samples were taken in the production workers category. The sampled dust resembled the inspirable dust fraction that is characterized by a 50% cut-off diameter of 30 μm, and passes

the mouth and nostrils when inhaled (ISO, 1981). In the samples gravimetric dust concentrations and endotoxin levels were determined using the LAL-test as described by Kateman et al. (1990). Details of the sampling strategy and methods as well as of the analyses used are given in chapter 5.

Table 7.1 Characteristics of animal feed production workers vs. non production workers and external controls: means and standard deviations

	animal feed workers	controls
n	265	175
age (year)	39.4 (11.2)	36.1 (10.2)
length (cm)	178 (7)	177 (7)
years smoked	16.3 (12.7)	13.1 (11.6)
pack-years	11.1 (10.5)	9.1 (10.4)
smokers	57%	54%
ex-smokers	23%	21%
non-smokers	20%	25%

The 265 exposed production workers were divided into 8 job title groups, with varying mean exposure to dust and endotoxin. Based on their job title mean exposure, the animal feed workers were divided into two inspirable dust exposure categories (job title mean < 5 mg/m^3 and > 10 mg/m^3). For endotoxin exposure three exposure categories were constructed, with job title means of < 15 ng/m^3; 30-40 ng/m^3; and 67 ng/m^3. Detailed information on exposure characteristics is given in chapter 5, and is summarized in table 7.2. Maintenance personnel was excluded from the study because of possible exposure to other agents than organic dust that may lead to respiratory disorders (welding fumes, metal dust etc.)

Table 7.2 Exposure categories for inspirable dust and endotoxin, job titles, and job title mean exposures

Exposure category	n*	job title mean exposure	job titles
Inspirable dust			
Low exposure	210	< 5 mg/m^3	crane drivers, facility operators, press operators,production managers, transport workers, packers, non production workers
High exposure	247	> 10 mg/m^3	unloaders, other production jobs
Endotoxin			
Low exposure	144	< 15 ng/m^3	facility operators, press operators, production managers, packers, non production workers
Intermediate exposure	274	30-40 ng/m^3	crane drivers, transport workers, other production jobs
High exposure	39	67 ng/m^3	unloaders

* n = number of cross-shift lung function change measurements

7.3.3 Symptoms

Using a self administered questionnaire, the prevalence was determined of respiratory and other symptoms developed while at work, and shortly after work. Symptoms were cough, phlegm, shortness of breath, chest tightness, irritation of the eyes, irritation of the nose, and sneezing.

7.3.4 Lung function

All 119 workers that took part in the lung function study worked in two shifts, from approximately 6 am to 2 pm and from 2 pm to 10 pm. All lung function measurements took place when the workers worked in the afternoon shift. The reason for this is that the circadian rhythm is in a dynamic phase in the early morning. Lung function increases relatively fast shortly after getting

up. Therefore, the lung function level will be strongly dependent on the moment of measurement. Although the size of the circadian rhythm is relatively small, we reduced the possibility of unwanted variation by measuring before and after the late shift. At midday the circadian rhythm is at its top and in a stable phase, and after the shift the lung function variables are decreasing, but at a much slower pace (Dimich & Sterling 1981, Lewinsohn et al. 1960).

Vicatest-V dry 'rolling seal' spirometers (Mijnhardt, Bunnik, the Netherlands) were used by trained interviewers and were calibrated twice daily. Measurements and procedures concerning data selection and BTPS correction were in accordance with the standards of the European Community for Coal and Steel (Quanjer, 1983). Variables recorded were the following: forced vital capacity (FVC); 1 second forced expiratory volume (FEV_1); maximum mid expiratory flow (MMEF); peak expiratory flow (PEF); and maximum expiratory flow rates at 75, 50 and 25% of the vital capacity (MEF_{75}, MEF_{50} and MEF_{25} respectively). Subjects were asked not to smoke one hour prior to the measurements.

7.3.5 Statistical analyses

The relationship between the exposure categories and respiratory disorders was studied using SAS software. Symptoms were related to exposure categories using maximum likelihood logistic regression models (SAS procedure CATMOD) to compute odds ratios after adjustment for potential confounders. For lung function analyses linear regression models were used to compute cross-shift and cross week lung function variables for exposure categories after direct adjustment for smoking habits.

7.4 Results

7.4.1 Work related symptoms

Work related symptoms were compared between animal feed production workers and the control group.

Table 7.3 shows prevalences and crude odds ratios of respiratory symptoms for both groups. Odds ratios that were adjusted for confounders (smoking habits and age) are also shown. Smoking habits were expressed as the number of pack-years (Σ(packs of cigarettes smoked daily * years smoked)). All symptoms were elevated in the exposed group, although only cough (adjusted odds ratio = 6.9), irritation of the nose (OR = 5.7) and sneezing (OR = 3.4) reached a significance level of less than 5% (all three: $p < 0.01$). The strongly elevated odds ratios clearly indicate direct respiratory and nasal irritation, with a prevalence ranging from 9% (cough) to 21% (sneezing). Symptoms after work showed considerably lower prevalences but comparable odds ratios. After adjustment for age and smoking habits only cough after work was significantly elevated (prevalence 5% for exposed workers and 0% for controls, OR > 9, $p < 0.01$).

Table 7.3 Prevalence of symptoms during work of animal feed production workers (n=265) vs. external controls and non-production animal feed workers (n=175)

	production workers n (%)	controls n (%)	odds ratio	odds ratio (adjusted)
cough	23 (9)	2 (1)	8.2**	6.9**
phlegm	15 (6)	5 (3)	2.0	2.0
shortness of breath	12 (5)	6 (3)	1.3	1.1
chest tightness	13 (5)	4 (2)	2.2	2.2
eye irritation	21 (8)	10 (6)	1.4	1.5
nose irritation	41 (15)	6 (3)	5.2**	5.7**
sneezing	55 (21)	13 (7)	3.3**	3.4**

** = $p < 0.01$ (one tailed)

When the 50 non production workers were excluded from the analyses the results remained similar. Exclusion of controls that had held production jobs in the past did not influence the results either.

A division of the animal feed workers into (dust or endotoxin) exposure categories did not lead to clear differences in work related symptoms. Although for the symptoms during work some results indicated exposure-response relationships, statistical significance was not reached because of the low prevalences. For the symptoms after work no indications for exposure response relationships were found.

7.4.2 Lung function

For the 119 production workers, a total of 457 cross-shift lung function changes were available. Since the maximum number of changes per worker was six (two weeks on Monday, Tuesday and Friday), the response was 457/714 or 64%. Non response can be explained by absence due to sickness leave, holidays, or days off, and by the considerable turnover of personnel between the measurement periods. Technical or organizational problems accounted for a small proportion of the non response, as did lung function measurements which failed to comply with the ECCS criteria.

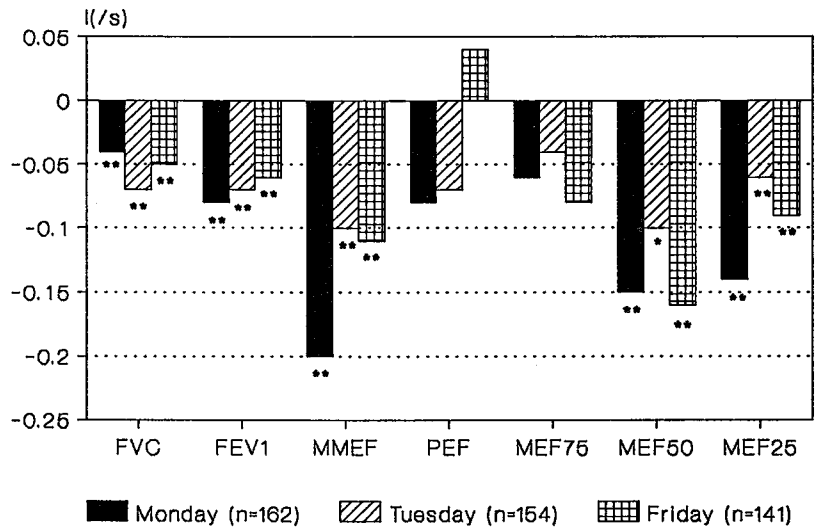

Figure 7.1 Mean cross-shift lung function changes on three weekdays.
* = $p < 0.05$
** = $p < 0.01$

Figure 7.1 shows mean absolute changes for all lung function variables on Mondays, Tuesdays and Fridays. As expected because of the circadian rhythm, all variables showed a decrease, with the exception of the peak flow on Friday. All decreases were significant for all three days, except the peak flow and the related MEF_{75}. The largest decreases appeared to be found on Mondays. The largest absolute decreases were found for the MMEF, ranging from a mean 100 ml/s on Tuesdays to 200 ml/s on Mondays. The largest mean relative changes ranged from 4.1% (Tuesdays) to 9.9% (Mondays) for the MEF_{25}.

Mean changes for the high dust group as related to the low dust category, and for the intermediate and high endotoxin group, as related to the low endotoxin category, were computed using linear regression models. A dichotomous variable indicating current smoking was included in the two models, because the decreases were considerably larger in smokers. Significant differences on the 5% level between smokers and non-smokers were found in both the dust and the endotoxin model for the FVC (approximately 30 ml); for the FEV_1 (40 ml); and for the MEF_{75} (approximately 190 ml/s).

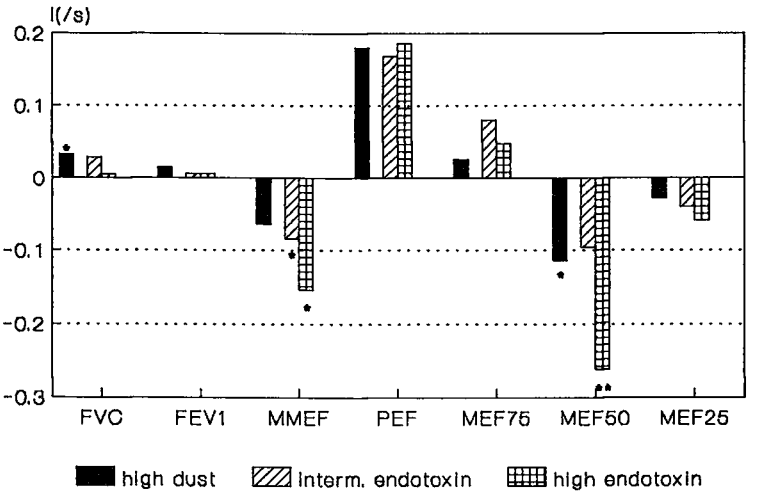

Figure 7.2 Differences in cross-shift lung function changes after adjustment for smoking habits. High dust category changes are relative to low dust category values. Intermediate and high endotoxin changes are relative to the low endotoxin category (n=457)
* = $p < 0.05$
** = $p < 0.01$

The results in figure 7.2 show that cross-shift decreases are dependent on exposure for the MMEF and MEF_{50}. MMEF decreases were significantly ($p < 0.05$) related to the endotoxin exposure categories with differences between low and intermediate exposure of 84 ml/s and between low and high exposure of 154 ml/s. The difference between the dust exposure categories (64 ml/s) failed to reach significance. For the MEF_{50} the differences between low and high dust and between low and intermediate endotoxin were comparable; 114 ml/s ($p < 0.05$) and 96 ml/s respectively. For the high endotoxin vs. the low endotoxin group, however, the difference of 262 ml was clearly larger ($p < 0.01$). The results for these two variables may indicate that the endotoxin exposure is more strongly related with lung function decreases than dust exposure.

In order to investigate exposure response relationships, all 457 cross-shift lung function changes were related to job title mean exposure levels of dust and endotoxin. Regression models with job title mean exposure levels and (dichotomous) current smoking habits as input were used to predict cross-shift changes of MMEF and MEF_{50}.

Results are shown in table 7.4. The estimated effects of 1 mg/m^3 dust and 1 ng/m^3 endotoxin were multiplied by the mean of 457 job title mean dust (10.1 mg/m^3) and endotoxin (29 ng/m^3) exposure levels, in order to compare the magnitude of lung function changes that could be attributed to dust and endotoxin exposure. It is clear from the table that the mean endotoxin exposure is related to larger cross-shift decreases than mean dust exposure.

Other lung function variables than the MMEF and MEF_{50} appeared not to be related to the group mean exposure levels. The apparent positive relationships between exposure and the peak flow and the related MEF_{75} (figure 7.2) were far from significant because of the (inherently) large variation of these variables.

Cross week lung function changes were computed by substracting the pre-shift Friday values from pre-shift Monday values. A total of 130 cross week changes were available for the analyses, from a theoretical 238 (two weeks, 119 workers). The reasons for 'non response' were discussed previously. Especially the large turnover played an important role; only 69 workers took part in both series. Results are shown in table 7.5, and indicate that except for the peak flow

and the related MEF_{75} all lung function variables decreased during the week. Changes in FEV_1, MMEF, MEF_{50} and MEF_{25} were significant on the 1% level.

Table 7.4 Estimated effects of endotoxin and dust levels on cross-shift changes of MMEF and MEF_{50}

	MMEF (SE) (ml/s)	MEF_{50} (SE) (ml/s)
change per 1 mg/m³ dust [1]	4.0 (1.7)*	7.4 (2.6)**
change per 1 ng/m³ endotoxin [1]	2.7 (1.0)**	4.1 (1.4)**
change per 10.1 mg/m³ dust [2]	40 (17)*	75 (26)**
change per 29.3 ng/m³ endotoxin [3]	79 (29)**	120 (41)**

* = $p < 0.05$
** = $p < 0.01$
[1] Estimated with linear regression models with job title mean exposures and (dichotomous) smoking habits as input (n=457).
[2] Mean of 457 job title mean dust exposure levels
[3] Mean of 457 job title mean endotoxin exposure levels

Table 7.5 Absolute and relative cross week lung function changes in production workers (n=130)

variable (%)	cross week change	sd	relative change
FVC (l)	0.00	0.19	0.0
FEV_1 (l)	-0.05**	0.19	-1.0
MMEF (l/s)	-0.18**	0.45	-4.6
PEF (l/s)	0.19	1.45	2.5
MEF_{75} (l/s)	0.01	1.11	0.4
MEF_{50} (l/s)	-0.19**	0.59	-4.2
MEF_{25} (l/s)	-0.08**	0.36	-5.3

** = $p < 0.01$

Although the results indicate that exposure is related to lung function decreases during the working week, no dose-response relationships could be detected when exposure categories were compared. The relatively small number of measurements may be a contributing factor to the apparent absence of exposure related cross week changes.

7.5 Discussion

As mentioned in the introduction, several studies on organic dust indicate acute respiratory effects. The results of this study support this general picture. Work related irritative symptoms such as cough, sneezing and nose irritation were clearly elevated, and both cross-week and exposure related cross-shift changes of MMEF and MEF_{50} were detected.

Although odds ratios of irritative respiratory symptoms were very clearly elevated, the prevalences of these symptoms in the exposed groups were relatively low. In the only (Yugoslavian) study in the animal food industry reported until now (Zuskin et al. 1989), the prevalences of symptoms during work were considerably higher. In the study group of 73 workers with approximately the same age distribution and smoking habits, 53% of the workers reported cough during work against 9% in our study. For eye irritation these prevalences were 53% and 8% respectively. There are no clear indications that the time weighted average exposure levels were different. Apart from possible differences in the composition of the dust, an explanation that can be thought of is the healthy worker effect. A cross-sectional analysis of our study group gave indications for a selection effect (chapter 6). Another explanation may be that the self-administered questionnaire has a low sensitivity compared to the usual oral interviews. The cross-sectional analysis of a larger group of animal feed workers which has been reported elsewhere (chapter 6) also indicated relatively low prevalences of chronic respiratory symptoms compared to other studies.

An important problem in the interpretation of lung function results is the dependency of measurements; 457 cross-shift measurements were taken from 119 workers. As the used statistical techniques used assume independent

measurements, significance testing must be interpreted carefully because p-values may be underestimated due to less variance. An examination of the data, however, did not reveal clear dependency, therefore we conclude that although the significance may be somewhat less, the general trend will not be effected. This is also true for the cross week changes, in which the dependency is less since a maximum of two cross week changes may be available for every subject.

Another potential problem is the non response, but as already mentioned the reasons were mainly external and generally not related to the objective of the study.

Nevertheless the results of the lung function measurements show that cross-shift changes of MMEF and MEF_{50} were clearly related to exposure. For cross-week changes the same variables are affected, but changes in FEV_1 and MEF_{25} were also detected. The FVC was not related to exposure, and the large variation in MEF_{75} and peak flow prevented detection of possible relationships with exposure.

This study indicates that the cross-shift lung function changes are more strongly related to endotoxin exposure than to dust exposure. This finding is in accordance with numerous experiments on endotoxin and lung function, indicating that endotoxin may lead to short term lung function (FEV_1) decreases (Castellan et al. 1987, Rylander et al. 1989). Also, in a field study in swine confinement workers (Donham et al. 1989), cross-shift FEV_1 (and MEF_{25}) changes were found to be related to area endotoxin concentrations. However, a major difference with the experiments and the swine confinement study mentioned was that in our study the exposure was related to MMEF and MEF_{50} and not to FEV_1. A comparison between effective doses of endotoxin for lung function changes is therefore not possible. Nevertheless the mean exposure level in our study (29.3 ng/m^3) was far below the estimated no effect level of 180 ng/m^3 in the swine confinement study. In this latter study, however, circadian rhythms were not taken into account, exposure measurements were area samples, and shift duration varied from 2 to 8 hours. No effect levels for FEV_1 changes that were estimated from experiments with cotton dust by Castellan et al. (1987) and by Rylander et al. (1985), however, were much lower and more in the range of our study: 9 ng/m^3 and 33 ng/m^3 respectively.

The results of this study reveal three major problems with both experimental and field studies on lung function changes caused by grain dust or other organic dusts. In the first place most studies do not explicitly take into account circadian rhythms of lung function variables. The results of our study indicate that considerable cross-shift decreases can be detected which can not be related to exposure levels. Although the timing of our measurements was favorable for the detection of decreases, changes caused by circadian rhythms are usually not excluded in other studies.

A second point of attention that arises from our study is that cross week changes could be clearly detected, even for variables that did not show cross-shift changes (FEV_1 and MEF_{25}).

Thirdly, most other studies are directed at changes in FEV_1, since this variable is a highly reproducible and proven sensitive indicator of respiratory disorders. The results of our study, however, indicate that cross-shift changes of MMEF and MEF_{50} can be detected without significant changes in FEV_1. Some authors have reported inverse relationships between FEV_1 and peak flow, dependent on effort (Krowka et al. 1987, Miller 1987). Since peak flow increased cross-shift, the absence of FEV_1 decline may be caused by a learning effect. This is, however, highly unlikely, since differences in peak flow were found to be dependent on exposure levels.

Another explanation may be that the combination of decreased levels of MMEF and MEF_{50} and the (though non significant) increase in peak flow is a very sensitive indicator for lung damage. The earliest change associated with flow limitation is thought to be slowing in the terminal portion of the spirogram, even when the initial part of the spirogram is unaffected (ATS 1991). Due to minor damage in the peripheral airways, the thoracic gas compression takes place in the upper airways, resulting in an increased peak flow and a decrease in mid expiratory flow variables. However, the relevance of the occurrence of this minor effect in the absence of FEV_1 changes in epidemiologic studies is unclear. For diagnostic use, the relevance of lowered parameters of the spirogram in the absence of FEV_1 changes is questioned (ATS 1987, 1991). It should be emphasized that the effects in this study relate to cross-shift changes, that either may or may not predict non-reversible lung function changes. The results of cross-

sectional analyses in this study clearly indicate the presence of chronic lung function changes, related to retrospective cumulative exposure levels (chapter 6).

In conclusion, the results of our study indicate acute respiratory effects of dust in animal feed mills. In general, the results are in concordance with other studies on grain dust that indicate work related symptoms and acute lung function changes (Zuskin et al. 1989, DoPico et al. 1983, Chan-Yeung et al. 1980). The effects appear to be more clearly related to endotoxin exposure than to dust exposure. To our knowledge only one field study at much higher exposure levels has shown this before, although there is a vast body of evidence from experiments (Rylander et al. 1985, Castellan et al. 1987).
The cross-sectional analysis of a larger group of animal feed workers also gave indications for respiratory disorders which was more clearly related to retrospective endotoxin exposure than to dust exposure (chapter 6). However, other components of the dust may have been a causal or a major contributing factor for the development of lung function changes. In the animal feed industry for example fungal counts in ambient air were more strongly related to endotoxin levels than to dust levels (chapter 5). Because endotoxin is a bacterial product, a general concept of 'microbiological activity' can be hypothesised as being related to respiratory disorders, without knowledge of the relative importance of possible active components. The finding that endotoxin exposure is related to other lung function variables than was found in experiments (Rylander et al. 1985, Castellan et al. 1987) supports this hypothesis.

This apparent partial inconsistency with the experiments also throws new light on the discussion on limit values for endotoxin exposure (Palchack et al. 1988, Milton et al. 1989, Jacobs 1989), since it indicates that other lung function variables than the FEV_1 may be more sensitive to exposure. Also, apart from the acute effects of endotoxin, a study in the cotton industry (Kennedy et al. 1987) as well as our cross-sectional analysis (chapter 6) give indications for endotoxin related chronic respiratory disorders. The discussion on effects of endotoxin and limit values will therefore become even more complicated than it already was.

7.6 Acknowledgements

This study was supported by a grant from the Directorate General of Labour from the Ministry of Social affair and Employment. We thank all workers who took part in the study for their cooperation, as well as Jan Boleij, Rudolf van der Haar, Hans Marquart, Anita Groenendijk, Mieke Lumens, Tonnie Zweers, Jos van Hutten, Reini Pater and Pieter Versloot.

8. General discussion and conclusions

8.1 Characteristics of the study design

Epidemiological studies are a powerful tool for the study of work related health problems. They can be designed for several objectives, such as identifying risk factors, estimating exposure-response relationships, estimating public health impact of occupational exposure, and studying the development and progress of health effects (Wegman 1987). The first objective of this study of animal feed workers was to verify (or falsify) foreign studies which indicated respiratory health effects in workers exposed to grain dust, and the identification of characteristics of exposure that could be considered risk factors. The second objective was the investigation of characteristics of the possible health effects, and exposure response relationships. Characterisation of exposure itself was a third goal; it yields information that may be of use for evaluating (public) health impact in the industry. Furthermore, the results of the extensive measurements of this study can be used for the development of concepts and methods for exposure assessment in an epidemiological study design (appendix 2).

The results of the analyses described in the previous chapters clearly show exposure related respiratory symptoms and lung function changes in the industry studied. The results confirm those of studies in grain handlers (see chapter 4) which also describe respiratory effects.

Estimation of the slope of exposure-response relationships, however, is a difficult task on the basis of epidemiologic research. With respect to chronic effects, quantification and generalisation of exposure-response relationships are limited by the inherent limitations of the observational design. Furthermore, the cross-sectional characteristics of the respiratory health measurement may hamper quantification, even when the exposure characteristics have been studied in great detail. Aspects that may play a role in this respect and will be discussed in this chapter are selection bias (healthy worker effect) and cohort effects. It must, however, be emphasized, that although quantification of exposure-response relationship is complex, it is not impossible, and the strength and magnitude of the relationships found are a major source of information for the identification of

risk factors and their relative importance. The design of this study implied extensive exposure measurements, to allow for maximal detective power when compared to earlier published work in this field.

With respect to the acute effects, the study yields information on exposure-response relationships as well. Limitations for generalisation which were mentioned above for chronic effects are of less importance in the study of short-term lung function changes. The observational design, however, has some limitations in comparison with controlled experiments. Apart from the healthy worker effect that may have resulted in a selected population with respect to susceptibility for acute effects, the main limitations are related to exposure characterisation.

As for the analysis of chronic effects, the statistical phenomenon of attenuation relates to downward bias of exposure-response relationships, caused by non-differential exposure measurement errors (see appendix 2). Furthermore, the interrelationship between dust and endotoxin concentrations limits independent analysis of both risk factors.

8.2 Bias and confounding

As mentioned above, several factors may limit the generalisation of study results. Exposure characterisation, selection bias, cohort effects and ethnicity are among the potentially relevant issues and need a more detailed discussion.

Exposure characterisation is a topic in epidemiologic research on work related disorders that, until recently, has received remarkably little attention in the literature. The early studies on grain dust and respiratory effects used the most simple exposure classification: the comparison of grain workers and controls. Exposure measurements were almost anecdotal. When performed, the measurement characteristics are arbitrary in several aspects. The often used stationary measurements can not be considered reliable estimators of personal exposure. Workers are usually mobile, and their work tends to be close to dust sources. On the other hand, workers may tend to leave rooms with massive dust exposure (Farant & Moore 1978).

Even when exposure measurements are performed with personal sampling, as in the animal feed study, several other issues remain. One is the choice of the sampling instruments. The inspirable dust fraction in this study was chosen because of the multitude of possible effects and receptive areas in the airways. The respirable fraction for example that was measured in the earlier studies accounts for a fraction of the total relevant exposure. The design and handling of the actual measurement device, as well as laboratory handling may introduce random and systematic errors, and therefore introduce both statistical noise and inaccuracies in exposure response relationships.

Another aspect in exposure assessment is the choice for gravimetric dust and endotoxin as exposure variables. As discussed in chapter 4, many other possible components are suggested to be related to respiratory diseases. At the time of the design of the study, only endotoxin was feasible in terms of probability as a causal agent and operational analytical methods, besides the well studied and 'easy to measure' gravimetric dust concentrations. Storage of aqueous extracts of all samples allows for further analysis in the future. Some of the most interesting dust components in this respect are probably allergens, since a recent study by Zuskin et al. (1992) has shown indications for immunological effects of dust exposure in animal feed workers. Another potentially relevant component may be ß-1,3-glucan, a fungal constituent that may lead to inflammatory responses in lung tissue (Rylander 1992).

A further point of attention is the measurement strategy, a concept that can be described by characteristics such as the number of measurements for every worker or job title, as well as the grouping strategy. An increasing number of publications (e.g. Smith et al. 1991, Esmen 1991, Kauppinen 1991), as well as appendix 2 illustrate the relevance of a well designed measurement strategy, both in terms of efficiency and efficacy. Loss of power and attenuation (chapter 8.1) are among the consequences related to a non optimal study design. In the animal feed study, exposure measurements were extensive; the exposure of the majority of workers was repeatedly measured. The modelling of exposure groups was therefore reliable when compared to other studies in this field. Modelling of retrospective exposure, however, was hampered by the absence of historic exposure measurements. Nevertheless, the retrospective analyses clearly

showed exposure response relationships, that were in agreement with a similar retrospective analysis of a Canadian grain workers cohort, for which historic exposure measurement results were available (Kennedy 1992, personal communication).

A major problem in epidemiological research on respiratory disorders is selection bias, caused by the healthy worker effect. Ogle (1885), the first author to describe this phenomenon, identified two kinds of selection. The first one takes place before accepting employment: people tend not to choose jobs that burden their respiratory system if they already suffer from airway diseases. Formal selection by occupational physicians is part of this primary selective mechanism, although in the Netherlands the number of people that are rejected by physicians is remarkably low, and rejection merely reflects work disablement in general instead of health restrictions related to job characteristics (de Kort & van Dijk 1990).

The second kind of selection takes places during employment. Workers tend to leave their job if their health is too much impaired for the job performed. Hollander et al. (1988) have demonstrated this phenomenon in a longitudinal study in a ship screw propeller industry in the Netherlands, where the workers who took part in the follow-up had better lung function values during the initial measurements, than those who left the industry. In chapter 4.4, several foreign studies in the grain industry are cited showing similar effects. Although exact data are not available, the large number of people in the Netherlands receiving disability pension due to respiratory disorders (26,600 in 1988 (CBS 1991a)) indicates that this phenomenon may be significant.

Both selective mechanisms may lead to serious problems in epidemiologic research. Cross-sectional studies may be severely biased, as was shown in a Dutch study in a milk powder plant where the exposed group had significantly better lung function variables than the controls, in spite of massive dust exposure (Smid & Huismans 1988). Studies in the grain industry (mentioned in 4.4) as well as the cross-sectional analysis of the animal feed worker data (6.5) have shown comparable phenomena related to lung function and respiratory symptoms. Retrospective and longitudinal studies in which exposure is quantified in a more detailed manner than by a dichotomisation as in the cross-sectional

studies mentioned, are less hampered by this healthy worker effect. The quantification of exact exposure response relationships, however, is considerably hampered by the unknown magnitude of selection.

A third problem that may influence the analysis of lung function data and prevalence of respiratory symptoms is the cohort effect. Van Pelt et al. (1990) have shown this in consecutive 3 yearly measurements in the Dutch Vlaardingen-Vlagtwedde study, which was aimed at identifying risk factors for COPD. Both lung function variables (FEV_1 and FVC) and respiratory symptom prevalence (dyspnoea) were found to be worse among the older cohorts. However, cross-sectional analysis using direct adjustment for age, as was performed in the animal feed study, is expected to be hardly influenced by cohort effects.

A fourth potential problem in epidemiological research on lung function and respiratory symptoms is ethnicity. Several authors have found differences in lung function between racial groups (Willems & Heederik 1984). Normal values as defined by the ECCS are considered valid for Caucasian people. In the animal feed study, non-Caucasians (people of African or Asian origin) were excluded from the analyses to prevent bias caused by racial differences. People from non-Dutch European nationalities, i.e. Spanish and Turkish people, were not excluded since they belong to the Caucasian race. A recent study in the grain industry, however, has revealed indications that ethnicity may be a confounding factor in the analysis of lung function data. In a study group of Saskatchewan grain silo workers, ethnicity was defined by the nationality of all four grandparents; the province of Saskatchewan was settled in the first decades of the 20th century by groups of immigrants who came mainly from northern European countries. A comparison between three ethnic groups revealed that, after adjustment for obvious confounders such as smoking habits, British people had a higher risk of airflow obstruction than German or Eastern European workers (Horne et al. 1989). The study, however, has some methodological drawbacks. The first is that the ethnic groups that were identified may have a tendency to retain some of their cultural differences, including smoking practices, sports habits, and dietary differences. Winter fruit consumption for example, was found to be positively related to lung function in Britain (Strachan

et al. 1991), and coffee consumption was found to be related to FEV_1 impairment (Cohen 1980). The second debatable element is the division into 'ethnic groups'. The German group for example consists of German, Austrian and Dutch workers, whereas the Polish workers are considered Eastern European, together with Russians and Ukrainians. Clearly, to a European audience, this is an arbitrary division. Although the results suggest genetic ethnic differences, the conclusions must be considered with caution. Culture related differences, however, are very clearly present in Europe. A WHO study revealed considerable differences in mortality rates for asthma, emphysema and bronchitis among males aged 45 and over, between different European countries (Melia & Swan 1986). Therefore, apart from the questions concerning the cause of differences, nationality or ethnicity can result in confounding in epidemiological studies. As far as our study concerns, this bias is probably small since the number of ethnic non-Dutch subjects in the study population was very small.

8.3 Conclusions

As mentioned in 8.1, the results of the study clearly indicate chronic and acute respiratory effects of animal feed dust exposure, as well as exposure-response relationships. The main drawbacks of the study design, as mentioned above, are the selection effect and statistical attenuation. Both phenomena will have biased the exposure-response relationships in a downward direction, but nevertheless were shown not to be able to hide the effects. When interpreting the results, however, with the aim of establishing no (or minor) effect levels, they must be accounted for.

The effects that were detected were acute and chronic lung function changes and increased prevalences of respiratory symptoms. Reported symptoms appeared to be a less sensitive effect indicator than lung function. The generally low reproducibility and sensitivity of questions on respiratory symptoms, as discussed in 2.3, will undoubtedly have been a major contributing factor for this finding. It may be concluded that, given these limitations, the group size was

marginally or too small, to allow for conclusions regarding the nature of the effects using questionnaires. A further explanation for the apparent smaller sensitivity compared to lung function results may be that the selective effect is more strongly influenced by perceived symptoms than by pre-clinical lung function changes.

Nevertheless, given these limitations, the analysis of reported symptoms indicate cough as the predominant symptom, for both the analyses of chronic and acute effects. Other symptoms reported during work were mainly irritative (nose irritation and sneezing).

Exposure related lung function changes were found for all flow variables studied, although FEV_1 and peakflow and the related MEF_{75} were most consistently related to several exposure indices. FVC was only (statistically significantly) related to current exposure, and not to retrospective exposure. Cross shift lung function changes were only found for MMEF and MEF_{50}, indicating consistent but clinically irrelevant effects. The role of these findings for the prediction of the development of respiratory diseases, however, is an intriguing question that can only be answered during the follow-up study of the animal feed workers.

The general picture that arises from analysis of both symptoms and lung function shows predominantly irritative responses during work, and a clinical picture that resembles chronic bronchitis. Symptoms that were indicative for asthma (symptoms, acute effects on peak flow) were not found to be significantly related to exposure.

The results of this study are in concordance with the vast amount of research on grain dust and lung function that was discussed extensively in chapter 4. The predominant effects of grain dust exposure also appear to be related to the clinical picture of chronic bronchitis. The other syndromes that were discussed, mainly allergic, asthmatic and toxic responses to grain dust, do not appear to play a significant role in the animal feed industry. Although a recent study in Yugoslavian animal feed mill workers has revealed indications for immunological reactions on several constituents of animal feed dust. However, the immunological reactions did not correlate with respiratory findings (Zuskin et al. 1992).

With respect to the aetiology of the respiratory effects, this study gives evidence for a significant role of endotoxins. Both for chronic and acute lung function effects, the endotoxin exposure was found to be a stronger predictor than dust exposure, both in terms of the magnitude and of the significance of the relationships. As discussed in chapter 7, several experimental studies and one field study have confirmed that endotoxin exposure may lead to cross shift lung function changes. The role of endotoxin as a cause of chronic lung function changes has been hypothesised previously in only one field study (Kennedy et al. 1987).

Although especially for acute lung function changes there is considerable support for the causal role of endotoxin, it can not be excluded that other (microbiological) factors may play a contributing role, since endotoxin concentrations were found to be more clearly related to fungal counts than to dust exposure (chapter 6.5). Considering that in research in this field only minor attention has been paid to other microbiological factors in organic dust, it may well be that future research will reveal a significant contribution of these other dust components. Recent research has focused on an other potentially relevant component: ß-1,3-glucan is a fungal constituent that may lead to inflammatory responses in lung tissue (Rylander 1992). It has been detected in buildings in which complaints about the indoor air had been numerous. Several respiratory symptoms were reported to be related to levels of ß-1,3-glucans (Goto et al. 1992). Interaction of endotoxin induced and ß-1,3-glucan induced inflammatory responses was found in animal studies, and was hypothesised in humans in environments with both fungi and endotoxin exposure (Rylander 1992).

8.4 Relevance for occupational health

The animal feed study adds information to the vast body of knowledge on respiratory effects of grain dust. From a viewpoint of public health, it is therefore of interest to draw conclusions from this scientific evidence, and estimate the impact of grain dust exposure to occupational health.

However, several gaps in existing knowledge hamper this process. The main obstacle is the lack of data on exposure related clinically relevant morbidity, as well as on mortality. The scientific evidence comprises merely information on accelerated lung function changes and reported symptoms. It is not clear to what extent this reflects, or may lead to morbidity and mortality. A complicating factor is the heterogeneity of clinical effects, ranging from acute toxic responses to slowly developing allergic disorders (chapter 4).

Furthermore, the relevance of acute lung function changes in predicting chronic disorders needs clarification. Although there is some evidence for this relationship (Chan Yeung et al. 1992), quantitative data are not available.

Another drawback is the lack of data on the extent of reversibility of grain dust related respiratory disorders, although it is generally accepted that a complete convalescence after exposure is hardly possible.

Although the data from the animal feed study do not allow for an estimation of public health impact in terms of clinically relevant morbidity (and mortality), it must be taken into account that, as discussed in 2.1, dust exposure in general is a significant causal factor in the aetiology of CNSLD. Evidence for the presence of clinically relevant respiratory disorders, as related to organic dust exposure, can be found in the work of Heederik et al. (1992). In a cross sectional analysis of the general population Zutphen study he related several indicators of respiratory disorders to exposure characteristics of the longest held occupation. The exposure characteristics were constructed by a job exposure matrix. Physicians diagnosed CNSLD, as well as treatment for chronic bronchitis or emphysema were significantly related to organic dust exposure. The prevalence of cough or sputum was also increased in the exposed workers. Other respiratory symptoms studied (mentioned in the BMRC (1960) questionnaire) did not show elevated odds ratios. Treatment for asthma was not found to be related to organic dust exposure. The results of this analysis on organic dust corroborate the findings of the analysis of chronic respiratory disorders in the animal feed workers, since they point towards chronic bronchitis as the predominant clinical picture. This finding is also generally supported by the literature on grain dust (8.3).

The results from the Zutphen study give strong indications that, even if their mutual relationship can not be quantified, lung function changes and increased respiratory symptoms may lead to clinically relevant morbidity and mortality.

8.5 Occupational exposure limits

8.5.1 Dust

The absence of information on clinical respiratory disorders as related to exposure hampers the establishment of health based occupational exposure limits. However, as mentioned above, the evidence of the presence of clinical effects is convincing. The establishment of (tentative) exposure guidelines is therefore of great importance, even when it should be done using information on subjective symptoms and lung function changes. As an approximation, the absence of statistically significant effects on lung function or reported symptoms can be used as an indicator for the establishment of a no adverse effect level. This method, however, should be used carefully, since insufficient population size, selection effects, and statistical attenuation may lead to a decreased detective power.

The use of animal studies has limited value for the estimation of no effect levels, mainly because of the heterogeneity of exposure (in terms of composition of the dust and particle size) which does not match with the usual procedures of animal studies that are performed with controlled exposure conditions. Similarly, there is no animal which satisfactorily mimics asthma, chronic bronchitis or pulmonary emphysema, so that pathological events in animals are not a faithful reflection of human pathofysiology. A further drawback is the general problem of generalising results of inhalation studies from animals to humans due to anatomical (particle deposition) and physiological differences. The inadequacy of animal studies for establishing limit values does, however, not imply that animal studies cannot be of great value in the search of active components and mechanisms for effects.

A further point of attention is the description of the dust for which an exposure limit should be established. The general term grain dust can be defined

operationally as the dust which is present in the ambient air of facilities in which a significant part of the working activities have to do with grains, pulses or oil seeds. This tentative definition comprises animal feed production and use, elevators for raw materials of animal feed, as well as several other processes in which dust is generated which is loosely called grain dust in the literature. The operational definition has the advantage that most of the relevant working conditions are covered. More precise definitions, such as the definition by the American Conference of Governmental Industrial Hygienists (dust from oats, wheat and barley, ACGIH 1992) will without doubt limit the applicability considerably. Furthermore, the available evidence does not support that different grains require different exposure limits (Chan Yeung et al. 1992). It must of course be emphasized that components of the actual dust may have separate exposure limits, such as pesticides, quartz, minerals etc. Endotoxin is clearly a component for which a seperate limit should be considered.

The dust that is usually called flour dust, originating from milled wheat and rye and present in flour mills and bakeries, may need to be distinguished from grain dust, since the allergic and asthmatic nature of the effects appear to be very prominent (chapter 4.4.4).

Using a level of no observed respiratory effects as a basis for an occupational exposure limit implies that respiratory effects are the most sensitive adverse effects, i.e. that other effects occur at higher exposure levels. Skin disorders which are possibly related to inhaled allergens (Thiel et al. 1970) are found in bakers exposed to flour dust. The proposed exclusion of flour dust from the definition of grain dust may overcome this problem. A Danish study indicating decreases of variables of sperm quality in 16 seaport grain elevator workers has several severe methodological drawbacks (Foldspang et al. 1987). Combined with the small group size and the fact that no other studies on this subject are published, it must lead to the conclusion that it needs not to be taken into account when establishing an exposure limit.

The evidence for carcinogenic effects of grain dust exposure is inconclusive (4.6). Although liver cancer in Europe, and lymphatic and hemopoietic malignancies and bladder cancer in Northern America appear to be elevated in some studies, the existing data are inconclusive and clearly not adequate for

establishing exposure limits. Since several chemical exposure factors are hypothesised as causes, such as pesticides in the American studies and mycotoxins in Europe, it is more practical to conduct research on these explicitly identified chemical factors and establish exposure limits based on carcinogenic effects. As long as the evidence for carcinogenic effects of grain dust does not allow to establish exposure response relationships, the proposed limit for grain dust could then be based on respiratory effects.

Apart from the animal feed study, some studies on grain dust have tried to identify dose-response relationships or no effect levels for chronic effects. In a recent literature survey, Chan Yeung et al. (1992) mentioned three studies in support of an exposure-response relationship and their proposed tentative exposure limit of 4 mg/m^3 total dust. The first study, by Corey et al. (1982), identified an inverse relationship between FEV_1 and respiratory flow and present respirable dust concentrations. Paradoxically, however, FEV_1 and FVC were found to increase with longer employment duration. Furthermore, lung function levels were not related to total dust levels.

A second study, by Enarson and colleagues (1985) used a nested case-control design in a six year longitudinal study. They concluded that a total dust level of 5 mg/m^3 or less was not associated with being identified as a 'case', i.e. belonging to the 10% of workers with the worst trend of FEV_1 during the study.

A more extensive analysis in the same cohort, by Huy et al. (1991), showed that workers with an 'average' exposure between 4 and 9 mg/m^3 were found to have lower values for FVC and FEV_1 when compared to grain workers exposed to $<$ 4 mg/m^3 and when compared to a reference population of civic workers.

It must, however, be emphasized that in this analysis, the group exposed to 'average' grain dust levels less than 4 mg/m^3 reported significantly more phlegm production and had a significantly lower FVC compared to the office workers. It is therefore apparent that adverse effects also take place at levels lower than 4 mg/m^3. A major problem when interpreting the results of this analysis is that average dust exposure was expressed as geometric job title mean exposure. When computing retrospective individual average exposures, however, an arithmetic mean of yearly geometric job title means was used. The (arbitrary) use of one year periods does not have physiological plausibility, since it

presumes that for periods of up to one year the effects are related logarithmically to the total body burden, whereas for time segments of one year, effects are related linearly to exposure.[1]

In the animal feed study, as was discussed in chapter 6, the analyses were performed under the hypothesis of dose-response relationships. Consequently, arithmetic means were used in the analyses using exposure estimates. The occurrence of a selection effect prevents comparison to external controls when estimating no effect levels. The analyses which were performed, both for chronic and acute effects, clearly showed differences between the low and high dust exposure categories. Since the low dust category comprised job titles with arithmetic mean inspirable dust exposure below 5 mg/m^3, it must be concluded that adverse effects at levels in excess of 5 mg/m^3 can not be ruled out. A tentative health based level of approximately 5 mg/m^3 corroborates the 4 mg/m^3 level that was proposed by Chan Yeung et al. (1992). As mentioned before, the selective effect and statistical attenuation require that safety factors are applied, since these two phenomena may bias exposure response relationship into a downward direction. Furthermore, when table 6.8 is applied to a working life of 40 years with an exposure of 5 mg/m^3, the estimated effect on FEV_1 would be approximately 150 ml/s (approximately 4% of the group mean FEV_1), but on MEF_{75} for example it would be 900 ml/s (approximately 12%). When using the same regression models (Houba et al. 1989), the estimated age related decrease of FEV_1 and MEF_{75} would be 1280 ml/s and 1800 ml/s respectively, therefore the decline FEV_1 and MEF_{75} would increase by 12% and 50% respectively. It must be taken into account that these figures relate to group mean levels, whereas the aim of establishing exposure limits is protection of the individual's

[1] The authors have identified four job title-time exposure categories, with geometric mean concentrations of among others 6.0 mg/m^3, 2.9 mg/m^3, and 1.1 mg/m^3. If a person A would be in the first category for one year, and in the third category for two years, the authors would compute his average exposure as (6.0 + 2 * 1.1)/3 = 2.7 mg/m^3. Person B that was in the second category for three years would be given an average exposure of 2.9 mg/m^3.
If the period for addition would have been three years, then B would still be given an average (geometric mean) exposure of 2.9 mg/m^3. A, however, has a geometric mean exposure of ln(((exp(6.0) + 2*exp(1.1))/3) = 4.9 mg/m^3.

health. Therefore, without applying a correction for selection and attenuation, the order of magnitude of estimated group mean effects on lung function are excessive, and clearly justify the application of a safety factor. A systematic theoretical or practical framework for estimating a safety factor using epidemiological data is currently not available. Taking into account the estimated excessive effects on MEF_{75} and the selection bias and attenuation effect, an arbitrary 'educated guess' would lead to safety factor between 2 and 5, and would therefore lead to a tentative exposure limit between 1 and 2.5 mg/m^3 as an eight hour average.

Finally, in the discussion on health based exposure levels, attention must be payed to the Swedish NIOH and US NIOSH document on the basis for an occupational standard for grain dust (Brown 1988). The conclusion of this extensive literature survey is that 'it is presently impossible to recommend an exposure limit at which all workers would be protected from adverse health effects', 'because exposure to grain dust results in an immunologic response in some individuals'. The author advocates reducing exposure levels in general, and preemployment screening to be used to counsel workers about their predisposition to adverse respiratory symptoms.

The results from the animal feed study, however, do not primarily point towards allergic mechanisms as playing an important role in the aetiology of dust related respiratory disorders (chapter 8.3). Furthermore, the literature on possible predisposing factors for immune system mediated adverse respiratory effects does not allow firm conclusions. In other words, the criteria that should be used for preemployment medical screening are not clear, making this alternative for standard setting not feasible for the current practice in occupational health. It is not clear whether this statement relates to an exposure level which prevents a reaction in sensitised workers, or to an exposure level which prevents sensitisation in healthy workers. In addition, the recommendation for reducing exposure levels in general to a level that is as low as reasonably achievable does not exclude the need for exposure limits. Since the allergy mediated effects do not appear to play an important role, a tentative exposure limit can still be proposed at between 1 and 2.5 mg/m^3, although it implies that it does not guarantee that all workers would be protected.

8.5.2 Endotoxin

The literature on possible limits of exposure to endotoxin has focused on exposure-response levels for acute lung function changes. As discussed in 7.4, no effect levels which were estimated from experiments with cotton dust were 9 ng/m^3 and 33 ng/m^3 (Castellan et al. 1987, Rylander et al. 1985). Palchack et al. (1988) proposed a TLV value of 30 ng/m^3 for large scale fermentation processes.

The results of our study indicate that both acute an chronic lung function effects could be found in the intermediate exposure group, as compared to the low exposure group. In analogy with the discussion on dust exposure limits above, a tentative no effect level would be the upper limit of the low exposure group, i.e. 15 ng/m^3. This level is in remarkable agreement with the experimental results. The two observational studies which were published and gave indications for exposure-response relationships (Kennedy et al. 1987, Donham et al. 1989) showed effects of much higher levels, but the study design (especially the exposure assessment strategy) and analysis lacked statistical precision compared to the animal feed study (as discussed in 6.5 and 7.4).

In analogy with the discussion on dust, application of table 6.8 shows that 40 years of exposure to 15 ng/m^3 may lead to an estimated effect on FEV_1 of approximately 200 ml/s. For MEF_{75} the effect would be 1200 ml/s. These estimates are in the same order of magnitude as those for a dust exposure of 5 mg/m^3. Taking again into account selection and attenuation leading to downward bias, a similar safety factor may be applied. Therefore, the tentative occupational exposure limit could be proposed at between 3 and 7.5 ng/m^3.

It must, however, be stressed that uncertainties about the relative activity of different types of endotoxin, as well as differences in laboratory practices (Milton et al. 1989), should lead to a careful use of the tentative limit. Furthermore, as discussed above, the evidence from the animal feed study does not exclude effects of endotoxin correlated (microbiological) factors in the dust that may lead to an overestimation of the endotoxin effect.

8.5.3 *Health impact for the animal feed industry*

As mentioned before the lack of data on clinically relevant morbidity hampers estimation of health impact in terms of respiratory disorders. It is, however, possible to estimate the number of people at risk of developing of rerspiratory disorders by using the proposed lower and upper limits of health based exposure limits for grain dust and endotoxin.

It is assumed that long term exposure to the proposed values leads to adverse health effects. Estimation of a long term mean exposure can be performed using the arithmetic mean exposure of job categories. Therefore, workers in a given job category with a mean exposure above the proposed limits, are considered to be at risk. As always, it must be taken into account that during their professional career workers may tend to shift to other job categories and even to other industries in which their exposure to agents that effect the respiratory system is lower, and reconvalescence may take place. The concept of eight-hour exposure limits, however, does not allow for this phenomenon, since lifetime dose limits, although they may be advocated from a health point of view, require massive administrative and enforcement efforts.

Table 8.1 shows arithmetic mean exposures to dust and endotoxin for the eight job categories that were identified, taken from 6.4.1. The mean exposure data were taken from 530 samples in 187 workers. The relative number of workers by job category are also given.

Table 8.1 Number of samples, number of workers, and arithmetic mean exposure to dust and endotoxin, by job category.

job category	n (samples)	N (%) (workers)	dust mg/m^3	endotoxin ng/m^3
unloaders	69	29 (16)	29.7	68
crane drivers	23	12 (6)	5.0	36
facility operators	63	20 (11)	1.7	6
press operators	54	17 (9)	3.0	7
production managers	42	16 (9)	3.8	12
transport workers	61	18 (10)	2.8	30
sackers	20	9 (5)	4.8	6
other	198	66 (35)	11.5	37

If the lower limits of the proposed health based limit values are applied (1 mg/m^3 dust and 3 ng/m^3 endotoxin), no job category shows mean exposure below the criterion. This would imply that, assuming a life long employment in the animal feed industry, no worker can be excluded from the risk of developing respiratory disorders.

If, however, the upper limits are applied, some job categories show average exposures below the proposed values of 2.5 mg/m^3 dust and 7.5 ng/m^3 endotoxin. As discussed before, the study group can be considered to be representative for the Dutch animal feed industry. Under this assumption, estimates can be made of the fraction of the total worker population that is at risk. If the upper dust exposure limit, is used 11% of the workers (facility operators) are exposed to lower average concentrations. This implies that for approximately 90% of the workforce the risk of the developing of respiratory disorders cannot be excluded. Using the proposed upper endotoxin limits, the facility and press operators as well as the sackers (25% of the study group) are not at risk. However, since two of these three categories still show exposures above the proposed upper dust exposure limit, their risk cannot be excluded.

Taking into account that the total exposed workforce in the animal feed industry is approximately 6000 workers (4.1), this would imply that, assuming life long employment, between 5400 and 6000 workers are at risk of the developing of respiratory disorders (i.e. that the risk for development of respiratory disorders can not be excluded), since their exposure is systematically in excess of tentative exposure limits.

8.6 Summarising conclusions

The results of this study lead to the following summarising conclusions:

- Workers in the Dutch animal feed industry are very frequently exposed to dust levels in excess of internationally recommended exposure limits for grain dust and the Dutch maximum allowed concentration for nuisance dust.

Endotoxin concentrations in airborne animal feed dust appear to be related to stages in the production process. Airborne endotoxin concentrations are more strongly related to fungal counts than to dust concentrations.

- A comparison between animal feed workers and external controls revealed an obvious healthy worker effect.
- Within the group of animal feed workers a strong negative association between (present and retrospective) exposure, and lung function was found.
- The effects on lung function were more strongly related to endotoxin exposure indices than to dust exposure indices, in terms of statistical strength and size of effects at average exposure levels.
- The predominant symptomatic and lung function effects indicate a clinical picture that seems related to chronic bronchitis.
- Cross shift changes of sensitive lung function variables were related to dust exposure and, more strongly, to endotoxin exposure.
- Primarily irritative respiratory symptoms and cross week lung function decreases were present in animal feed workers.
- Tentative health based exposure limits for grain dust and endotoxin can be derived from the results of this study and existing literature. Depending on the safety factor to compensate for methodologically induced downward bias of the exposure-effect relationships, it is estimated that for 90 to 100% of all animal feed workers the risk of respiratory disorders can not be excluded. This would relate to 5400 to 6000 workers.
- The value of this study, in terms of exposure-response quantification, can be significantly improved by a follow-up study in the same cohort of animal feed workers.
- The present knowledge of respiratory effects of grain dust exposure can be significantly improved by further research into pathophysiological mechanisms and relevant dust components, as well as into clinically relevant respiratory morbidity and mortality.

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Summary

Chapter 1 summarises the background of the study. Nonmalignant respiratory disorders account for a significant part of sick leave diagnoses (19%), disability pension (3.4%) and mortality (7%). The rate of chronic obstructive pulmonary diseases has risen substantially during the last 10 to 20 years. Allthough smoking is a major external cause for the increasing mortality and morbidity, other environmental factors also play an important and probably underestimated role in the aethiology of COPD. Knowledge on occupational dust related respiratory diseases can be found in the work of famous sixteenth and seventeenth century authors, such as Agricola and Ramazzini.

Allthough the role of organic dust in this field has never been questionned, scientists (and legislative authorities) have not been able to establish guidelines or limits for occupational exposure.

This thesis is an epidemiological study and deals with the relationship between grain dust and endotoxin, and spirometric lung function changes and respiratory symptoms. The study was performed in the animal feed industry, in which approximately 6000 workers are exposed to grain dust. Grain dust was broadly defined as the dust present in working environments in which grain and related products are handled and processed. It is estimated that outside the agricultural sector, 20,000 to 35,000 workers are exposed to grain dust in the Netherlands.

Chapter 2 is a discussion on methodological issues concerning epidemiological research on respiratory disorders, and on aethiology and terminology of respiratory disorders.

It is estimated that occupational exposure may account for an excess mortality for bronchial carcinoma of up to 10%, and for at least 10% of all clinically relevant CNSLD morbidity. The concept of CNSLD (chronic non specific lung diseases) is used predominantly in the Netherlands, and comprises bronchitis, asthma and emphysema. Other concepts, more generally accepted, divide this group into asthma on the one hand, and chronic bronchitis and emphysema on the other. This latter group is also known as COPD or COLD (chronic obstructive pulmonary (lung) diseases).

Spirometric lung function tests, and especially FVC and FEV_1, meet most of the requirements for epidemiological research, in terms of acceptability, simplicity, objectivity and reproducibility. Standardised questionnaires, however, lack reproducibility, and therefore require study groups of sufficient size. The general problem of validity, agreement between 'the disease' and the test value, is overcome by using both lung function tests and questionnaire score as operationally defined effect variables.

The etiology of CNSLD can be explained by two different hypothesis. The so-called Dutch hypothesis states that all constituents of CNSLD are interrelated, and that the development of CNSLD is genetically predisposed. The British hypothesis, however, states that exogenous stimuli may lead to chronic imflammatory responses, and thus to bronchitis.

Chapter 3 describes the history and present characteristics of the animal feed industry. The industry is relatively young; industrial production did not start until the 1930's and 40's.

Generally the production process can be represented in four steps; grinding, mixing, addition of vitamins and additives, and pressing into pellets. The process is highly mechanised.

Raw materials may be grains, other vegetable products, and waste products from the food industry. The relative amount of grains has been steadily decreasing since industrial production started. The proportion is now approximately 10% in the Netherlands, allthough it may be considerably more in other EC countries. The production volume in the Netherlands is about 15 million tons/year, in approximately 350 facilities, employing over 10,000 workers. Due to upscaling and a stabilised production volume, the number of facilities and employees are expected to decrease in the coming years.

An overview of the literature on grain dust and health is given in chapter 4. Exposure to grain dust may include exposure to grain; non grain plant matter; fungi, moulds and spores; mycotoxins; bacteria and their chemical components and excreta; mites and other animal matter; pesticides; and inorganic matter. A

limit value for exposure to grain dust has not been established in the Netherlands.

Non respiratory effects of exposure to grain dust include skin disorders. Mortality and cancer incidence studies give some indications for an increased risk of primary liver cancer, lung cancer, lymphatic and hemopoietic malignancies and bladder cancer. Three respiratory syndromes are more or less clearly defined.

Extrinsic allergic alveolitis (EAA) is characterised by respiratory and systemic symptoms, appearing some hours after exposure. Grain fever is characterised by systemic effects during or up to 4-8 hours after exposure. In contrast to EAA the mechanism is not allergy mediated. Bakers asthma is an IgE mediated bronchial asthma, frequently found in bakers and millers.

Epidemiological research has revealed that symptoms and spirometric lung function are frequently related to both long term and acute exposure to grain dust. The nature of respiratory effects is not unequivocal, allthough the obstructive effects appear to be most pronounced. The symptoms which point towards chronic bronchitis appear to prevail. The role of immunological mechanisms or bronchial hyperreactivity is not yet clarified.

Chapter 5 is an overview of measurements of exposure to dust, endotoxin and fungi in the Dutch animal feed industry. 79 stationary and 530 personal dust samples were taken in eight animal feed production facilities. The stationary sampled showed gravimetric eight hour mean total dust concentrations ranging from 0.2 to 25 mg/m^3 (Geometric Mean 1.3 mg/m^3). Concentrations of smaller particle fractions were considerably lower. Personal inspirable dust concentrations were considerably higher than stationary concentrations, and were strongly related to job titles. Inspirable dust concentrations ranged from 0.2 to 450 mg/m^3 (GM = 2.4 mg/m^3). After adjusting for differences between inspirable and total dust, 25% of the measurements exceeded the Dutch MAC for nuisance dust, and 42% exceeded the ACGIH Threshold Limit Value for grain dust (4 mg/m^3). Endotoxin concentrations ranged from 0.2 to 1870 ng/m^3 inspirable dust (GM = 5.1 ng/m^3). Endotoxin was less prevalent in respirable dust than in larger dust fractions. Concentrations in dust appeared to be related to

different stages in the production proces. Colony forming units (CFU) of fungi ranged from 130 to 15300 CFU/m^3 (GM = 2300 CFU/m^3), and were in parallel measurements more strongly related to endotoxin concentrations than to dust concentrations.

A cross-sectional analysis of 315 workers in 14 animal feed mills is given in chapter 6. Primary aims were to explore relationships between exposure to organic dust and chronic lung function changes. The study comprised monitoring dust and endotoxin exposure, spirometric lung function measurements (FVC, FEV_1, mean midexpiratory flow, and flow volume parameters) and a questionnaire for respiratory symptoms. The exposure was measured in two periods, during spring and autumn. The average 8-hour personal inspirable dust exposure was 9 mg/m^3 grain dust (ranging from 0.2-150 mg/m^3), and 25 ng/m^3 endotoxin (0.2-470 ng/m3), based on the 530 measurements described in chapter 5. On the basis of these measurements and the occupational history of the workers, the number of years 'worked in dust' and an estimate of the cumulative dust and endotoxin exposure was calculated. The prevalence of most chronic respiratory symptoms tended to decrease with increasing years of exposure. The healthy worker effect is probably responsible for this finding. In general, a strong negative association between most of the exposure variables and lung function was found. The endotoxin exposure was more strongly related to decreases in lung function than the dust exposure. The lung function changes occur at endotoxin levels lower than previously reported in field studies.

In chapter 7, an analysis is given of symptoms during work and acute lung function changes. Reported respiratory and related symptoms during work were compared between 265 exposed animal feed workers and a control group consisting of 175 external controls and non exposed workers in the animal feed industry. Symptoms indicating respiratory and nasal irritation were significantly increased in the animal feed workers. Prevalences ranged from 9% (cough) to 21% (sneezing). Reported cough after work was also significantly increased. In 119 workers, a total of 457 cross shift spirometric lung function changes were measured. Almost all lung function variables showed a decrease during the work shift, as could be expected since the circadian rythm is in a downward phase

during the measurement period (2 PM - 10 PM). When the workers were grouped into dust and endotoxin exposure categories according to their job title, an exposure-response trend was seen for MMEF and MEF_{50}. The effect of endotoxin was stronger than that of dust, both in magnitude and significance. For the same lung function variables and for FEV_1 and MEF_{25} a significant cross week change was also detected. The results of this study are in concordance with other studies that indicate acute effects on lung function and elevated prevalences of respiratory symptoms during work caused by exposure to grain dust.

A general discussion of the animal feed study is given in chapter 8. Sources of bias may be related to exposure characterisation, selection, ethnicity of the study group and cohort effects. In our study, the latter two are probably of minor importance. Despite the (other) sources of bias, the study clearly indicates exposure related lung function changes and increased symptom prevalences. It is evident that these effects may be related to clinically relevant morbidity and mortality.

Using data from our study and from the literature, tentative exposure limits for grain dust are proposed between 1 and 2.5 mg/m^3, and for endotoxin between 3 and 7.5 ng/m^3 8 hour time weighted average.

The results of this study lead to the following summarising conclusions:

- Workers in the Dutch animal feed industry are very frequently exposed to dust levels in excess of internationally recommended exposure limits for grain dust and the Dutch maximum allowed concentration for nuisance dust.
  Endotoxin concentrations in airborne animal feed dust appear to be related to stages in the production process. Airborne endotoxin concentrations are more strongly related to fungal counts than to dust concentrations.
- A comparison between animal feed workers and external controls revealed an obvious healthy worker effect.
- Within the group of animal feed workers a strong negative association between (present and retrospective) exposure, and lung function was found.
- The effects on lung function were more strongly related to endotoxin exposure indices than to dust exposure indices, in terms of statistical strength and size of effects at average exposure levels.

- The predominant symptomatic and lung function effects indicate a clinical picture that seems related to chronic bronchitis.
- Cross shift changes of sensitive lung function variables were related to dust exposure and, more strongly, to endotoxin exposure.
- Primarily irritative respiratory symptoms and cross week lung function decreases were present in animal feed workers.
- Tentative health based exposure limits for grain dust and endotoxin can be derived from the results of this study and existing literature. Depending on the safety factor to compensate for methodologically induced downward bias of the exposure-effect relationships, it is estimated that for 90 to 100% of all animal feed workers the risk of respiratory disorders can not be excluded. This would relate to 5400 to 6000 workers.
- The value of this study, in terms of exposure-response quantification, can be significantly improved by a follow-up study in the same cohort of animal feed workers.
- The present knowledge of respiratory effects of grain dust exposure can be significantly improved by further research into pathophysiological mechanisms and relevant dust components, as well as into clinically relevant respiratory morbidity and mortality.

Samenvatting

Hoofdstuk 1 is een overzicht van de achtergronden van het onderzoek. Niet maligne respiratoire aandoeningen zijn in Nederland de oorzaak van een belangrijk deel van het ziekteverzuim (19% van de diagnoses), arbeidsongeschiktheid (3,4%) en mortaliteit (7%). Het absolute en relatieve aantal chronisch obstructieve longaandoeningen is gedurende de laatste 10 à 20 jaar aanzienlijk gestegen. Hoewel rookgewoonten een belangrijke externe oorzaak vormen voor de toegenomen mortaliteit en morbiditeit, hebben andere omgevingsfactoren een belangrijke en waarschijnlijk onderschatte rol gespeeld bij de toename van CARA. Kennis op het gebied van respiratoire aandoeningen in relatie tot het beroep kan reeds worden gevonden in het werk van zestiende en zeventiende eeuwse auteurs als Agricola en Ramazzini. Hoewel de rol van organisch stof op dit gebied nooit is betwijfeld, zijn wetenschappers en wetgevende instanties niet of nauwelijks in staat geweest richtlijnen of grenswaarden te formuleren voor beroepsmatige blootstelling.

Dit proefschrift beschrijft een epidemiologisch onderzoek naar de relatie tussen blootstelling aan graanstof en endotoxine, en spirometrische longfunctieveranderingen en respiratoire symptomen. Het onderzoek werd verricht in de mengvoederindustie, waar circa 6000 werknemers zijn blootgesteld aan graanstof. Graanstof is vrij algemeen gedefinieerd als het het stof dat aanwezig is in de werkomgevingen waar graan en vergelijkbare produkten worden verwerkt. Naar schatting zijn, buiten de agrarische sector, 20.000 tot 50.000 mensen in Nederland blootgesteld aan graanstof.

Hoofdstuk 2 is een bespreking van methodologische aspecten van epidemiologisch onderzoek naar respiratoire aandoeningen, en van de aetiologie en terminologie op dit gebied. Naar schatting wordt tot circa 10% van de longkankersterfte veroorzaakt door beroepsmatige blootstelling. Minimaal 10% van de klinisch relevante morbiditeit als gevolg van CARA is waarschijnlijk aan expositie in het beroep gerelateerd.

Het begrip CARA (chronische aspecifieke respratoire aandoeningen) wordt vooral in Nederland gebruikt, en omvat bronchitis, astma en emfyseem. Andere,

meer algemeen gebruikte indelingen maken onderscheid tussen enerzijds astma, en anderzijds chronische bronchitis en emfyseem. Deze laatste groep is bekend onder de term chronisch obstructieve long (pulmonaire) aandoeningen (COLD of COPD).

Spirometrische longfunctietesten, en vooral FVC en FEV_1 voldoen aan de meeste vereisten voor epidemiologisch onderzoek, zoals een goede acceptatie, eenvoud, objectiviteit en reproduceerbaarheid. Bij gestandaardiseerde vragenlijsten echter is de reproduceerbaarheid doorgaans laag, waardoor de eisen die aan groepsgrootte worden gesteld worden verzwaard. Het algemene probleem van validiteit, overeenkomst tussen 'de aandoening' en de meetuitkomst, wordt vermeden door een operationele definitie van 'de aandoening' in termen van longfunctie of vragenlijstscore.

Voor het ontstaan van CARA bestaan twee aetiologische hypothesen. De zogenaamde Dutch hypothesis gaat uit van een interrelatie tussen alle vormen van CARA, en van genetische predispositie voor de ontwikkeling van CARA. De Britse hypothese echter stelt dat met name exogene stimuli kunnen leiden tot chronische ontstekingsreacties in de luchtwegen, en daardoor tot bronchitis.

In hoofdstuk 3 is een overzicht opgenomen van de geschiedenis en de hedendaagse kenmerken van de mengvoederindustrie. Deze is relatief jong; de industriële produktie van mengvoeder kwam pas in de jaren dertig en veertig op gang.

Het produktieproces is eenvoudig en kan in vier stappen worden beschreven: malen, mengen, toevoeging van vitaminen en additieven en persen tot brokjes (pellets). Het proces is grotendeels gemechaniseerd en geautomatiseerd.

De gebruikte grondstoffen zijn vooral granen, andere plantaardige produkten, en bijprodukten van de levensmiddelenindustrie. Het aandeel van granen is in de tijd sinds de industriële produktie op gang kwam geleidelijk gedaald tot momenteel circa 10% in Nederland. In andere EG landen is dit aandeel doorgaans hoger. Het totale produktievolume in Nederland is circa 15 miljoen ton per jaar, geproduceerd in 350 bedrijven met meer dan 10.000 werknemers. Als gevolg van schaalvergroting in de produktie en een stabilisatie van de afzet zal het aantal bedrijven en het aantal werknemers in de komende tijd (verder) dalen.

Hoofdstuk 4 omvat een literatuuroverzicht met betrekking tot graanstof en gezondheid. Graanstof kan bestaan uit verschillende componenten. Naast delen van het graan zelf bestaat het uit ander plantaardig materiaal, schimmels en schimmelsporen, mycotoxinen, bacteriën en excreta daarvan, mijten en ander dierlijk materiaal, pesticiden, en niet organisch materiaal. Een grenswaarde voor blootstelling is in Nederland niet vastgesteld.

Niet respiratoire effecten van blootstelling aan graanstof betreffen onder meer huidaandoeningen. Studies van kanker mortaliteit en morbiditeit geven indicaties voor verhoogde risico's voor primaire leverkanker, longkanker, neoplasmata van het lymfatisch en bloedvormend weefsel, en blaaskanker.

Drie min of meer duidelijk omschreven respiratoire syndromen worden in verband gebracht met blootstelling aan graanstof. Extrinsieke allergische alveolitis (EAA) wordt gekenmerkt door respiratoire en systemische symptomen die tijdens of enkele uren na blootstelling voorkomen. Bij graankoorts is er sprake van systemische effecten die vier tot acht uur na blootstelling optreden. In tegenstelling tot EAA is het mechanisme niet allergisch. Bakkersastma is een IgE gemedieerde bronchiale astma die vaak wordt gevonden bij aan meelstof blootgestelden.

Uit epidemiologisch onderzoek is gebleken dat respiratoire symptomen en longfunctie vaak samenhangen met momentane en met langdurige blootstelling aan graanstof. De aard van de effecten is niet eenduidig, maar obstructieve effecten lijken de overhand te hebben. Symptomen die wijzen op chronische bronchitis worden vaak gevonden. De rol van immunologische mechanismen en bronchiale hyperreactiviteit is nog niet opgehelderd.

Hoofdstuk 5 is een overzicht van de gemeten blootstelling aan stof, endotoxinen en schimmels in de Nederlandse mengvoederindustrie. 79 stationaire en 530 persoonlijke metingen werden verricht in acht mengvoederbedrijven. Bij de stationaire metingen werden 8 uurs gemiddelde concentraties van 0.2 to 25 mg/m^3 totaal stof gevonden, met een geometrisch gemiddelde van 1,3 mg/m^3. De concentraties van de kleinere stoffracties waren aanzienlijk lager. De persoonlijke metingen varieerden van 0,2 tot 450 mg/m^3 inspirabel stof (GM = 2,4 mg/m^3), en waren sterk geassocieerd met de functie van de betrokken

werknemers. Na correctie voor verschillen tussen inspirabel en totaal stof bleek dat 25% van de waarden lag boven de Nederlandse MAC waarde voor hinderlijk stof, en 42% boven de Amerikaanse TLV waarde voor graanstof van 4 mg/m^3. De blootstelling aan endotoxine varieerde van 0,2 tot 1870 ng/m^3 in de inspirabele fractie (GM = 5.1 ng/m^3). De concentratie endotoxine was lager in respirabel stof dan in de overige fracties. De concentraties endotoxine in stof waren geassocieerd met de verschillende fasen in het produktieproces. Het aantal kiemvormende eenheden (KVE) van schimmels varieerde van 130 tot 15300 KVE/m^3 (GM = 2300 KVE/m^3), en was in parallelle metingen sterker gecorreleerd met endotoxine concentraties dan met stofconcentraties.

Een dwarsdoorsnede onderzoek onder 315 werknemers in 14 mengvoederfabrieken is beschreven in hoofdstuk 6. Onderwerp was de relatie tussen blootstelling aan organisch stof, en chronische longfunctieveranderingen en respiratoire symptomen. Er werden metingen verricht van blootstellingsniveau's van stof en endotoxine, en van spirometrische longfunctievariabelen en respiratoire symptomen. De blootstelling werd gedurende twee perioden gemeten, tijdens het voor- en najaar. De gemiddelde 8 uurs blootstelling was 9 mg/m^3 inspirabel graanstof (variërend van 0,2 tot 150 mg/m^3), en 25 ng/m^3 endotoxine (0,2-470 ng/m^3). Op basis van deze metingen en gegevens over functies die de deelnemers in het verleden hadden uitgeoefend, werden als onafhankelijke variabelen het aantal blootgestelde jaren en de cumulatieve stof- en endotoxineblootstelling berekend. De prevalentie van de meeste symptomen daalde met het toenemend aantal blootgestelde jaren, hetgeen vermoedelijk door een selectie effect verklaard kan worden. Voor wat betreft longfunctiewaarden werd in het algemeen een duidelijk negatieve associatie met de blootstellingsvariabelen gevonden. De endotoxine blootstelling hing sterker samen met longfunctiedalingen dan de stofblootstelling. De longfunctieveranderingen werden gevonden bij lagere endotoxineconcentraties dan tot nu toe in de literatuur werd beschreven.

In hoofdstuk 7 wordt een analyse beschreven van respiratoire symptomen tijdens het werk en acute longfunctieveranderingen. De symptoomprevalentie werd vergeleken tussen 265 blootgestelde werknemers, en een controlegroep die

bestond uit 175 externe controles en werknemers in de mengvoederindustrie die doorgaans niet waren blootgesteld. De symptomen die wezen op respiratoire en neus irritatie waren significant verhoogd in de blootgestelde groep. De prevalenties varieerden van 9% (hoesten) tot 21% (niezen). Klachten over hoesten na het werk kwamen eveneens significant vaker voor. Bij 119 werknemers werden in totaal 457 longfunctieveranderingen gedurende de werkdag gemeten. Bijna alle variabelen vertoonden een daling tijdens het werk, zoals overigens op grond van de circadiane ritmen kon worden verwacht (gemeten werd gedurende de avondploeg van ca. 14.00 tot 22.00 uur). Wanneer de deelnemers op grond van hun functie werden gegroepeerd in categorieën van stof- en endotoxineblootstelling, bleek er een blootstellings-respons trend te bestaan voor MMEF en MEF_{50}. Het effect was groter voor endotoxine dan voor stof, zowel voor grootte als voor de sterkte van het verband. Voor dezelfde longfunctievariabelen, en voor FEV_1 en MEF_{25}, werd een significante daling over de week aangetoond.

Een algemene discussie naar aanleiding van het onderzoek is opgenomen in hoofdstuk 8. Verstorende invloeden die de generaliseerbaarheid van de uitkomsten van de studie zouden kunnen beïnvloeden hebben te maken met karakterisering van de blootstelling, selectie, etniciteit en cohorteffecten. In het onderzoek zijn de laatste twee waarschijnlijk van ondergeschikt belang. Ondanks de invloed van de andere twee mechanismen levert het onderzoek duidelijke aanwijzingen op voor longfunctiedalingen en verhoogde symptoomprevalenties die met de blootstelling samenhangen. De uitkomsten van het onderzoek kunnen worden gebruikt voor de vaststelling van indicatieve blootstellingslimieten. Voor stof wordt een acht uurs gemiddelde waarde tussen 1 en 2,5 mg/m^3 voorgesteld, en voor endotoxine een limiet van 3 tot 7,5 ng/m^3.

De samenvattende conclusies van het onderzoek zijn de volgende:

- Werknemers in de Nederlandse mengvoederindustrie worden veelvuldig blootgesteld aan stofconcentraties boven internationaal vastgestelde limieten voor graanstof, en boven de Nederlandse grenswaarde voor hinderlijk stof.

- Endotoxine concentraties in stof in mengvoederbedrijven lijken gerelateerd te zijn aan fasen in het produktieproces. Endotoxineconcentraties zijn sterker gecorreleerd met schimmelkiemtallen dan met stofconcentraties.
- Een vergelijking tussen werknemers in de mengvoederindustrie en controles wijst op een duidelijk healthy worker effect.
- In de onderzoekspopulatie bestaat een sterke negatieve associatie tussen (huidige en retrospectieve) blootstelling en longfunctie.
- De effecten op de longfunctie hangen sterker samen met endotoxineblootstelling dan met expositie aan stof. Dit geldt zowel voor de sterkte van het verband als voor de grootte van de effecten bij gemiddeld blootstellingniveau.
- Symptomatische en longfunctie effecten wijzen in de richting van van een klinisch relevant effect waarin kenmerken van bronchitis overheersen.
- Veranderingen van daarvoor gevoelige longfunctievariabelen gedurende de werkdag hangen samen met blootstelling aan stof, en in sterkere mate met blootstelling aan endotoxine.
- In de onderzoekspopulatie worden voornamelijk irritatieve symptomen en een daling van longfunctievariabelen gedurende de werkweek aangetroffen.
- Uit de resultaten van het onderzoek kunnen indicatieve gezondheidsgerelateerde grenswaarden voor blootstelling worden afgeleid. Afhankelijk van de veiligheidsfactor die wordt gebruikt om een mogelijke onderschatting van blootstellings respons relaties te compenseren, kan worden geschat dat voor 90 tot 100% van de werknemers een risico voor respiratoire aandoeningen aanwezig is.
- De waarde van het onderzoek kan in belangrijke mate worden vergroot door een vervolgstudie in hetzelfde cohort.
- De bestaande kennis op het gebied van respiratoire effecten van blootstelling aan graanstof kan worden vergroot door nader onderzoek naar pathofysiologische mechanismen en relevante stofcomponenten. Ook onderzoek naar klinisch relevante respiratoire morbiditeit en mortaliteit is van groot belang.

Appendices

Appendix 1.

Enumeration of viable fungi in occupational environments A comparison of samplers and media[1]

Tjabe Smid, Erik Schokkin, Jan S.M. Boleij, Dick Heederik

A1-1 Summary

The performance of sampling for fungi using four widely used microbiological air sampling devices and three broadspectrum media was compared in seven occupational settings. Total colony forming units counts ranged from 10 to 3700 CFU/m^3.

The sampling efficiency of the Andersen N-6 sampler, Slit sampler and Reuter centrifugal air sampler (RCS) was comparable, whereas the SAS sampler underestimated CFU counts by approximately 50%. The correlation between the RCS results and the other three samplers used was lowered compared to their mutual correlations. There were no large differences in performance of the three media tested: Malt Extract Agar, Dichloran Glycerol (Hocking & Pitt 1980) Agar and Dichloran Rose Bengal Chloramphenicol Agar. Coefficients of variation of duplicate samples were large compared to commonly used methods for sampling dust and chemicals.

It is concluded that the N-6 and slit sampler perform best, although RCS is useful in probes because it is easy to handle. The three media tested yielded similar results. Further testing of DG-18 is recommended as fast growing genera are inhibited and thus counting colonies is easier.

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[1] Am Ind Hyg Assoc J 50 (1989) 235-239

A1-2 Introduction

In recent years the role of microbial factors in the pathogenesis of work related respiratory disorders has been increasingly recognized.

Exposure to airborne fungal particles is a well known phenomenon in several work environments, such as grain handling facilities (Palmgren et al. 1984, Hill et al. 1984) paper mills (Heederik et al. 1987), fruit warehouses (Ström & Blomquist 1986), agricultural environments (Malmberg et al. 1985, Donham 1986), and air conditioned office environments (Edwards 1980). These exposures may lead to respiratory disorders such as acute inflammatory reactions and allergic alveolitis (Malmberg et al. 1985, Lacey et al. 1972, Belin 1985). Several methods for measuring airborne concentrations of viable fungal particles have been described. In a recent report the ACGIH committee on bioaerosols gave an overview of recommended samplers for indoor environments. As a culture medium for detection and enumeration of fungi, malt extract agar is recommended (Burge et al. 1987).

The purpose of this study was to make a statistical comparison of four widely used sampling devices as well as three broad spectrum media for sampling airborne fungi in the low to medium range in occupational environments.

A1-3 Methods

General strategy

Sampling was conducted in seven occupational environments. In three environments two sampling sessions were completed in the morning and the afternoon of the same day, so that a total of ten sessions was available for statistical analysis.

During each session four sampling devices were used, each with three different media. All measurements were conducted in duplicate and took place within one hour.

Sampling locations

Measurements were conducted in a flour mill, in two locations of an animal feed production facility in two locations of a municipal archive building, and in two microbiological laboratories.

In the flour mill mainly wheat and rye flour were produced. Two measurement sessions were staged in the packing department where bags were filled. Preliminary sampling showed that predominant genera collected were Penicillium, Cladosporium, Eurotium and Endomyces.

In the animal feed production facility materials such as cassave wheat, soy, and derivates from vegetable oil and starch production were used to formulate animal feed. Preliminary sampling indicated that the predominant genera were Aspergillus, Penicillium, Eurotium and Scopulariopsis. Two sampling sessions were staged on top of the silos which were filled with raw materials, and one in the packing department where bags were being filled.

In the municipal archive books up to 800 years old were stored, some of those covered with fungi. On two different floors a measurement session was conducted. Preliminary sampling indicated that the predominant fungal genera were Penicillium, Cladosporium and Alternaria.

In two microbiological laboratories three measurement sessions took place near working-tables. No preliminary information about genera was available.

Sampling devices

Four widely used sampling devices were used: The Reuter Centrifugal Air Sampler (RCS), Andersen single stage (N-6) impactor, Surface Air Sampler (SAS) and Slit to agar sampler.

I. Reuter Centrifugal air sampler

The RCS (Biotest, Frankfurt, FRG) is a portable air sampler which collects fungi on a medium coated plastic strip which is placed into the sampling drum. The plastic strip is divided into 34 square sections.

The airborne fungi are drawn into the sampling device by fan and impacted against the medium strip. The inlet velocity is given as being 40 L/min, and can

not be calibrated. A study by Macher & First indicated that the RCS generally sampled at a slightly lower airflow rate (Macher & First 1983).

II. Andersen N-6 impactor

The standard (six stage) Andersen sampler (Andersen Samplers, Atlanta, USA) is a sieve type cascade impactor that collects air through six stages (Anders 1958). The N-6 modification, as described by Jones et al. (1985) and recommended in an earlier publication of the ACGIH-committee on bioaerosols (Morey et al. 1987), consists of only stage 6 and the base plate.

The N-6 sampler was used in series with a gasmeter (GI.6, Meterfabriek Dordrecht, the Netherlands) and a Charles Austin (F-65) pump. The flow rate (28.3 L/min) was calibrated with a calibrated rotameter.

III. Surface Air System Sampler (SAS)

The portable battery-operated SAS sampler (Pool Bioanalyse Italiana, Milan, Italy) is a sieve type impactor, which collects air through 220 holes at a quoted flow rate of 180 L/min. Fungi are impacted on a 50 mm Rodac plate. A suction fan and a solid state timer are built into the sampler body.

A study on the performance of the SAS showed that the measured air flow rates might be 10-20% higher than the quoted values (Lach 1985).

IV. Slit sampler

The Slit sampler used was a slit-to-agar biological sampler (Apparatenfabriek van Doorn, De Bilt, the Netherlands). The sampler draws air at 30 L/min through a slit orifice and the fungal spores are impacted directly onto a rotating petri dish containing medium.

As with the N-6 sampler, the slit sampler was used in series with a gas meter and a calibrated vacuum pump.

Culture media

Three culture media were compared in this study; malt extract agar, dichloran glyceral 18 agar (DG-18), and dichloran rose bengal chloramphenicol agar (DRBC).

I. Malt extract agar (MEA)

Malt extract agar is widely used in aerobiological studies as a broadspectrum medium for fungi collection and is recommended by the ACGIH committee for indoor environments (Burge et al. 1987, Blomquist et al. 1984a, Blomquist et al. 1984b).

The malt extract agar consisted of malt extract, 30 g/L; peptone, 5 g/L; and agar, 15 g/L. The final pH was 5.4. The composition differed from the ACGIH advised malt extract agar that is comprised of malt extract, 30 g/L; peptone, 1 g/L; dextrose, 20g/L; and agar, 15 g/L (10).

II. Dichloran Glycerol (18) Agar (DG-18)

DG-18 is a relatively unknown medium that was developed to be selective for xerophilic fungi, but proved to be a general broad spectrum medium in food analysis (Hocking & Pitt 1980). The dichloran restricts the growth of fast growing genera like Rhizopos and Mucor, facilitating the counting of colonies. The quantities of the ingredients of DG-18 differed slightly from the original description by Hocking & Pitt (1980); it was comprised of: dextrose, 10 g/L; peptone, 5 g/L; NaH_2PO_4, 1 g/L; $MgSO_4$, 0.5 g/L; dichloran, 0.002 g/L; chloramphenicol, 0.1 g/L; glycerol, 220 g/L; and agar, 15 g/L. The final pH was 5.6.

III. Dichloran Rose Bengal Chloramphenicol agar (DRBC)

As Malt extract agar, rose bengal agar is widely used in aerobiological studies. It is recommended by NIOSH for indoor environments (Morring et al. 1983). As in DG-18, fast growing genera are inhibited. As the rose bengal decomposes in light it has to be handled in the dark. The DRBC consisted of: dextrose, 10 g/L; peptone, 5 g/L; NaH_2PO_4, 1 g/L; $MgSO_4$, 0.5 g/L; rose

bengal, 0.025 g/L; dichloran, 0.002 g/L; chloramphenicol, 0.1 g/L; and agar 15 g/L. The final pH was 5.6.

General procedures

All samples were taken approximately 1.5 meter above the floor, within 2 square meters during each session. Every trial lasted less than one hour, in which duplicate measurements were taken for every combination of sampler and medium. Sampling time and sampling volumes are presented in table A1-I. Between measurements, all samplers were swabbed with 70% ethanol. All petri dishes and strips were incubated at 24°C for four days and then counted. Results were expressed in colony forming units per cubic meter (CFU/m^3). Results for the two sieve type impactors were corrected for multiple hits, using positive hole conversion factors as given by the manufacturers.

Table A1-I. Sampling Time and Sampled Volume by Sampler*

sampler	N6	SAS	Slit	RCS
time	2 (6) min	20 (40) sec	2 (6) min	1 (4) min
volume (L)	57 (170)	60 (120)	60 (180)	40 (160)

* times and volumes between brackets were used in the municipal archive

Statistical evaluation of the data was performed using SAS software (1985). Comparison between measurement techniques was accomplished using three criteria: reproducibility of duplicate samples, sampling efficiency, and mutual correlations.

A1-4 Results

If one or both plates or strips of a duplicate measurement was overgrown by fast growing colonies and the counting of colonies was impossible, the

measurement was excluded from the data. For this reason and because of technical failures, 17 of 120 duplicate measurements (10 trials, each with 4 samplers and 3 media) were not available for statistical evaluation (14%).
All CFU concentrations were between 10 and 3700 CFU/m^3. Pooled data showed a distribution that was skewed to the right and resembled a log-normal distribution. Depending on type of sampler and medium geometric mean CFU concentrations were between 180 and 465 CFU/m^3, while geometric standard deviations ranged from 3.2 to 7.4.

Reproducibility of duplicate samples can be expressed as a coefficient of variation (CV) (Quality Assurance Handbook 1986). CV values for all combinations of sampler and medium are given in table A1-II. All values were between 24 and 51%. No general differences in reproducibility between samplers or between media could be detected.

Table A1-II. Coefficients of Variation (CV) of duplicate samples broken down by Sampler and Medium (%)*

sampler	medium		
	MEA	DG-18	RBA
RCS	37 (10)	36 (9)	24 (10)
N-6	40 (8)	35 (10)	29 (10)
SAS	27 (7)	51 (7)	33 (7)
Slit	36 (8)	38 (9)	36 (8)

* The sample size is given between brackets

An analysis of variance on log transformed CFU concentrations was conducted to find out whether differences in yield (efficiency) could be explained by choice of medium or sampler. The data in table A1-III show that 'trial' is the most important source of variation, indicating large differences in CFU concentrations between measurement sites. The choice of sampler was an important source of variation as well, indicating significant differences in yield between samplers. No indications for significant differences in yield between the three types of media tested were found.

Table A1-III. Analysis of Variance on All log-transformed Data

Source of variance	df	MS	F	p (F>0)
trial	9	18.44	20.3	0.0001
sampler	3	3.14	3.5	0.02
medium	2	0.74	0.8	0.44
residual	88	0.91		

df = degrees of freedom
MS = mean square
F = F-value

Inclusion of an interaction term between medium and sampler in the analysis of variance showed no significant increase in explained variance, indicating that in this study relative efficiency of samplers was not dependent on the medium used.

Mean CFU counts per sampler and per medium were computed, using the population marginal means method (Searle e al. 1985) to estimate missing data.

Results are given in table A1-IV, in which the log-transformed values were transformed back to normal values.

Table A1-IV. Estimated Mean Yield in CFU/m^3 for each Sampler and Medium, based on an analysis of variance (Anova Model)

sampler (n)	CFU/m^3	medium	CFU/m^3
RCS(30)	330	MEA(40)	260
N-6(30)	275	DG-18(40)	260
SAS(30)	135*	RBA(40)	205
Slit(30)	275		

* significantly lower than RCS ($p < 0.01$) and SAS and N-6 ($p < 0.05$) (two tailed t-test on log-transformed data)

From the estimated values it is clear that the SAS-sampler shows a significantly lower yield than the other three sampling devices, whose yields are similar.

No clear-cut differences in yield between the media used was found.

When three trials that accounted for 10 out of 14 missing measurements were removed from the data, similar results were found, indicating that estimating missing values did not result in serious estimation errors.

To investigate mutual correlations, Pearson correlation coefficients were computed on log-transformed data for all pairs of samplers, by medium. Table A1-V shows that the correlation of RCS with the other samplers is low, while the mutual correlation of the other three samplers seems to be higher at least on the MEA and the DG-18 medium. If large mutual correlation is considered to be an indication of accuracy, the RCS is less accurate then the other three samplers.

A similar comparison of media by sampler showed no differences in correlations.

Table A1-V Pearson Correlation Coefficients of Log Transformed CFU/m^3 Counts for All Pairs of Samplers, broken down by Medium

medium	sampler	SAS	slit	RCS
MEA	N-6	.89+(6)	.96*(7)	.75+(8)
	SAS		.95*(6)	.84+(7)
	slit			.51 (8)
DG-18	N-6	.95+(7)	.95*(9)	.58 (9)
	SAS		.94*(7)	.84+(6)
	slit			.67 (7)
DRBC	N-6	.76 (7)	.99*(8)	.86*(10)
	SAS		.67 (7)	.82+(7)
	slit			.97*(8)

\+ = $p < 0.05$
* = $p < 0.01$

A1-5 Discussion

The aim of this study was to compare the general quantitative performance of four widely used samplers and three media in occupational situations with fungi levels in the low to medium range. However, when exposure to fungi is predominated by a few species, special characteristics of one or more species, like shape and size of the CFU-particles or ability to grow on the media tested may limit the validity of the conclusions of the study (Burge et al. 1987).

Compared to commonly used sampling methods for dust and chemical components, the reproducibility of duplicates was low, as indicated by CV values ranging from 24 to 51%. This might be explained by the short sampling times indicating that, in situations with low CFU concentrations longer sampling times may provide more accurate data. Since estimating CFU concentrations may be especially important in situations where higher levels of airborne fungi are present the short sampling times (an the resulting large CV values) mandate a well designed sampling strategy. In any case, duplicate or even triplicate sampling is essential, as is advised by the ACGIH committee on bioaerosols (Burge et al. 1987).

Comparison of samplers showed that the yield of all samplers was comparable, with exception of the SAS which underestimated CFU concentrations by about 50%. This result is in accordance with the findings of the ACGIH Committee stating the efficiency of the SAS as approximately 40% of the slit sampler. Where the ACGIH committee stated the efficiency of the N-6 as 'nearly as efficient as slit', this study found the efficiency of these two samplers to be identical.

The analysis of mutual correlations showed the RCS to have lower correlations with the other methods than the mutual correlations of the other three samplers. Since no differences in CV between samplers were found, the RCS was found to be less reliable when different situations were compared.

Performance of the other two samplers was comparable, indicating that both the N-6 and the slit sampler were found to be the most accurate in this study. The RCS, although less reliable, may be useful in probes, since it is small, portable and battery-operated.

Comparison of media showed that both DG-18 and DRBC had a similar performance to MEA, which is recommended by the ACGIH committee on bioaerosols. When ease of use is considered, a disadvantage of DRBC is that it has to be handled in the dark. Counting colonies on DG-18, however, is easier since fast growing genera are inhibited.

A1-6 Conclusion

The choice of sampler medium in practice depends on requirements for accuracy and ease of handling. This study shows that in general occupational situations N6 and slit sampler were the most accurate. The RCS, although slightly less reliable, may be useful in probes, since it is easier to handle.

There were no indications that the other media tested performed better than the ACGIH-advised malt extract agar, although DG-18 might be an alternative to MEA since its performance was found to be identical to MEA and counting of colonies was easier. Further testing of this relatively unknown medium is considered to be worth while.

A1-7 Acknowledgements

This study was financed by the Department of Social Affairs and Employment of the Netherlands. We thank Peter Attwood, Reini Pater, Pieter Versloot, Bert Brunekreef, Arnoud Verhoeff, Joop van Wijnen, Ellen van Reenen, Miquette Lulofs, David Drown and Jos van Hutten for their advice and assistance.

A1-8 Errata[1]

In the paper "Enumeration of Viable Fungi in Occupational Environments: A comparison of Samples and Media", we stated that Dr. Macher and Dr. First found that the RCS impactor sampled at a slightly lower airflow rate. However, Dr. Macher kindly pointed out that this is incorrect. Macher & First (1983) indicated that the total sampling rate of the RCS is somewhat lower than the calculated sampling rate of the manufacturer (210 $\pm$ 17 L/min versus 280 L/min) but much higher than the manufacturer's stated airflow rate (40 L/min). They confirmed the results reported previously by Clark et al. (1981). Furthermore, the results of both studies indicate that the effective sampling rate of the RCS depends on the particle size. Clark et al. (1981) reported that the effective sampling rate for particles $>$ 5 μm varied between 50-170 L/min. For smaller particles the effective sampling rate was lower than 40 L/min. Macher & First (1983) found that the collection efficiency of particles of 5 μm was approximately 60%. For smaller particles the collection efficiency decreased clearly. However, the manufacturer advises users to assume that the "effective" airflow rate is 40 L/min, irrespective of the particle size. In view of the results presented by Macher & First (1983) and by Clark et al. (1981), it is more correct to calculate the yield of the RCS using the actual flow rate. This means that the yield obtained with the RCS, as reported in our study, will be a factor 2.5 (Macher & First 1983) or even 5 (Clark et al. 1981) lower. However, this does not alter the conclusion of the study.

J.S.M. Boleij

[1] Am Ind Hyg Assoc J 51 (1990) 296

A1-8 References

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Appendix 2.

Use and analysis of exposure monitoring data in occupational epidemiology[1] An example of an epidemiological study in the Dutch animal food industry

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A2-1 Abstract

No general theoretical framework exists for exposure monitoring strategies for epidemiological purposes nor for the analysis of these data. It is however, recognized that the variation in exposure over time and between workers is an important element of consideration in the design of measurement strategies and the analysis of exposure data. In this paper the partitioning of exposure variability is discussed for two types of monitoring strategies. First a monitoring strategy is considered in which all members of the study population are monitored on various occasions. In this strategy the ratio of within and between worker variance gives information about the magnitude of underestimation of the exposure-response relationship. The second strategy discussed is one based on 'homogeneous' exposure categories. This strategy is illustrated with a study among animal feed workers in the Netherlands. It is shown that the grouping which results from this strategy can be applied for epidemiological purposes successfully if the ratio of the within and between category variance in exposure is relatively small. This implies that it is important to optimize the ratio of these variances in a given study population instead of composing strictly homogeneous exposure categories.

In both strategies the analysis of variance of repeated exposure measurements on the same individuals play a crucial role. It is recommended that repeated

[1] Appl Occ Environ Hyg 6 (1991) 458-464

measurements at the same individual should to be taken by routine. And the analysis of variance should be used as a tool to analyse such data in order to optimalise a measurement strategy or a categorization of the population in exposure groups.

A2-2 Introduction

In the past studies of exposure-response relationships in the work environment were focused on situations in which a substantial exposure and a resulting substantial health risk occurred. Rare monocausal diseases associated with a specific agent were easily detected, e.g. pleural mesothelioma caused by asbestos. Simple approaches of exposure assessments based on accidentally collected information generally are not sufficient any more. However, overlooking the literature one tends also to conclude that exposure assessment for prospective epidemiologic studies is still in its infancy. Only few examples are described of epidemiologic studies with a priori designed exposure assessment strategies.

The possibility to correctly identify associations between exposure and health effects is partly determined by the health relevance of the exposure measurement. Exposures and health effects are usually linked through the concept of dose.

It is obvious that knowledge of the disease process should influence environmental monitoring strategies. However in many situations, a priori information about disease processes and thus the relevant dose is not present. For instance for grain dust, very limited information exists about the relevant exposure in relation to the development of lung function changes. One therefore often assumes that cumulative exposure is the best exposure index to estimate and temporal patterns in exposure are neglected. The most useful approach in such cases is to compare different exposure indices in the analysis.

A2-3 Exposure assessment strategies

In occupational epidemiological studies several methods are being used to characterize the exposure of the population under study. Several authors provide a hierarchy of exposure characterization methods for use in epidemiological studies (Vihma 1981, Checkoway et al. 1987) with exposure measures ranging from specific exposure measurements to semi-quantitative estimation of specific exposure (ranking) and surrogates as occupational title, occupational status, department or sector of industry. Basically two approaches are applied which consider measured exposure information. In the first all population members are monitored. In the second only sub-groups, sometimes referred to as homogeneous exposure groups, are being monitored. The composition of homogeneous sub-groups can be made on the basis of information on a subject's job, or by an estimation technique. Semi-quantitative exposure estimation is the process of estimating a subject's exposure on the basis of present knowledge on e.g. tasks performed, and processes applied. This second method is often applied because it is simple, comprehensive and efficient and because it leads to a reduction in measurement effort when applied in combination with strategies in which the exposure is actually measured (Kromhout et al. 1987). In this case the grouping takes place before measurements will be taken. An alternative is an a posteriori grouping based on randomly sampled exposure measurements. For the purpose of this paper no distinction will be made between a priori and a posteriori grouping strategies.

Examples of studies in which detailed quantitative exposure information is gathered are given by studies among workers exposed to ionizing radiation in nuclear power plants and other nuclear facilities. Personal monitoring is done permanently during working hours because personal dosimetry is a technically simple method for this particular exposure. The exposures can be expressed as doses from external sources to various parts of the body or the entire body, the skin of the entire body and extremities.

More often strategies are applied in which exposure is established for subgroups of workers. Such procedures result in a considerable reduction of measurement effort, because only a sample of workers needs to be monitored.

This is favorable when sampling techniques are time consuming and expensive to apply. An early description of such a strategy has been given by Oldham & Roach (1952) and Ashford (1958). They described the statistical background of the measurement strategy applied in the Pneumoconiosis Field Research of the British Coal Board. Colliery populations were divided into homogeneous subgroups on the basis of their occupation, place of work, and shift. The so called "man-shift". This meant that dust exposure measurements were taken for a random sample of all workers employed during a certain shift. Time exposed was measured for all workers based on colliery records. The cumulative exposure was calculated as the product of the exposure measured for a certain occupational group and the time exposed. The sampling effort was allocated to certain man- shifts based on several factors such as the standard deviation in exposure for the particular homogeneous subgroup, the labor turnover of a group, and the number of men belonging to the group. An underlying assumption of this sampling strategy is that the exposure of an individual worker is supposed to be equal to the shift average of the whole group. Surveys in different occupational exposed populations have shown that considerable inhomogeneity between workers may exist (Kromhout et al. 1987, Rappaport 1985). Main reasons for this inhomogeneity are an inappropriate definition of the subgroup and differences in work practice and environmental conditions. The latter may for instance occur when a broad definition of a subject's function or job is used while workers with the same job or function perform different tasks.

A2-4 Misclassification of exposure

In epidemiology non-differential and differential misclassification are distinguished. Both are forms of information bias (Rothman 1986). In case of non-differential misclassification, misclassification is independent of the health effect. Differential misclassification of exposure can lead to under- as well as overestimation of the effect of exposure on health, dependent on type of study and exact form of differential misclassification (Rothman 1986). Generally, non-differential misclassification of exposure leads to underestimation of the

effect of exposure on the health parameter studied. Large differences in concentrations of an air pollutant in time and space lead to a large inter- and intra-individual (or day-to-day) variation in the workers exposure. This can be regarded as a specific form of non-differential misclassification.

First the influence of the non-differential exposure estimation error will be illustrated for a situation in which repeated exposure measurements are present for all the members of the population under study and no control group is present. Consider an epidemiological study in which the mean personal exposure to some agent has to be related to a health endpoint. A limited number of measurements per worker is at its best an approximation of that persons long term exposure if a considerable within person or day to day variation in exposure exists. It has been shown that this within person variation in exposure can result in a considerable bias of the exposure-response relationship (Armstrong 1982, Ulvarsson 1983). The statistical background has been recognized in the statistical literature and is referred to as attenuation (Cochran 1968, Snedecor & Cochran 1980). The bias that occurs when a dependent variable measured on a continuous scale is regressed on an imperfectly measured exposure variable can be described by the ratio λ of the within-and between worker variability in exposure:

$$b = \beta(1 + \lambda)^{-1} \qquad (1)$$

b = expected value of the empirical regression coefficient of a dependent variable Y on an independent variable x which is an imperfect measure of the independent variable X,

β = the true regression coefficient of Y on X,

$\lambda = \sigma^2_w/\sigma^2_b$

σ^2_w = within subject variance,

σ^2_b = between subject variance.

If repeated exposure measurements on an individual are present, it is possible to estimate the within and between worker components of variance by an

analysis of variance (ANOVA). The lay-out of an analysis of variance table together with the estimated parameters can be found in the literature (Searle 1971) and is given in the appendix (A2-A).

If for instance $\lambda=1$, which means that the within and between subject variances in exposure are equal, the empirical regression coefficient underestimates the true coefficient by 50 percent. The formula clearly shows that the underestimation is a function of both the within and the between worker variance in exposure. In a situation with a high within worker variability of the exposure we still get almost unbiased regression coefficients as long as the between worker variability in exposure for instance in the population is a multitude of the within worker variability in exposure. This implies that we cannot formulate a strict rule for an acceptable or required within or between worker variance. The ratio between the two variance component determines the magnitude of the underestimation rather than the absolute values. Before starting a large epidemiological study information about the partitioning of the exposure variability is necessary for further planning. Suppose we want to study a population with a large λ or a relatively large within worker variance in exposure. The exposure measurement strategy can than be modified in three ways to avoid bias of the exposure-response relationship. First one can increase the measurement effort to attain a reliable estimate of the personal exposure with a minimal bias due to within person variance in exposure. This effect can be calculated according to the mathematics described by Liu et al. (Liu et al. 1978):

$$k = \{p/(1-p)\}.\lambda \quad (2)$$

in which k is the number of repeated measurements per worker and p is the bias in a regression coefficient. For instance when $\lambda = 6$ one needs at least $k = 54$ measurements per worker in order to reduce the bias in a regression coefficient to maximally 10% ($p = 0.90$). Secondly, if this first approach leads to a measurement effort which is too large, repeated measurements in a sub sample of workers can be performed to get a precise estimate of within and between person variability after which a correction for attenuation can be applied (Armstrong & Oakes 1982, Heederik & Miller 1988). Thirdly a completely

different study design can be chosen, for instance by maximizing the contrast in exposure by means of an external control group (which basically means increasing the between worker variance in exposure).

In a grouping strategy one assumes that subjects in an exposure group have a comparable exposure profile and that the mean exposure can be used to describe the exposure of all group members. However, occupational hygiene surveys showed that considerable differences in mean exposure may exist between workers. This implicates that for some members of the homogeneous exposure group we under- or overestimate their actual exposure. In analogy with the previous section it seems clear that the underestimation of a exposure-response relationship is a function of the within and between group variation in exposure. A within group variance which is larger than the between group variance means that the exposure distributions of the groups are strongly overlapping and that the grouping did not led to any distinction in exposure between groups compared to the whole population. If the between group variance is larger than the within group variance distributions do not or only partially overlap. An example of the magnitude of underestimation of the exposure-response relationship has been reported by Heederik and Miller (1988) in an epidemiological study among British coal miners. They estimated that up to 30% of the total variance of the cumulative dust exposure might be due to inhomogeneity of exposure groups. Correction for this inhomogeneity showed that the exposure-response relationship between cumulative coal dust exposure and eight year lung function changes was underestimated by approximately 200%.

This suggests that in general for both strategies underestimation of an exposure-response relationship is a function of two variances, the within and between person or group variance, and that the underestimation cannot be minimized by an absolute criterion for one of the two. The two basic monitoring strategies distinguished require a specific procedure in the analysis of the data. In the first strategy, in which all population members are being monitored, most emphasis has to be given to the calculation of within- and between worker variance and the ratio of these two to establish the potential bias in the relationship between exposure and response due to attenuation. The second approach, based on monitoring of homogeneous sub samples of workers, also

requires the calculation of within- and between worker variances but this time the between worker variance is the primary aim of the analysis of variance because it gives an impression of inhomogeneity of an exposure group and can give information about the overlap between exposure groups. A comparison of the variance ratio λ of the whole group with the variance ratio of subsets of workers gives information about the homogeneity of subgroups relative to the entire population. The calculation is similar to the calculation of the within and between worker variance (see Appendix A2-A). A similar comparison can be based on the 95% confidence limits of the distribution of worker mean exposures $R_{.95}$, as a measure of homogeneity of the group (Rappaport 1988). Partitioning of exposure variability into between and within exposure group variance can be derived by a nested type of analysis of variance.

A2-5 The animal food industry example

In the Netherlands approximately 6000 people are employed in animal feed production. Several raw materials are used such as grain, pulses, and waste products of the vegetable oil and starch industry. Since 1985 a large scale study is in progress. The primary aim of this project was to study the influence of occupational dust exposure in animal feed mills on respiratory symptoms and lung function. Dust exposure of workers in eight production and storage facilities was studied during 1 to 24 days, in most cases in two periods (spring and autumn). For five facilities repeated measurements on the same worker were available. The aims of the exposure measurements were to study:

- if and how the population could be divided into 'homogeneous' exposure groups;
- if and how these data could be used to calculate cumulative exposure estimates and;
- if and how the measurements in eight facilities could be used to estimate the exposure of workers of in total fourteen facilities.

The facilities differed in a number of characteristics such as age, number of employees, and technology applied. All workers were a priori grouped into eight occupational categories according to their job title: unloaders, crane drivers, operators, press operators, production managers, expedition workers, sackers, and other production workers. The exposure categories consisted of one or more job titles with similar facility tasks and working environments. Personal inspirable dust sampling was performed on workers from all occupational categories. The dust was sampled during full shift periods of approximately eight hours, in the workers' breathing zone. The endotoxin concentration was determined in all samples according to methods described in the literature (Kateman et al. 1990). Lung function was measured of 315 workers from in total fourteen animal feed production mills according to the European Committee of Coal and Steel protocol (Kateman et al. 1990).

The data were analysed with SAS statistical software. Analyses of variance were performed using Proc GLM. The within and between worker mean squares were recalculated to within and between worker geometric standard deviations according to the formulas in the appendix (A2-A). On the basis of the between worker variance, a $R_{.95}$ as a measure of homogeneity of the group, was calculated (Rappaport 1988, Rappaport 1991). The within and between occupational category variances were calculated with a nested analysis of variance (Proc NESTED) in which the occupational group was the nesting variable. The relationship between the current and the cumulative dust exposure and endotoxin exposure and lung function was studied in a multiple regression analysis after a correction for age, standing height and pack years smoked (Proc REG).

The results for the dust and endotoxin concentration are presented in table A2-I and A2-II. Tabular analysis as well as the analyses of variance with the dust and endotoxin concentration as dependent variables and occupational category and feed mill as explanatory variables showed that occupational category explained most of the variance in dust as well as endotoxin concentration (Table A2-III). This meant that (1) in the eight mills monitored more or less the same pattern of exposure existed for the various occupational categories, that (2) differences in mean exposure between mills were small, despite differences in

age and technology level, and that sampling would not necessarily have to be expanded to all fourteen mills. This last point is clearly illustrated by a breakdown of the exposure to occupational groups and to facility. The differences in geometric mean dust and endotoxin exposure between the highest and lowest exposed occupational group were respectively a factor 12 and 24 while for the facilities this was maximally a factor 2 to 4. Because the facilities had a different age and technology level, the results also indicated that the current exposure could probably serve as an acceptable estimate of a previous exposure.

Table A2-I. Characteristics of personal dust concentrations by job category

job category	n	j	AM	GM	GSD	range
			(mg/m^3)	(mg/m^3)		(mg/m^3)
unloaders	69	29	30.0	9.8	4.6	0.2-450
crane drivers	23	12	6.1	2.5	4.7	0.2-27
facility operators	63	20	1.4	0.8	2.7	0.2-9.4
press operators	54	17	4.2	1.3	3.2	0.2-100
production managers	42	16	3.8	1.6	3.5	0.2-49
expedition workers	61	18	2.1	1.2	2.8	0.2-14
sackers	20	9	6.6	3.4	2.9	1.1-45
other	198	121	11.3	3.2	4.9	0.2-250
total	530	242	9.8	2.4	4.7	0.2-450

GM = geometric mean
GSD = geometric standard deviation
n = number of exposure measurements
j = number of workers measured at least at one occasion

Table A2-II. Characteristics of personal endotoxin measurements by job category

job category	n	j	AM (mg/m^3)	GM (mg/m^3)	GSD	range (mg/m^3)
unloaders	69	29	102	28.5	5.4	0.2-1150
crane drivers	23	12	49.6	10.6	8.7	0.2- 420
facility operators	63	20	3.9	1.2	4.3	0.2- 50
press operators	54	17	9.4	2.3	4.2	0.2- 160
production managers	42	16	15.2	2.5	5.0	0.3- 160
expedition workers	61	18	29.7	2.4	5.2	0.2- 880
sackers	20	9	9.3	3.7	3.8	0.5- 75
other	198	121	40.9	7.0	6.0	0.2-1870
total	530	242	37.1	5.1	6.7	0.2-1870

GM = geometric mean
GSD = geometric standard deviation
n = number of exposure measurements
j = number of workers measured at least at one occasion

Table A2-III. Analysis of variance of log-transformed personal dust and endotoxin concentration (n=530)

factor		dust		endotoxin	
	df	MS	F	MS	F
job category	7	28.2	15.8*	37.2	14.3*
facility	7	11.6	6.5*	18.8	7.2*
residual	515	1.8	2.6		

df = Degrees of freedom
MS = Mean squares
F = F-test
* = $p < 0.001$

An analysis of variance showed that for the total population the between worker variation in exposure, expressed as a GSD was somewhat larger than the within worker variation both for dust and endotoxin ($\lambda < 1$) (table A2-IV). A

similar analysis of variance for each occupational group revealed that the within person variation in exposure (or day to day variability) was equal to or considerably greater than the inter-personal variability in exposure for most occupational groups. Crane drivers formed a clear exception for both endotoxin and dust. A more detailed analysis of the exposure of crane drivers showed that some of them had specific tasks of short duration which led to a relatively high exposure and thus inhomogeneity within the group of crane drivers. The group was also very small and exclusion of one outlier increased the λ and reduced the $R_{.95}$ drastically. 'Other production workers' showed large differences in dust exposure within the group, probably because they were too heterogeneous in personal mean exposures due to shifting of tasks. However, in most cases the $R_{.95}$ for each occupational group appeared to be smaller than the $R_{.95}$ for the total group. This indicated that differences between workers within an occupational group were smaller than the differences between workers in the whole population. This implies that the occupational group mean exposure was probably an acceptable exposure estimate for each member of that group, except for 'crane drivers' (dust and endotoxin) and 'other production workers' (dust only). Reduction of the $R_{.95}$ for most occupational groups compared to the overall $R_{.95}$ suggests that calculation of a cumulative exposure based on occupational categories could still be useful. This was also confirmed by the nested analysis of variance. This analysis resulted in a ratio of the within and between exposure group variance which was 1.24 for dust and 0.97 for endotoxin. This means that within exposure group variance was larger than between exposure group variance for dust exposure. For endotoxin exposure within exposure group variance was smaller than between group variance suggesting that a breakdown to occupational categories was more informative than in case of dust. Therefore, the calculation of the cumulative dust and endotoxin exposure was based on the different occupational categories ever performed, the arithmetic average dust exposure in that particular occupational category and the time spent in that occupational category. Differences in mean exposure for workers with the same occupational category but working in different mills were neglected.

Table A2-IV. Within and between worker variation, expressed as a GSD, and $R_{.95}$ for log transformed personal dust and endotoxin exposure per occupational category. Only workers with $k>1$ repeated measurements have been taken into account

		dust exposure				endotoxin exposure			
	j^1	GSD_w	GSD_b	λ	$R_{.95}$	GSD_w	GSD_b	λ	$R_{.95}$
all workers	120	2.88	3.08	0.89	82	3.88	4.02	0.95	234
unloaders	15	4.05	1.55	10.0	6	4.30	2.46	2.6	34
crane drivers	5	3.07	3.37	0.8	117	4.20	4.75	0.8	450
facility operators	13	2.39	1.73	2.5	9	3.26	2.11	2.5	19
press operators	12	2.50	2.12	1.5	20	2.80	2.67	1.1	48
production managers	10	2.29	2.66	0.7	47	3.22	3.40	0.9	125
expedition workers	14	2.51	1.30	12.2	3	4.69	1.83	6.6	11
packers	3	3.16	1.40	11.4	4	4.86	1.01	>100	1.04
other production	48	3.04	3.13	0.95	88	4.05	3.29	1.4	107

j^1 = number of persons measured more than one occasion ($k>1$)
GSD_w = within worker geometric standard deviation
GSD_b = between worker geometric standard deviation
λ = variance ratio of within and between worker variance
$R_{.95}$ = ratio of 5 and 95% confidence interval of between worker variance

Statistically significant relationships were found between most of the lung function variables and the cumulative dust as well as endotoxin exposure after correction for the confounding variables. Such relationships were also found for the current exposure to dust and endotoxin. The results of the analysis with cumulative exposure are given in table V. The relationship between the endotoxin exposure and lung function appeared to be a statistically stronger related to the endotoxin exposure than to the dust exposure.

Table A2-V. Relationship between lung function and cmulative dust and endotoxin exposure among animal feed production workers adjusted for age, standing height, and pack years with linear regression (n=263).

	cumulative dust exposure		cumulative endoxin exposure	
	β_{dust}	SE	$\beta_{endotoxine}$	SE
FVC (l)	-0,0011	0,0014	-0,0003	0,0006
FEV_1 (l)	-0,0021	0,0013	-0,0009	0,0006
FEV_3 (l)	-0,0012	0,0014	-0,0004	0,0006
PEF (l/s)	-0,0206**	0,0049	-0,0084**	0,0021
MEF_{75} (l/s)	-0,0141**	0,0045	-0,0067**	0,0019
MEF_{50} (l/s)	-0,0059	0,0032	-0,0032*	0,0014
MEF_{25} (l/s)	0,0005	0,0015	-0,0003	0,0006
MMEF (l/s)	-0,0028	0,0026	-0,0019	0,0011

* $p < 0.05$
** $p < 0.01$
β regression coefficient
SE standard error

A2-6 Discussion and conclusions

Large variations in exposure in time and between workers are an important source of difficulties in the design and analysis of exposure-response relationship because they can be responsible for considerable misclassification. Theory showed that misclassification of exposure can result in underestimation of the relationship between an exposure and some health endpoint. The magnitude of this bias is determined by the ratio of the within and between worker variance in exposure in a study in which all workers have been monitored. The magnitude of bias can be calculated on the basis of the variance ratio of within and between worker variance in exposure. Several strategies are recommended to reduce this bias.

For grouping strategies no strict criteria can be given for a required level of homogeneity. The inhomogeneity which is acceptable in a specific situation is in analogy determined by the magnitude of the variation in exposure in the whole population and the variation within the exposure groups. It is therefore not

necessary to define absolute criteria for homogeneity. Criteria need to be developed for the acceptable ratio of the within and between group variances.

Some elements of the theory were illustrated with a study in the Dutch animal food industry. Clear differences in exposure existed between some of the a priori defined occupational categories. These differences were such that differences within an occupational category due to different mills explained only a limited part of the total variance in the exposure. However, the analyses of the exposure data were performed before the lung function results were analysed. The initial analysis of exposure data was meant to decide on further steps to be taken. It was planned that additional measurements would be taken if considerable differences between mills would exist or differences within occupational groups would exist. Cumulative exposure could have been calculated in a different way, based on the occupational categories performed in a certain facility where the tasks were performed. For the sub-sample of 8 facilities cumulative exposure was calculated in this alternative way. Similar exposure-response relationships were found suggesting that the assumptions made were acceptable.

The between worker variance for the subgroups was reduced compared to the between worker variance for the whole group. This showed that the grouping lead to a reduction of exposure variability. The fact that clear exposure-response relationships were found of the dust and endotoxin exposure with lung function suggests that an acceptable ratio between these two variance components was present.

It is recommended to establish the within and between group variance in exposure with an analysis of variance using repeated measurements on the same individual. The succes of different groupings can be judged by comparing ratios of within and between group variances of the alternative groupings. The optimal grouping of the population can than be selected for the further epidemiologic analysis of the data.

A2-7 Acknowledgements

The animal feed production industry study was granted by the Dutch Directorate General of Labor of the Ministry of Social Affairs and Employment.

This analysis was conducted in the framework of the EC Conserted Action "Retrospective evaluation of Occupational Exposures in cancer epidemiology".

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Appendix A2-A.

An analysis of variance of between and within worker variance components using repeated measurements on the same worker can be performed with various procedures in SAS such as Proc GLM, Proc NESTED and Proc VARCOMP. The results are presetend schematically in the table below. The between and within group variances can be calculated in a similar way with group and worker as an explanatory variable in the model depending on the procedure used.

Analysis of variance (ANOVA) of repeated exposure measurements on the same worker (Searle 1971)

Variation source	SS	df	MS	estimated parameter
between worker	SS_b	(n-1)	$SS_b/(n-1)$	$\sigma^2_w+k\sigma^2_b$
within worker	SS_w	n(k-1)	$SS_w/n(k-1)$	σ^2_w

SS sum of squares
MS mean of squares
df degrees of freedom
σ^2_w = within subject variance
σ^2_b = between subject variance
k = number of repeated measurements per worker

Several variables can be calculated from the outcomes of an analysis of variance:

$GSD_w = \exp(\sigma^2_w)^{½} = \exp(MS_w)^{½}$

$GSD_b = \exp(\sigma^2_b)^{½} = \exp\{(MS_b - MS_w)/k\}^{½}$

$R_{.95} = \exp(\sigma_b \cdot 3.92)$

if the number of repeated measurements differs from worker to worker k is approximated by:

$$k' = [t-\{k_{(1)}^2 + k_{(2)}^2 + \ldots + k_{(n)}^2\}/t]/n-1$$

in which:

t total number of observations
k(1) number of observations on day 1
n maximum number of monitoring days

or can be approximated by:

$$k'' = \{df_{\text{within persons}} / (df_{\text{between persons}} + 1)\} + 1$$

Curriculum Vitae

Tjabe Smid werd geboren op 22 december 1955 in Groningen, waar hij de lagere school en het Atheneum doorliep. In 1974/75 studeerde hij aan de Technische Hogeschool Delft, in de richting Vliegtuigbouwkunde. Vanaf 1975 studeerde hij aan de Landbouwuniversiteit, waar in 1983 het doctoraalexamen Humane Voeding werd behaald. Doktoraalvakken, in de sociaal wetenschappelijke differentiatie, waren voeding, gezondheidsleer en methoden en technieken van het sociale onderzoek. Van 1984 tot 1988 was hij werkzaam als wetenschappelijk medewerker bij de vakgroep Gezondheidsleer van de Landbouwuniversiteit, waar hij onder andere het onderzoek verrichtte dat in dit proefschrift is beschreven. In 1988 trad hij, als arbeidshygiënist, in dienst van de KLM. In 1991 behaalde hij het diploma Master of Safety, Health and Environment aan de Technische Universiteit Delft. Sinds 1990 is hij werkzaam als hoofd arbeidsveiligheid van de Dienst Arbeidsomstandigheden van de KLM.