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Airborne Dust and Aeroallergen Concentrations
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AIRBORNE DUST AND AEROALLERGEN CONCENTRATIONS IN A PONY BARN UNDER TWO MANAGEMENT SYSTEMS

By

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ABSTRACT

AIRBORNE DUST AND AEROALLERGEN CONCENTRATIONS IN A PONY BARN UNDER TWO MANAGEMENT SYSTEMS

By

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Airborne dusts and aeroallergens are major etiologic factors in the pulmonary hypersensitivity that results in obstructive pulmonary disease in horses. Airborne dust concentration (ADC) was measured in a conventional (CS) and a recommended (RE) management system using an Andersen cascade impactor in the box-stall, and a Marple cascade impactor attached to the halter. Aeroallergens implicated in COPD were measured by radioallergosorbent-inhibition immunoassay. The total and respirable ADC were significantly higher in CS than in RE. Under CS, but not under RE, the total and respirable ADC in the breathing zone was manyfold higher than the stall. Major aeroallergens were significantly higher in CS than RE. Measurement of ADC using personal samplers indicates that the high inhalation challenge in the breathing zone is not reflected in measurements of stall air quality. When compared to CS, RE poses forty-fold less respirable dust burden in the breathing zone and a decreased aeroallergen challenge.

Dedicated to my family, in appreciation

of their love, support, and encouragement.

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CHAPTER 1

INTRODUCTION

Airborne dusts and aeroallergens are considered to be major factors in the etiology of chronic obstructive pulmonary disease (COPD) in horses. This condition, also known as "heaves," is common in stabled horses. Clinical signs of COPD are intermittent and thought to result from a reversible pulmonary hypersensitivity reaction to inhaled barn dusts in susceptible horses (Alexander 1959, Cook and Rossdale 1963, Lowell 1964, Gerber 1973, Halliwell et al. 1979, McPherson et al. 1979b).

Signs regress when horses are removed from the enclosed areas and kept on pasture (Derksen et al. 1987). Certain management regimes have been suggested to produce a low dust and aeroallergen concentration, and be suitable for the stabling of COPD-susceptible animals (Grunig et al. 1989, Thomson and McPherson 1984, Clarke 1987, Beech 1989).

The concentration of airborne dusts in riding stables has been measured with stationary dust samplers (Crichlow, Yoshida, and Wallace 1980, Zeitler 1985). In these previous studies, airborne dust levels were measured from ambient air within the barn using stationary filter-type samplers positioned in the corridors, not in the stall area. These samplers do not measure the dust concentration within the horse's breathing zone, which may vary from the "average" dust concentration measured with the stationary dust sampler, depending on the activity and the position of the horse's head.

The direct immunochemical measurement of the aeroallergen concentration in horse barns has not been previously reported. Levels of viable airborne microbes in horse barns have been measured using viable culture analysis, and the species of bacteria and fungi cultured from airborne dust samples depends on the management system practiced (Clarke, Madelin, and Allpress 1987). Viable culture analysis of airborne dust samples tend to underestimate the actual spore prevalence when compared to paired volumetric spore counting methods (Burge et al. 1977). Furthermore, the antigenicity of bacteria and fungi does not rely on microbial viability, and measurement of viable airborne microbes may underestimate the levels of antigenic dust particles.

In this thesis, I describe the gravimetric size-selective sampling of airborne dusts in two different management systems. The first is a conventional management system, which induces clinical signs of COPD in susceptible animals. Ponies were bedded on straw and fed poor-quality hay. The second system is the recommended environment (RE) based on recommendations for a management system suitable for the housing of COPD-susceptible animals. Ponies were bedded on wood shavings and fed a complete pelleted diet.

The airborne dust sampling was performed with both a stationary Andersen cascade impactor sampler in the corner of the stall, and a personal Marple cascade impactor sampler attached to the ponies' halter. Airborne dust samples were collected for immunochemical analysis using a volumetric air filter sampler. Levels of various aeroallergens in the same environments were measured using a radioallergosorbent inhibition (RAST) immunoassay following descending elution of the bulk airborne dust sample (Swanson, Agarwal, and Reed 1985, Campbell et al. 1989).

The hypotheses to be tested were:

1) that the alteration of the management system significantly changes the total and respirable airborne dust concentrations in the stall;

2) that the alteration of the management system significantly changes the aeroallergen concentrations in the stall;

3) that the total and respirable airborne dust concentrations within the pony's breathing zone significantly change following alteration of the management system;

4) that the total and respirable airborne dust concentrations in the stall are significantly different from those measured in the breathing zone under each management system.

CHAPTER 2

LITERATURE REVIEW

Description of chronic obstructive pulmonary disease

Clinical signs in advanced cases of COPD include chronic coughing, decreased exercise tolerance, dyspnea with a double expiratory movement, and a variable nasal discharge (Cook 1976, McPherson and Thomson 1983). Chronic obstructive pulmonary disease is characterized by recurrent pulmonary function abnormalities, including decreased dynamic compliance, increased pulmonary resistance (Robinson and Sorenson 1978, Willoughby and McDonnel 1979, McPherson et al. 1979b), and an increased intrapleural pressure change during tidal breathing (Littlejohn and Bowles 1980). These changes are thought to be due to a decrease in small airway diameter as a result of bronchospasm and plugging of the bronchiolar lumens with mucus and exudate (Murphy, McPherson, and Dixon 1980). A significant impairment of gas exchange, as shown by the nitrogen washout technique, together with decreased partial pressure of arterial oxygen, has been reported in COPD-affected horses during disease episodes (Muylle and Oyaert 1973).

On histopathologic examination of the lungs of COPD-affected animals, there are chronic inflammatory changes in the bronchioles, with plugs of mucus or neutrophils or both in the lumina of the small airways. There is also goblet and squamous cell hyperplasia or metaplasia or both and this is often accompanied by alveolar septal thickening and acinar overinflation (Gerber 1973, Breeze 1979, Viel 1983). More recent reports on the histopathologic pulmonary lesions in COPD-affected horses (Kraup, Drommer, and Deegen 1990, Kraup, Drommer, Damsch, and Deegen 1990) state that there are localized areas of epithelial hyperplasia with loss of ciliated cells, as well as areas of peribronchiolar inflammation containing focal alveolar emphysema and fibrosis. Other features of the diseased airways include: goblet cell multiplication; bronchiolar accumulations of mucus and inflammatory cells, especially neutrophils and degranulated mast cells; and decreased numbers of non-ciliated bronchiolar epithelial (Clara) cells. These authors report positive correlations between the severity of clinical signs and the pathomorphologic lesions of both ciliary loss and the extent of bronchiolar alterations in severely affected horses (Kraup, Drommer, and Deegen 1990, Kraup, Drommer, Damsch and Deegen 1990). Chronic obstructive pulmonary disease may affect horses of two years and older and the disease prevalence increases with advancing age. No sex or breed predisposition has been reported (McPherson et al. 1979b). Chronic obstructive pulmonary disease is considered to be the most frequent cause of loss through disease in the Swiss horse population (Gerber 1973), and the most common cause of chronic coughing in horses in the United Kingdom and Western Europe, where most horses are stabled in winter (McPherson and Thomson 1983).

The appearance of clinical signs is associated with the inhalation of dusts from feed and bedding (Lowell 1964, Gerber 1973, Halliwell et al. 1979, McPherson et al. 1979b). Clinical signs regress when horses are removed from the enclosed areas and kept on pasture (Derksen et al. 1987), or after removal of access to hay and straw. However, the horses remain sensitive to some constituent of agricultural dusts, and COPD signs will recur following dust exposure (Johnson et al. 1960, Schatzmann et al. 1974, Meister, Gerber and Tschundi 1976, Thomson and McPherson 1984).

Composition of agricultural dusts

Agricultural dusts are variable, complex mixtures of organic and inorganic materials including: animal derived components, e.g., dander, hair, urine, and feces; feed grains and parts of plants themselves, e.g., hay and pollen grains; insect parts and feces; fungal spores, hyphae, sporangia, and mycotoxins; and bacteria and bacterial endotoxins (Donham 1986). Lacey (1986) reported that the actual constituents of the dust found in livestock buildings are variable and depend on the type of operation as well as the source and conditions under which the feed and bedding materials are grown, harvested, and stored. Hays can be described with a semi-quantitative classification as good, moldy, or very moldy based on the number and types of spores contained, which depends on the water content of the material at the start of storage. The microflora of hay stored at above 40% water is dominated by thermotolerant actinomycetes and fungi, especially *Aspergillus fumigatus*, *Micropolyspora faeni*, and *Thermoactinomyces vulgaris* (Lacey 1986).

Possible adverse effects of airborne dust on the respiratory function

A variety of mechanisms has been suggested to explain the adverse effects on the pulmonary system of high airborne dust levels.

Antigenic capabilities of the dust components

An allergic reaction to some dust antigens is considered to be important in the pathophysiology of COPD (Alexander 1959, Cook and Rossdale 1963, Lowell 1964). IgG antibodies to thermophilic molds and fungi are detected more frequently in the serum of COPD-affected horses than in normal animals (Amundsson, Gunnarsson, and Johannesson 1983, Lawson et al. 1979). However, the presence of these antibodies is not restricted to COPD-affected animals, and more likely reflects the degree of antigen exposure due to different management practices, or advancing age (Clarke 1986). This agrees with Burrell and Rylander (1981), who suggested that serum precipitins have no pathogenic role in the development of hypersensitivity pneumonitis in humans, but that the precipitins may be used to identify provocative agents in the environment to which the patient has been exposed. Intradermal antigen provocation in horses also demonstrates the allergenic properties of the dust components, without necessarily indicating that there is a pulmonary hypersensitivity to these aeroallergens (McPherson and Thomson 1983, McPherson et al. 1978). Intradermal skin tests performed with the suspected antigens result in dual responses of predominantly Type III (IgG mediated or Arthus reaction), but also type I (IgE mediated or immediate) reactions (Lowell 1964, McPherson et al. 1979a, Lawson et al. 1979, Eriksen 1986, Sasse 1986). One report suggests that hyposensitization, based on the results of positive intradermal reactions, results in clinical improvements in horses with COPD (Beech and Merryman 1986). This approach is only of value in Type I hypersensitivities, as it encourages IgG formation, and may exacerbate Type III hypersensitivities (Halliwell et al. 1979). Horses with COPD have a significantly higher IgA:albumin ratio in the tracheal fluid compared to normal animals. This may indicate an involvement of the lower respiratory tract local humoral immune system in these COPD-affected horses (Mair, Stokes, and Bourne 1988).

Aerosolized challenge of horses with COPD using specific antigens, including *Micropolyspora faeni* (McPherson et al. 1979a) and *Aspergillus fumigatus* (Lawson et al. 1979), produces measurable increases in maximal intrapleural pressure change. Aerosolized *M. faeni* challenge of COPD-affected ponies increases pulmonary resistance but does not affect the pulmonary mechanical properties of normal ponies (Derksen et al. 1988).

Decreased particle removal by mucociliary clearance

Exposure of horses to high airborne dust concentrations for prolonged periods resulted in decreased mucociliary clearance, probably due to mechanical overload (Cross and Halliwell 1991). Progressive pulmonary accumulations of airborne particles after long-term exposure have been described in other species (Snipes 1989).

Direct damage to mucosal membranes of the respiratory tract by dust and aerosols

Neuronally mediated alterations in breathing patterns and activation of neurohormonal inflammatory processes are elicited by inhaled toxins or their metabolites. Inhaled dusts may act as irritant stimuli and trigger type C sensory nerves to release neuropeptides, which may then initiate neurogenic inflammation (Cross and Halliwell 1991).

Alternative pathway activation of complement by thermophilic actinomycetes

The thermophilic actinomycetes have been reported to be specifically capable of affecting the respiratory system via alternative pathway activation of complement. The activation of complement by the alternative pathway leads to the generation of chemotactic factors for neutrophil movement into the area (Schatz, Patterson, and Fink 1977). Activated complement stimulates circulating neutrophils to adhere to vessel walls and release tissue-injuring oxygen radicles (Roth and Carpenter 1989).

Cell-mediated immunity induction by thermophilic actinomycetes

Thermophilic actinomycetes in airborne dusts serve as adjuvants for the induction of specific cell-mediated immunity (Schatz, Patterson, and Fink 1977).

Macrophage stimulation by thermophilic actinomycetes

Alveolar macrophages are stimulated by thermophilic actinomycetes in inhaled dust to produce activated proteases, inactivated antiproteases, and oxygen-derived free radicles, and to generate neutrophil chemoattractants. The tissue damage caused by inhaled agents may be a result of an imbalance between the injurious mechanisms initiated by the inhalants and the defensive cytoprotective mechanisms of the lung (Cross and Halliwell 1991).

Exogenous enzyme release

Certain airborne microbes, including *Bacillus* spp and *Streptomyces* spp, are capable of releasing exogenous proteolytic and elastase enzymes (Karol 1989). Elastase activity that is not inhibited by protease inhibitors may result in damage to the alveolar septa (Rom and Crystal 1991). Strains of both *Bacillus* and *Streptomyces* spp have been isolated from hay (Lacey 1974), equine transtracheal washes (Mansmann and Strous 1976), and direct culture of equine lung tissue (Grunig et al. 1986).

Threshold limiting values

The concentration of an agent that results in an adverse effect is called the threshold limiting value (TLV) (First 1989). The TLV of the various components of dust required to develop clinical signs of COPD has not been reported. Each dust component will have its own TLV, and may act additively or synergistically with other dust components. It is difficult to identify responses to specific individual agents in agricultural dusts (Flaherty 1982, Donham 1986). The total airborne dust concentration is important and industrial bronchitis is more prevalent in workers exposed to dust levels greater than 10 mg/m^3, regardless of whether the particles are of organic or inorganic origin (Morgan et al. 1982). The relationship of changes in pulmonary function to the concentration of inhaled airborne dusts is influenced by host factors, environmental conditions, and the presence of other potentially hazardous airborne contaminants.

Host factors that may affect the TLV for dust include age, duration, and severity of COPD (Thomson and McPherson 1984), and previous respiratory tract infections (Clarke 1987b). It has been suggested that mechanisms that decrease the clearance of inhaled material lead to retention, prolonged antigenic stimulation, and hence hypersensitivity (Mirbahar and Eyre 1986). The balance between airborne particle deposition and clearance determines the concentration of particles retained in the lung. Clearance mechanisms of inhaled aerosols include solubilization and absorption, mucociliary transportation, and alveolar macrophages. Deposited inert, insoluble, inhaled dust is cleared within hours from the tracheobronchial region by mucociliary transport towards the larynx. Particulate dust is removed at a much slower rate from the non-ciliated gas exchange areas by phagocytic alveolar macrophages, by dissolving in situ, or by passage through the alveolar walls to the lymphatics (Lippmann 1989, Schlesinger 1989). Alveolar clearance is reported to be a function of the time since exposure, the nature of the particles, and the pulmonary dust load (Le Bouffant 1971). These processes are summarized in Figure 1 (Schlesinger 1989).

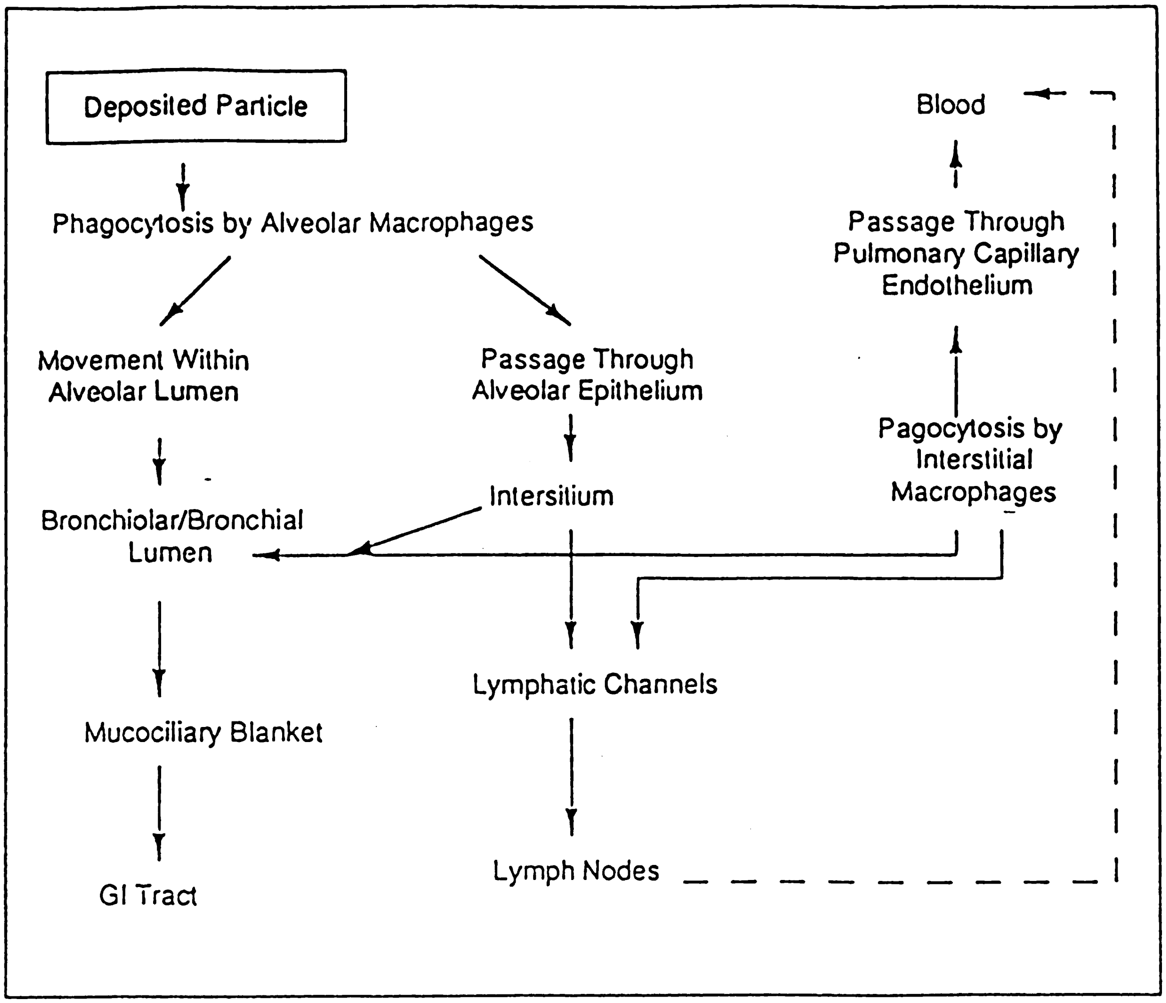

Figure 1: Flow chart showing the non-absorptive clearance pathways for particles depositing in the pulmonary region (dissolution not depicted) (Schlesinger 1989).

The importance of the difference between clearance and deposition rates in the determination of a pathologic or physiologic response to inhaled aerosols was emphasized by Brain and Valberg (1979), who pointed out that if the clearance efficiency for a particle decreases from 99 to 98%, retention doubles. This results in a 100% change in the retention from a 1% change in the clearance (Brain and Valberg 1979). Thus, host differences in the clearance rates can greatly influence the TLV for inhaled dusts.

Other environmental aerosols can have an additive or synergistic action on the effect of airborne dust and act to reduce the concentration of dust required to produce an effect, i.e., reduce the TLV of airborne dust. Environmental factors that increase nonspecific airway reactivity by non-immunologic means include extremes of relative humidity and temperature (Sheppard 1986). Levels of other irritating substances, e.g., ammonia, which could alter the TLV of other dust components, have been measured in horse stalls (Sasse 1985, Lawrence et al. 1988). These substances may be adsorbed onto dust particles and be deposited in the lower respiratory tract (Donham 1986).

Factors affecting particle deposition in the respiratory tract

The airborne dynamic behavior of a particle in the airways depends on its physical characteristics of size, shape, and density rather than on its microscopic size. These dynamic qualities are described in terms of the particle aerodynamic diameter, which is defined as the diameter of a unit density sphere having the same terminal velocity as the particle in question (Lippmann 1970). The aerodynamic diameter of a particle will determine its ability to remain airborne and also its site of deposition within the respiratory tract. Once a particle contacts any portion of the lung surface, it is not resuspended, and so is deposited or filtered from the pulmonary air (Muir 1972).

On the basis of particle aerodynamic diameter, airborne dust can be divided into fractions that are likely to reach and deposit in different regions of the respiratory tract, allowing an estimation of the concentration that will reach the target region. The total dust concentration gravimetrically determined in the ambient air may be misleading, as it is affected by the presence of a few large particles that increase the measured airborne dust concentration even though these large particles will not reach the target area. This use of total dust concentration alone would result in the dust concentration bearing no relation to the inhalation hazard (Lippmann 1970).

Particles are deposited in the respiratory tract by four mechanisms (Schlesinger 1989).

Impaction

When there is a rapid change in the direction of bulk airflow (curvilinear motion) large particles continue in the original airflow direction due to their large momentum and impact on the opposing wall of the airway. Smaller particles with negligible inertia perfectly follow the air streamlines and move around the obstacle. Impaction is the main mechanism by which particles of aerodynamic diameter greater than 2.0 μm are deposited in the upper respiratory tract and at or near tracheobronchial bifurcations (Lippmann 1989).

Sedimentation

When the gravitational forces on an airborne particle overcome the upward air resistance forces, the particle falls out of the airstream at a constant rate (terminal settling velocity) and sediments onto the lower airway or alveolar surface (Muir 1972). Sedimentation is proportional to particle size and density, and is decreased with a shortened residence time in the airways. Sedimentation is an important method of removal of particles with aerodynamic diameter between 0.5 μm and 2.0 μm that penetrate to the smaller bronchi and bronchioles where air velocity is relatively low (Schlesinger 1989, Lippmann 1989).

Diffusion

This is a major mechanism for the deposition of very small particles with aerodynamic diameter less than 0.2 μm in those airways with low bulk flow rates, e.g., bronchioles and alveoli. Diffusion is a result of the random motion of a particle due to bombardment by the surrounding air molecules, and is inversely proportional to its diameter, but independent of its density (Muir 1972). Diffusion may result in the particle contacting and adhering to all surfaces of the airway walls.

Interception

This occurs when part of a fibrillar particle contacts the airway wall. Deposition due to interception increases as the fiber length increases and as the airway diameter decreases (Schlesinger 1989).

Respiratory tract geometry also has an effect on particle deposition. The cross-sectional area of an airway is determined by its diameter and this determines the airflow velocity for a given inspiratory flow rate. Airway diameter also determines the displacement required for a particle to contact and deposit on a surface. A decrease in the path length from the trachea to the terminal bronchioles increases the deposition in the latter. This regional deposition pattern is more evident for larger particles, which are subject to impaction and sedimentation. Small particles tend to deposit evenly in all lobes in proportion to the relative ventilation of the area rather than in response to path length (Raabe et al. 1982, Schlesinger 1989).

Ventilation patterns also alter the sites and relative amounts of regional deposition of inhaled dust particles. Increased inspiratory air flow rates increase the airborne particle deposition due to impaction, but also decrease the time available for sedimentation, and may thus result in the decreased deposition of larger particles in the smaller airways and pulmonary region (Bennett et al. 1985, Schlesinger 1989). For a given minute ventilation, an increase in tidal volume compared to a more rapid shallow breathing pattern will result in the deeper penetration and the increased deposition of airborne particles in the more distal regions (Muir 1972). An extended respiratory pause will increase the particle residence time at low flow rates and increase the deposition of particles due to sedimentation and diffusion deposition (Schlesinger 1989).

Factors affecting the environmental airborne dust or aerosol concentration in stables

An aerosol is defined as "any system of liquid droplets or solid particles dispersed in air, of fine enough particle size, and consequently low settling velocity, to possess considerable stability as an aerial suspension" (Hatch and Gross 1964). A more general description of an aerosol is "any atmosphere containing particles which remain airborne for a reasonable length of time and [the term] is used to describe all particles that can be inhaled whether they are therapeutic, industrial, or of natural origin such as bacteria, fungi, and pollen" (Muir 1972). The aerial dust or aerosol concentration is a result of an equilibrium between the rates of particle release and removal. The steady state concentration for an aerosol in a building (gaseous, dust or microbial particles) is given by the following equation (Wathes 1983).

$$C = 1/q\ (R/V + Ci \cdot qv)$$

C = concentration of pollutant (units/volume)

q = clearance by all routes including ventilation (qv) and sedimentation (1/hour)

R = rate of emission of pollutant (units/hour)

V = volume of building (m^3)

Ci = concentration of pollutant at air inlet (units/m^3)

The rates of release of contaminants will depend both on the source concentration and the degree of agitation of this material. The main sources of airborne dusts in stables are hay and bedding (McPherson et al. 1979b). Bedding materials of peat moss and shredded newspaper release fewer fungal and actinomycete spores than wood shavings or straw (Clarke 1987). Multiplication of thermotolerant and thermophilic microbes is enhanced in poorly ventilated, heavily insulated stables (Clarke and Madelin 1987a) and these conditions will increase the source concentration of dust particles. Increases in the level of activity within the stables, e.g., stall cleaning or feeding, increase the concentration of aerial dusts (Crichlow et al. 1980, Zeitler 1984). The main method of airborne dust removal from a building is via ventilation. Sedimentation is only important for the removal of larger particles. The rates of sedimentation for various sizes of dust particles are given in Table 1 (Clarke 1986).

Particle size (aerodynamic diam.)	**Example**	**Time to fall 2 meters**
50 μm	Pollen grain or large fungal spore	28 sec
5 μm	Aspergillus/ Penicillium spores	45 min
1 μm	Actinomycete spores	16 hrs

Table 1: Relationship of particle aerodynamic diameter and the time taken for airborne particles to fall 2 meters (Clarke 1986).

Measurement of environmental airborne dust concentration

Assessment of the health hazard posed by various aerosols requires a knowledge of the total concentration distribution of airborne particles and their likely site of deposition in the respiratory tract (Lippmann and Harris 1962).

The respiratory tract can be divided into two regions based on the size of airborne particles that are likely to reach that anatomical area: 1) the extrathoracic region, composed of the head and nasopharyngeal airways, which are penetrated by particles of aerodynamic diameter less than 200 μm; and 2) the thoracic region, which is composed of both the tracheobronchial region, which can be penetrated by particles of diameter less than 10 μm, and the alveolar region penetration by particles less than 5 μm. The fraction of the total aerosol that enters the nose and mouth is termed the "inspirable dust" fraction (International Standards Organization 1983, American Conference of Governmental Industrial Hygienists 1984). The "respirable dust" fraction is defined as the portion of the inhaled dust that penetrates to the non-ciliated portions of the lung (Hatch and Gross 1964, Lippmann 1989). This classification system is described in Figure 2 (Lippmann 1989).

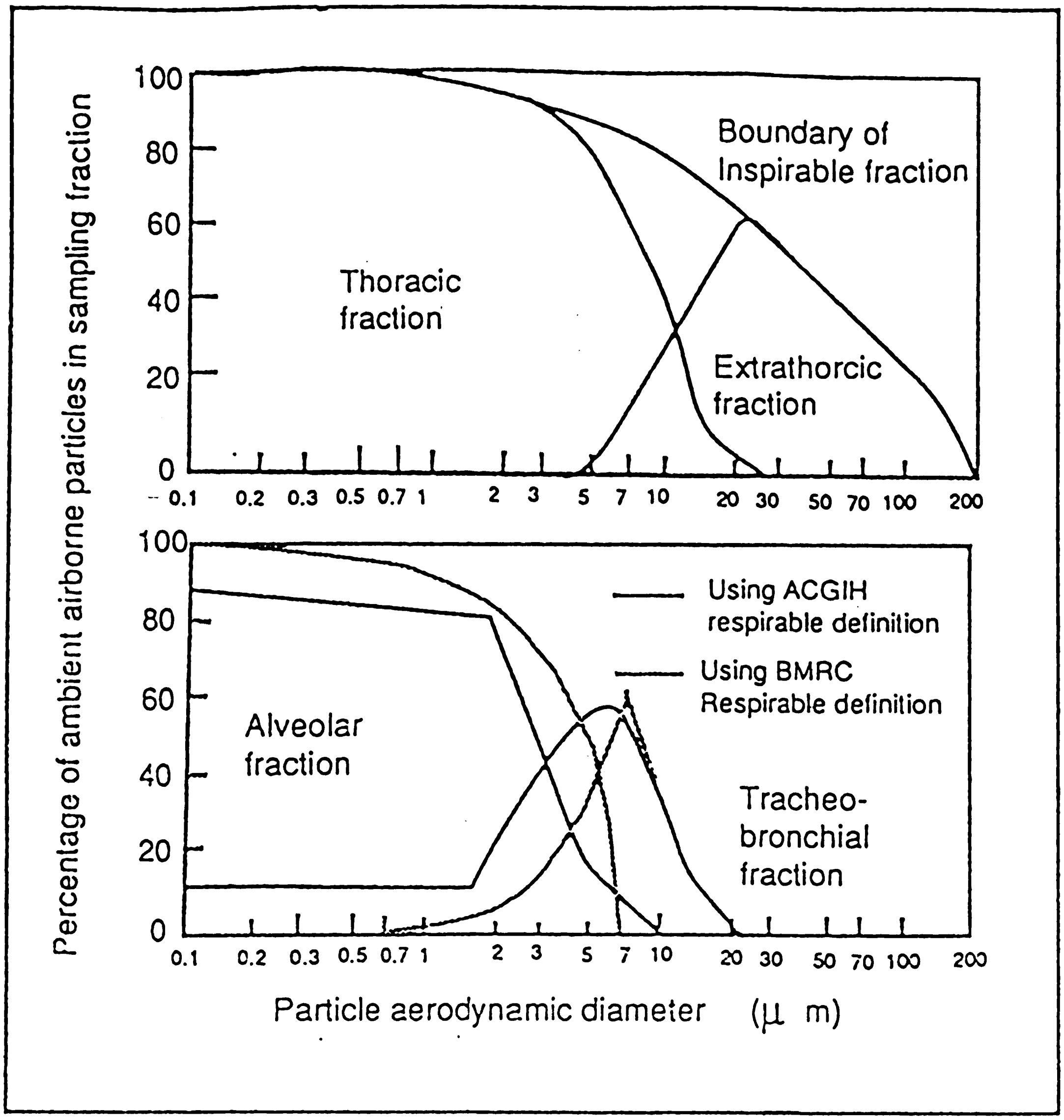

Figure 2: Classification of airborne particles based on their theoretical sites of deposition in the respiratory tract according to recommendations of the International Standards Organization (Lippmann 1989).

Respirable dust concentration is considered a good index of the respiratory hazards posed by airborne dust inhalation (Lippmann 1989, Air Sampling Procedures Committee 1984). Respirable dust concentration can be measured using air sampling devices that divide AD into fractions based on aerodynamic diameter. Alternatively, the respirable dust concentration can be estimated following the sampling of total airborne particulate mass sampling, if the particle size distribution is constant and the fraction of the total dust that is respirable is known. Agricultural or barn dusts have variable composition and size distribution (Lippmann 1970). Respirable dust sampling of agricultural dust is essential in order to estimate the potential hazards posed by different environments on the pulmonary system.

Multi-stage impactor samplers with cut-off characteristics like those of the upper respiratory tract and tracheobronchial airways, followed by an efficient final stage, provide air concentration data relative to health hazard with minimal sampling and analytical effort (Figure 3) (Andersen 1983). In these multi-stage impactor samplers, an aerosol passes through a jet in which it is assumed that the airflow is laminar and that all the particles within the jet have uniform velocity. The outflow of the air stream is directed towards a flat plate (the impaction plate), which deflects the flow through 90 degrees. Large particles with sufficient inertia are unable to follow the changed airflow direction and impact onto the plate. Smaller particles are able to follow the streamline and remain airborne. Hence, the impactor has separated the airborne particles into two size ranges, those greater than a certain size range that are removed from the airstream, and the remaining airborne particles of smaller aerodynamic diameters. In a multi-stage impactor, the aerosol moves through a succession of impaction plates with progressively narrower jet diameters. The flow rate through the series of impactor plates is constant, but the air velocity increases as it moves through the impactor, resulting in the impaction of progressively smaller particles and the separation of all airborne particles (solid and liquid) based on aerodynamic diameter.

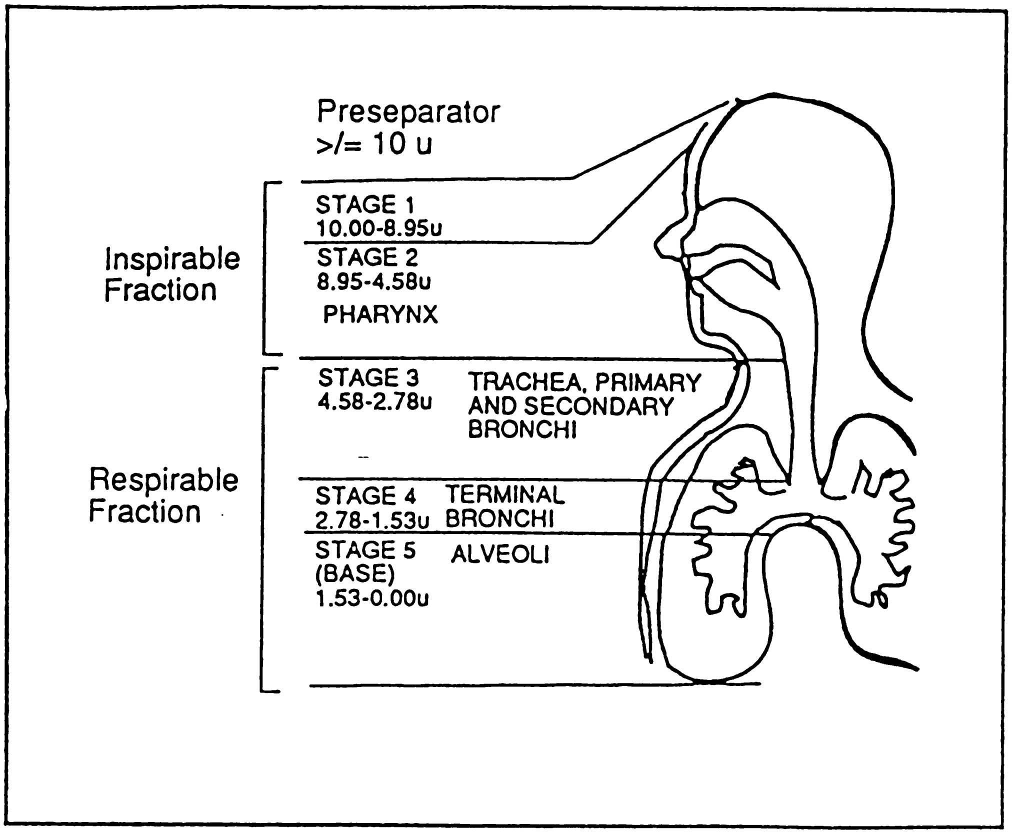

Figure 3: The stages of the Andersen cascade impactor simulating the anatomical regions of the human respiratory tract. The range of particle aerodynamic diameter that is collected on each stage is shown. (Adapted from Andersen 1983.)

The theoretical considerations of utilizing cascade impactors to sample airborne dust concentrations have been elucidated. Curvilinear motion is characterized by a dimensionless number called Stokes number (St), which is defined for an impactor as the ratio of the particle stopping distance at the average nozzle exit velocity (U) to the nozzle radius (Dj/2).

$$St=\frac{\rho D_p^2 CV}{9\mu W}$$

where: ρ = particle density

D_p = particle Stokes diameter

C = Cunningham slip factor

V = mean velocity in the jet

μ = air viscosity

W = jet diameter or width

As St approaches zero, the particles track the streamlines perfectly; whereas the particles resist changing their directions with the gas streamlines (i.e., the particles' inertia increases) as the St increases. The stopping distance, or inertial range, represents the distance that the particle continues to travel in its original direction once the airstream in which it is moving is abruptly turned 90 degrees (Hind 1982).

The most important characteristic of an impactor stage is its collection efficiency curve. This curve is the fraction of particles of a given size collected from the incoming aerosol stream as a function of particle size. Collection efficiency is governed by the St. Impactors with a sharp cut-off curve are ideal for size classification. If the cut-off curve is sharp, all particles larger than a certain aerodynamic diameter are collected and all those with a smaller aerodynamic diameter pass through. The collection efficiency curve can be characterized by the St that gives 50% collection efficiency. The steeper the curve, the greater the collection efficiency. The point on each collection curve that corresponds to the 50% efficiency is referred to as the cut-off point diameter (D50), which assumes that the mass of particles larger than the cut-off size that get through equals the mass of particles below cut-off size that are collected. The D50 of each stage must be sufficiently distinct to prevent overlap of collection curves and distortion of the size distribution curves (Hind 1982). Ideally, each particle is assigned to its proper size class, but in the real situation, there is some misclassification and particles are assigned to the wrong classes (Watson, Lioy, and Mueller 1989).

Errors associated with the size-selective sampling of airborne dust using multi-stage impactors

There is often a large discrepancy between the theoretical collection efficiency and the experimental measurements (Rao and Whitby 1978). The collection efficiency is the product of the impaction efficiency and the adhesion efficiency (Rao and Whitby 1977). The adhesion efficiency is the fraction of particles that adheres to the surface after impaction. This adherence depends on the characteristics of the particle and the collecting surface (Cushing, McCain, and Smith 1979). The impactor theory assumes that all impacted particles will adhere, but particles may either bounce off the plate, or they may initially adhere and be blown off later (Hind 1982). The physical nature of the particles also limits the collection of particles onto a solid surface. Liquid particles may coalesce, volatile particles evaporate, and solid particles may agglomerate (adherence of particles to each other) or break up.

The difference between the actual and theoretical efficiencies can be accounted for by interstage (wall) losses, particle bounce and re-entrainment, electrostatic effects (Cushing, McCain, and Smith 1979), and effects of stage loading (Knuth 1976).

Interstage losses

Interstage or wall losses are due to the deposition of particles on surfaces other than the impaction plate. Wall losses are sensitive both to the surface properties of the aerosol (associated with particle water content and roughness), and the sampling conditions (relative humidity) (Mitchell, Costa, and Waters 1988). These losses tend to decrease with smaller particle sizes and are negligible for particles less than 2 μm diameter, and can be attributed to particle settling, diffusion, and electrostatic forces (Cushing, McCain, and Smith 1979). Wall losses are high for particles greater than 5 μm diameter in multijet cascade impactors in which the ratio of wall-to-jet area is large, and the aerosol pathway between stages is more tortuous than in single jet designs (Mitchell, Costa and Waters 1988). Rao and Whitby (1978) report high wall losses for particles of greater than 10 μm diameter, where the major portion of the loss occurred on the jet plate of the stage that collected the major portion of the test aerosol, and a visible deposit developed at the inlet side of the jet. Interstage losses cannot be theoretically predicted, and have to be determined experimentally (Marple and Willike 1976).

Particle re-entrainment

Particles can re-entrained into the airstream after bouncing off, or being blown off the collection plate. For cascade impactors, this results in the collection of large particles on later stages intended for collection of smaller particles, and results in the size distribution being shifted toward smaller particles. The degree of re-entrainment depends on the type of particle and the nature of the impaction surface. If the particles are sufficiently soft, e.g., liquid, or the surface is sufficiently adhesive, e.g., coated with grease, then adhesion is maximal and re-entrainment is decreased. Particle rebound has significant effects on stage collection efficiency and D50 (Knuth 1976). When sampling with uncoated, bare metal plates the collection curve of the Andersen impactor is distorted due to particle bounce, especially for the stages that collect small particles at high velocities (Rao and Whitby 1977, Esmen and Lee 1980).

Effect of stage loading

Solid particles have maximum re-entrainment with a dry, hard surface, but the collection efficiency curves for oil-covered glass plate agree well with the theoretical efficiency curves (Marple and Willike 1976). When glass fibers are used as the collection surface, there is a significant reduction in particle bounce, but the character of the collection curves is changed so as to reduce the size resolution of impaction (Rao and Whitby 1977). Particle re-entrainment due to rebound from impaction surfaces has not been theoretically quantified and results in serious errors when the size distribution is calibrated from theoretical efficiency curves.

The collection efficiency of the various stages varies with time, depending on the relative change of the impaction surface with particle accumulation directly below the jet (Knuth 1976). The particle loading may increase the efficiency if the loading increases the surface roughness (Rao and Whitby 1978). The collection efficiency need not be 100% in order to be a useful measurement, providing that the efficiency is constant, known, and accounted for when concentrations are calculated. However, the efficiency does not remain constant over time as loading of the medium changes the efficiency (Lippmann 1989). The upper limit for any one size range of the Andersen cascade impactor given by the manufacturer is 10 mg of particulate matter. Overloading can be detected by visual inspection (Andersen Samplers Inc. 1983). The ring that can be seen encircling the collection area beneath the jet is made up of the particles that are either swept away by airflow from the central deposit, or have rebounded slightly on impaction and have precipitated further away where the air velocity is lower.

Electrostatic effects

The electrostatic charges of environmental airborne particles are not predictable, and are assumed negligible in the impactor theory.

Measurement of aeroallergen

Chronic obstructive pulmonary disease is thought to result from a reversible pulmonary hypersensitivity reaction in response to the inhalation of specific dust aeroallergens. There is no universally acceptable method of aeroallergen sampling and measurement due to the wide diversity of environmental aerosols and differences in their aerodynamic size, physical properties, and intrinsic qualities, e.g., viability.

All aeroallergen measurement methods involve an initial collection process or method of trapping airborne particles, followed by a sample analysis stage (Solomon 1984). Aeroallergen sample collection devices are based on four methods of collection.

"Gravity sampling," or fallout onto a fixed horizontal sticky surface or culture medium

Fallout sampling results in the preferential collection of larger particles and the exclusion of particles less than 10 μm. This method is suitable for qualitative surveys or trend data on the more common pollens and larger fungal spores. However, errors may arise as air movement influences the collection efficiency, and also the volume of air contributing to the sample is uncertain (Solomon 1984, Chatigny et al. 1989).

Filtration using a suction device and filters of definable effectiveness

This method allows volumetric air sampling, and when a glass filter of nominal pore size of 0.1 μm is utilized the sample will contain the majority of airborne dust. However, particle morphology is difficult to assess on samples collected onto filters, and these filtered samples are only suitable for further culture analysis of bacterial and fungal spores that can withstand desiccation (Chatigny et al. 1989). Wind orientation will bias collection efficiency, and the suction devices using filters are more suited for indoor air sampling rather than for sampling under outdoor conditions with varying prevailing winds (Solomon 1984).

"Rotating arm impactors," or impaction onto rapidly moving surfaces

In this method, airborne particles are collected onto a narrow sticky surface that is rotated rapidly through the air (Solomon 1984). The collection efficiency of this type of sampler is high for larger particles (>15 μm), but there is only 25% collection efficiency for particles of 10 μm diameter, and only 5% for 5 μm particles, including most fungal spores (Chatigny et al. 1989).

Impaction of accelerated particles onto collection points

In an impactor, the particles are aspirated into a flow channel, accelerated, and then forced to negotiate a sharp bend. Larger particles impact onto the collecting surface. This collection method is efficient for particles of 5-10 μm and smaller (Andersen 1958, Solomon 1984, Chatigny et al. 1989).

Analysis of the collected samples will take one of three forms:

1) Direct microscopic enumeration of individual particles: This direct counting method is not dependent on the growth potential of a sample, but requires distinctive particle form to allow adequate particle recognition. Spores of *Aspergillus* spp, *Penicillium* spp and related genera are not easily distinguished from each other or

from oil droplets and ash particles, leading to problems during microscopic analysis of aeroallergens (Chatigny et al. 1989).

2) Culture and identification of colonies: The detection of viable microbes usually requires that the collected cells be allowed to multiply to readily observable colonies, with possibly further isolation. Airborne particles can be either collected directly onto semi-solid media or filter, or into a liquid before transfer onto the nutrient agar. Each particle that contains one or more viable bacterium or fungus produces one colony and is referred to as a colony-forming unit. The counting of colonies produced on semi-solid media requires fairly elaborate processing, including specific requirements for materials, space, sterile procedures, and careful sequential observations (Solomon 1984, Chatigny et al. 1989). The growth potential and germination rate of microbes are determined by many variables including viability, nutritional requirements, and inter- and intra-species competitive inhibition (Burge et al. 1977). Problems encountered while using the culture and identification method of aeroallergen analysis include: the differential growth rates of microbes leading to overgrowth of the more fastidious or slower growing species; lack of growth due to deficient substrates and non-ideal culture conditions; antagonistic interactions resulting in the underestimation of the number of colonies of certain species, e.g., *Micropolyspora faeni* growth can be inhibited by *Bacillus* spp., and overgrown by *Thermoactinomyces* spp. (Lacey 1986). Cultural recoveries tend to underestimate the actual spore number, and culture plate data has been reported to progressively underestimate an increasing spore count when compared to paired volumetric spore counting methods (Burge et al. 1977). The presence of dust mites may cause further

inaccuracies in the culture analysis of aeroallergens. Dust mites feed on fungal spores and decrease the number of viable fungal spores. Hay samples that contain large numbers of dust mites and/or partially digested fungal spores in fecal pellets must be considered potentially allergenic (Hockenjos, Mumeuoglu, and Gerber 1981, Clarke 1986). The antigenicity of bacteria and fungi does not rely on microbial viability, and measurement of viable airborne microbes may underestimate the levels of antigenic dust particles.

3) Immunochemical or direct analysis of bulk airborne dust samples following descending elution: These methods utilize antigen or antigen-specific antibodies adsorbed or covalently bound to a solid surface, and either radiolabelled or enzyme-linked antibody to detect any bound antigen or specific antibody (Gleich et al. 1974, Agarwal et al. 1981, Reed 1982, Campbell et al. 1989). This method allows analysis of dust samples without regard to the particle viability or defined form, e.g., animal dander (Brown et al. 1987) or arthropods (Swanson et al. 1985). Such analysis is only useful if the identity of the allergen is either known or suspected (Chatigny et al. 1989). However, the specificity of the assays is critical as the air filter collections contain both allergenic and non-allergenic proteins (Swanson et al. 1985).

Influence of housing and management on airborne dust

Clinical improvement of horses affected with COPD has been reported to follow managemental changes and environmental control (Thurlbeck and Lowell 1964, Eyre 1972, Cook 1976, Thomson and McPherson 1983). Certain management regimens have been suggested to produce a low dust/aeroallergen concentration, and be suitable for the stabling of COPD-susceptible animals (Beech 1989). Clarke (1987) reported that bedding materials that can be used as an alternative to straw, e.g., wood shavings or shredded paper, are much "cleaner" than straw when first put into the stables. Airborne particle counts of barn environments where shavings are used as a bedding are between 6-40 particles/ml air, compared to particle counts of 167-724 particles/ml air for barns using straw bedding (Clarke 1987). This means that certain materials are likely to produce a low airborne dust and spore concentration and pose less of a respiratory challenge to COPD-susceptible ponies. This is supported by reports that "low-dust" housing (conditions where horses are bedded on wood shavings, fed a pelleted diet with good quality straw, and kept outside for 6-8 hours daily) do not induce clinical signs of COPD. The same animals develop signs of dyspnea when kept under conventional housing conditions that are poorly ventilated, and while bedded on straw and fed good to medium quality hay. Clinical signs regress after horses are returned to the "low-dust" environment (Grunig et al. 1989). The remission of signs of COPD in 4-24 days after the horses are removed from a conventional management system and housed in a "controlled" environment using both a shredded paper bedding and a complete cubed diet has been reported. The actual time interval for animals to become asymptomatic after these environmental changes are implemented is correlated with the age of animal, duration of illness, and the severity of respiratory signs as measured by increase in the non-elastic work of breathing (Thomson and McPherson 1984).

Airborne dust levels have a profound influence on the respiratory health of the horse and influence the course of infectious respiratory disease in the horse. Burrell

(1986) reported significantly longer duration of lower respiratory tract inflammation among horses bedded on a "dusty" bedding of straw compared to those on a "cleaner" bedding of shredded newspaper. Clarke and Madelin (1987a) compared the levels of tracheal mucopus during outbreaks of Equid Herpesvirus-1 infection in different stabling environments. They reported increased amounts of tracheal mucopus in horses stabled in badly ventilated areas with heavy fungal contamination, compared to those animals stabled in well-ventilated stables.

The level of airborne dust in the horse's environment can be expected to decrease as a result of implementing managemental changes. A system for objective assessment of the degree of fungal spore contamination of bedding and feed materials has been devised (Clarke and Madelin 1987b) and marketed (Equigiene, Wrington, Avon, England). This method utilizes the microscopic particle identification and enumeration of a dust sample collected in a hand-held volumetric slit sampler (Buckard single stage impactor air sampler). A portion of the material is agitated to release dust and the sample collected over a short time period. The type and number of dust particles collected allows assessment of the quality of the materials that are intended for use in the stable (Clarke and Madelin 1987b). More sophisticated volumetric air sampling methods can assess the air quality and airborne dust levels within the stables or barn, and the results can be used to monitor any benefits of increased ventilation or managemental changes.

Previous measurements of airborne dust and aeroallergen concentrations

The concentration of airborne dusts in riding stables has been measured with stationary dust samplers (Crichlow, Yoshida, and Wallace 1980, Zeitler 1985). Mean concentrations of airborne dust of 0.41 mg/m^3 (coefficient of variation 31.7%) when the barn doors were kept closed during winter, and 0.25 mg/m^3 when the barn was unoccupied and all doors and windows were kept open during summer, were reported. In the Crichlow, Yoshida, and Wallace study, airborne dust levels were measured from ambient air within the barn using stationary filter-type samplers attached to vacuum pumps positioned in the corridor at least 5 meters from the nearest horse stall at 1.5 meter height. Filters were collected every four days and the total dust concentration calculated. The fractions of respirable particles measured during winter was 40%, and these proportional values did not alter with changes in the total dust concentration. The fraction of respirable particles measured during summer was lower than the winter values at 8.5% (Crichlow, Yoshida, and Wallace 1980). Zeitler (1985) sampled the airborne dust concentration using a Portikon dust sampler in a closed barn during winter where horses were bedded on straw and fed hay and found average airborne dust concentrations of 0.63 ± 0.13 mg/m^3. The airborne dust samplers were positioned in the corridors between stables at a height of one meter. Size-selective airborne dust sampling was performed using an Andersen cascade impactor also positioned in a corridor. This author reports a diurnal variation in respirable airborne dust particles, which form 80-90% of total airborne dust concentrations at night compared to only 30% of the dust being in the respirable range during the morning stable work period. Buildings that are well managed and with ample ventilation have lower airborne dust concentrations (average total dust concentration 1.95 mg/m^3) than poorly ventilated, less well-managed systems (average total dust concentration 3.13 mg/m^3) (Zeitler 1985). Improvement in ventilation rates, e.g., opening the barn doors, significantly decreases the airborne dust concentration. Decreased concentrations were reported for the quiet periods during the night (Crichlow et al. 1980, Zeitler 1985). An increase in activity within the barn, e.g., cleaning or feeding, results in a large increase in airborne dust concentration, with reported values of 0.8 mg/m^3 during the day compared to 0.1 mg/m^3 during the night (Crichlow, Yoshida, and Wallace 1980) or values of 2.25 mg/m^3 during the day compared to 0.39 mg/m^3 during the night (Zeitler 1985).

The concentrations of total airborne dusts of 0.41 mg/m^3 (Crichlow, Yoshida, and Wallace 1980), 0.55-3.63 mg/m^3 (Sasse, Boerma and Smolders 1986), and 0.63 mg/m^3 (Zeitler 1985) reported in horse barns or riding stables are lower than the threshold limiting value of 10 mg/m^3 for nuisance particulates established for humans during an eight-hour work shift (American Conference of Governmental Industrial Hygienists 1977). However, this applies to inert, insoluble, airborne dusts, and not to airborne particulates that cause allergic reactions.

During winter periods the horses may be subjected to continuous exposure to high dust levels as they may be kept indoors for 24-hour periods, rather than an 8-hour shift. Moreover, these previous results are from airborne dust samplers positioned in the corridor and not in the stall area. The stationary aerial dust samplers do not measure the dust concentration within the horse's breathing zone.

The horse could be temporarily subjected to very high dust concentrations, depending on its level of activity and the position of its head.

The direct immunochemical measurement of the aeroallergen concentration in horse barns due to the presence of airborne bacteria and fungi has not been reported. Campbell and coworkers (1989) have reported the levels of various aeroallergens in dairy barns measured using a direct immunochemical method. Substantial concentrations of *A. fumigatus, M. faeni, T. vulgaris,* and mites were reported (Campbell et al. 1989). Aeroallergen concentrations in horse barns have been previously measured using indirect methods that rely on microbial variability or particle identification. The species of bacteria and fungi cultured from airborne dust samples depends on the management system practiced; increased numbers of thermotolerant and thermophilic species are present in poorly-ventilated, well-insulated barns (Clarke, Madelin, and Allpress 1987). The number of airborne bacterial colony forming units is decreased if livestock buildings are well ventilated (Curtis et al. 1975). The flora cultured from equine transtracheal aspirates have been reported to represent the microbial population of the inhaled air, and to vary with the type of stabling and management procedures practiced (Mansman and Strous 1976, Sweeny, Beech, and Roby 1985).

Objectives of the present study

Two aspects of the horse barn environmental quality that are important in the development of COPD are the aeroallergen concentration and the airborne dust concentrations in the horse's breathing zone. The objectives of this study were to determine if a change in management system from the conventional system, where horses were bedded on straw and fed medium to poor quality to the recommended system, utilizing pelleted feed and woodshaving bedding, would significantly alter the airborne dust aeroallergen concentrations both in the stall and in the pony's breathing zone. The airborne dust concentrations in the stall and in the pony's breathing zone were compared. We have previously demonstrated that this conventional management system results in COPD-susceptible animals developing clinical signs, and we have used the recommended system as a suitable environment in which to keep some of our COPD-susceptible horses.

The hypotheses to be tested were:

1) that the alteration of the management system significantly changes the total and respirable airborne dust concentrations in the stall;

2) that the alteration of the management system significantly changes the aeroallergen concentrations in the stall;

3) that the total and respirable airborne dust concentrations within the pony's breathing zone significantly change following alteration of the management system;

4) that the total and respirable airborne dust concentrations in the stall are significantly different from those measured in the breathing zone under each management system.

CHAPTER 3

MATERIALS AND METHODS

Introduction

The purpose of the investigation reported in this thesis was to compare the airbone dust and aeroallergen concentration under two different management systems, and to compare the airborne dust concentrations in the stall and in the pony's breathing zone.

Gravimetric size-selective sampling of airborne dusts in both management systems was performed using both a stationary sampler in the corner of the stall and a personal dust sampler attached to the ponies' halter. The levels of the aeroallergens implicated in the development of COPD in both these environments were also measured in conjunction with Mayo Clinic Allergy Research Laboratory.

Environmental management

Sampling was conducted in a four-stall barn attached to our laboratory (Figure 4). The stalls faced out onto a large open area. Two ponies were kept in each stall at all times. The barn was constructed of a steel frame with a concrete block base and aluminum siding lined with plasterboard. The stalls were divided by wood plank walls, and each stall had a steel bar door. The area has an insulated ceiling and a concrete floor. A mechanical ventilation system consisting of an inlet fan (CRB centrifugal fan and/or Reznor furnace SCE 350) operating at 3000 cubic feet per minute and an exhaust fan were the only sources of ventilation. The main entrance to the barn was a large door, which allowed entry of a forklift truck, and was only opened during stall cleaning and feeding, and a separate door to allow entry of personnel. Stalls were cleaned out once daily in the morning. Attention was paid to the removal of all soiled or damp bedding, and development of deep litter was avoided. A constant water supply and *ad libitum* feed were provided. The feed was added twice daily, in the morning and late afternoon.

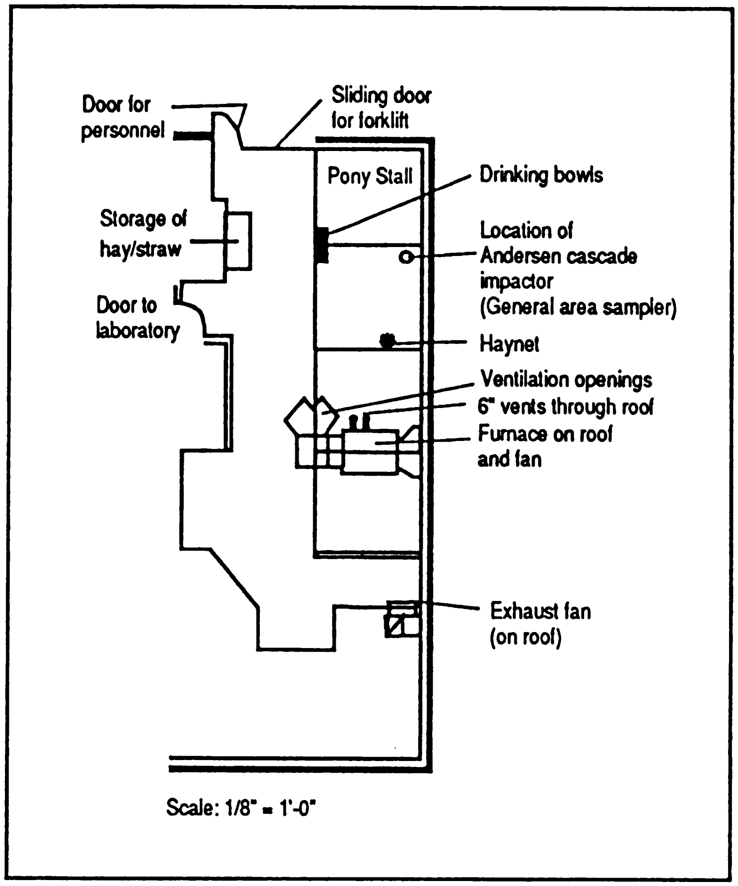

Figure 4: Floor plan of the pony barn and the adjoining building, including details of the ventilation system.

Management systems chosen for study

Management system I: Conventional management system

Ponies were bedded on straw and fed poor-quality hay. Some bales of hay and straw for use that day were stored in the vicinity.

Management system II: Recommended management system

Ponies were bedded on high-quality wood shavings and fed a complete pelleted diet (Purina horse chow pellets). No materials were stored in the barn.

The conventional system has been used to induce signs of COPD in our horses and ponies with a history of heaves. The recommended management system for the housing of COPD-susceptible animals is based on recommendations made by previous authors (Beech 1989, Clarke 1987, Grunig et al. 1989, Thomson and McPherson 1984). Each management system was introduced at least one week prior to sampling in order to establish steady-state airborne dust levels. All animals in the barn were managed in the same manner.

Airborne dust concentration sampling procedures

Use of cascade impactor dust samplers

An Andersen particle fractionating cascade impactor and a Marple personal cascade impactor were used for the size-selective volumetric sampling of airborne dust concentrations in the pony barn. Both samplers are multijet, multistage impactors with removable stainless steel collection stages with tapered orifices arranged in a radial pattern. The orificies are progressively smaller from top to bottom stage. Each stage of the sampler has a characteristic aerodynamic diameter cut-off point and progressively smaller particles impacted on successive stages. A back-up filter collects sub-micrometer particles.

Operation of the Andersen and Marple cascade impactors

Preweighed collection plates were placed on each stage. Cellulose filter collection materials were used for the Andersen cascade impactor (Andersen Samplers, Inc., Atlanta, GA), and mylar media (Andersen Samplers, Inc., Atlanta, GA) for the Marple personal sampler (Sierra Instruments). After sampling was completed, the sampler was disassembled and the collection plates and back-up filters removed and replaced with fresh, preweighed collection media. The collection materials were desiccated before and after the sampling period as some particles and the cellulose filter materials were hygroscopic (Andersen Samplers Inc. 1983). The mylar collection material has the advantage over the hygroscopic cellulose filters of a low moisture absorbency and decreased requirement for repeated desiccation. However, the mylar filters required an equilibration period of at least 24 hours in the laboratory environment with relative humidity less than 50% to ensure a constant mass. The mass of airborne particles collected on each stage was measured using a balance.

Stall airborne dust sampling

Sampling of the general area of the stall was conducted with an Andersen four stage Hi-Volume Fractionating Sampler (Model 65-800) cascade impactor (Andersen Samplers, Inc., Atlanta, GA) mounted on a volumetric air sampler (Air Sentinel TM; Quan-Tec-Air,Inc., Rochester, MN). The dust sampler was operated on a shelf in the corner of the stall, at an air inlet height of 1.5 meters. The sampler operated at a flow rate of 220 L/min. Dust was collected onto desiccated, pre-weighed cellulose filters (GMW-65-350, General Metal Works, Inc., Village of Cleves, OH) onto 4 stages. The aerodynamic diameter of the cut-off levels for the dust collected on each stage is shown in Table 2. The upper two stages collected the non-respirable dust, which meant that samples of airborne dust, as collected with this stationary Andersen were only considered to be respirable if they were collected on stages with a cut-off diameter of aerodynamic diameter less than 4.58 μm. The very fine dust was collected onto a back-up filter of a polytetrafluoroethylene (PTFE) membrane (0.3 μm pore size) (Quan-Tec-Air, Inc.).

After sampling for a selected time period (see below under "sampling periods"), the sampler was disassembled in the laboratory, the dust samples were labeled, placed in the incubator, and then the sampler was loaded with pre-weighed dessicated collection discs and back-up filter, before being carried to the collection site. The dust samples were re-desiccated to a constant weight (minimum 48 hours in an incubator at 72 °C), and reweighed to allow measurement of the net particulate accumulation. Filter weighing was made to an accuracy of ± 1.0 mg and a precision of 0.5 mg on a Mettler PM 100 microbalance (USA Mettler Instruments Corporation, Hightstown, NJ) and was conducted in a perspex hood to decrease the effects of ambient air currents on the large filters.

Breathing zone airborne dust sampling

Dust within the ponies' breathing zone was measured with a 6-stage Marple Personal Cascade Impactor (Sierra Instruments Series 290, Sierra Instruments Inc., Carmel Valley, CA) attached to the halter at the angle of the jaw. The Genesis air sampler pump (Model gn-5, Ametek, Inc., Largo, FL) to operate the sampler was attached to a girth belt or surcingle and operated at 2 L/min. The pump flow was regularly calibrated using the Mini-buck calibrator (Model M-5, A.P. Buck, Inc., Orlando, FL). The airborne dust was collected onto 7 stages according to particle aerodynamic diameter (see Table 2). The upper two stages were considered to collect non-respirable dust. Airborne dust samples collected using this personal sampler categorized respirable dusts as those with aerodynamic diameter less than 6 μm. The samples were collected onto pre-weighed mylar media filters (C-290-MY, Sierra Instruments Inc.), which had been sprayed with a light coating of silicone grease to avoid possible particle bounce, and a back-up PTFE membrane filter (Quan-Tec-Air, Inc.). After a 24-hour equilibration period, the mass of particulates collected was measured using a Mettler AE 163 microbalance (USA Mettler Corp.) with a sensitivity of 0.01 g and precision of 0.02 g.

	Stage #	Cascade Impactor Type	
		Andersen	**Marple**
Non-respirable	1	> 8.95 μ	> 10.00 μ
	2	> 4.58 μ	> 6.00 μ
Respirable	3	> 2.78 μ	> 3.50 μ
	4	> 1.53 μ	> 1.55 μ
	5	-----	> 0.93 μ
	6	-----	> 0.52 μ
	Base	0	0

Table 2: Comparison of collection stage cut-off levels for the Andersen and Marple cascade impactors. The Andersen sampler divides the airborne dust onto 5 collection stages depending on particle aerodynamic diameter, whereas the Marple sampler has 7 collection stages.

Sampling periods

The sampling periods were selected based on pilot sampling protocols to ensure that an adequate measurable mass of dust was collected on each stage without exceeding the sampler's capacity and resulting in collection errors due to overload. The sampling times for operation of the samplers were as follows:

Conventional system (Stall and breathing zone measurements)	Day	7:00 a.m. - 1:00 p.m. 1:00 p.m. - 7:00 p.m.
	Night	7:00 p.m. - 7:00 a.m.
Recommended environment (Stall measurements only)	Day	7:00 a.m. - 7:00 p.m.
	Night	7:00 p.m. - 7:00 a.m.
Recommended environment (Stall and breathing zone measurements)	24 hrs	7:00 a.m. - 7:00 a.m.

In order to ensure that the capacity of the sampler was not overloaded during the sampling of the conventional environment, the daytime sampling period was divided into two 6-hour sections. This was not necessary for the recommended environment, where sampling was divided into two 12-hour periods. The daytime period described included all of the feeding, cleaning, and personnel movement. Each sampling protocol was started on Wednesday and continued for 7 days.

Sampling was initially conducted during summer using both the recommended and conventional management systems. Because of sampling problems, insufficient samples were obtained from the Marple personal impactor in the recommended environment. For this reason, the studies of the recommended environment were repeated during the following winter. The Andersen impactor was not available at that time so two Marple impactors were used simultaneously to measure both the breathing zone and stall dust levels.

The airborne dust concentration was calculated as follows:

$$\text{Dust concentration (mg/m}^3\text{)} = \frac{\text{mass of dust collected on relevant stages}}{\text{sampling time x flow rate}}$$

Comparison of Marple and Andersen sampler collection characteristics

An additional protocol was necessary in order to attribute any differences in the results obtained from the two samplers to a real difference in the environments measured rather than to methodological differences in the collection efficiencies of the different samplers. In this protocol, the airborne dust collection efficiencies of the two samplers were compared by running both samplers simultaneously, when both were stationary and measuring the same environment. The samplers were placed side by side approximately one meter above the floor in the center of the stall. The samplers were positioned one meter apart to avoid artifacts due to the "vacuum-cleaning" effect of the high-volume sampler filtering out the airborne dust, which would have resulted in an artificially low airborne dust concentration entering the smaller Marple sampler. All stalls were managed by the conventional management system, and the ponies were removed from the stall immediately prior to the start of sampling, and replaced after each sampling period was completed. Paired dust samples were collected for 6 hours on five consecutive days by the methods described previously.

Aeroallergen concentration sampling procedures

Airborne dust sample collection

A volumetric air sampler (Air Sentinel TM; Quan-Tec-Air, Inc., Rochester, MN) was placed on a shelf in the corner of the stall. Airborne dust particles were collected onto filters composed of PTFE membrane with a pore size of 0.3 μm with a fibrous backing layer (Quan-Tec-Air, Inc.). Samples were collected for 30 minutes at a calibrated flow rate of 3 L/second. Thirty-minute sampling periods, to represent a 24-hour period, were randomly spread over 5 days to avoid excessive influence of any daily variations in dust load or composition. After the sampling was completed, these filters were then labelled, and stored in individual sealed, plastic bags for further analysis at Mayo Clinic Allergy Research Laboratory.

Preparation of sample for analysis

The dust samples were prepared for the aeroallergen analysis by radioallergosorbent inhibition (RAST) immunoassay (Swanson, Agarwal, and Reed 1985, Campbell et al. 1989). The PTFE membrane, along with its deposited airborne dust sample, was separated from its fibrous backing and extracted overnight in 2-3 mls of 0.1 M phosphate buffer, 0.2% bovine serum albumin, pH 7.6, and 50% glycerine. The sample was then decanted into a Brinkmann vial (Brinkmann Instruments, Westbury, NY) and centrifuged at 10,000 rpm for five minutes to remove the solid particles. The supernatant was then decanted into a clean vial and stored at -20°C until analyzed (Campbell et al. 1989).

The allergens that were tested included those that are frequently implicated in the aetiopathogenesis of COPD by previous authors (Table 3) and included:

1) those that produce COPD signs after experimental inhalation challenge (McPherson et al. 1979a);

2) those that result in serum precipitins (Eriksen 1975, Lawson et al. 1979, Sasse 1975, Hockenjos, Mumcuoglu, and Gerber 1981, Zeitler 1984);

3) some of the bacteria and fungi that have been isolated from transtracheal washes (Mansmann et al. 1976);

4) some of the bacteria and fungi that have been cultured from air in stables (Clarke et al. 1987);

5) other aeroallergens, including those identified in swine barns (Donham et al. 1989) and dairy barns (Campbell et al. 1989).

The aeroallergens tested were:

Thermophilic fungi and actinomycetes:

Micropolyspora faeni

Aspergillus fumigatus

Thermoactinomyces vulgaris

Mites:

Lepidoglyphys destructor (storage mite)

Dermatophagoides pteronyssinus (house-dust mite)

	McPherson et al. 1979a	Eriksen 1975	Lawson et al. 1979	Sasse et al. 1975	Zeitler 1984	Mansmann et al. 1975	Clarke et al. 1987	Hockenjos et al. 1981	Donham 1986	Campbell et al. 1989
Micropolyspora faeni	x	x	x	x	x		x		x	x
Thermoactinomyces vulgaris	x	x	x	x	x		x		x	x
Aspergillus fumigatus	x	x	x		x	x			x	x
Streptomyces viridis						x	x		x	
Alternaria spp		x					x			x
Penicillium spp		x				x	x		x	
Mucor spp						x	x			
Cladosporium spp	x	x				x	x			
Lepidoglyphys destructor		x		x				x		x
Dermatophagoides spp		x		x				x		x

Table 3: Allergens in stable dust that have been implicated in the development of chronic obstructive pulmonary disease.

Measurement of filter eluates by radioimmunoassay

Quantification of the specific suspected allergens in the air filter eluates was performed by plate-RAST inhibition assays. The preparation of the solid-phases reference allergen was as follows: the various reference allergens (as per Campbell et al. 1989) were coated onto the surface of Immulon-2 microtitre Removawell strips (Dynatech Laboratories Inc., Alexandria, Va). Each well received 100 μl of reference allergen containing 1.0 μg of protein in 200 mmol/L of sodium bicarbonate buffer titrated with 200 mmol/L of sodium carbonate to pH 9.2. The plates were incubated at room temperature in a humid box and washed three times with RAST wash buffer (0.1 mol/L of phosphate buffer, 1% Tween 20) using a Nunc Immuno-wash 12 plate washer (Nunc-Inter Med, Copenhagen, Denmark). A standard inhibition curve was generated with a reference allergen standard.

The measurement of the allergenic activity of the air filter eluates was performed as follows: fifty μl aliquots of the test allergen at various concentrations were placed in the allergen-coated microtitre wells along with 50 μl of specific human antisera (as per Campbell et al. 1989) diluted appropriately. The plates were incubated at room temperature overnight in a humid box, washed five times, and 100 μl (20 ng) of 125^{I}-antihuman IgE or protein A for IgG antibodies were added to each well. The plate was incubated again at room temperature in a humid box for 18 hours. The plate was subsequently washed five times, and the removawell (Dynatech Laboratories, Inc.) strips were broken apart into 16 x 125 mm glass test tubes and counted in a gamma scintillation counter. Plate-RAST inhibition results were evaluated by regression analysis with a Hewlett-Packard 984B computer (Hewlett-Packard Co., Palo Alto, CA).

Statistical analysis of results

The results from the airborne dust and aeroallergen concentration calculations were tested for a normal distribution by the outliers test and the homogeneity of variances. Non-parametric statistical tests were selected for analysis of results, as the data were not normally distributed. The Mann Whitney-U test (equivalent to the students t test for independent samples) was used to compare results of the total airborne dust concentrations in the two management systems, the respirable airborne dust concentrations in the two systems, and the specific aeroallergen concentrations in the two systems. The total airborne dust concentration in the breathing zone (measured by the Marple personal cascade impactor) was compared to the total airborne dust concentration in the stall (measured by the Andersen cascade impactor) by the signed rank test as these were paired simultaneous samples. The signed rank test was also used to compare results of the respirable airborne dust concentration in the pony's breathing zone compared to the respirable airborne dust concentration in the stall. The results of the additional sampling protocol, where the collection efficiencies of the Andersen cascade impactor and the Marple personal sampler were compared when both were operating as stationary samplers, were analyzed using the signed ranks test. The confidence limits for all the statistical analyses was set at 95%.

CHAPTER 4

RESULTS

Effect of changes in management on the airborne dust concentrations in the stall

The total and respirable airborne dust concentrations in the stall were measured using an Andersen cascade impactor in the corner of the stall at the height of the pony's head. The mean total airborne dust concentrations were significantly higher when the barn was managed by the conventional method than when managed by the recommended method (Figure 5; Table 4). In the recommended management system, the total airborne dust concentration was 28% of the levels measured in the conventional management system. This was the case during both the daytime and the nighttime sampling periods. In both management systems, the mean total airborne dust concentration was significantly higher during the daytime period (7:00 a.m. - 7:00 p.m.), than during the nighttime period (7:00 p.m. - 7:00 a.m.). For both management systems, the total airborne dust concentrations measured at night were approximately 62% of the daytime values. During the week of sampling in the conventional system, the total airborne dust levels rose from Saturday to Monday, and then decreased to a midweek level (Figure 6). This elevation in total airborne dust over the weekend was most obvious in the samples collected during the night.

Total airborne dust concentrations remained at lower, fairly constant levels throughout the week in the recommended environment (Figure 7).

The mean concentrations of respirable airborne dust in the stall measured over 24 hours and during the daytime period were significantly higher in the conventional system than in the recommended system. In the recommended system, the 24-hour respirable airborne dust concentrations were 46% of those measured in the conventional system. The nighttime respirable dust concentrations did not differ significantly between the two management systems (Figure 8; Table 5). Despite the large, statistically significant diurnal variations in the total dust levels measured in both management systems, there were no diurnal changes in the levels of respirable dust in either management system. The respirable airborne dust concentrations measured in the stall under the conventional system increased towards the end of the week. This trend was not noted for the recommended environment (Figure 9).

Respirable dust formed a larger percentage of total dust in the recommended system than in the conventional system. In each system, the percentage of the total airborne dust that was respirable remained constant over 24 hours and there was no significant difference between daytime and nighttime values of the percentage respirable dust (Figure 10; Table 6).

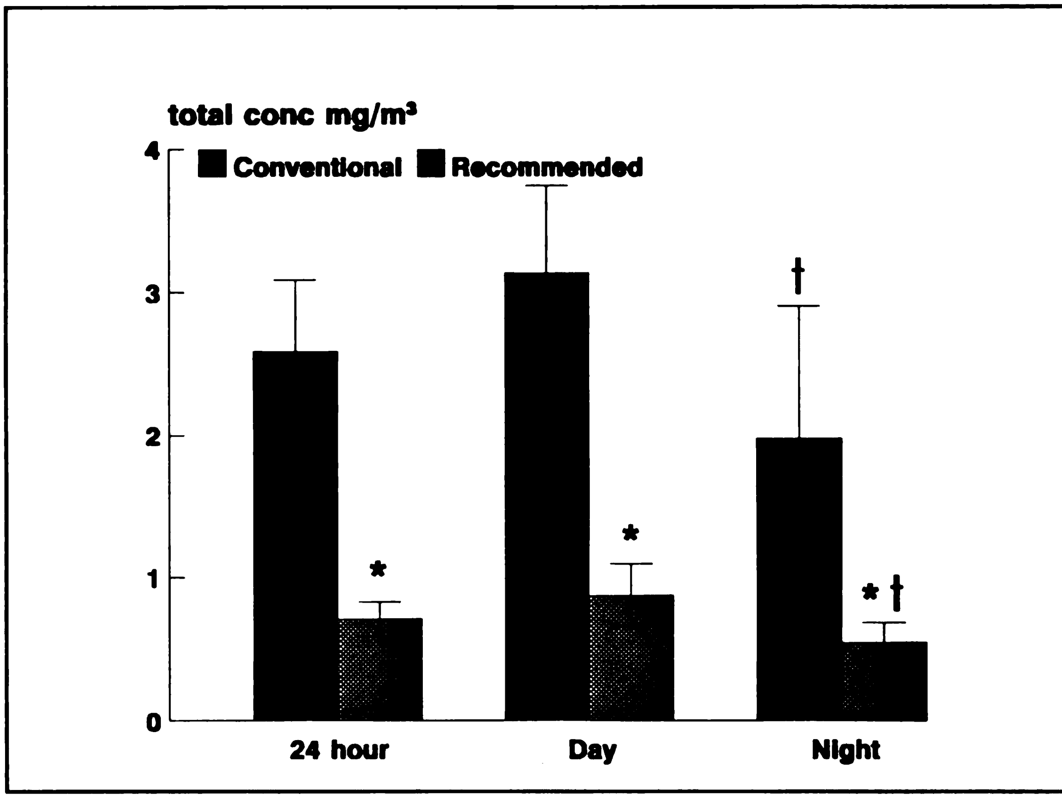

Figure 5: Comparison of the total airborne dust concentrations in the pony barn under the conventional and the recommended management systems (mean ± S.D.). The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The differences in the total airborne dust concentration between the two management systems were statistically significant; $P < 0.05$ (*). Under both management systems, dust levels at night were lower than those during the day; $P < 0.05$ (†).

	Total ADC 24 hrs	Total ADC day	Total ADC night
CS	2.587 ± 0.50 mg/m^3	3.136 ± 0.61 mg/m^3	1.975 ± 0.93 mg/m^3†
RE	0.709 ± 0.12 mg/m^3*	0.874 ± 0.22 mg/m^3*	0.545 ± 0.14 mg/m^3†*

Table 4: Total airborne dust concentrations (Total ADC) in the pony barn under the conventional (CS) and the recommended (RE) management systems during different time periods (mean ± S.D.). The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The differences in the total airborne dust concentration between the two management systems were statistically significant; $P < 0.05$ (*). Under both management systems, dust levels at night were lower than those during the day; $p < 0.05$ (†).

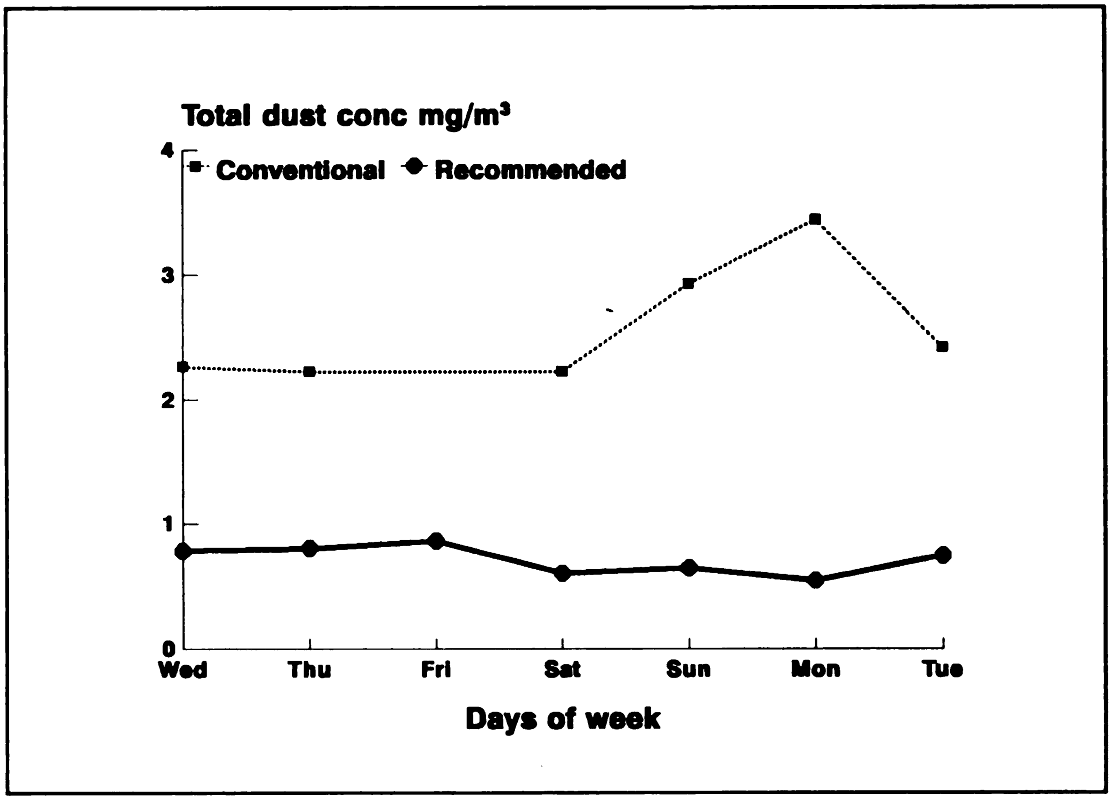

Figure 6: Twenty-four hour total airborne dust concentrations in the stall. Daily values are shown for each management system. Sampling started on Wednesday after a three-day equilibration period following the initiation of the new system. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings.

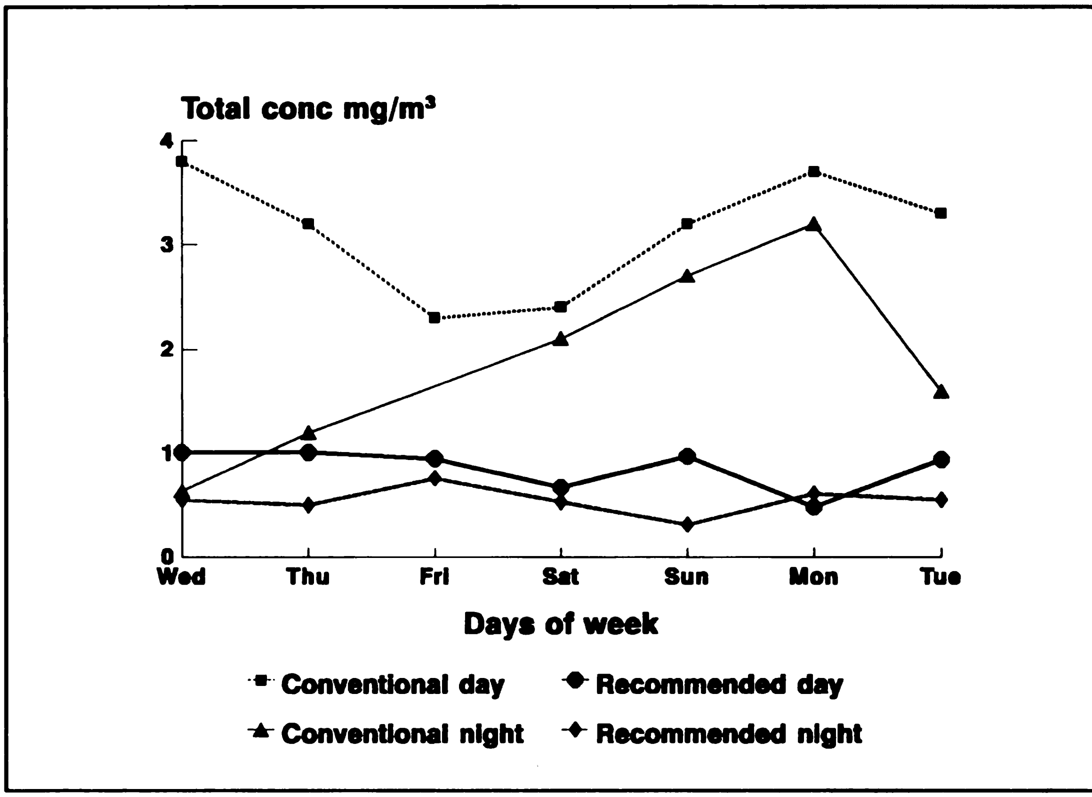

Figure 7: Twelve-hour total airborne dust concentrations in the stall. Daily values are shown for each management system. The daytime samples were collected from 7:00 a.m. to 7:00 p.m., during which time all cleaning and feeding activities were conducted. The nighttime samples were collected from 7:00 p.m. to 7:00 a.m. Sampling started on Wednesday in each system after a three-day equilibration period following the initiation of the new system. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings.

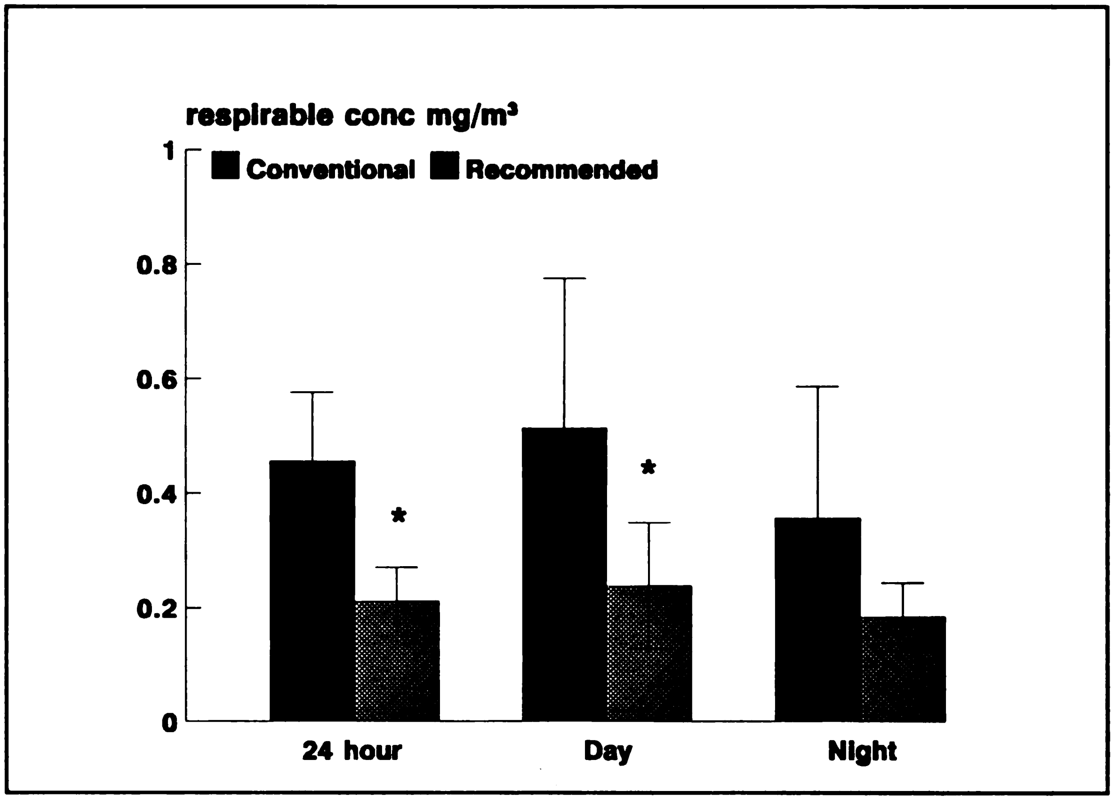

Figure 8: Comparison of the respirable (aerodynamic diameter < 4.58 μ) airborne dust concentrations in the stall under the conventional and recommended management systems (mean ± S.D.). Dust levels were measured with the Andersen cascade impactor. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. Respirable airborne dust concentrations measured during the day (7:00 a.m. to 7:00 p.m.) and over the 24-hour period differed significantly between the two management systems; P < 0.05 (*).

	Resp. ADC 24 hrs	Resp. ADC day	Resp. ADC night
CS	0.456 ± 0.12 mg/m^3	0.514 ± 0.26 mg/m^3	0.356 ± 0.23 mg/m^3
RE	0.211 ± 0.06 mg/m^{3*}	0.238 ± 0.11 mg/m^{3*}	0.183 ± 0.06 mg/m^3

Table 5: Comparison of the respirable (aerodynamic diameter < 4.58 μ) airborne dust concentrations (Resp. ADC) in the stall under the conventional (CS) and recommended (RE) management systems during different time periods (mean ± S.D.). Dust levels were measured with the Andersen cascade impactor. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. Respirable airborne dust concentrations measured during the day and over the 24-hour period differed significantly between the two management systems; $P < 0.05$ (*).

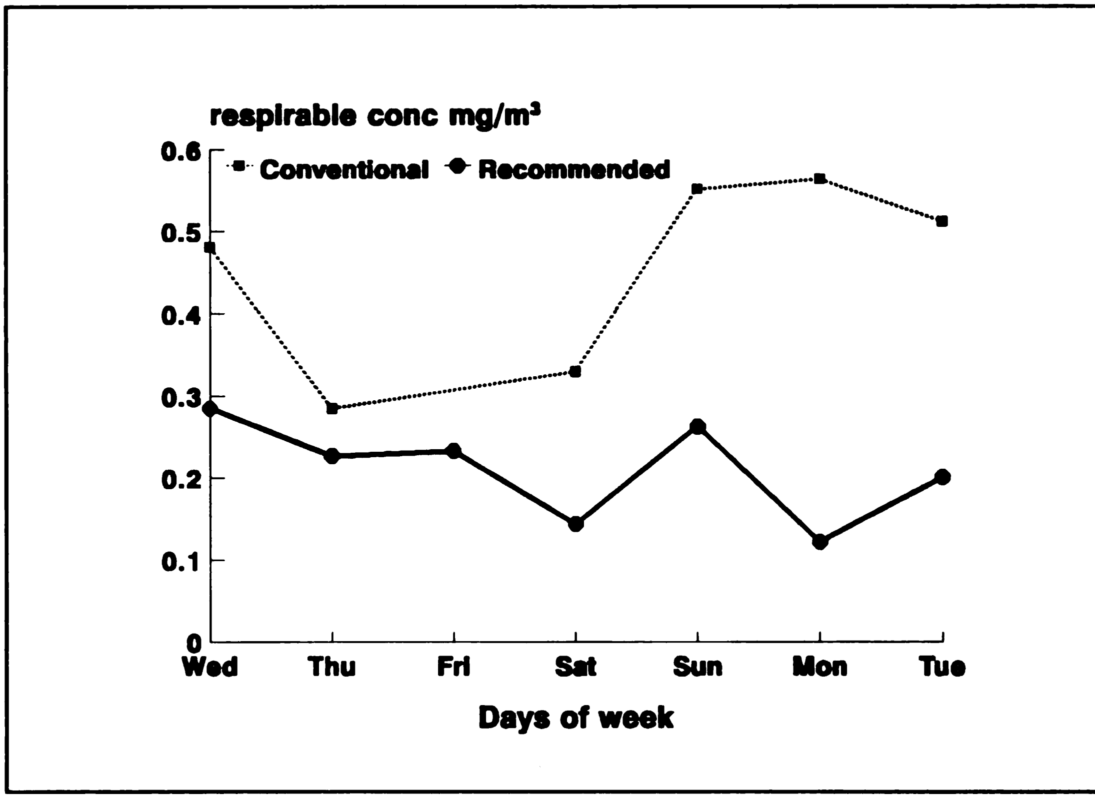

Figure 9: Twenty-four hour respirable airborne dust concentrations measured in the stall by the Andersen cascade impactor. Daily values are shown for each management system. Sampling started on Wednesday after a three-day equilibration period following the initiation of the new system. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings.

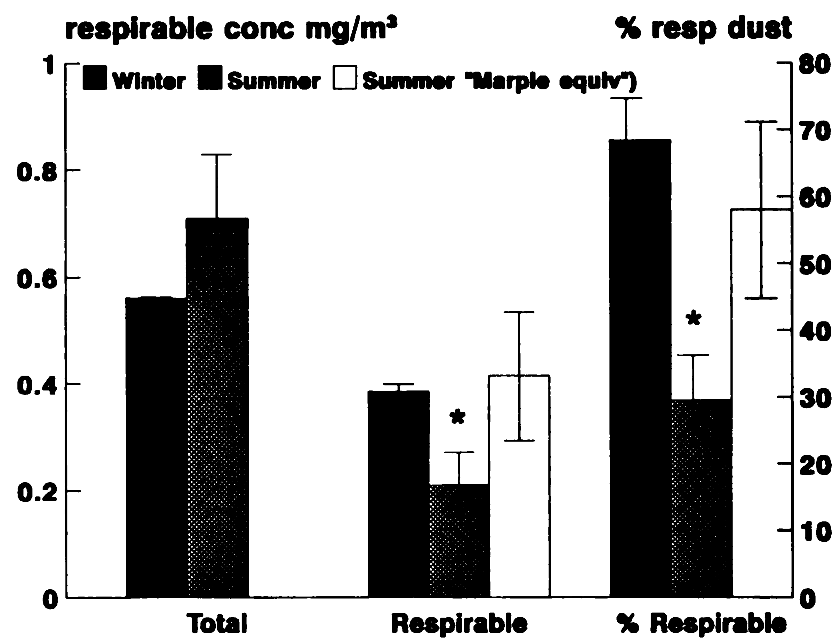

Figure 10: The percentage of the total airborne dust concentration that was respirable (aerodynamic diameter < 4.58 μ) in the stall under the conventional and recommended management systems (mean ± S.D.). Dust levels were measured with the Andersen cascade impactor. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The differences between the two management systems were statistically significant for each time period; $P < 0.05$ (*). In each system the percentage of the total dust that was respirable remained constant over the 24-hour period.

	%Resp.ADC 24 hrs	%Resp. ADC day	%Resp. ADC night
CS	17.52% ± 3.5	15.80% ± 6.0	17.36% ± 6.4
RE	29.51% ± 6.7*	26.38% ± 9.9*	33.83% ± 9.5*

Table 6: The percentage of the total airborne dust concentration that was respirable (%Resp. ADC) (aerodynamic diameter < 4.58 μ) in the stall under the conventional (CS) and recommended (RE) management systems (mean ± S.D.). Dust levels were measured with the Andersen cascade impactor. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The differences between the two management systems were statistically significant for each time period; P < 0.05 (*).

Effect of changes in management on the airborne dust concentrations in the pony's breathing zone

The total and respirable airborne dust concentrations in the pony's breathing zone were measured with a Marple cascade impactor attached to the pony's halter. The mean total and respirable breathing zone dust concentrations were thirty-eight times higher when the barn was managed by the conventional method than when managed by the recommended method (Figure 11; Table 7). Respirable dust formed approximately half of the total dust in both management systems. Under the conventional system, the total and respirable airborne dust concentrations were significantly higher during the day than at night, but the percentage of dust that was respirable did not alter (Figure 12; Table 8). Owing to the low total airborne dust concentration in the breathing zone and the low flow rate of the Marple sampler, it was not possible to collect enough dust in 12 hours to distinguish differences in daytime and nighttime dust concentrations under the recommended system. The patterns of dust concentration observed throughout the week in the stall were also apparent in the breathing zone (Figure 13).

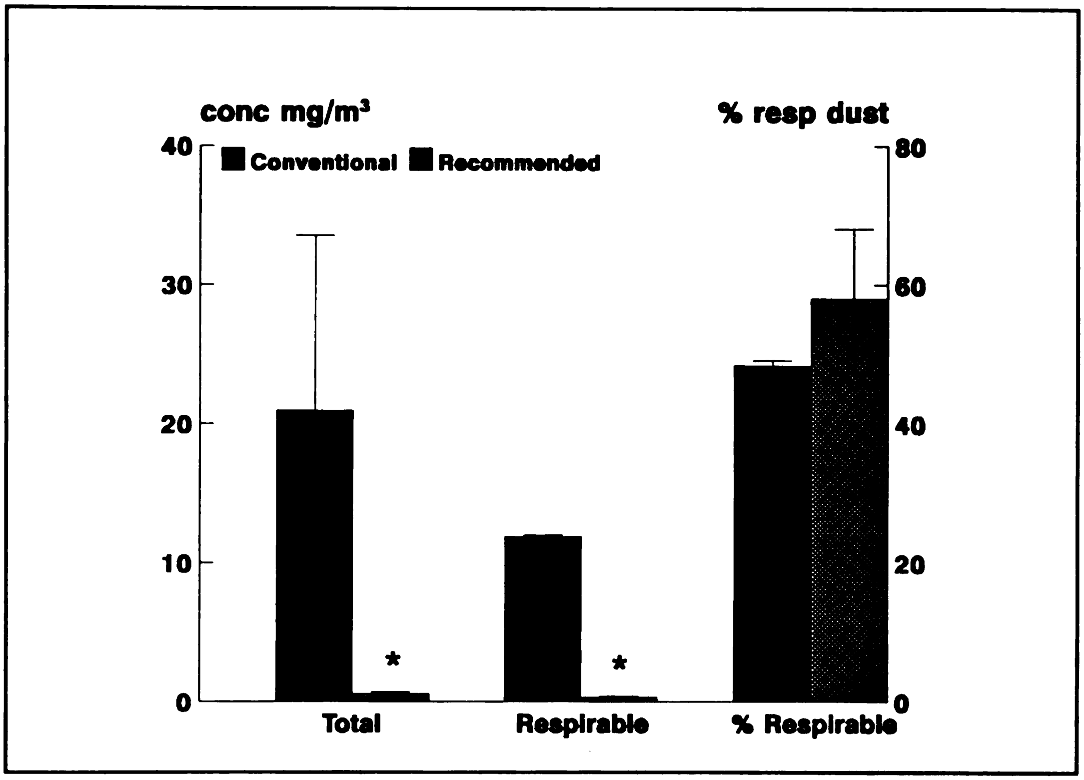

Figure 11: Total and respirable airborne dust concentrations and percentage of total dust that was respirable in the breathing zone under the conventional and the recommended management systems (mean ± S.D.). Dust levels were measured with the Marple impactor and respirable particles were < 6.0 μ. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The differences between the two management systems in the total and respirable airborne dust concentrations were statistically significant; $P < 0.05$ (*).

	Total ADC 24 hrs	Resp. ADC 24 hrs	%Resp. ADC 24 hrs
CS	20.95 ± 12.6 mg/m^3	11.89 ± 0.1 mg/m^3	48.34% ± 1.1
RE	0.551 ± 0.17 mg/m^{3}*	0.312 ± 0.09 mg/m^{3}*	58.0% ± 10.3

Table 7: Total and respirable airborne dust concentrations (ADC) and percentage of total dust that was respirable in the breathing zone under the conventional (CS) and the recommended (RE) management systems (mean ± S.D.). Dust levels were measured with the Marple impactor and respirable particles were < 6.0 μ. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The differences between the two management systems in the total and respirable airborne dust concentrations were statistically significant; P < 0.05 (*).

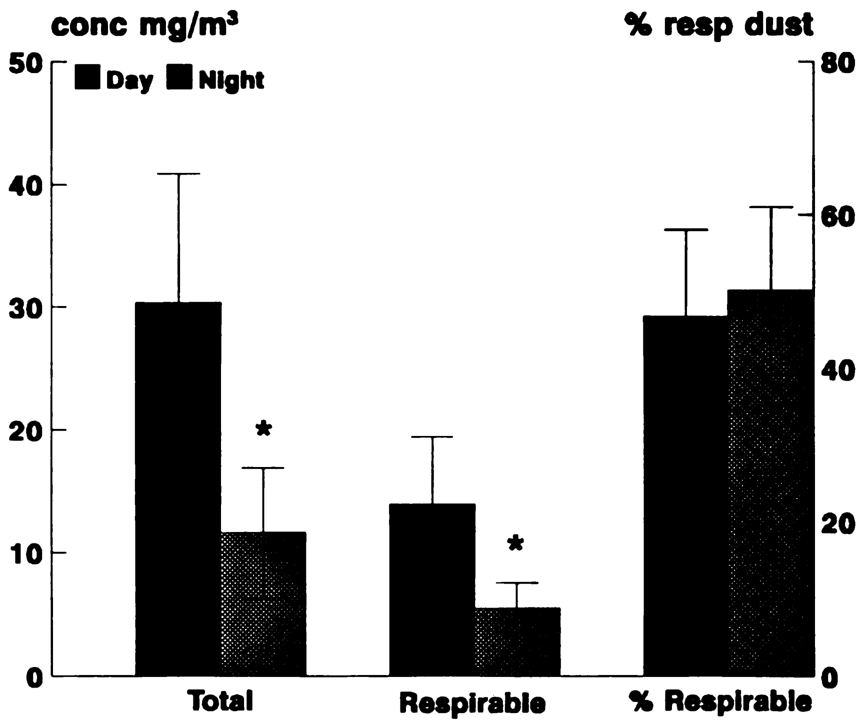

Figure 12: Day and night levels of total and respirable airborne dust and the percentage of total dust that was respirable in the breathing zone under the conventional management system (mean ± S.D.). Dust levels were measured with the Marple impactor and respirable particles were < 6.0 μ. The daytime samples were collected from 7:00 a.m. to 7:00 p.m., during which time all cleaning and feeding activities were conducted. The nighttime samples were collected from 7:00 p.m. to 7:00 a.m. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The differences between the day and night concentrations of total and respirable dust were statistically significant; $P < 0.05$ (*).

	Total ADC 24 hrs	Resp. ADC 24 hrs	%Resp. ADC 24 hrs
Day BZ CS	30.35 ± 10.5 mg/m^3	13.92 ± 5.5 mg/m^3	46.78% ± 11.24
Night BZ CS	11.62 ± 5.3 mg/m^{3*}	5.52 ± 2.0 mg/m^{3*}	50.11% ± 10.92

Table 8: Day and night levels of total and respirable airborne dust concentrations (ADC) and percentage of total dust that was respirable in the breathing zone (BZ) under the conventional management system (CS) during the different time periods (mean ± S.D.). Dust levels were measured with the Marple impactor and respirable particles were < 6.0 μ. The daytime samples were collected from 7:00 a.m. to 7:00 p.m., during which time all cleaning and feeding activities were conducted. The nighttime samples were collected from 7:00 p.m. to 7:00 a.m. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The differences between the day and night concentrations of total and respirable dust were statistically significant; P < 0.05 (*).

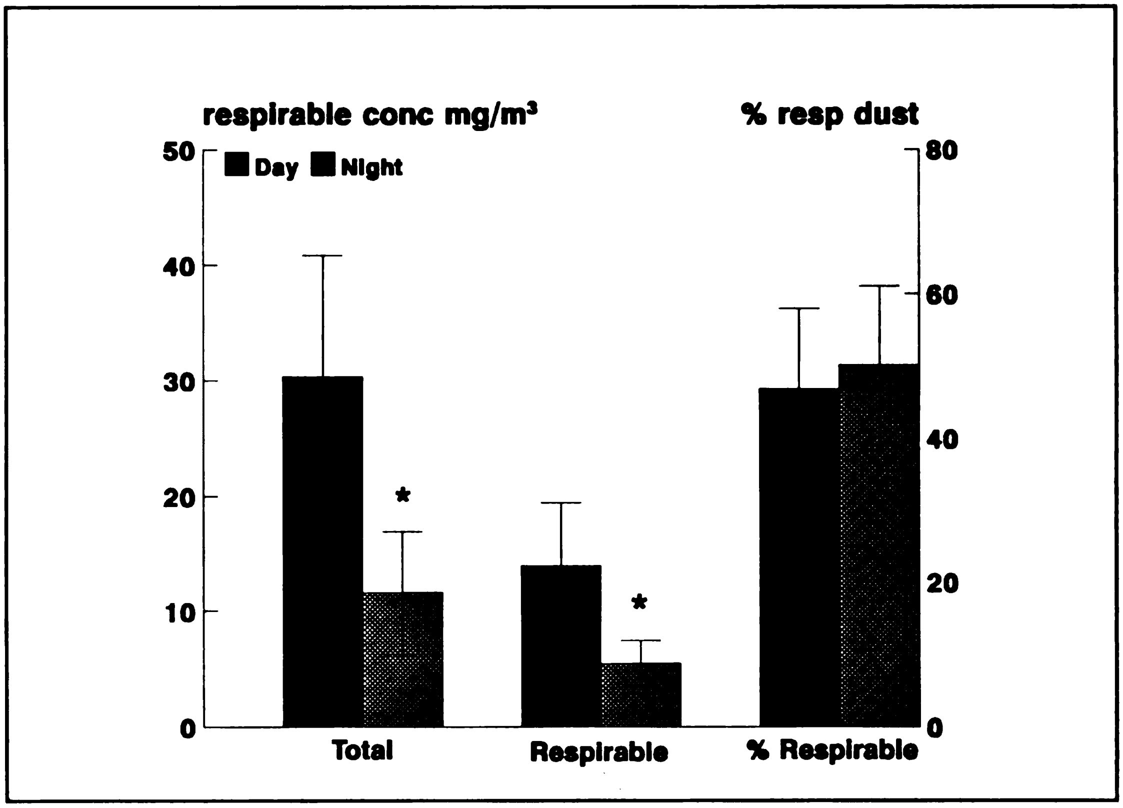

Figure 13: Twelve-hour total airborne dust concentrations measured in the stall and breathing zone under the conventional management system. Daily values are shown. The airborne dust in the stall was measured with an Anderson impactor in the corner of the stall at the height of the pony's head. The airborne dust concentration in the breathing zone was measured with a Marple personal cascade impactor attached to the pony's halter. The daytime samples were collected from 7:00 a.m. to 7:00 p.m., during which time all cleaning and feeding activities were conducted. The nighttime samples were collected from 7:00 p.m. to 7:00 a.m. Sampling started on Wednesday in each system after a three-day equilibration period following the initiation of the new system. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding.

Comparison of total and respirable airborne dust concentrations measured simultaneously by the Andersen impactor and the Marple personal impactor

In order to compare the dust levels measured with the Andersen impactor with those measured with the Marple impactor, it was necessary to establish the relationship between the sampling efficiencies of the two impactors. For this reason, the two impactors were used simultaneously as stationary samplers to measure airborne dust concentrations in the stall under the conventional management system. The mean total airborne dust concentrations measured by the two samplers did not differ. However, the respirable airborne dust concentrations and percentage respirable dust measured by the two different samplers were significantly different (Table 9). The latter result was not surprising, because the cut-off point for respirable dust differed between the two samplers. The Andersen cascade impactor classified all airborne particles less than 4.58 μ as respirable, while the Marple cascade impactor had 6.0 μ and less as the cut-off diameter for respirable dust. Therefore, a greater proportion of the total airborne dust was classified as respirable by the Marple sampler than by the Andersen sampler.

A conversion factor was required to compare the respirable levels measured with the Andersen sampler with those measured by the Marple sampler. It was assumed that the aerodynamic size and mass distribution profile remained constant over the sampling period. Using the paired daily results of respirable dust concentrations from the simultaneous sampling with both the Andersen and Marple samplers, the ratio of the Andersen respirable dust concentration to the Marple respirable dust concentration was calculated. The mean ratio was 0.509 ± 0.23. The same procedure was repeated for percentage respirable dust and the ratio was 0.509 ± 0.27.

	Total ADC 24 hrs	Resp. ADC 24 hrs	%Resp. ADC 24 hrs
Andersen CS[a]	2.338 ± 0.60 mg/m^3	0.715 ± 0.28 mg/m^3	31.28% ± 4.8
Marple CS[b]	2.366 ± 0.84 mg/m^3	1.505 ± 0.61 mg/m^{3*}	63.3% ± 4.9*

Table 9: Comparison of the total and respirable airborne dust levels in the stall under the conventional management system (CS) during the simultaneous sampling during summer. An Andersen sampler and a Marple sampler were used to measure the airborne dust concentrations (ADC) within the stall (mean ± S.D.). The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. [a] = airborne dust sampling conducted during summer with an Andersen sampler; [b] = simultaneous airborne dust sampling conducted during summer with a Marple sampler. The differences between the two samplers in the measurement of respirable and percentage respirable dust were statistically significant; $P < 0.05$ (*).

Comparison of airborne dust concentrations in the stall under the recommended management system in summer and winter

In the recommended management system, there was no significant difference between the total airborne dust concentrations in the stall measured during the summer using an Andersen cascade impactor, and those measured during the winter with a Marple cascade impactor. However, the respirable airborne dust concentrations and the percentage of the total dust that was respirable measured by the two different samplers were significantly different (Table 10). If the difference had been due simply to differences in the cut-off points of the two impactors, the ratio of Andersen (summer) to Marple (winter) respirable dust concentrations should not differ from the ratio calculated when the two samplers were used in the same environment (see previous section). The mean ratio, Andersen (summer)/Marple (winter) was 54.81, not significantly different from the simultaneously measured Andersen/Marple ratio of 50.89. Therefore, these differences were attributed to the different cut-off points for respirable dust in the two samplers, rather than being a real difference in concentrations.

To eliminate the difference in the aerodynamic diameter cut-off level for respirable dust between the two impactors, the individual Andersen results were multiplied by the ratio determined in the previous section in order to provide "Marple equivalent" values (Figure 14; Table 10). After conversion, there was no significant difference in either the stall concentrations of respirable dust or in the percentage of respirable dust measured in the recommended environment in summer and in winter.

	Total ADC 24 hrs	Resp. ADC 24 hrs	%Resp. ADC 24 hrs
Summer Andersen[a]	0.709 ± 1.12 mg/m^3	0.211 ± 0.06 mg/m^{3*}	29.5% ± 6.7*
Summer "Marple Equiv"[b]	n/a	0.414 ± 0.12 mg/m^3	58.0% ± 13.3
Winter Marple[c]	0.561 ± 0.19 mg/m^3	0.385 ± 0.14 mg/m^3	68.4% ± 6.3

Table 10: Seasonal comparisons of the total and respirable airborne dust concentrations and percentage of total dust that was respirable in the stall under the recommended (RE) management system (value ± S.D.). The Andersen sampler was used during the summer and the Marple sampler during the winter. The respirable concentrations within the stall measured by the Andersen impactor are given as actual results and as converted "Marple equivalent" values. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. [a] = airborne dust sampling conducted during summer with an Andersen sampler; [b] = Andersen results converted to "Marple equivalents"; [c] = airborne dust sampling conducted during winter with a Marple sampler. The differences in the respirable airborne dust concentrations, and the percentage of respirable airborne dust differed significantly between the summer and winter only prior to conversion of the summer data to "Marple equivalents"; $P < 0.05$ (*).

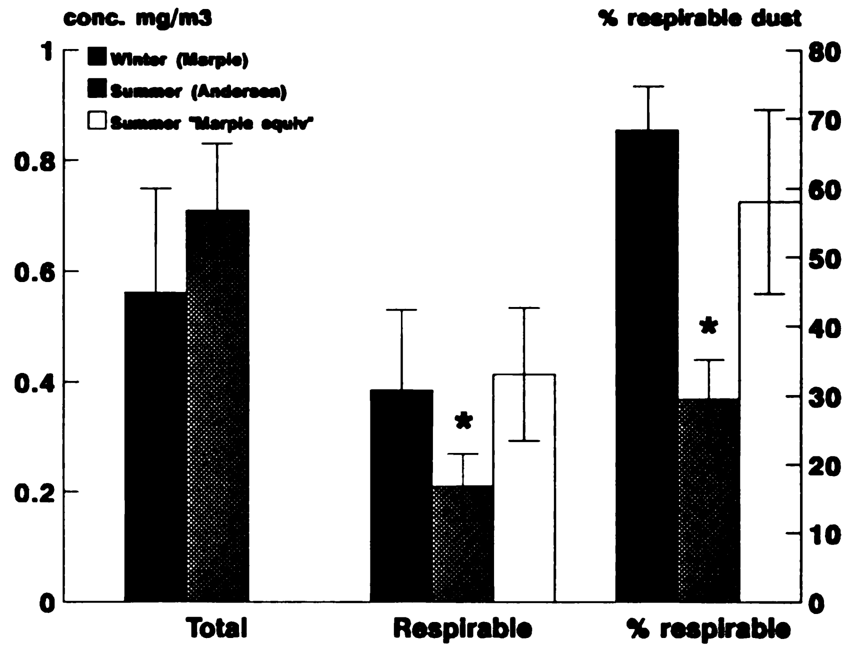

Figure 14: Seasonal comparisons of the total and respirable airborne dust concentrations and percentage of total dust that was respirable in the stall under the recommended management system (mean ± S.D.). The Andersen sampler was used during the summer and the Marple sampler during the winter. The respirable concentrations within the stall measured by the Andersen impactor are given as actual results and as converted "Marple equivalent" values. The differences in the respirable airborne dust concentration, and the percentage of the total airborne dust that is considered respirable differed significantly between the summer and winter only prior to conversion of the summer data to "Marple equivalents"; $P < 0.05$ (*). The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings.

Comparison of airborne dust concentrations in the stall and breathing zone under the two management systems

The mean total airborne dust concentration in the pony's breathing zone under the conventional management system was 20.95 ± 12.6 mg/m^3. This value was significantly higher, by a factor of 8, than the simultaneously measured stall levels (Figure 15; Table 11). Even though total dust levels decreased at night, the difference between stall and breathing zone persisted. In contrast, under the recommended management system, the total airborne dust concentrations in the stall (0.561 ± 0.20 mg/m^3) and breathing zone (0.551 ± 0.17 mg/m^3) did not differ significantly.

The respirable airborne dust concentrations measured by the Andersen cascade impactor used in the conventional management system were converted (as described in the previous section) to the Marple-equivalent values for statistical analyses. The mean 24-hour respirable dust concentrations sampled in the pony's breathing zone under the conventional management system were ten times higher than the levels in the stall (Figure 16; Table 12). Although the stall levels of respirable dust did not differ between day and night, the breathing zone levels decreased significantly at night. In contrast, the mean 24-hour respirable airborne dust concentrations in the stall (0.385 ± 0.14) and breathing zone (0.312 ± 0.09) under the recommended management system were not significantly different.

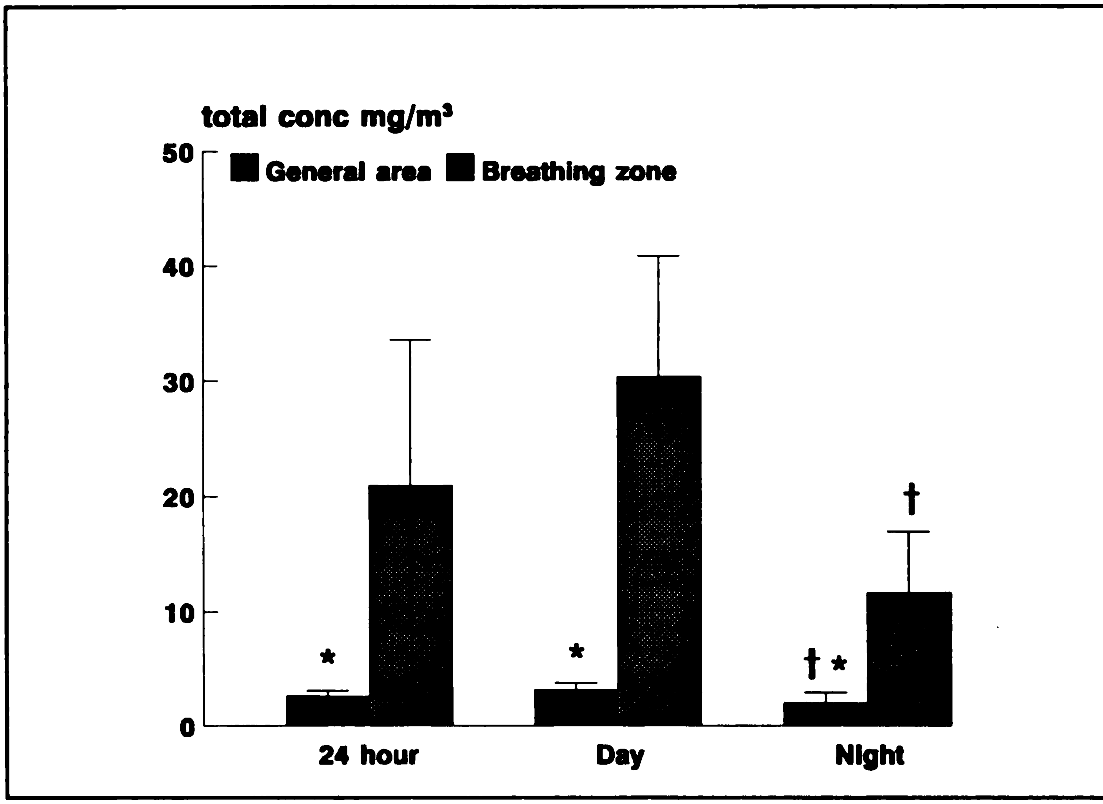

Figure 15: Total airborne dust concentrations in the stall and in the pony's breathing zone under the conventional management system (mean ± S.D.). The airborne dust in the stall was measured with an Andersen cascade impactor in the corner of the stall at the height of the pony's head. A Marple personal cascade impactor attached to the pony's halter was used to measure the airborne dust concentration in the pony's breathing zone. The barn was managed by the conventional management system, which induces signs of COPD in susceptible ponies, and utilized hay feed and straw bedding. The differences in the total airborne dust concentration between the two regions were statistically significant at all time periods; $P < 0.05$ (*). The daytime values of total airborne dust were significantly different from the nighttime values in each region; $P < 0.05$ (†).

	Total ADC 24 hrs	Total ADC day	Total ADC night
Stall	2.587 ± 0.50 mg/m^3	3.136 ± 0.61 mg/m^3	1.975 ± 0.93 mg/m^3†
BZ	20.95 ± 12.6 mg/m^3*	30.35 ± 10.5 mg/m^3*	11.62 ± 5.3 mg/m^3*†

Table 11: Total airborne dust concentrations (ADC) in the stall and in the pony's breathing zone (BZ) under the conventional management system (mean ± S.D.). The airborne dust in the stall was measured with an Andersen cascade impactor in the corner of the stall at the height of the pony's head. A Marple personal cascade impactor attached to the pony's halter was used to measure the airborne dust concentration in the pony's breathing zone. The barn was managed by the conventional management system, which induces signs of COPD in susceptible ponies, and utilized hay feed and straw bedding. The differences in the total airborne dust concentrations between the two regions were statistically significant at all time periods; $P < 0.05$ (*). The daytime values of total airborne dust were significantly different from the nighttime values in each region; $P < 0.05$ (†).

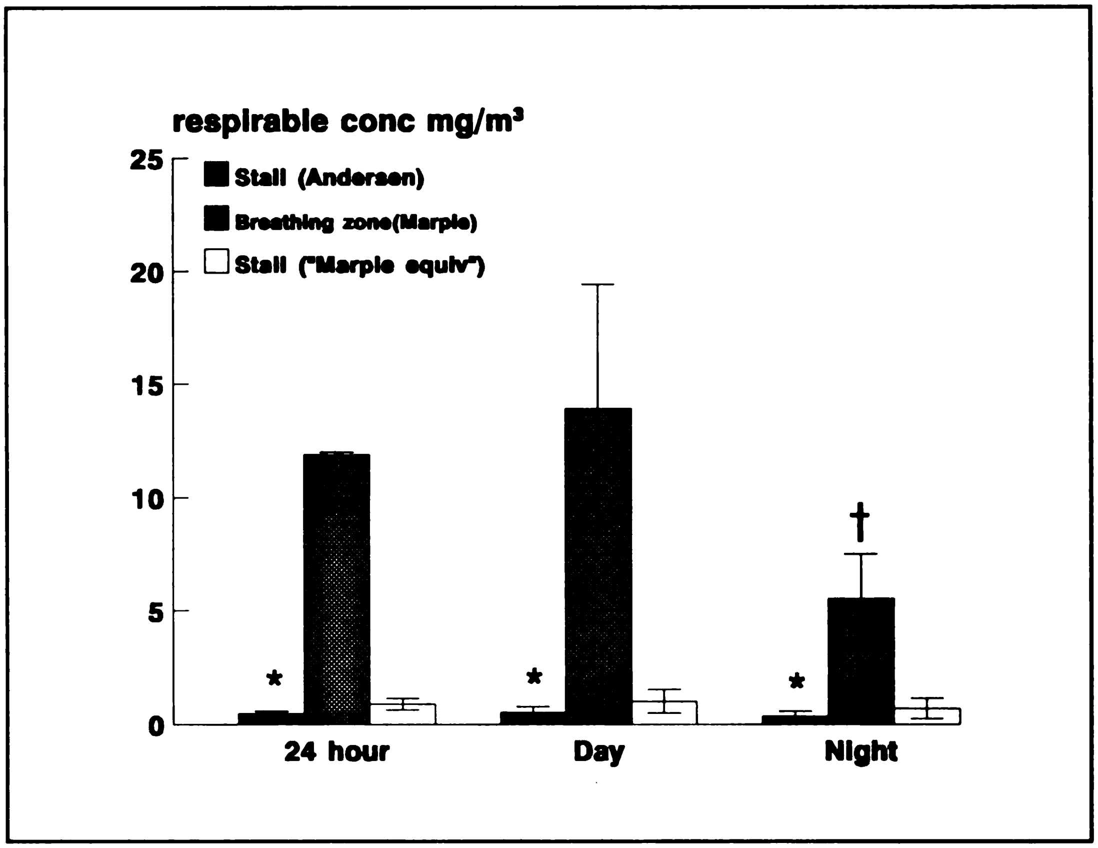

Figure 16: Respirable airborne dust concentrations in the stall and in the pony's breathing zone under the conventional management system (mean ± S.D.). The concentration in the stall were measured with an Andersen cascade impactor in the corner of the stall at the height of the pony's head. Values are expressed as actual results and as converted "Marple equivalents." A Marple personal cascade impactor attached to the pony's halter measured the breathing zone concentrations. The differences in the respirable airborne dust concentration between the two regions were statistically significant at all time periods both before and after conversion to "Marple equivalents"; $P < 0.05$ (*). The daytime values of respirable airborne dust were significantly different from the nighttime values only in the breathing zone; $P < 0.05$ (†).

	Resp. ADC 24 hrs	Resp. ADC day	Resp. ADC night
Stall	0.456 ± 0.12 mg/m^3*	0.514 ± 0.26 mg/m^3*	0.356 ± 0.23 mg/m^3*
Stall Marple equiv.	0.896 ± 0.26 mg/m^3*	1.010 ± 0.51 mg/m^3*	0.699 ± 0.45 mg/m^3*
BZ	11.89 ± 0.1 mg/m^3	13.92 ± 5.5 mg/m^3	5.52 ± 2.0 mg/m^3†

Table 12: Respirable airborne dust concentrations in the stall and in the pony's breathing zone (BZ) under the conventional (CS) management system (mean ± S.D.). The airborne dust concentration in the stall was measured with an Andersen cascade impactor in the corner of the stall at the height of the pony's head. Values are expressed as actual results and as converted "Marple equivalents." A Marple personal cascade impactor attached to the pony's halter was used to measure the airborne dust concentration in the pony's breathing zone. The barn was managed by the conventional management system, which induces signs of COPD in susceptible ponies, and utilized hay feed and straw bedding. The differences in the respirable airborne dust concentration between the two regions were statistically significant at all time periods, both before and after conversion to "Marple equivalents"; $P < 0.05$ (*). The daytime values of respirable airborne dust were significantly different from the nighttime values only in the breathing zone; $P < 0.05$ (†).

Comparison of the aeroallergen concentrations in the stall under the conventional and recommended management systems

The mean concentrations of the major aeroallergens that are suspected as being involved in the aetiopathogenesis of COPD were significantly higher in the conventional system than the recommended management system. The concentrations of *Micropolyspora faeni, Aspergillus fumigatus,* and *Lepidoglyphys destructor* aeroallergens were similar to each other, and their airborne concentration in the stall under the conventional management system was double that in the recommended system (Figure 17; Table 13). *T. vulgaris* levels were lower than those of the other aeroallergens and also decreased in the recommended environment.

The aeroallergen concentration in each management system was calculated in terms of the mass of aeroallergen per unit mass of airborne dust (Table 14). With the exception of *T. vulgaris*, the concentration of aeroallergen per unit mass of total airborne dust was lower in the conventional system than in the recommended system. When only dust of respirable size was considered, the mass of aeroallergen per unit mass of respirable dust was similar in both management systems.

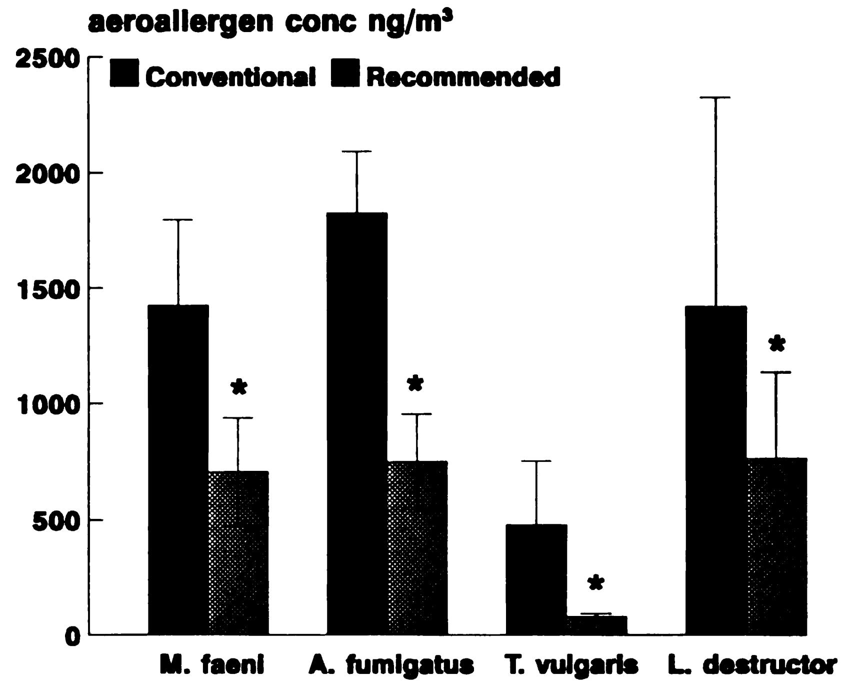

Figure 17: Aeroallergen concentrations in the stall under the conventional and the recommended management systems (mean ± S.D.). The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The aeroallergens were volumetrically filtered using a sampler in the corner of the stall, and their concentrations were measured using the radioallergosorbent inhibition immunoassay. The differences in aeroallergen concentrations between the two management systems were statistically significant; $P < 0.05$ (*).

	CS	RE
Micropolyspora faeni	1423 ± 374 ng/m^3	705 ± 233 ng/m^{3*}
Aspergillus fumigatus	1823 ± 268 ng/m^3	748 ± 204 ng/m^{3*}
Thermoactinomyces vulgaris	502 ± 273 ng/m^3	79 ± 15 ng/m^{3*}
Lepidoglyphys destructor	1420 ± 905 ng/m^3	761 ± 373 ng/m^{3*}

Table 13: Aeroallergen concentrations in the stall under the conventional (CS) and the recommended (RE) management systems (mean ± S.D.). The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The aeroallergens were volumetrically filtered using a sampler in the corner of the stall, and their concentrations were measured using the radioallergosorbent inhibition immunoassay. The differences in the aeroallergen concentrations between the two management systems were statistically significant; $P < 0.05$ (*).

	M. faeni		*A. fumigatus*		*L. destructor*		*T. vulgaris*	
	CS	RE	CS	RE	CS	RE	CS	RE
Aeroallergen conc. (ng/m^3)	1423	705	1823	748	1420	761	502	79
Aeroallergen mass/ total dust mass (ng/mg)	550	994	705	1055	549	1073	194	111
Aeroallergen mass/ respirable dust mass (ng/mg)	3121	3341	3998	3545	3114	3606	1101	374

Table 14: Aeroallergen concentrations in the stall under the conventional (CS) and the recommended (RE) management systems (mean value over 24 hours ± S.D.). Aeroallergen concentrations are ng allergen per m^3 of air sampled, ng allergen per mg of total airborne dust, and as ng allergen per mg of respirable airborne dust. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The aeroallergens were volumetrically filtered using a sampler in the corner of the stall, and their concentrations were measured using the radioallergosorbent inhibition immunoassay.

CHAPTER 5

DISCUSSION

In this study, the airborne dust and aeroallergens levels were measured in a horse barn under a conventional management systems, which utilized straw bedding and hay feed, and in a recommended management system with wood shaving bedding and a complete pelleted diet with no exposure to hay or straw. This second system has been recommended as a suitable management system for the housing of ponies susceptible to COPD (Grunig et al. 1989, Thomson and McPherson 1984, Clarke 1987, Beech 1989). The study demonstrated that the recommended environment management system was more suitable for the housing of COPD-susceptible animals than the conventional management system. Before discussing the results of the study, it is important to consider the limitations of the measurement techniques used, and their impact on the data obtained.

The use of cascade impactors to measure airborne dust concentration

Cascade impactors were used for the gravimetric determination of the airborne dust concentration in both management systems. Cascade impactors divide the total airborne dust into fractions based on particle aerodynamic diameter, which allows the measurement of the airborne concentration of particles in a specific size range. The gravimetric determination of airborne dust concentration using cascade impactors could be considered superior to the alternative "particle counting" methods, as the cascade impactor results are reported as a mass per unit volume. This allows the estimation of an inhalation challenge or dose in mg rather than as a count.

In the present investigation, both an Andersen and a Marple cascade impactor were used. Different collection periods were required for both the Andersen sampler operating with a flow rate of 220 l/min and the Marple sampler operating at 2 l/min. The exact coordination of the times required to simultaneously collect adequate samples was not possible in the recommended management system. When measuring in areas of high airborne dust concentrations, such as in the pony barn during cleaning and feeding, an appropriate sampling period must be established to ensure accurate sample collection. If the sampling period is too short, not enough dust is collected onto each stage, and the total airborne dust concentration cannot be calculated. However, the cascade impactors have upper limits of collection and if these are exceeded the aerodynamic sizing of the stages is not valid. Although exceeding these upper limits may not alter the total airborne dust concentration measurement, it may affect the separation of total dust into its respirable fractions. These sampling errors were avoided by establishing suitable sampling periods in pilot protocols during which the stages of the cascade impactor were examined for evidence of particle bounce or unusual deposition patterns on the filters.

One of the problems encountered in the use of the two samplers was the different cut-off limits for respirable and non-respirable dust. A conversion factor was therefore calculated before the respirable airborne dust concentrations from the two samplers were compared. As pointed out in the results section, this conversion factor required that the distribution profile of the mass of airborne particles in certain aerodynamic size ranges remained fairly constant over the sampling period. This is because the ratio of total to respirable dust that was calculated using the results of one sampling period is used in calculations for other sampling periods and is assumed to be the same during other sampling periods in the same management system.

The Marple personal cascade impactor was more convenient to use and more suited for the sampling in the stalls than the large, stationary Andersen cascade impactor. The small, compact size and light weight of the Marple sampler meant that it was easy to handle and to transport to and from the area where the filters were weighed. The Andersen sampler required a secure, level surface on which to operate, whereas the Marple sampler could be suspended from the ceiling or attached to a cord. The Andersen sampler required a protective grille to prevent the sampling head from being knocked off the vacuum pump, and the arrangement was less rugged than the Marple sampler. The vacuum pump for the Marple sampler was battery operated, which was more suited for use in livestock buildings than the Andersen sampler, which required an electrical outlet. In addition, the filters for use on the two samplers were different. The Marple mylar collection filters were much smaller (34-mm diameter) than the Andersen filters, and although the mylar filters required at least 24 hours in the laboratory environment with a relative humidity of less than 50% before weighing, they were not hygroscopic like the cellulose Andersen filters, and did not require desiccation. The Andersen sampler used large (300-mm diameter) cellulose filters that were hygroscopic and required 24-hour drying periods before and after sampling, and the availability of additional heating/drying equipment. The large, floppy Andersen filters had to be weighed in a enclosed area so that the air currents did not lift the filters and affect the measured weight of the collected dust.

The Marple cascade impactors have the additional advantage over the Andersen samplers of being suitable for the sampling of airborne dust concentration in the breathing zone. Both cascade impactors were suitable for use in an enclosed area of the size of the pony barn as they did not violate the atmosphere that they were measuring. There was no "vacuum-cleaning" effect of the Andersen sampler, which could occur if the sampler pumped and effectively cleaned a large volume of air (Cohen et al. 1983). This was confirmed by the very similar results obtained during the period of simultaneous sampling using both the Andersen and Marple samplers in the stall situated one meter apart (Table 9). If there had been a "vacuum-cleaning" effect of the high-volume Andersen sampler, then the Marple sampler would have measured a lower airborne dust concentration than the Andersen sampler. The sampling procedures did not require excessive personnel movement and any attention that was required (changing of the filters every 6 or 12 hours) did not disturb the ponies. These protocols did not impose additional safety risks to the ponies or handlers.

Airborne dust concentration measurements

The results of the present study showed that the airborne dust concentrations were higher a) in the conventional management system than in the alternative recommended environment management system; b) during the day than at night in both management systems; and c) in the breathing zone than in the stall under the conventional management system. These results were not surprising, as the airborne dust concentrations within a barn are the result of an equilibrium between the rates of particle release from the source material and particle removal from the area.

There are three main methods of decreasing the airborne dust concentration in a building. The first is to decrease the contaminant release potential by modifying the source materials. This was the method employed in this study, where the inhalation challenge of airborne dust and aeroallergens was decreased by changing the bedding and feed materials from the hay and straw used in the conventional system to the wood shaving bedding and pelleted feed in the recommended system. The second method of decreasing the airborne dust concentration in a building is to decrease the rate of particle release by decreasing the degree of agitation of the source materials. When a pony agitates hay when eating, many dust particles are suspended. This results in very high airborne dust concentrations in the region of the pony's muzzle as well as elevated dust concentrations in the stall. By contrast, eating the relatively dust-free pellets releases less dust particles. The third method of decreasing airborne dust concentrations is to increase the particle clearance rates by increasing the building ventilation rates. Ventilation removes air with its suspended dust load from the barn and is the most effective method to clear fungal and actinomycete spores, which sediment slowly due to their small aerodynamic diameter (Clarke 1986). An increase in the building ventilation rate will decrease the ADC in the stall during the CS by removing these small spores. The building ventilation rates were kept constant in this study, and all variables, other than the bedding and feed materials, were controlled. The constant ventilation rate throughout the protocol was confirmed by the lack of difference in the airborne dust concentration in the stall when the recommended management system was compared during the summer and winter.

Airborne dust concentrations in the stall under the two management systems

The conventional management system had significantly higher total and respirable airborne dust concentrations than the recommended management system. There was no seasonal difference in the total or respirable airborne dust concentrations measured within the stall under the recommended management system. The mean total airborne dust concentration in the conventional management system reported in this study was 2.587 ± 0.5 mg/m^3. These results are higher than those reported in previous studies (Crichlow, Yoshida, and Wallace 1980, Zeitler 1985, Sasse, Boerma, and Smoulders 1986). The total dust levels in the recommended environment of the present study (0.709 ± 1.12 mg/m^3) were three-fold less than the levels of total dust measured in the conventional management system and in the same range as those reported previously (Crichlow, Yoshida, and Wallace 1980, Zeitler 1985).

These earlier studies measured stall levels during conventional-type management systems that utilized hay and straw. The increased dust concentration under the conventional management system in the present study could be partly accounted for by the use of a notably moldy hay, which was selected for this protocol because of its dusty properties. This increased source potential would result in increased airborne dust concentrations. Also, the previous studies did not position the samplers within the stall and the increased distance of the sampler inlet from the source of the airborne dust would result in the measurement of lower airborne dust levels. Zeitler (1985) reported mean airborne dust concentration of 0.64 mg/m^3 with a range of 0.47 - 0.84 mg/m^3 in winter in a poorly managed stable that practiced no quality control of its hay and straw, and lower levels in a better managed stable with increased ventilation. In Zeitler's study, two different stable complexes with different management system were compared. The applications of the conclusions from Zeitler's study are limited, because the two stables being compared varied from each other in ventilation rates and cleaning routines and methods, as well as the feed and bedding materials selected. This means that the difference in the airborne dust concentrations could not be attributed to the alteration of any single factor. In the present study, the only factors that were allowed to change between the two management systems were the feed and bedding materials utilized; thus, the significant differences between the two management systems were likely to be due to the changes in the bedding and feed materials.

Crichlow and coworkers (1980) reported a mean airborne dust concentration of 0.41 mg/m^3 and a range of 0.19 -0.91 mg/m^3 of which respirable dust was constant at between 32-42%. They did not compare these values to other establishments or attempt to alter the management system.

Airborne dust concentrations in the stall under both management systems during the daytime and nighttime sampling periods

In both management systems, the total airborne dust concentrations were significantly higher during the day than at night. The levels were three-fold higher in the conventional management system than in the recommended management system throughout the 24-hour period. The daytime sampling period included all the activities in the stall, including cleaning, feeding, and personnel movement. This agitation of the materials would release and suspend the particles, and result in increased airborne dust concentrations until the dust either settles out or is removed by the building's ventilation system. The nighttime sampling period was the quiet period with minimal personnel activity.

The 12-hour total airborne dust concentrations from the present study were in the same range or higher than the results from other authors. Crichlow et al. (1980) reported that the daytime airborne dust concentrations were higher (0.474 ± 0.21 mg/m^3) than the nighttime airborne dust concentrations (0.257 ± 0.18 mg/m^3), with peaks reported at the time of stall cleaning. This is a 50% decrease in total airborne dust concentrations, which is similar to the 60% decrease from day to nighttime values in the present study.

Zeitler (1985) also reported increased airborne dust concentration during morning stable work of 2.25 ± 9.8 mg/m^3 compared to night levels of 0.39 mg/m^3. The elevations in airborne dust during the morning cleaning were only 1.95 mg/m^3 in well-managed stables with improved ventilation and thorough stall cleaning by trained staff. Clarke (1987) also reported a decrease in the airborne dust concentrations during the bedding down when using wood shavings rather than straw as a bedding material.

The respirable airborne dust concentrations remained the same during the daytime and nighttime sampling periods in each management system. This is in contrast to the total airborne dust concentrations, which were 1.6 times higher during the day than at night. The mean respirable airborne dust concentrations in the conventional system were double those in the recommended management system. However, this difference was only statistically significant between the daytime samples.

The percentage of the total dust that was respirable did not alter in each management system during the 24-hour period. This means that the factors that decreased the airborne dust concentration at night were not size-selective. If the airborne particle clearance was due to the sedimentation of airborne particles alone, then the percentage of respirable particles would increase during the quiet periods, because the larger particles sediment out faster than the smaller spores, and the small particles would remain suspended. This is in contrast to the results of Zeitler (1985), who reported that the percentage of total dust that was respirable increased from a maximum of 30% during the morning to 90% at night. These differences between the two studies are to be expected if the building ventilation rates are different. The ventilation rates in the current study were adequate for particle clearance and probably higher than those in Zeitler's study. Therefore, in the latter study particle sedimentation would be more important in airborne particle clearance and may account for the bigger daytime versus nighttime differences and the abundance of smaller airborne particles.

Airborne dust concentrations in the pony's breathing zone

The instantaneous increase in airborne dust concentrations that occurs when the pony agitates dusty hay during feeding are more likely to be measured by a personal sampler placed close to the nostril region than by a stationary stall sampler placed away from the dust source. The Marple personal sampler attached to the halter has an increased sampler sensitivity compared to the Andersen stationary sampler, and better measures the inhalation challenge presented to the horse. Continuous sampling, with the inclusion of the peaks and troughs that occurred throughout the day and night, increased the sampling sensitivity of both impactors during the present study.

When comparing the total and respirable airborne dust concentrations in the breathing zone to those measured simultaneously in the stall, the levels were significantly higher in the region of the pony's nostrils than in the stall under the conventional system. This difference between the airborne dust concentrations in the two regions can be explained by 1) release of particles close to the sampler when the pony agitates hay, 2) a dilution effect as the airborne dust disperses from the source, and 3) the removal of air carrying dust as a result of the building ventilation rates. The result of the present study clearly showed that when the building ventilation rates are high, the airborne dust concentration measured in the stall will grossly underestimate the breathing zone airborne dust challenge. It is important for horse owners to realize that high ventilation rates do not remove the dust burden from the pony's nostrils as it feeds on dusty hay. Changing the source material is the only way to decrease dust levels in the breathing zone. The latter point is emphasized by the observation that the breathing zone and stall levels of total and respirable airborne dust measured simultaneously under the recommended management system did not differ significantly. This is in contrast to measurements made in the conventional system, and resulted from the lower dust potential of the bedding and feed materials, which did not produce the instantaneous very high airborne dust concentrations that occur when dusty hay is agitated. The building ventilation rate is not as important as the alteration in the source materials to reduce the airborne dust concentration in the stall. This is important, as very few horse housing units have adequate ventilation (Webster et al. 1987, Jones et al. 1987), especially during the cold months when buildings tend to be closed up to decrease heat loss. A low airborne dust concentration in the horse's breathing zone should be the aim of management alterations. The use of bedding and feed materials that provide a low source concentration of airborne dust is a more reliable method of maintaining a low airborne dust concentration in the stall and breathing zone to decrease the inhalation challenge, than merely increasing the building ventilation rates.

The impact of changing source materials on the dust levels in the breathing zone was demonstrated by the 38-fold higher levels of both the total and the respirable airborne dust concentrations in the breathing zones when the conventional system was compared to the recommended management system (Figure 11; Table 7). This means that the dust levels in the region of the ponies' nostrils when eating pellets and on wood shaving bedding were only 3% of the dust concentration when they were fed hay and bedded on straw.

Effect of changes in management on the aeroallergen concentrations

The reduction in the levels of aeroallergens in the stall paralleled the reduction in airborne dust levels when the management systems were changed. The concentration of airborne aeroallergens under the conventional management system was approximately double the concentration in the recommended environment. Unlike respiratory infections, where the viability and infectivity of the causative organism is important, the incidence and severity of COPD is more closely related to the quantity of allergenic material inhaled and the sensitivity of the individual horse to the specific allergen (Webster et al. 1987). The conventional management system posed a significantly higher inhalation challenge than the recommended management system for those aeroallergens measured. The levels of these aeroallergens, even the lower levels measured in the recommended environment, are well up in the range that can cause asthma in sensitized human subjects (Reed 1992, personal communication).

Both *Micropolyspora faeni* (McPherson et al. 1979a) and *Aspergillus fumigatus* (Lawson et al. 1979) are considered important in the etiology of COPD signs in horses. Mites and their feces are reported to be involved in the development of asthmatic signs in humans (Platts-Mills 1992) and are suspected to be involved in the development of COPD in horses (Hockenjos et al. 1981). *Thermoactinomyces vulgaris* is reported to be important in the development of COPD (Lawson et al. 1979, McPherson et al. 1979a), but, compared to the other aeroallergens, low levels of this actinomycete were measured in the present study. This is in contrast to results from Clarke (1987) where *T. vulgaris* was reported to be the most prolific actinomycete present. *T. vulgaris* is reported to be found on degrading wood chips and may have been more frequent if a deep litter system had been allowed to develop in the recommended management system. These fungal and actinomycete spores are of respirable size (*M. faeni* has an aerodynamic diameter 1.91 μ, *A. fumigatus* has a diameter of 3.11 μ, and *T. vulgaris* has an aerodynamic diameter of 0.58 μ ([Lacey and Dutkiewicz 1976]).

These airborne allergen concentrations were considered in terms of the inhalation challenge dose posed by the two management systems. The inhalation challenge doses were calculated, using the mean hourly aeroallergen concentrations measured in the present study, for a horse with a minute ventilation of 60 l/min, that remained in the stall for a 24-hour period. Although the aeroallergen concentration was measured in the stall, the challenge dose is likely to be higher in the breathing zone, because the relationship of the airborne dust in the stall and the breathing zone almost certainly also holds true for the aeroallergen level in these two regions. In addition, aeroallergens are of a size that would be in the respirable fraction. Hence, the aeroallergen dose was calculated for the breathing zone (Table 14), using firstly the ratio derived from the total airborne dust concentrations (derived from Table 9 and Table 10), and secondly using the ratio from the respirable airborne dust concentrations (Table 9 and Table 11). The formulas used were as follows:

$\dot{V}_{day}$ = volume of air inhaled per 24 hours at minute ventilation rate of 60 L/min (m^3)

$[A]_{stall}$ = aeroallergen concentration in stall (ng/m^3)

$[A]_{BZ}$ = aeroallergen concentration in breathing zone (ng/m^3)

$[Dust]_{stall}$ = airborne dust concentration in stall (ng/m^3)

$[Dust]_{BZ}$ = airborne dust concentration in breathing zone (ng/m^3)

$$\dot{V}_{day} = \frac{60 \times 60 \times 24}{1000}$$

$$\text{Inhaled aeroallergen dose} = \dot{V}_{day} \cdot [A]_{stall}$$

$$\text{Assuming} \quad \frac{[Dust]_{BZ}}{[Dust]_{stall}} = \frac{[A]_{BZ}}{[A]_{stall}}$$

$$\text{Then} \quad [A]_{BZ} = \frac{[Dust]_{BZ}}{[Dust]_{stall}} \times [A]_{stall}$$

$$\text{Then the inhaled dose in the breathing zone} = \dot{V}_{day} \cdot [A]_{BZ}$$

If it is assumed that the aeroallergens are in the respirable dust fraction only, then the inhaled dose in the breathing zone

$$= \dot{V}_{day} \cdot [A]^{BZ} \cdot \frac{([Dust]^{BZ})total}{([Dust]^{BZ})resp.}$$

The differences between the two management systems became even more apparent when the calculated aeroallergen challenge doses in the breathing zone were compared. The potential aeroallergen challenge in the breathing zone was 16 times greater under the conventional management system than the recommended management system, calculated from the ratio of the total airborne dust concentrations, and 30 times higher under the conventional system when the values were calculated using the ratios of the respirable dust concentrations in the two regions. These differences in the inhalation challenges during a single 24-hour period posed by the two management systems, especially when the levels in the breathing zone are considered, serve to emphasize the importance of utilizing materials with a low dust-producing potential to produce a low-challenge environment.

	M. faeni		*A. fumigatus*		*L. destructor*		*T. vulgaris*	
	CS	RE	CS	RE	CS	RE	CS	RE
Aeroallergen concentration (ng/m^3)	1423	705	1823	748	1420	761	502	79
Aeroallergen inhalation challenge (mg/day)[a]	0.123	0.061	0.158	0.065	0.123	0.066	0.043	0.0072
Aeroallergen inhalation challenge in breathing zone related to total airborne dust concentration (mg/day)[b]	0.996	0.060	1.279	0.064	0.996	0.065	0.348	0.0071
Aeroallergen inhalation challenge in breathing zone related to respirable airborne dust concentration (mg/day)[c]	1.591	0.049	2.044	0.053	1.591	0.053	0.556	0.006

Table 15: Calculated aeroallergen inhalation challenge in the stall and breathing zone under the conventional (CS) and the recommended (RE) management systems. The values were calculated for a horse with a mean minute ventilation of 60 L/min. The conventional management system, which induces signs of COPD in susceptible ponies, utilized hay feed and straw bedding. The recommended management system is the method suggested for the housing of COPD-susceptible ponies and involved the use of pelleted feed with the ponies bedded on wood shavings. The aeroallergens were volumetrically filtered using a sampler in the corner of the stall, and their concentrations were measured using the radioallergosorbent inhibition immunoassay. [a] = challenge calculated using aeroallergen concentration x daily tidal volume; [b] = aeroallergen inhalation challenge in the breathing zone calculated using the ratio of the total airborne dust concentrations in the breathing zone and in the stall; [c] = aeroallergen inhalation challenge in the breathing zone calculated using the ratio of the respirable airborne dust concentrations in the breathing zone and in the stall.

CHAPTER 6

CONCLUSIONS

1. The change of management system from the conventional system using hay feed and straw bedding to a recommended method, which utilized wood shaving bedding and a pelleted diet, more than halved the total and respirable airborne dust concentrations in the stall.

2. The levels of the major aeroallergens involved in the development of chronic obstructive pulmonary disease were double in the conventional system compared to the recommended management system. However, both management systems produced aeroallergen concentrations that are in the range considered to cause asthma in sensitized humans.

3. Altering the feed and bedding materials decreased the airborne dust concentrations in the pony's breathing zone by 38-fold, even though the building ventilation rates remained unchanged. The total and respirable airborne dust concentrations in the breathing zone under the recommended management system were each only 3% of the concentrations measured under the conventional management system. This indicates the importance of changing the feed

and bedding materials to alter the airborne dust concentrations even though the ventilation rates remained unchanged.

4. The airborne dust concentrations measured in the stall under the conventional system were 8-12 times lower than the simultaneous concentrations measured with a personal sampler in the region of the pony's nostrils. The measurements in the stall underestimated the inhalation challenge as they did not include the high instantaneous concentrations when the pony feeds on dusty hay. This difference in concentrations between the two regions was not apparent under the recommended management system, where the low dust potential of the pellets did not produce the peak airborne dust concentrations.

5. The calculated aeroallergen inhalation dose in the pony's breathing zone, when related to the total dust concentration, was 15-20 times higher in the conventional system than in the recommended system, and when related to the respirable airborne dust concentration was 30 times higher in the conventional system than in the recommended system.

REFERENCES

REFERENCES

Aerosol Technology Committee. Guide for respirable mass sampling. *Am Ind Hyg Assoc J* 31: 133-136, 1970.

Agarwal MK, Yunginger JW, Swanson MC, Reed CE. An immunochemical method to measure atmospheric allergens. *J Allergy Clin Immunol* 68: 194-200, 1981.

Air Sampling Procedures Committee. *Particle size - selection sampling in the workplace*. Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 1985.

Alexander AF. Chronic alveolar emphysema in the horse. *Am Rev Resp Dis* 80: 141-146, 1959.

American Conference of Governmental Industrial Hygienists [ACGIH] Technical Committee on Air Sampling Procedures. Particle size - selective sampling in the workplace. *Ann Am Conf Govt Ind Hyg* 11: 21, 1984.

American Conference of Governmental Industrial Hygienists. *Documentation of threshold limit values.* 3rd edition, 4th printing. Cincinnati, OH, pp 227-230, 1977.

Andersen AA. A new sampler for the collection, sizing and enumeration of viable airborne particles. *J Bacteriol* 76: 471-484, 1958.

Amundsson T, Gunnarsson E, Johannesson T. "Haysickness" in Icelandic horses: precipitin tests and other studies. *Equine Vet J* 15: 229-232, 1983.

Andersen Samples Inc. *Particle Fractionating Sampler*. Atlanta, GA, 1983.

Beech J. Managing horses with chronic obstructive pulmonary disease. *Vet Med* June; 620-626, 1989.

Beech J, Merryman GS. Immunotherapy for equine respiratory disease. *Equine Vet Sci* 6: 6-10, 1986.

Bennett WD, Messina MS, Smaldone GG. Effects of exercise on deposition and subsequent retention of inhaled particles. *J Appl Physiol* 59: 1045-1054, 1985.

Brain JD, Valberg PA. Deposition of aerosol in the respiratory tract. *Am Rev Respir Dis* 120: 1325-1373, 1979.

Breeze RG. Heaves. *Vet Clin North Am, Large Anim Pract.* 1: 219-230, 1979.

Brown M, Aalberse R, Platts-Mills T, Chapman M. Monoclonal immunoassay for quantitative analysis of *fel-d* I (Cat-1) in house dust extracts. *J Allergy Clin Immunol* 79: 221 (abstract), 1987.

Burge HP, Boise JR, Rutherford JA, Solomon WR. Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. *Mycopathologia* 1: 27-33, 1977.

Burrell R, Rylander R. A critical review of the role of precipitins in hypersensitivity pneumonitis. *Eur J Respir Dis* 62: 332-343, 1981.

Burrell MH. Aetiological aspects of respiratory disease. *Annual Report of Animal Health Trust* 54-57, 1986.

Campbell AR, Swanson MC, Fernandez-Caldas E, Reed CE, May JJ, Pratt DS. Aeroallergens in dairy barns near Cooperstown, New York and Rochester, Minnesota. *Am Rev Resp Dis* 140: 317-320, 1989.

Chatigny ME, Macher JM, Burge HA, Solomon WR. Sampling airborne microorganisms and aeroallergens. *Air Sampling instruments for evaluation of atmospheric contaminants.* 7th ed. Hering SV (ed). Cincinnati, OH: American Governmental Industrial Hygienists, pp 199-220, 1989.

Clarke AF. A review of environmental and host factors in relation to equine respiratory disease. *Equine Vet J* 19: 435-441, 1987.

Clarke AF. Air hygiene of stables and chronic pulmonary disease of the horse. PhD Thesis, Department of Animal Husbandry, School of Veterinary Science, University of Bristol, 1986.

Clarke AF, Madelin TM. The relationship of air hygiene in stables to lower airway disease and pharyngeal lymphoid hyperplasia in two groups of thoroughbred horses. *Equine Vet J* 19: 524-530, 1987a.

Clarke AF, Madelin T. Technique for assessing respiratory health hazards from hay and other source materials. *Equine Vet J* 19: 442-447, 1987b.

Clarke AF, Madelin TM, Allpress RG. The relationship of air hygiene in stables to lower airway disease during an outbreak of Equid Herpesvirus-1 infection. Equine infectious disease V. *Proceedings Fifth International Conference.* University of Kentucky Press, 1987.

Cohen BS, Harley NH, Martinelli CA, Lippmann M. Sampling artifacts in the breathing zone. *Aerosols in the mining and industrial work environments. I. Fundamentals and status.* Marple VA, Liu BYH (eds). Ann Arbour Science Publications, 1983, 347-359, 1983.

Cook WR. Chronic bronchitis and alveolar emphysema in the horse. *Vet Rec* 99: 448-451, 1976.

Cook WP, Rossdale PD. The syndrome of "broken wind" in the horse. *Proc Royal Soc Med* 56: 972-977, 1963 .

Crichlow EC, Yoshida K, Wallace K. Dust levels in a riding stable. *Equine Vet J* 12: 185-188, 1980.

Cross CE, Halliwell B. Biological consequences of general environmental contaminants. *The lung: scientific foundations.* Crystal RG, West JB (eds). New York: Raven Press, Ltd., 1961-1973, 1991.

Curtis SE, Drummond JG, Kelley KW, Grunloh DJ, Meares VJ, Norton HW, Jensen AH. Diurnal and annual fluctuations of aerial bacteria and dust levels in enclosed swine houses. *J Ani Sci* 41: 1502-1511, 1975.

Cushing KM, McCain JD, Smith WB. Experimental determination of sizing parameters and wall losses of 5 source-test cascade impactors. *Environ Sci Technol* 13: 726-731, 1979.

Derksen FJ, Robinson NE, Armstrong PJ, Stick JA, Slocombe RF. Airway reactivity in ponies with recurrent airway obstruction (heaves). *J Appl Physiol* 53: 719-725, 1985.

Derksen FJ, Robinson NE, Scott JS, Stick JA. Aerosolized *Micropolyspora faeni* antigen as a cause of pulmonary dysfunction in ponies with recurrent airway obstruction (heaves). *Am J Vet Res* 496: 933-938, 1988.

Derksen FJ, Scott JS, Slocombe RF, Robinson NE. *Micropolyspora faeni* causes airway inflammation but not hyperresponsiveness in sensitized ponies. *J Appl Physiol* 62: 1398-1404, 1987.

Donham KJ. Hazardous agents in agricultural dusts and methods of evaluation. *Am J Ind Med* 10: 205-220, 1986.

Donham KJ, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish swine confinement buildings. *Br J Ind Med* 46: 31-37, 1989.

Esmen NA, Lee TC. Distortion of cascade impactor measured size distribution due to bounce and blow off. *Am Ind Hyg Assoc* 41: 410-419, 1980.

Eriksen L. Studies on *Micropolyspora faeni* and chronic obstructive pulmonary disease (COPD). *Lung function and respiratory diseases in the horse*. (International Symposium in Hanover, Germany, June 27-29, 1985), 32-34, 1986.

Eyre P. Equine pulmonary emphysema: a bronchopulmonary mould allergy. *Vet Rec* 91: 134-140, 1972.

First MW. Air sampling and analysis for contaminants: an overview. *Air sampling instruments for evaluation of atmospheric contaminants*. 7th ed. Hering SV (ed). Cincinnati, OH: American Governmental Industrial Hygienists, 59-71, 1989.

Flaherty DK. Mechanisms of host response to grain dust. *Ann Am Conf Gov Ind Hyg* 2: 197-205, 1982.

Gerber H. Chronic pulmonary disease in the horse. *Equine Vet J* 5: 26-33, 1973.

Gleich GL, Larson JB, Jones RT, Baer H. Measurement of the potency of allergen extracts by their inhibitory capacities in the radioallergosorbent test. *J Allergy Clin Immunol* 53: 158-169, 1974.

Grunig G, Hermann M, Howald B, Winder C, von Fellenberg R. Partial divergence between airway inflammation and clinical signs in equine pulmonary disease. *Equine Vet J* 21: 145-148, 1989.

Grunig G, von Fellenberg R, Maier R, Corboz L. Elastase-producing microorganisms in horse lungs: their possible role in the pathogenesis of chronic pulmonary disease in the horse. *Equine Vet J* 18: 396-400, 1986.

Halliwell EW, Flieschman JB, Mackay-Smith M, Beech J, Gunson DE. The role of allergy in chronic pulmonary disease of horses. *J Am Vet Med Assoc* 174: 277-281, 1979.

Hanna CJ, Eyre P, Wells PW, McBeath DG. Equine immunology 2: immunopharmacology-biochemical basis of hypersensitivity. *Equine Vet J* 14: 16-24, 1982.

Hatch TF, Gross P. Pulmonary deposition and retention of inhaled aerosols. New York: New York Academic Press, 1964.

Hind WC. *Aerosol technology: properties, behavior and measurement of airborne particles*. John Wiley and Sons, Inc, 117, 1982.

Hockenjos von P, Mumcuoglu Y, Gerber H. Zur möglichen aetiologischen Bedeutung von Heumilben für allergisch bedingte Lungenkrankheiten des Pferdes. *Schweiz Arch Tierheilk* 123: 129-136, 1981.

International Standards Organization. *Air quality - particle size fraction definitions for health related sampling*. ISO/TR, 7708, 1983.

Johnson HW, Churchill EA, Delahanty DD, Fowler ME. Respiratory problems of the horse; their diagnosis and treatment. *Proceedings of the Sixth Annual American Association of Equine Practitioners Convention* 119-140, 1960.

Jones RD, McGreevy PD, Robertson A, Clarke AF, Wathes CM. Survey of the designs of racehorse stables in the south west of England. *Equine Vet J* 19: 454-457, 1987.

Karol MH. Immunologic responses of the lung to inhaled toxicants. *Concepts in inhalation toxicology*. McClellan RO, Henderson RF (eds). New York: Hemisphere Publishing Corp, 1989.

Kaup FJ, Drommer W, Deegen E. Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD) 1: alterations of the larger conducting airways. *Equine Vet J* 22: 343-348, 1990.

Kaup FJ, Drommer W, Damsch S, Deegen E. Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD) 2: pathomorphological changes of the terminal airways and the alveolar region. *Equine Vet J* 22: 349-355, 1990.

Knuth RH. Comments on inertial impactors calibration and use. *Aerosol measurement*. Aerosol measurement workshop, University of Florida, 108-116, 1976.

Knutson EO, Lioy PJ. Measurement and presentation of aerosol size distribution. *Air sampling instruments for evaluation of atmospheric contaminants*. 7th ed. Hering SV (ed). Cincinnati, OH: American Governmental Industrial Hygienists, 59-71, 1989.

Lacey J. Collection, detection and identification of agents in farm dust implicated in respiratory disease. *Am J Ind Med* 10: 311-313, 1986.

Lacey J. The microbiology of hay and straw. *Aspergillosis and Farmers lung in man and animal*. DeHaller F, Suter F (eds). Berne: Hans Huber, pp 16-23, 1974.

Lacey J, Dutkiewicz J. Methods of examining the microflora of mouldy hay. *J Appl Bact* 41: 13-27, 1976.

Lawson GHK, McPherson EA, Murphy JR, Nicholson JM, Wooding P, Breeze RG, Pirie HM. The presence of precipitating antibodies in the sera of horses with chronic obstructive pulmonary disease (COPD). *Equine Vet J* 11: 172-176, 1979.

Lawrence LM, Bump KD, McLauren DG. Aerial ammonia levels in horses stalls. *Equine Practice* 10: 20-23, 1988.

LeBouffant L. Influence de la nature des poussieres et de la charge pulmonaire sur l'epuration. *Inhaled particles III.* Walton WH (ed). London: Unwin Bros, 227-237, 1971.

Lippmann M, Harris WB. Size-selective samplers for estimating "respirable" dust concentrations. *Health Physics* 8: 155-163, 1962.

Lippmann M. Respirable dust sampling. *Am Ind Hyg Assoc J* 31: 138-159, 1970.

Lippmann M. Size-selective health hazard sampling. *Air Sampling instruments for evaluation of atmospheric contaminants.* 7th ed. Hering SV (ed). Cincinnati, OH: American Governmental Industrial Hygienists, 163-198, 1989.

Littlejohn A, Bowles F. Studies on the physiopathology of chronic obstructive pulmonary disease in the horse. III. The intrathoracic pressure. *Onderstepoort Journal of Veterinary Research* 47: 193-196, 1980.

Lowell FC. Observations on heaves: an asthma-like syndrome in the horse. *J Allergy* 35: 322-328, 1964.

Mair TS, Stokes CR, Bourne FJ. Increased local IgA production in chronic obstructive pulmonary disease. *Equine Vet J* 20: 214-216, 1988.

Mansmann RA, Strouss AA. Evaluation of transtracheal aspiration in the horse. *J Am Vet Med Assoc* 16: 631-633, 1976.

Marple VA, Willike K. Inertial impactors. *Aerosol measurement* (Aerosol measurement workshop, University of Florida, 1976), 90-107, 1976.

McPherson EA, Lawson GHK, Murphy JR, Nicholson JM, Fraser JA, Breeze RG, Pirie HM. Chronic obstructive pulmonary disease (COPD) in horses: identification of affected horses. *Equine Vet J* 10: 47-53, 1978.

McPherson EA, Lawson GHK, Murphy JR, Nicholson JM. Chronic obstructive pulmonary disease (COPD) in horses: aetiological studies: responses to intradermal and inhalation antigenic challenge. *Equine Vet J* 11: 159-166, 1979a.

McPherson EA, Lawson GHK, Murphy JR, Nicholson JM, Breeze RG, Pirie HM. Chronic pulmonary disease (COPD): factors influencing the occurrence. *Equine Vet J* 11: 167-171, 1979b.

McPherson EA, Thomson JR. Chronic obstructive pulmonary disease in the horse 1: nature of the disease. *Equine Vet J* 15: 203-206, 1983.

Meister U, Gerber H, Tshundi P. Die arterielle Blutgasanalyse in der Diagnostik und Überwagung chronisch lungenkranker Pferde. *Schweiz Arch Tierheilk* 118: 99-104 (cited by Thomson and McPherson 1984), 1976.

Mirbahar KB, Eyre P. Chronic obstructive pulmonary disease (COPD) in horses. *Vet Ann* 26: 146-155, 1986.

Mitchell JP, Costa PA, Waters S. An assessment of the Andersen Mark-II cascade impactor. *J Aerosol Sci* 19: 213-221, 1988.

Morgan WKC. Occupational bronchitis. *Eur J Resp Dis* 63: 117-124 (Suppl 123), 1982.

Muir DCF. Deposition and clearance of inhaled particles. *Clinical aspects of inhaled particles*. Muir DCF (ed). Philadelphia, PA: FA Davis Co, 1-20, 1972.

Murphy JR, McPherson EA, Dixon PM. Chronic obstructive pulmonary disease (COPD): effects of bronchodilator drugs on normal and affected horses. *Equine Vet J* 12: 10-14, 1980.

Muylle E, Oyaert W. Lung function tests in obstructive pulmonary disease in horses. *Equine Vet J* 5: 37-44, 1973.

Platts-Mills TAE. Mechanisms of bronchial reactivity: the role of immunoglobulin E. *Am Rev Respir Dis* 145: S44-S47, 1992.

Raabe OG. Deposition and clearance of inhaled aerosols. *Mechanisms in respiratory toxicology*. Witshi H, Nettesheim P (eds). Boca Ranton, FL: CRC Press, 27-76, 1982.

Rao AK, Whitby KT. Non ideal collection characteristics of single stage and cascade impactors. *Am Ind Hyg Assoc J* 38: 174-179, 1977.

Rao AK, Whitby KT. Non-ideal collection characteristics of inertial impactors. II. Cascade impactors. *J Aerosol Sci* 9: 87-100, 1978.

Reed CE. Measurement of airborne antigens. *J Allergy Clin Immunol* 70: 38-40, 1982.

Reed CE. [Personal Communication, 1992; Mayo Clinic, Rochester, MN]

Robinson NE, Sorenson PR. Pathophysiology of airway obstruction in horses: a review. *J Am Vet Med Assoc* 171: 299, 1978.

Rom WN, Crystal RG. Consequences of chronic inorganic dust exposure. *The lung: scientific foundations*. Crystal RG, West JB (ed). New York: Raven Press, 1885-1897, 1991.

Roth RA, Carpenter LJ. Potential role of phagocytic cells as mediators of chemically induced lung injury: use of isolated lung preparations in evaluating pneumotoxicity. *Concepts in inhalation toxicology*. McClellan RO, Henderson RF (eds). New York: Hemisphere Publishing Corp, 445-475, 1989.

Sasse HHL, Boerma S, Smolders EAA. The relationship between pulmonary function tests and other parameters. Results of a research project into the etiology of COPD in horses. *Lung function and respiratory diseases in the horse* (International symposium in Hanover, Germany, June 27-29, 1985), 46-48, 1986.

Sasse HHL, Boerma S, Smolders EAA. Zur Ätiologie chronischer Atemweg-erkrankungen beim Pferd: Veterinärmedizinische und landwirtschaftliche Aspekte in Grossmanegen. Arbeitstagung der Fachgruppe "Pferdekrankheiten," Münster, Westfalen, 29 May - 1 June 1985, pp 79-86.

Schatz M, Patterson R, Fink J. Immunopathogenesis of hypersensitivity pneumonitis. *J Allergy Clin Immunol* 60: 27-37, 1977.

Schatzmann U, Straub R, Gerber H, Lazary S, Meister U, Sporri H. Die Elimination von Heu und Stroh als Therapie chronischer Lungenerkrankungendes Pferdes. *Tier Prax* 2: 207-214 (cited by Thomson and McPherson 1984), 1974.

Schlesinger RB. Deposition and clearance of inhaled particles. *Concepts in inhalation toxicology*. McCllellan RO, Henderson R F (eds). New York: Hemisphere Publishing Corp, 163-192, 1989.

Sheppard D. Mechanisms of bronchoconstriction from nonimmunologic environmental stimuli. *Chest* 90: 584,587, 1986.

Sierra Instruments, Inc. *Instruction Manual Series 290 Marple personal cascade impactor*. Bulletin No. 290I.M. -3-82. Carmel Valley, CA: Sierra Instruments, 1982.

Solomon WR. Sampling Airborne Allergens. *Annals of Allergy* 52: 140-147, 1984.

Steel RGD, Torrie JH. *Principles and procedures of statistics - a biometrical approach*. 2nd ed. New York: McGraw-Hill, Inc, p 92, 1980.

Swanson MC, Agarwal MK, Reed CE. An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pig antigens. *J Allergy Clin Immunol* 76: 724-729, 1985.

Sweeney CR, Beech J, Roby KAW. Bacterial isolates from tracheobronchial aspirates of healthy horses. *Am J Vet Res* 46: 2562-2565, 1985.

Thomson JR, McPherson EA. Chronic obstructive pulmonary disease in the horse 2: therapy. *Equine Vet J* 15: 207-210, 1983.

Thomson JR, McPherson EA. Effects of environmental control on pulmonary function of horses affected with chronic obstructive pulmonary disease. *Equine Vet J* 16: 35-38, 1984.

Thurlbeck WM, Lowell FC. Heaves in horses. *Am Rev Resp Dis* 89: 82-88, 1964.

Viel L. Structural-functional correlations of the lung in horses with small airway disease. PhD Thesis. University of Guelph, Canada, 1983.

Viel L. Diagnostic procedures, prognosis and therapeutic approaches of chronic respiratory diseases in horses. *Can Vet J* 26: 33-35, 1985.

Wathes CM, Jones CDR, Webster AJF. Ventilation, air hygiene and animal health. *Vet Rec* 113: 554-559, 1983.

Watson JG, Lioy PJ, Mueller PK. The measurement process: precision, accuracy and validity. *Air sampling instruments for evaluation of atmospheric contaminants.* 7th ed. Hering SV (ed). Cincinnati, OH: American Governmental Industrial Hygienists, 51-58, 1989.

Webster AJF, Clarke AF, Madelin TM, Wathes CM. Air hygiene in stables 1: effects of stable design, ventilation and management on the concentration of respirable dust. *Equine Vet J* 19: 448-453, 1987.

Willoughby RA, McDonell WN. Pulmonary function testing in horses. *Symposium on Equine Respiratory Disease* 171-196, 1979.

Zeitler M. Untersuchungen über Staub in Pferdeställen, sowie über dessen allergene Wirkung auf das Pferd. *Dust in Animal Houses.* Symposium of the International Society of Animal Hygiene, Hannover, 13-14 March, pp 108-111, 1984.

Zeitler MH. Konzentration und Korngrößenverteilung von luftgetragenen Staubpartikeln in Pferdeställen. *Berl Münch Tierärztl Wschr.* 98: 241-246, 1985.