*Review*
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# Investigating the Link between Particulate Exposure and Airway Inflammation in the Horse

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Inhalant exposure to airborne irritants commonly encountered in horse stables is implicated in the pathogenesis of inflammatory airway disease (IAD) and recurrent airway obstruction (RAO), non-infectious, inflammatory pulmonary disorders that impact the health and performance of horses across all equine disciplines. IAD and RAO have overlapping clinical, cytological, and functional manifestations of the pulmonary response to organic dust and noxious gases encountered in the barn environment. Study of these diseases has provided important but incomplete understanding of the effect of air quality upon the respiratory health of horses. In this review, the principles of particulate exposure assessment, including health-related aerosol size fractions and size-selective sampling, the factors influencing air quality in equine environments, and the effect of air quality on the equine respiratory tract are discussed. The objective of this review is to provide the reader with a summary of the most common chronic inflammatory airway diseases in the horse and the principles of air sampling that are essential to the planning, interpretation, and assessment of equine respiratory health-related exposure studies.

**Key words:** Aerosol sampling; Dust; Endotoxin; Inflammatory airway disease; Recurrent airway obstruction.

The response of the pulmonary immune system to inhaled environmental factors underlies a number of diseases, including asthma[1,2] and occupational respiratory diseases[3] in humans, hypersensitivity pneumonitis in cattle,[4] allergic asthma in cats,[5] and inflammatory airway disease (IAD) and recurrent airway obstruction (RAO) in the horse. IAD and RAO are common chronic inflammatory pulmonary disorders that impact the health and performance of horses across all equine disciplines. The two conditions have overlapping clinical, cytological, and functional features that arise from the pulmonary response to organic dust and noxious gases encountered in the barn environment.

**Abbreviations:**

| | |
|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists |
| BALF | bronchoalveolar lavage fluid |
| $d_{ae}$ | aerodynamic diameter |
| FEV1 | forced expiratory volume in one second |
| HDS | hay dust suspension |
| IAD | inflammatory airway disease |
| IOM | Institute of Occupational Medicine sampler |
| LPS | lipopolysaccharide |
| RAO | recurrent airway obstruction |
| TLR | toll-like receptor |

## Inflammatory Airway Disease

Characterized by cough, poor performance, and excess mucus in the airways, IAD is the most common chronic airway disease of athletic horses. The disease has been identified as the second most common cause of veterinary care and lost use in young racing Thoroughbreds,[6] and increased tracheal mucus accumulations have been documented in roughly 20% of this population[7] and mature pleasure horses alike.[8] Although presumptive diagnosis of IAD is often based upon history, physical examination, and airway endoscopy, clinical signs are usually subtle and can be difficult to differentiate from cases of primary respiratory infection. Diagnosis is confirmed by demonstration of increased nucleated cell count and increased percentages of neutrophils, mast cells, or eosinophils in bronchoalveolar lavage fluid (BALF) or impaired lung function in the absence of fever or signs of systemic illness.[9]

The degree of airway inflammation detected in IAD is usually mild; however, even a mild level of pulmonary inflammation exerts a negative effect on the performance of intensely exercising horses because of impaired gas exchange.[10–12] Correspondingly, tracheal mucus accumulation has been identified as a risk factor for poor performance.[13–15] Thoroughbred race horses exhibiting tracheal mucus scores ≥2 out of 5[14] and sport horses with tracheal mucus scores ≥3 out of 5[15] have been demonstrated to be more likely to perform poorly than horses without increased mucus accumulations.

Most commonly, IAD is described as a mild to moderate BALF neutrophilia. However, IAD phenotype can vary, with young horses often exhibiting a cytologic profile marked by increased BALF eosinophils and mast cells, suggesting hypersensitivity.[9,16,17] Distinct cytokine patterns between cytologic phenotypes further support differing pathophysiologic mechanisms: BALF mastocytosis is associated with a mixed T helper lymphocyte type 1/ type 2 profile with

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increases in both interferon-γ and interleukin-4 messenger RNA expression, whereas BALF neutrophilia is accompanied by increases in interleukin-1β expression.[17,18] Both mastocytic and eosinophilic IAD have been associated with airway hyper-responsiveness and poor performance.[16,19,20] The heterogeneity in disease phenotype suggests similarly heterogeneous etiologies, but the specificity of response to diverse inciting factors remains speculative.

Exposure to airborne dust in the barn environment appears to play a major role in IAD pathogenesis. Barn dust contains particularly high levels of organic particulates including mite debris, microbes, and vegetative material with varying content of endotoxin and β-glucans.[a,21–25] Development of airway inflammation in otherwise healthy horses has been demonstrated upon introduction to barn confinement,[26–29] and higher dust environs have been associated with an increased degree of airway inflammation,[a,b,30–32] as have those with higher respirable endotoxin concentrations.[a,b] Exposure to gaseous ammonia has also been shown to induce airway inflammation, with naturally occurring exposures >2 ppm linked to neutrophilic inflammation.[a] Though the barn environment has thus been strongly implicated in the development of IAD, the pathogenesis of the disease remains largely unknown.

## Recurrent Airway Obstruction

Similarly, the barn environment plays an integral role in the development and exacerbation of RAO. Commonly known as heaves, RAO is characterized by coughing and increased respiratory effort because of the development of severe airway inflammation, mucus accumulation, and reversible airway obstruction upon exposure to organic dust.[28,29,33,34] Clinical signs include cough, weight loss, and respiratory embarrassment that ranges from exercise intolerance to marked respiratory distress at rest.[33] Airway neutrophilia as demonstrated by recovery of markedly increased percent and number of non-degenerate neutrophils in BALF defines RAO cytologically.[28,29,33,35,36] The respiratory distress of horses experiencing RAO exacerbation is associated with significant changes in pulmonary function caused by mucus accumulation, airway wall thickening, and bronchoconstriction of peripheral airways.[28,29,33,34,37,38] Pulmonary functional measurements reveal significantly increased lung resistance, transpulmonary pressures, and functional residual capacity with decreased dynamic lung compliance and arterial oxygen tension and accompanying chronic respiratory acidosis.[33,34,38] Forced expiratory maneuvers reveal significant reductions in forced expiratory volume in one second ($FEV_1$), peak expiratory flow, and forced expiratory flow.[34] Reversibility of airway obstruction is a cardinal feature of RAO and is often documented to confirm diagnosis. IV administered bronchodilators such as atropine[39] and *N*-butylscopolammonium[40,41] or aerosolized bronchodilators such as ipratropium bromide[42] and albuterol[43] result in rapid improvement in lung resistance and transpulmonary pressure.

## Pulmonary Responses to Inhalation Exposures

### *Organic Dust: Organic Dust Refers to Particulates of Animal, Plant, Insect, or Bacterial Origin*

Most of the current understanding of the horse's response to organic dust has been gained from hay and straw challenge studies comparing the responses of healthy horses to horses with RAO. When subjected to organic dust challenge by exposure to straw and moldy hay, healthy mature horses respond with airway neutrophilia,[b,27,28,37,44,45] and this recruitment of neutrophils appears to be dose-dependent.[b] Healthy horses exposed to straw and moldy hay do not develop clinical signs, changes in pulmonary function,[33,37,45] or changes in arterial blood gases.[38] Trends for increased bronchial responsiveness[a] and increased airway smooth muscle turnover[37] have been observed in healthy horses after high organic dust exposures. In racing Thoroughbreds, higher particulate levels are associated with an increased risk of visible tracheal mucus accumulation.[46] Similarly, in young Thoroughbreds entering race training, BALF eosinophilia has been associated with respirable dust exposure.[30]

When organic dust challenges are performed in horses with RAO, the responses are more pronounced and share characteristics of pulmonary function impairment documented in asthmatics and chronically exposed agricultural workers. After exposure to moldy hay and straw, RAO-susceptible horses exhibit increased respiratory rate and effort as well as abnormalities upon auscultation of the airways, including crackles and wheezes.[38] Endoscopy reveals significantly increased tracheal mucus accumulation, and cytologic evaluation of BAL fluid recovered demonstrates profound airway neutrophilia by 6 hours.[28,29,37] The magnitude of this airway neutrophilia is far greater than that seen in organic dust-challenged healthy control horses: reported post-exposure BALF neutrophil proportions range from 69.5%[28] to 79.3%[29] in RAO-susceptible horses and 18%[28] to 25.4%[29] in control horses. Unlike challenge of healthy horses, exposure of RAO-susceptible horses to straw and dusty hay does not change any indices of airway smooth muscle remodeling.[37] Instead, RAO airway smooth muscle was characterized by established hypertrophy and hyperplasia,[37] suggesting that smooth muscle remodeling occurs early in the course of the disease, as is the case in human asthma.[47] After straw and moldy hay challenge, pulmonary function measurements in horses with RAO reveal significant impairment in lung function. Changes include increases in lung elastance, resistance, and transpulmonary pressures, and decreased dynamic lung compliance,[37,38] with accompanying decline in arterial $O_2$ tension and increased arterial $CO_2$ tension.[38,44] When dust exposure is minimized, RAO horses enter remission, with resolution of clinical and cytologic signs and improvement in lung resistance and transpulmonary pressure measurements.[28,29,35,36,48–55]

In an attempt to standardize exposure challenges, suspensions and aqueous extracts of organic dust have been used in equine, human, and rodent studies. A series of reports from Pirie and colleagues elucidated several aspects of the horse's response to organic dust using an experimentally produced hay dust suspension (HDS) and differing fractions of the HDS.[24,56–58] Both control and RAO-susceptible horses develop an inflammatory response to inhalation challenge with HDS with dose-dependent recruitment of neutrophils to the airway: both BALF neutrophil numbers and ratios increase as increasing concentrations of HDS are delivered to the airway.[56] The BALF neutrophilia is more pronounced in horses with RAO and is accompanied by mucus accumulation and airway obstruction as evidenced by increased measures of lung resistance. No such change in function is observed in control horses.[56] Neither group exhibited any clinical or respiratory signs after challenge. Additionally, challenge with HDS failed to produce any change in airway reactivity in control or RAO horses.[57] Similar airway neutrophilia and pulmonary function changes were observed after inhalation challenge with an experimental fungal-lipopolysaccharide-silica microsphere suspension in RAO horses.[59] In control horses, neutrophil recruitment to the airway is observed, but no change in lung function occurs. Comparison of HDS to aqueous dust extracts that do not contain particulates has shown that particulates augment the pulmonary response.[56]

***Endotoxin.*** Endotoxin is an important mediator of the lung's response to organic dust. As part of the cell wall of gram-negative bacteria, endotoxin is present in variable concentrations in organic dust.[60] Molecularly, it is a lipopolysaccharide (LPS) with profound inflammatory effects. Upon inhalation, endotoxin binds LPS-binding protein and this complex associates with CD-14 and toll-like receptor 4 (TLR-4), initiating a signaling cascade in macrophages, dendritic cells, and epithelial cells within the airway. Ultimately nuclear factor-κB is activated and up-regulates production of inflammatory mediators such as interferon-γ, interleukin (IL)-8, IL-12, and tumor necrosis factor-α, stimulating and directing adaptive immune responses.[61,62]

In horses, experimental inhalation exposures to increasing doses of purified LPS up to 2,000 μg do not result in any appreciable clinical signs or airway mucus accumulation in healthy controls or horses with RAO.[44] However, even with the absence of clinical signs, both RAO and healthy control horses exhibit a dose-dependent BALF neutrophilia, with a response threshold <20 μg of LPS in horses with RAO and between 20 and 200 μg in healthy horses. At a dose of 2,000 μg LPS, BALF neutrophil numbers increase 50-fold in horses with RAO.[44] Both RAO and control horses demonstrate a decrease in BALF macrophages, perhaps analogous to the proposed macrophage apoptosis induced by LPS inhalation in humans.[57] At high doses, lung resistance increases in horses with RAO, whereas no change in lung function is seen in healthy controls.[44] The difference between RAO and healthy horses' response thresholds resembles that seen when comparing asthmatics to healthy subjects, where doses as low as 20 μg inhaled LPS result in bronchoconstriction in asthmatics whereas the threshold in healthy volunteers is 80 μg.[63]

In addition to experimental exposure studies, epidemiologic evidence of the respiratory response to endotoxin has also been established. A large prospective cohort study in Australia documented an increased risk of increased neutrophil proportions in tracheal wash aspirates in otherwise healthy racehorses subjected to high endotoxin exposures.[a] Likewise, associations have been demonstrated between home endotoxin levels in settled dust and severity and frequency of clinical signs of asthma in people.[64] Occupational endotoxin exposures have been correlated with declines in $FEV_1$ and forced vital capacity in swine and poultry workers,[65,66] and higher endotoxin exposures have been associated with increased clinical signs of asthma and decreased $FEV_1$ in children.[61,67]

Exposure challenges with HDSs and organic dust extracts that contain depleted or attenuated LPS activity confirm the importance of endotoxin in organic dust-induced airway inflammation. Attenuation of LPS activity in HDS severely blunts the neutrophilic airway responses in both RAO and healthy horses and abolishes the lung dysfunction observed in RAO horses. Restoration of original LPS activity with purified endotoxin restores the airway cellular and functional responses.[57] Similarly, in asthmatics sensitized to house dust antigens, house dust extracts depleted of LPS fail to induce significant changes in $FEV_1$, in contrast to the marked and immediate decline triggered by unmodified house dust extract.[63] Furthermore, the response to corn dust extract is severely dampened in endotoxin-resistant mice.[68]

However, failure of purified endotoxin solutions to fully duplicate the clinical, cytologic, and functional responses to organic dust inhalation indicates that endotoxin is not solely responsible for the development of clinical disease. While HDS inhalation results in significant lung dysfunction in RAO horses, none of the individual fractions of HDS produce this response when administered separately.[58] Purified LPS inhalation does not elicit mucus accumulation in the trachea of RAO horses, even at a dose of 2,000 μg; however, even 5 hours of exposure to straw and moldy hay does.[44] In the latter study, respirable endotoxin concentrations at the breathing zone of the horses measured 3.95 $ng/m^3$.[44] With a resting minute ventilation of 78 L/min,[69] the average horse inhales roughly 112 $m^3$ of air in a 24 hour period, corresponding to a respirable endotoxin dose of only 0.44 μg in a 24 hour period. While aqueous hay dust extract contains nearly all the endotoxin activity of HDS, the airway neutrophilia it elicits is significantly augmented by the inclusion of the particulate fraction.[58] Similarly, washed HDS particulates combined with purified LPS prompts neutrophil recruitment to the airways, but the magnitude of the response is significantly less than that seen after exposure to HDS.[57] The dose of soluble purified LPS required to induce airway inflammation far exceeds the dose of LPS that is delivered in HDS and encountered in the short term straw and moldy hay challenges, yet the latter exposures elicit a greater inflammatory response.[44,57] Alveolar macrophages treated with HDS responded with significantly higher tumor necrosis factor-α production than when treated with LPS alone.[70] This potentiation of LPS activity by other elements of organic dust is also apparent in cases of asthma, where allergen exposure mediates an increase in LPS-binding protein and soluble CD-14, increasing the magnitude of the response.[63]

A key effect of endotoxin exposure on the airway that is largely unexplored in equine medicine is the relationship between endotoxin exposure and allergic sensitization. Evidence from both human epidemiology and mouse sensitization models points toward a complex relationship. While endotoxin exposure in the face of existing asthma exacerbates clinical signs , adequate endotoxin exposure early in life has been associated with decreased risk of atopic asthma.[61,71] Furthermore, children from farm backgrounds, who are presumably exposed to high levels of endotoxin, have been found to have 30–50% lower incidence of hay fever and atopy.[61] Atopic asthma in Swedish children has been linked with certain TLR polymorphisms that confer reduced responsiveness to endotoxin.[71,72] Mouse models of sensitization also support the potential protective effect of endotoxin, indicating LPS modulates the immune response to sensitization protocols depending on time, dose, and route of administration. In models using inhaled sensitization protocols, challenge with pure allergen devoid of endotoxin content fails to elicit an immune response, whereas inclusion of low doses of LPS during sensitization directs an allergen-specific Th2 response and airway hyper-responsiveness. Higher doses of LPS initiate a Th1 response but do not prevent airway hyper-responsiveness. However, if the low dose endotoxin inhalation exposure occurs repeatedly before sensitization, the allergic response is diminished.[72] TLR-4 deficient mice subjected to sensitization protocols using inhaled OVA and LPS, regardless of endotoxin dose, fail to demonstrate significant antigen-bearing dendritic cell migration from the airway, and markedly decreased airway inflammation is seen.[73] Although it is unlikely that young horses will be subjected to conditions of sufficient hygiene to prevent exposure to endotoxin, further investigation of the apparent synergy between particulate antigens and endotoxin in organic dust exposures in the horse will provide further insight into the molecular events that determine the airway response.

## Exposure Assessment

### *Health-Related Aerosol Size Fractions*

As they are inhaled, particles either remain airborne or leave the airstream and deposit on the surfaces of the airways because of impaction, settling, interception, diffusion, and electrostatic interactions. The anatomical point where a particle leaves the airstream is determined by characteristics of the airflow, governed largely by geometry of the airway, the rate and pattern of breathing, and particle characteristics. For particles larger than roughly 0.3 μm deposition is essentially determined by aerodynamic diameter ($d_{ae}$), which describes the diameter of a spherical particle of unit density that settles with the same terminal velocity as the particle in question.[74]

Large particles have a higher probability of deposition earlier in the airway, and therefore, a lower probability of penetration to the distal airways. The American Conference of Governmental Industrial Hygienists (ACGIH) describes three fractions for health-related aerosol sampling to predict the likelihood of penetration to each region of the human respiratory tract based upon $d_{ae}$: the inhalable fraction, the thoracic fraction, and the respirable fraction. These fractions are defined by curves that estimate penetration efficiency in the human airway as a function of $d_{ae}$. Because these definitions are continuous curves over the range of particle $d_{ae}$ equal to or <100 μm, described by unwieldy equations, the fractions are generally discussed according to the $d_{ae}$ at which penetration is 50%. This parameter is also referred to as the 50% cut-point. Table 1 summarizes the 50% cut-points of the particulate size fractions.

Additionally, the Environmental Protection Agency has two criteria for evaluation of atmospheric particulates: PM10 and PM2.5. With a 50% cut-point of 10 μm, PM10 is described by a curve that approximates the thoracic criteria, and it is intended to be a measure of particles that can penetrate to the tracheobronchial regions of the lung. PM2.5 has a steep curve with a cut-point of 50% collection efficiency at $d_{ae}$ of 2.5 μm.[75] Figure 1 displays the penetration efficiency curves of the three ACGIH and two Environmental Protection Agency particle size criteria.

It is likely that significant differences exist between the size-dependent penetration of particulates into the equine lung and the human lung. Because the inhalable, thoracic, and respirable curves are based upon experimental and theoretical data focusing on the average adult man, it is tempting to assume that 50% penetration efficiencies for each region will occur at higher $d_{ae}$ in the horse. However, many factors render this simplistic supposition inappropriate. First, horses are obligate nasal breathers, indicating the potential for significant divergence of the inhalable curve from that

**Table 1.** Health-related aerosol size fractions.

| Size Fraction | 50% Cut-point (μm) |
|---|---|
| Inhalable | 100 |
| Thoracic | 10 |
| Respirable | 4 |
| PM10 | 10 |
| PM2.5 | 2.5 |

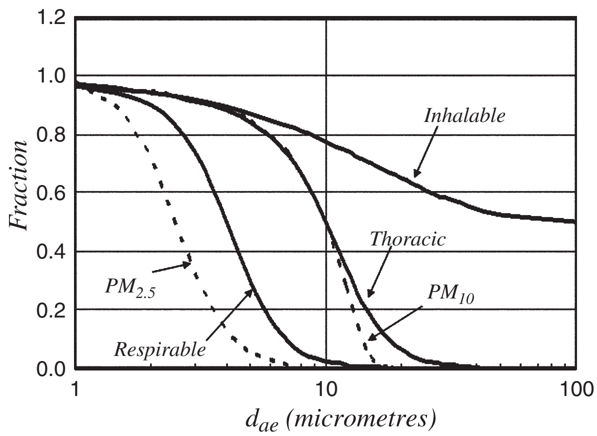


**Fig 1.** Particle size-selective criteria for health-related aerosol sampling. Adapted with permission from Vincent JH. *J Environ Monit* 2005;7:1045 with permission of The Royal Society of Chemistry (http://dxdoi.org/10.1039/10.1039/B509617K).

of humans, which assumes mouth breathing. Furthermore, the equine nasal passages are potent conditioners of inhaled air[69] and may also prove particularly efficient at achieving deposition of inhaled particulates. Important differences also exist between human and equine respiratory anatomy. Horses are able to vary the path of airflow from an extremely acute angle between the nasopharynx and trachea to an angle approaching 180° by flexing or extending head and neck, respectively. The impaction of particles upon the surfaces of the pharynx would be significantly reduced with extension of the head, as occurs during strenuous exercise. The resting tidal volume in the average man that is assumed in the ACGIH particle penetration models is roughly 0.5 L,[75] whereas in the average horse at rest, tidal volume is 5.6 L and can increase up to 13 L or more during strenuous exercise.[69] Similarly, the minute respiratory volume in the average man is roughly 6 L/min. In the horse at rest, minute respiratory volume averages 78 L/min. Detailed knowledge of the relative differences in tracheal, bronchial, and bronchiolar diameters is necessary to translate these differences in volumetric flow to differences in linear flow. Linear flow rates have considerable effect upon the likelihood of particle impaction,[74] so calculating these differences would allow development of a model to predict particle penetration in the equine lung. Unfortunately, sufficiently detailed anatomic descriptions of airway dimensions in the horse have not been published. In the absence of experimental data to define equine health-related size fractions, it may be best to include two or more health-related size fractions when evaluating the effect of air quality upon equine health.

To assess inhalation exposure, it is also important to consider the amount of an aerosol present, its biologic and chemical properties, and the point of the respiratory tract it is likely to deposit.[75] The ultimate effect that a particle has upon the health of the exposed subject depends upon if and where it contacts the airway surface. For example, insoluble particles deposited along the trachea and major bronchi are quickly expelled by mucociliary clearance, whereas particles deposited within the respiratory bronchioles and alveoli are cleared much more slowly by phagocytosis.[75] Even upon phagocytosis by airway macrophages, inhaled particulates may still exert biologic effect through activation of the signaling network of the pulmonary immune system.[1] Extremely fine aerosols may mediate the death of macrophages upon phagocytosis or escape phagocytosis and gain access to the blood stream directly through the alveolar wall.[76] For these reasons, smaller particles are usually considered the most detrimental. However, soluble particles or particles with soluble, biologically active coatings may exert detrimental health effects upon deposition anywhere along the respiratory tract. Consequently, knowledge of the expected behavior and health effects of the aerosol under study determines the size fraction of interest.

The size criteria set forth by the ACGIH attempt to describe the probability of penetration to each region of the airway. With aerosol sampling techniques that approximate the appropriate convention, the concentration or dose of an aerosol that reaches the site of its biologic activity within the airway can be estimated.

## *Size-selective Sampling*

Various size-selective samplers have been designed to collect either the inhalable, thoracic, or respirable fraction. Sampler performance in approximating the defined curves will depend upon sampling rate, wind speed, orientation of inlet, physical interaction with the subject, and characteristics of the sampled aerosol. The operational characteristics for several commercially available samplers for each size fraction are discussed.

***Inhalable Fraction.*** The performances of several samplers historically used to collect "total particulates" have been compared to the inhalable criteria. Ironically, most total particulate samplers significantly underestimate the inhalable fraction. Instead, personal size-selective samplers designed to match the inhalable criteria have been designed. A comprehensive evaluation of inhalable sampler performance has shown that the Institute of Occupational Medicine (IOM) inhalable sampler and the capteur individuel de poussière inhalable sampler provide the best approximation of the inhalable particulate curve.[77]

The IOM personal sampler (Fig 2) is used to sample at a flow rate of 2 L/min. The sample is collected on a 25 mm filter secured in the sampling cassette. Gravimetric analysis is performed by weighing the filter and assembled cassette before and after sampling and calculating the difference. Therefore, any particulates collected in the cassette, not just those that adhere to the filter, are measured. The IOM sampler appears to most closely match the inhalable curve under most conditions.

The capteur individuel de poussière operates at 10 L/min and relies upon rotational forces to pull air


**Fig 2.** Photograph of Institute of Occupational Medicine (IOM) sampler. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

through porous polyurethane foam in which the particles are retained. Larger particles fall into a cup during sampling, so both the foam and the collecting cup are weighed before and after sampling.

***Thoracic Fraction.*** Because of a lack of thoracic limit values, selection of and performance information for the thoracic fraction is relatively lacking. In a 1999 report, Maynard evaluated the performance of six personal thoracic samplers and found that the GK2.69 cyclone provided the closest match to the thoracic definition, followed by the SIMPEDS cyclone. Additionally, a modified IOM inhalable sampler approximated the thoracic curve fairly well.[78]

The GK2.69 cyclone operates as a thoracic sampler at a sampling flow rate of 1.6 L/min and has been adopted by the National Institute of Occupational Safety and Health for the airborne sampling of metalworking fluids.[78] Particulates are aspirated, enter a cyclone separation chamber where larger particles are removed from the airstream, and smaller particulates pass through a vortex finder to be deposited onto a 37 mm filter.

The SIMPED cyclone approximates the thoracic convention at a sampling flow rate of 0.8 L/min.[78] Again, air is directed through a cyclone separation chamber where larger particles leave the airstream, analogous to the extra-thoracic airway. Particulates that pass through the vortex finder are deposited on a 25 mm filter.

Inserting a 10 mm thick circular section of polyurethane foam with diameter 17.5 mm and pore density of 45 pores/in can modify the IOM sampler to approximate the thoracic convention.[78] Sampling is performed at 2 L/min.

***Respirable Fraction.*** Historically, personal measurements of respirable exposure have been performed with the 10 mm Dorr-Oliver cyclone. Though it has been demonstrated to consistently under-sample in comparison to the ACGIH respirable criteria, the Dorr-Oliver cyclone remains the sampler recommended by Occupational Safety and Health Administration for use in confirming regulatory compliance. The National Institute of Occupational Safety and Health manual of analytic methods also recognizes the Higgins-Dewell cyclone and the SKC aluminum cyclone for sampling respirable particulates not otherwise regulated. Finally, the multi-inlet cyclone described by Gautam and Sreenath was shown to perform in close agreement with the ACGIH curve and is available as the GS-3 respirable cyclone.[79] All of the cyclones operate by removing larger particles from the airstream by the effect of centrifugal motion before entrained particles reach the collecting filter. The collection efficiency curves and optimal sampling rates differ.

The 10 mm Dorr-Oliver cyclone approximates the ACGIH curve at a flow rate of 1.7 L/min, with a 50% cut-point at $d_{ae}$ = 4.0 µm. Particulates are collected onto 37 mm filters in standard 3-part cassettes.

The aluminum cyclone (Fig 3) closely matches the current ACGIH respirable curve with 50% cut-point at $d_{ae}$ = 4.0 µm at a sampling flow rate of 2.5 L/min, collecting the respirable fraction on 37 mm filters housed in standard conductive cassettes. Alternatively, the aluminum cyclones are capable of sampling onto 25 mm filters.[75]

At 2.75 L/min, the GS-3 cyclone has a 50% cut-point of 4 µm as specified by the ACGIH curve, but tends to over-sample at smaller $d_{ae}$ and under-sample at larger $d_{ae}$.[79]

**Fig 3.** Photograph of aluminum cyclone. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

### *Integrated Filter Sampling Versus Real-Time Particulate Measurements*

***Integrated Filter Sampling.*** Forming the backbone of personal exposure assessment, integrated filter sampling gravimetrically determines particulate concentrations by comparing the pre-sampling weight of a filter with the weight after the collection of a particulate sample using the appropriate size-selective instrument. Additionally, filter sampling allows microscopic, elemental, and biologic analysis of collected particulates in order to further characterize aerosol-associated health hazards. In equine applications, this has included measurement of endotoxin content of respirable and total dust,[22,23,80,81] β-glucan content,[23,81] and aeroallergen levels.[25] Additionally, further analyses such as chemical composition, source apportionment, and identification of novel allergens will be important to more completely characterize the particulates present in horse barns.

Integrated filter sampling allows air sampling to be performed at the breathing zone, providing a more accurate estimation of the individual's exposure when compared to stationary area measures (Fig 4). Because they cannot account for particles generated or re-suspended by personal activity, area particle measures consistently underestimate the concentrations present in the breathing zone.[75,82,83]

Conventional filter sampling provides only a cumulative average particulate concentration for the sampling period, is frequently plagued by sample collection below the limit of detection, and is ill-suited for the investigation of specific tasks' or activities' impact upon particulate levels.

**Fig 4.** Photograph of horse equipped for air sampling at the breathing zone. Black arrow: IOM sampler[c]; White arrow: aluminum cyclone[c]; Personal sampling pump (AirCheck 2000[c]). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

***Real-Time Particulate Measurements.*** Total mass concentration of an aerosol can be determined directly by integral concentration detectors that translate principles such as vibrational frequency shift, beta-attenuation, or most commonly, light scatter measured in real-time into mass concentration data output.

Light scattering principles are used in a number of instruments to measure various characteristics of aerosols. In the case of mass concentration determination, nephelometers such as the DustTrak[d] (Fig 5) measure the light scatter of an aerosol cloud (of a given size distribution) and mass concentration is inferred. For aerosols of differing particle size distributions, recalibration of the nephlometer is required for accurate mass concentration data, but in practice this constraint is frequently neglected. Additionally, intensity of scattered light can be attenuated because of light absorption, introducing possible errors in the resulting data caused by differences in the particle refractive index.[84]

Two approaches allow real-time size-selective mass concentration measurements. The first approach utilizes size-selective pre-samplers to limit access to detection chamber based upon size. The second approach uses instrumentation that provides measure of both particle size and number to allow size distribution analysis. Pre-selectors such as cyclones or impactors are commonly used in real-time instruments to allow measurement of the ACGIH size fractions. As with gravimetric sampling, the performance of these samplers can be greatly impacted by deviations in sampling flow rate, sampling conditions such as wind speed, and aerosol characteristics.

Alternatively, more sophisticated equipment can be used to determine the size distribution of the aerosol in question. This can be an important, but often neglected, step in determining appropriate sampling protocols in new settings. Three detection principles are used for direct size distribution measurements: par-

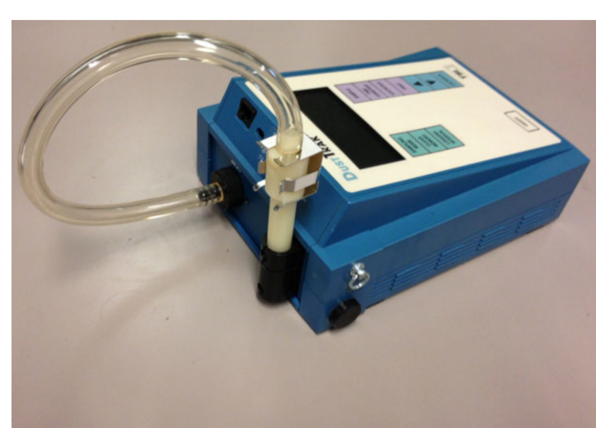


**Fig 5.** Photograph of DustTrak[d] equipped with 10 mm Dorr-Oliver cyclone for real-time measurement of respirable particulates. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ticle-light interactions, particle inertia, and electrical mobility.

In optical particle counters, such as the MetOne HHPC-6 Airborne Particle Counter,[e] the light scattering properties of each individual particle are measured as indication of particle size, allowing the construction of particle size histograms. Appropriate calibration is necessary for accurate conversion of light scatter measures to particle size, and the number of data channels provided by the instrument determines the resolution capability. The latter concern has been greatly improved with advances in data storage and analysis capabilities. Because particles are individually evaluated, simultaneous arrival and measure of two or more particles will result in erroneous interpretation of light scatter.[84] Therefore, use of such instruments in environments with high particulate concentrations may result in misleading data. Inlet efficiency and classification of non-spherical particles can also significantly impact the quality of data.

Particle size, or more accurately, $d_{ae}$ can also be obtained from measures of particle inertia. Instruments such as the Aerodynamic Particle Sizer measure the time required for a particle to traverse a known distance (time of flight) as an indication of particle size with a measurement range of 0.5 to 30 μm. Again, the quality of data obtained is largely determined by calibration factors and the precision with which particles are accelerated.[84] As with the optical particle counter, simultaneous presence of more than one particle in the flight chamber can result in incorrect measure of flight time and therefore particle size.

Major advantages are offered by direct, real-time particulate measurements in horse stables. Lower limits of detection are provided,[83] and peak particulate levels can be determined and associated with specific activities or events within the barn.[85] Nephlometers and optical particle counters have been used to map particle concentrations and numbers within racing stables to evaluate the effect of stable design and season.[86]

Combined integrated and direct measures of particulate matter concentrations provide the most comprehensive characterization of an aerosol environment and assessment of risk. Numerous and varying sources of particulate matter exposure can then be identified temporally, chemically, and biologically. Such complete characterization can greatly improve and facilitate mitigation and treatment efforts.

## *Factors Influencing Air Quality in Equine Environments*

When horses are confined to stalls, they are exposed to airborne irritants at much higher concentrations than those encountered at pasture.[22,87] Multiple studies have demonstrated that the concentrations of such particulate matter and endotoxin are heavily influenced by management practices within the barn, including feed and bedding used, activity within the barn, and barn characteristics. Tables S1 and S2 summarize the methods and results of dust and endotoxin measurements under various equine management conditions.

***Combined Effects of Feed and Bedding.*** The combined effects of feed and bedding upon particulate matter and endotoxin concentrations have been widely studied by comparing "conventional" management practices of bedding on straw and feeding hay to "low dust environments," in which horses are fed complete pelleted feed and bedded on wood shavings. Woods and colleagues demonstrated that switching from poor quality hay and straw bedding to complete pelleted feed and wood shavings reduced area respirable particulate concentrations by more than 50%. Moreover, breathing zone total and respirable particulate levels were reduced to 3% of the exposure measured under the conventional management practices.[25] Additionally, concentrations of selected aeroallergens, including *Aspergillus fumigatus* and *Lepidoglyphys destructor* (storage mite), in conventionally managed stalls were found to be roughly twice that of stalls in which horses were bedded on wood shavings and fed pelleted feed.[25] Similar findings are reported for breathing zone endotoxin concentrations, with the highest total and respirable endotoxin levels measured when feeding hay, bedded on straw, in a stall with sub-optimal ventilation. Over a 5-fold decrease in total endotoxin concentrations and >10-fold decrease in respirable endotoxin concentrations were achieved by bedding on shavings and feeding silage while maximizing the ventilation of the stall.[22]

***Effect of Feed.*** A large amount of the variation in horses' exposure to particulates can be explained by variation in the feed. Though hay is considered a mainstay of conventional feeding programs, its use is associated with increased particulate matter exposures because of release of insect, fungal, and plant fragments as well as inorganic dust from even the best quality hay. Changing a horse's diet from dry hay to haylage can achieve a 60–70% reduction in respirable particulate exposure.[83] Similar mitigation of particulate matter exposure can be realized by completely immersing dry hay immediately before feeding: breathing zone mean respirable particulate concentrations were reduced by 60% after completely immersing the hay.[85] Similarly, soaking hay was shown to halve respirable particle numbers.[32] When horses were fed a complete pelleted feed in place of poor quality hay, total and respirable particulate concentrations were reduced by 70 and 99%, respectively.[88]

Several studies have quantified the release of particulates from feedstuffs under controlled laboratory settings to compare dust generation independent of horse activities and barn ventilation. Hay composition and preparation significantly affect particulate and mold contamination. Use of *Trifolium repens* (white clover) and *Lolium perenne* (winter ryegrass) have been shown to minimize release of respirable particulates and mold colony forming units when harvested appropriately.[89] Late harvest, second cutting hay baled at 85% dry matter,[90,91] and haylage[89–91] provide the lowest potential for respirable particulate and fungal release. Choice of concentrate can also impact particulate matter exposure. Commercially available pelleted feed generated 10–12 times lower particulate matter concentrations than uncleaned oats, while cleaning oats or steam flaking barley effectively reduces particulate matter release by 80%.[92] Rolled barley and oats release nearly 60 times the number of respirable particles and more than 10 times the number of *Aspergillus fumigatus* colony forming units when compared to commercially produced molasses-treated concentrates.[93]

Although fewer studies have measured horses' exposure to endotoxin, choice of feed plays a major role. Similar to particulate matter exposures, endotoxin concentrations measured at the breathing zone of horses fed poor quality hay were significantly higher than those of horses fed a complete pelleted feed.[88] In fact, significant correlations between endotoxin and particulate matter exposures have been established, with particulate matter concentration accounting for >50% of the variation in endotoxin levels.[22,23,30] Moreover, the endotoxin content of dust collected from the breathing zone of horses has been found to be strongly correlated with the endotoxin content of the feed.[88]

***Effect of Bedding.*** Choice of bedding material also impacts endotoxin and particulate exposures within the stall. A significant effect of bedding material on particulate matter concentrations has been demonstrated: area PM10 concentrations were found to be higher in stalls bedded with straw compared to stalls bedded with wood shavings, wheras use of straw pellets was associated with the lowest PM10 concentrations.[94] Clements reported higher respirable dust concentrations at the breathing zone for horses bedded on straw compared with horses bedded on wood shavings, but the difference was not statistically significant.[83] Likewise, respirable endotoxin concentrations measured at the breathing zone of horses bedded on straw were found to be roughly 1.5 times those measured while horses were bedded on sawdust.[f]

***Relative Effects of Feed versus Bedding.*** Those study designs that allowed interpretation of the relative significance of feed choice versus bedding choice indicate that feed has a greater relative impact on particulate and endotoxin exposures. Changing the feeding regimen from hay to haylage reduced mean respirable dust concentrations by nearly 70%, whereas the replacement of straw bedding with shavings only reduced mean respirable dust concentrations by roughly 30%.[83] Similarly, breathing zone endotoxin concentrations were greater when horses were fed hay than when they were fed pellets, but no significant difference in exposure was found between bedding on straw versus wood chips.[88]

***Effect of Activity.*** Activity within the barn can have a significant effect on air quality as well. Particulate matter concentrations within the stall peak during cleaning,[f,85,94] with smaller particle sizes taking longer to return to baseline.[g] Activity and management in adjacent stalls can also have a strong impact on particulate concentrations. Clements and Pirie demonstrated a 19-fold increase in respirable particulates in the stall being cleaned, and 9-fold increase in the respirable particulate concentration in the adjacent stall that shared airspace above the stall walls. Furthermore, a 71% reduction in respirable particulates was seen when the adjacent stall was changed from the conventional straw and hay management to wood shavings and haylage.[85] Particulate concentrations measured at the breathing zone of barn workers have indicated association between maximum exposures and cleaning activities, particularly sweeping the aisle.[23] Daytime total and respirable particulate concentrations have been found to be nearly double the concentrations measured overnight, independent of management system,[25] further reinforcing the impact of activity within the barn on particulate exposures. Similarly, higher endotoxin concentrations have been demonstrated to coincide with feeding and stall cleaning.[f]

Although individual horse behavior would be expected to significantly impact exposure to particulates, it has received little attention in veterinary literature. Measurement of inhalable dust at the breathing zone of both horses and barn workers in four stables with varying management practices revealed that horse exposures were nearly 3 times greater than that of barn workers.[23] This difference suggests that horse activity can generate exposures of greater magnitude than even those experienced by workers while sweeping aisles and mucking stalls. Furthermore, breathing zone measures of respirable and inhalable particulates have been found to vary significantly horse-to-horse despite identical management.[82] Future use of real-time particulate monitors at the breathing zone of the horse while recording activity with a video camera or written notes may help to resolve the impact of particular activities.

***Effect of Ventilation and Ambient Environmental Conditions.*** Barn ventilation and the surrounding environment also have an important impact on air quality in the barn, but less data exists to evaluate these factors independently. Demonstrated effects of season and stable construction upon particulate concentrations have been interpreted as surrogates for the effect of level of ventilation. However, results are conflicting and often rely on isolated measures of particulate concentration to evaluate the effect of each season. Higher area total particulate and PM10 concentrations but lower respirable concentrations have been reported when winter sampling was compared to summer at a number of horse barns in the mid-west region of the United States.[95] Conversely, evaluation of total and respirable particulate concentrations in a single racing stable in Sweden revealed higher total and respirable dust concentrations during the winter sampling period.[96] Season and stable construction were found to impact particulate concentrations and number when three barns were evaluated at an American Thoroughbred race track: the barn judged subjectively to have the least natural ventilation had significantly higher PM10 and PM2.5 measurements.[86] Particle mass concentrations were lowest in the summer, but particle number variation depended upon particle size.[86] In another study, area respirable particulate concentrations were found to be highest during summer months and positively correlated with ambient temperatures, while PM10 was highest in the winter months.[82] The same study demonstrated that area PM10 concentrations were significantly reduced when ventilation within the barn was maximized by opening barn doors, regardless of season. However, breathing zone particulate measures are not affected by season or temperature, but instead significantly influenced by horse behavior and method of feeding hay.[82] Sources of particulate matter from outside the barn such as road traffic can also impact particulate concentrations within the barn.[86]

In summary, air quality within horse confinement settings is determined by the sources of potential contaminants, such as feed and bedding, activity within the barn, ventilation, and proximity to external sources of particulates, such as traffic pollution. Within an existing barn, such management practices as feeding a complete pelleted feed and removing horses from the barn during cleaning can minimize horse exposure to poor air quality. New constructions should seek to maximize the natural ventilation of the building as well as the distance from potential pollution sources such as busy roads.

## Conclusions

Complex interactions between the various components of organic dust most likely determine the inflammatory and functional status of the equine respiratory tract. Persistence in the quest to further characterize the effect of air quality upon the equine respiratory tract through simultaneous respiratory health and exposure assessments holds promise for improved outcomes in equine respiratory medicine as well as advancing our understanding of the interaction between the pulmonary immune system and the environment. Data collected from experimental exposure trials and large-scale epidemiological studies should allow the determination of threshold limit values for exposures to total and respirable particulates and endotoxin that minimize the risk of airway inflammation in the horse. These values can then be used to judge the clinical significance of exposure measures in various management systems and environmental mitigation strategies.

## Footnotes

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1**. Particulate measurements in horse barns.
**Table S2**. Endotoxin measurements in horse barns.