DRAYTON SOUTH

Equine Health Impact Assessment

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Drayton South Coal Project

Equine Health Impact Assessment

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EXECUTIVE SUMMARY

A comprehensive review of the scientific literature was undertaken to evaluate the potential effects of dust, noise, vibration and blasting on equine health. This review found that horses are subjected to high levels of dust, noise and vibration as part of their normal progression from stud farm to racing stable. Dust exposure was found to be very high, primarily in stabled horses as occurs for example during race training, yearling preparation, and hospitalisation of horses. Despite this dust exposure having an adverse impact on equine health, horses are able to compete to the best of their ability. A key finding from the literature review was that endotoxins attached to dust particles played a key role in initiating respiratory disease in horses. As a result, studies were undertaken to evaluate the endotoxin levels of dust potentially generated by the Project. Noise level exposure was similarly found to be very high, as documented by studies on noise levels undertaken for example on race days. Vibration, in particular that associated with road or air transport, is regularly encountered by horses and there has been no demonstrated adverse effects. There is some evidence that suggests low level whole body vibration can have beneficial effects when applied using scientific principles.

The findings from the literature review enabled accurate interpretation of the potential effects of the Project in regard to dust, noise and vibration. Comparison with data generated in the EA assessments, being the air quality and greenhouse gas impact assessment and the acoustic impact assessment indicated that the levels of dust, noise and vibration that the horses would be exposed to as a result of the project would be far less than that which they are exposed to in a breeding and racing career. While the impact of blasting is difficult to evaluate experience suggests that horses will rapidly become accustomed to this.

Based on the literature review and evaluation of data generated in the EA assessments it was concluded that in terms of dust, noise, vibration and blasting, the Project will have no detrimental effects on the health of horses on the adjoining properties. This includes both adult horses and foals that are either permanent or temporary residents of the properties.

Specific recommendations in regarding to ongoing assessment of any impact on equine health include continuous monitoring of dust levels throughout the duration of the Project as well as assessing the initial impacts of blasting from the Project on horse behaviour.

N Kannegieter

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

1 INTRODUCTION

The author has been engaged by Hansen Bailey Environmental Consultants (Hansen Bailey) on behalf of Anglo American Metallurgical Coal Pty Ltd (Anglo American) to complete an equine health impact assessment for the Drayton South Coal Project (the Project). The purpose of the assessment is to form part of an Environmental Assessment (EA) being prepared by Hansen Bailey to support an application for Project Approval under Part 3A of the *Environmental Planning and Assessment Act 1979* (EP&A Act) to facilitate the continuation of the existing Drayton Mine by the development of an open cut and highwall coal mining operation and associated infrastructure within the Drayton South area.

In October 2011, Part 3A of the EP&A Act was repealed. However, the Project has been granted the benefit of transitional provisions and as a result, is a development to which Part 3A applies.

The purpose of this report is to provide an assessment as to whether the predicted air quality, noise and vibration impacts from the Project are likely to have a detrimental effect on the health of horses on neighbouring properties.

1.1 PROJECT DESCRIPTION

Drayton Mine is managed by Anglo Coal (Drayton Management) Pty Ltd which is owned by Anglo American. Drayton Mine commenced production in 1983 and currently holds Project Approval 06_0202 (dated 1 February 2008) which expires in 2017, at which time the operation will have to close.

The Project will allow for the continuation of mining at Drayton Mine by the development of open cut and highwall mining operations within the Drayton South mining area while continuing to utilise the existing infrastructure and equipment from Drayton Mine.

The Project is located approximately 10 km north west of the village of Jerrys Plains and approximately 13 km south of the township of Muswellbrook in the Upper Hunter Valley of NSW. The Project is predominately situated within the Muswellbrook Shire Local Government Area (LGA), with the south west portion falling within the Singleton LGA. **Figure 1** illustrates the location of the Project. The Project is located adjacent to two thoroughbred horse studs, two power stations and several existing coal mines.

The Project will extend the life of Drayton Mine by a further 27 years ensuring the continuity of employment for its workforce, the ongoing utilisation of its infrastructure and the orderly rehabilitation of Drayton Mine's completed mining areas.

Anglo American is seeking Project Approval under Part 3A of the EP&A Act to facilitate the extraction of coal by both open cut and highwall mining methods within Exploration Licence (EL) 5460 for a period of 27 years. The Project Application Boundary (Project Boundary) is shown on **Figure 1**.

The Project generally comprises:

- The continuation of operations at Drayton Mine as presently approved with minor additional mining areas within the East, North and South Pits;
- The development of an open cut and highwall mining operation extracting up to 7 Mtpa of ROM coal over a period of 27 years;
- The utilisation of the existing Drayton Mine workforce and equipment fleet (with an addition of a highwall miner and coal haulage fleet);
- The Drayton Mine fleet consists of at least a dragline, excavators, fleet of haul trucks, dozers, graders, water carts and associated supporting equipment.
- The use of the Drayton Mine existing voids for rejects and tailings disposal and water storage to allow for the optimisation of the Drayton Mine final landform;
- The utilisation of the existing Drayton Mine infrastructure including the Coal Handling and Preparation Plant (CHPP), rail loop and associated loadout infrastructure, workshops, bath houses and administration offices;
- The construction of a transport corridor between Drayton South and Drayton Mine;
- The utilisation of the Antiene Rail Spur off the Main Northern Railway to transport product coal to the Port of Newcastle for export;
- The realignment of a section of Edderton Road; and
- The installation of water management (including a licence water discharge point and pumping station adjacent to the Hunter River) and power reticulation infrastructure at Drayton South.

The conceptual layout of the Project is shown in **Figure 2**.

1.2 DIRECTOR-GENERAL'S ENVIRONMENTAL ASSESSMENT REQUIREMENTS

All applications for Project Approval under Part 3A of the EP&A must be accompanied by an EA prepared in accordance with the Director-General's Environmental Assessment Requirements (EARs). This equine health impact assessment, which forms part of the EA, addresses the EARs concerning equine health. **Table 1** lists the EARs that are relevant to and addressed by this assessment.

Table 1
Director-General's Environmental Assessment Requirements

Key Issue	Requirement
Air Quality	A quantitative assessment of the potential air quality and odour impacts of the project on both people and livestock
Noise & Blasting	A quantitative assessment of the potential blasting impacts on people, livestock and property

This assessment only addresses these EARs to the extent that they apply to horse health. The impacts on humans and property are not addressed in this report.

1.3 RELATED STUDIES

The studies which are to be read in conjunction with this assessment include the following:

- The EA air quality and greenhouse gas impact assessment;
- The EA acoustic impact assessment; and
- The EA agricultural impact statement.

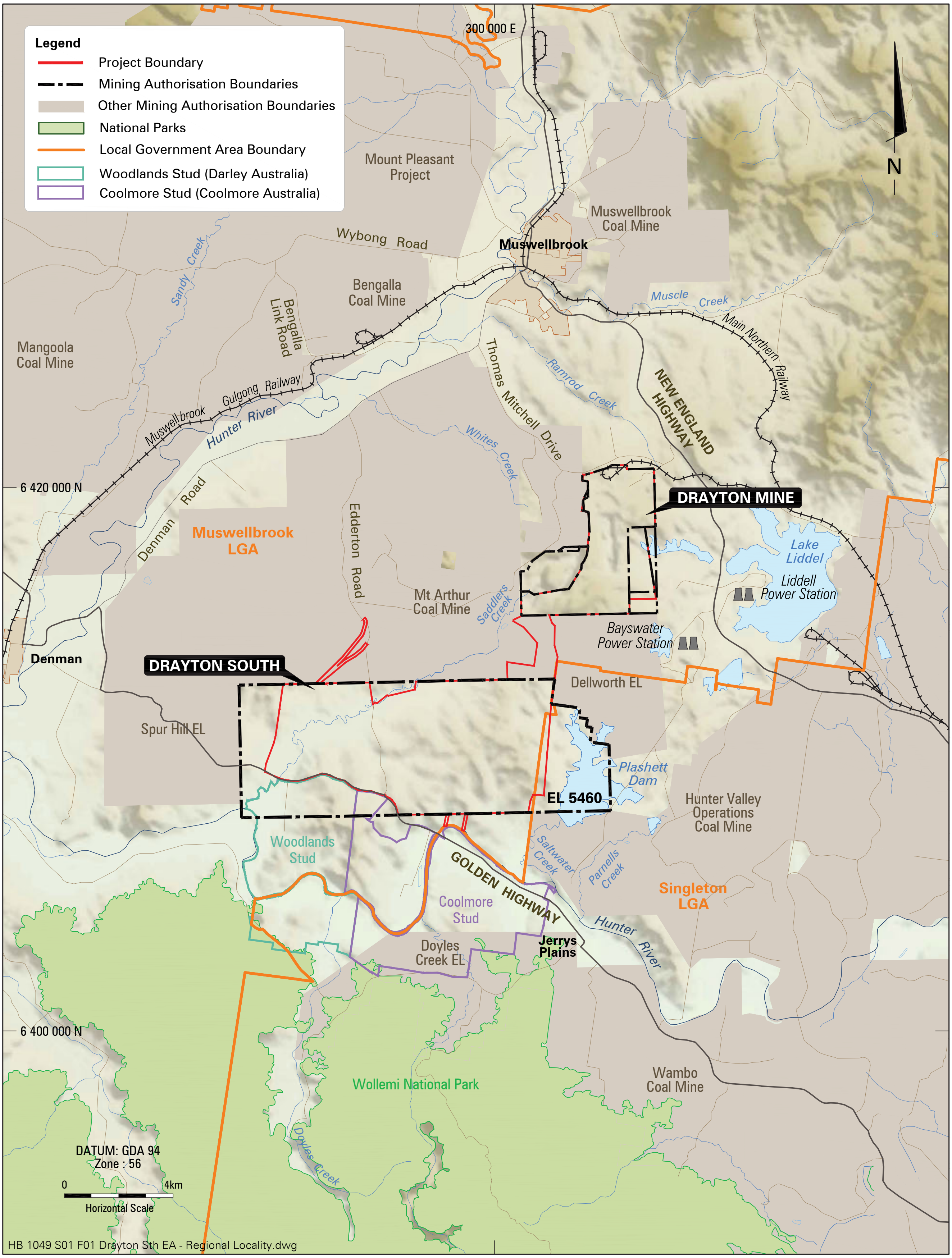
1.4 REPORT STRUCTURE

The report is structured as follows:

- **Section 1** is the introduction;
- **Section 2** describes the assessment approach;
- **Section 3** outlines the key findings of the literature review;
- **Section 4** discusses the potential for health issues resulting from dust generated by the Project;
- **Section 5** discusses the potential for health issues resulting from noise generated by the Project;
- **Section 6** discusses the potential for health issues resulting from vibration generated by the Project
- **Section 7** provides recommendations from the horse health assessment;
- **Section 8** provides a conclusion; and
- **Section 9** lists relevant references.

The report also includes the following appendices:

- **Appendix 1** Horse Health Literature Review – The effects of dust on the equine lower respiratory tract
- **Appendix 2** Comparative review of air quality backgrounds near horse breeding and racing areas prepared by PAEHolmes, 2012
- **Appendix 3** Results of Endotoxin Studies



DRAYTON SOUTH COAL PROJECT

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Regional Locality Plan

FIGURE 1

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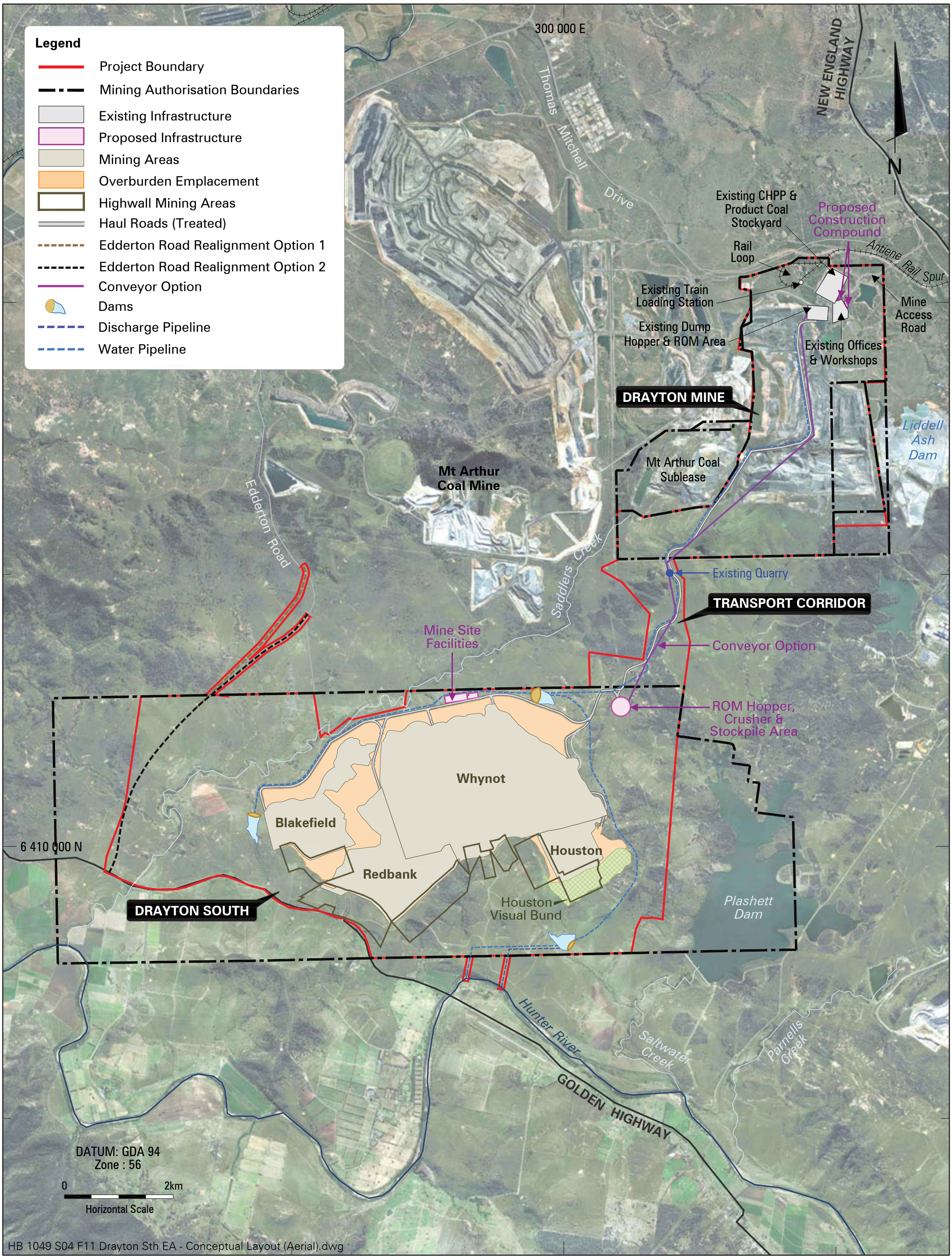
Hansen Bailey



DRAYTON SOUTH COAL PROJECT

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Conceptual Project Layout

FIGURE 2

2 ASSESSMENT APPROACH

The objective of the equine health impact assessment was to establish an understanding of the levels of dust that horses may be exposed to during the various stages of their life cycle in order to understand their level of sensitivity and then draw comparison to the predicted dust levels for the Project to determine whether there is likely to be any potential health impacts for horses being bred on the neighbouring thoroughbred breeding operations of Coolmore Australia and Darley Australia. As part of the assessment, the sensitivity of horses to noise and vibration impacts from blasting was also assessed. This involved a literature search regarding the hearing ability of horses and their response to noise as well as an evaluation of the effect of ground vibration on horses. Predicted data from the Project could then be evaluated for any potential impact on horse health.

The scope of this assessment included the following:

- Address the Director-General's Environmental Assessment Requirement concerning horse health, issued on 3 August 2011;
- Conduct a review of relevant literature and veterinarian databases with regard to the effects of dust, noise and vibration on horses;
- Conduct a review of the existing air quality of the region and a comparative review of this with other well-known racing and breeding locations in Australia and around the world;
- Undertake soil and dust sampling and analysis to evaluate endotoxin levels likely to be present in dust generated from the Project;
- Analyse the results of the air quality modelling undertaken for the Project to determine whether the predicted levels of dust are likely to have an impact on equine health;
- Analyse the results of the noise and vibration assessment undertaken for the Project to determine whether the predicted noise and vibration levels are likely to have an impact on equine health; and
- Provide recommendations and management measures as relevant.

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3 LITERATURE REVIEW

3.1 OVERVIEW

A targeted literature review was undertaken in three main areas to determine the sensitivity of horses to dust, noise and vibration. The findings of each are provided in the sections below.

3.2 DUST LITERATURE REVIEW

A comprehensive literature review was undertaken to determine the levels of dust and endotoxin that horses may be exposed to during rearing and during a racing career. The reason for this was to establish baseline levels of dust exposure in the environment of these horses. While dust is recognised as a potential irritant to the respiratory tract, the response to dust is mostly the result of endotoxins as well as other material such as bacteria or fungi attached to the dust. This is a particular problem in stabled horses, as occurs during race training or yearling preparation.

The following databases were searched in compiling the literature review:

a) CAB abstracts (1990 to present)
b) PUBMED
c) Science Direct
d) Wiley Online Library.
e) Medline (1950 to present)
f) Personal data base N Kannegieter
g) Web of Science
h) Cambridge Journal Online

The review found that there is very little published scientific information which evaluates the effects of dust of topsoil or crustal origin on the health of pastured horses. In contrast there is a large amount of data that examines the effects of dust from bedding, feed and the environment on stabled horses. The review evaluated the available data in both areas. There is also a vast amount of information regarding the effects of dust and pollution on human health. As this was not the focus of the study and there would appear to be sufficient differences between equine and human responses to dust exposure, this area was not reviewed as reliable conclusions cannot be made from the human literature in regard to equine respiratory tract health and disease.

The primary aim of the review was to provide information on known sources of dust as well as the composition and effect of this dust on the equine lower respiratory tract (LRT). Considerable data has been presented in regards to dust in a stabled environment even though this may not seem totally applicable to the situation in pastured horses as generally exists in the horse studs immediately adjacent the Project. This has been done for two reasons. Firstly to provide data on “occupational” background dust levels experienced by horses that undertake any athletic career and secondly to examine the effects that different levels and types of dust may have on the LRT.

The full literature review regarding the effects of dust on equine health is attached in Appendix 1.

Definitions

For the purposes of this report some definitions are presented below.

There is considerable imprecision in use of the term (dust) in both the human and veterinary literature. Many articles describe studies evaluating "dust"; however the composition of this dust can vary widely. This makes comparison between reports and evaluation of data very difficult. In many papers the composition of the dust is not defined at all.

The report of Reed et al (2006) provides full details of regulatory standards in relation to human health and dust in agricultural industries from an Australian perspective and can be referred to human health guidelines if required.

Inspirable Dust - Inspirable (inhalable) dust is defined as a material that may be deposited anywhere along the respiratory tract, where the aerodynamic diameter of the dust may range from 0 to 100 µm (ACGIH, 2005).

Respirable Dust - Respirable dust is defined as the proportion of airborne dust levels that when inhaled may penetrate to the unciliated airways of the lung. The median diameter of the dust particles is 4.25 µm. Respirable dust fraction is defined by ISO 7708 (AS2985, 2004) (Reed et al 2006)

Particulate matter is derived from several sources:

- *Background crustal dust from local and distant areas.*
- *Biologically derived;*
  - *'hay dust' at the horse stud infrastructure, and*
  - *pollen, and plant and insect fragments in ambient air blown from local and distant areas.*
- *Combustion derived particulates (e.g. automobile exhaust, emissions from industrial boilers and other processes, smoke from domestic and grass/bush fires).*
- *Dust associated with the Project (e.g. overburden and coal dust).*

Dust may often be a combination of several of the above sources.

3.2.1 Key Findings

The literature review identified a number of research studies that provided good data with which to compare the potential effects of the Project. The key points from this research are summarised below:

a) There is likely to be a poor correlation between humans and horses in regards to the adverse effects of dust pollution on health.

b) Horses are exposed to a large amount of dust in their lives particularly when performing as athletes. The primary sources of dust are bedding, hay and feed.

c) The major causes of adverse effects from dust exposure on horses in any environment is not the particulate matter as such but rather the endotoxins, bacteria and fungi etc. that are attached to the particulate matter.

d) Horses have a highly refined respiratory tract that greatly protects against contamination of the upper and lower respiratory tracts. They also have excellent mucocillary clearance mechanisms which when combined with the advantages of postural drainage provide a very efficient and effective means of clearing the lower respiratory tract of particulate matter (PM) or foreign material.

e) Despite exposure to high levels of dust, horses can compete to the best of their ability.

f) Dust that does not have high levels of endotoxin associated with it (e.g. nuisance or crustal dust) does not appear to increase the incidence of Inflammatory Airway Disease in horses.

Following the literature review, it was concluded that the very high amount of dust that horses are exposed to, both as a result of being fed hay and in particular being kept in a stabled environment, is an “occupational hazard”. There are undoubtedly effects of this “dust” on the respiratory tract, particularly Inflammatory Airway Disease. However, it is well documented that the effects of dust are primarily a result of endotoxins attached to the dust particle, rather than the inorganic dust component itself.

3.2.2 Key Data from Literature Review

The literature review also identified a number of research studies that provided good data with which to compare the potential effects of the Project. The key points from this data are summarised below:

a) The minimal total airborne endotoxin concentration likely to cause lower respiratory tract disease in normal horses most likely exceeds 20 ng/m^3 (0.02 µg/m^3) (McGorum et al, 1998).

b) Total dust concentration in normal pasture environment for horses has been measured at 0.17 mg/m^3 (170 µg/m^3), respirable dust at 80 µg/m^3 and endotoxin levels of 0.00129 µg/m^3 in total airborne dust (McGorum et al, 1998).

c) The recommended maximum value for inhalable dust in a stable is probably in the order of 2.5 to 3.0 mg/m^3 (2500-3000 µg/m^3). Concentrations of respirable dust in stables range from 0.15 to 9.28 mg/m^3 (150–9280 µg/m^3) with a recommended maximum value of 0.23 mg/m^3 (230 µg/m^3) (Cargill 1999).

d) Racehorses spend most of their time (up to 22 hours) in looseboxes and are exposed to aerosolised foreign dusts and gases almost continuously and any effects these agents have would likely be cumulative. There can be peaks of dust exposure up to 50 times greater than background levels depending on type of bedding, feed, activity of the horse and stable management (Malakides and Hodgson 2003).

e) Forty percent of horses develop Inflammatory Airway Disease within the first 2 weeks of entering racetrack stables for training. This represents a very high percentage of affected horses that are clinically well and preparing for the high stresses of racing (Malakides and Hodgson 2003).

f) More than a third of horses entering racetrack stables had some form of airway inflammation prior to transport to racetrack stables but these horses were not necessarily at greater risk of continuation of inflammation or further respiratory disease (Malakides and Hodgson 2003).

g) Contrary to expectation, many dust-generating activities and sources are not associated with Inflammatory Airway Disease (Malakides and Hodgson 2003).

3.2.3 Inflammatory Airway Disease

The most common disease of the lower respiratory tract of horses in Australia is Inflammatory Airway Disease (IAD). This is a relatively recently categorised condition that covers most LRT diseases of horses apart from Chronic Obstructive Pulmonary Disease (COPD). In Australia well over 20-55% of horses in any form of athletic endeavour may have IAD. The incidence in pastured horses is much lower. IAD is a very general response to a wide variety of stimuli. Causes include viruses, bacteria, fungi, stable dust, etc. The effects that IAD have on horse health and performance vary greatly from no effect through to death in cases that become complicated. IAD is primarily a disease of stabled horses. IAD is considered to be more a response to the presence of irritant material in the lower airways, rather than the more severe allergic response seen in COPD. No doubt the precise definition of both these diseases will evolve with future research and as the links between IAD and COPD, if any, are further evaluated.

N Kannegieter

As IAD is a non-specific response to general irritants, this is potentially a disease that can have an increased incidence directly as a result of increased exposure to dust. The concentration and composition of dust that the horses might be exposed to in a paddock situation needs to be evaluated before any conclusion can be drawn as to the potential risk of IAD in pastured horses.

The report of Malakides and Hodgson (2003) provides comprehensive information on IAD in horses. They found that endotoxin was primarily responsible for IAD. The source of endotoxin is from degenerate g*ram-negative bacteria and as such endotoxin is ubiquitous in nature and is commonly present on surfaces of plants and animals. Variable concentrations of endotoxins are found in agricultural environments such as housing for pigs, chickens, cows and horses, as well as sources such as bedding, hay, grains, straw, and bird droppings. Most environments in which horses have contact potentially can be contaminated with dusts with adherent bacteria and their cell-wall fragments, which subsequently may become aerosolised and inhaled. Endotoxin from dust sources (such as feed, bedding and sources outside boxes) in stables is associated with IAD in thoroughbred racehorses in Australia.*

Malikides and Hodgson (2003) also performed a number of experiments and concluded that 40.3% (range 30-50%) developed IAD within the first 2 weeks of entering racetrack stables for training. This represents a very high percentage of affected horses that are clinically well and preparing for the high stresses of racing. In half these horses the problem persists and may develop into clinical disease as a result of training and management stresses. More than a third of horses entering racetrack stables for training had some form of airway inflammation prior to transport to racetrack stables. The conditions that young racehorses are placed in and their health management (particularly with regard to regular anthelmintic treatment) prior to transport to racetrack stables may influence whether or not horses arrive with some form of IAD. However, the high proportion of young racehorses that develop IAD prior to arrival at racetrack stables are not necessarily at greater risk of continuation of inflammation or further respiratory disease.

A further key conclusion was that contrary to expectation, many dust-generating activities and sources were not associated with IAD.

3.2.4 Rhodococcus equi (Rattles)

Rattles is a common LRT disease in foals and is currently particularly prevalent in the Hunter Valley of NSW (Muscatello et al 2006). There is an increased incidence of the disease associated with dry dusty conditions and dust particles may carry the R Equi bacteria considerable distances. The most common source of R Equi is considered to be the manure from “carrier” mares which is then compacted into soil and in dry dust conditions can be inhaled by foals. As the foal’s immune system is poorly developed, they are susceptible to the disease.

3.2.5 Equine Exposure to Dust

Horses are exposed to large amounts of dust from many sources. These are well reviewed in the report of Malikides and Hodgson (2003) as follows:

> *Factors Contributing to Dust Exposure - In order to be inhaled and cause an effect, dusts must be aerosolised such that solid particles (dusts or smoke) or liquid droplets (mists) of sufficiently small diameter maintain stability as a suspension in air. In racehorse environments this is achieved by machinery (eg., mechanical walkers), ventilation air, or movement of humans and horses in and around stables. Particles that are aerosolised (eg., large viruses, fungal spores and small to medium sized bacteria, fine feed or bedding dust, smoke and motorised pollution) usually range between 0.1 to 10 μm in diameter. They are cleared from air via direct removal by ventilatory airflow (particularly small fungal spores), removal of pathogenic potential while airborne (eg, death of infectious agent) or by reaching an equilibrium and subsequently falling out of the air. Although aerosolised bacteria and viruses may die within seconds, viruses being particularly sensitive to changes in relative humidity, many species still maintain their pathogenicity, antigenicity and ability to induce airway inflammation. This is an important mechanism for certain bacteria, which after death release endotoxin and (1→3)-β-D-glucan, potent pro-inflammatory agents when inhaled into airways. In general, clearance of airborne dusts takes many hours whereas gases such as ammonia or ozone diffuse through air and can remain airborne in still air for much longer. Racehorses, who spend most of their time (up to 22 hours) in looseboxes, are therefore exposed to aerosolised foreign dusts and gases almost continuously and any effects these agents have would likely be cumulative. However, it is important to note that these "steady state" dust levels do not reflect the true levels to which racehorses are exposed due to large variation induced by horse behaviour in loose boxes, horse and human activity in and outside looseboxes, and stable ventilation, bedding type and management. This large variation in concentrations of dusts can occur within horse looseboxes, between looseboxes in the same stable and particularly around the horse's head. Airborne concentrations of dusts in looseboxes, (mostly small fungal and actinomycete spores), regardless of quality of ventilation, can be 2-50 times higher when bedding in looseboxes is being changed and cleaned. On average, this increase in concentration is higher when horses are bedded on straw rather than wood shavings or paper. As well, short periods lying and resting on dust rich bedding exposes horses to massive quantities of infectious and non-infectious particles. Sweeping laneways or stable corridors, catching and moving horses, delivery of feed and bedding to stables, and proximity to roads and urban environments also contribute to increasing exposure of horses to airborne dusts.*

Work by McGorum et al (1998) gives a good indication of base line levels for dust in a pasture management system for horses although the pasture setting was in Edinburgh (UK) so dust levels may be low compared with general Australian conditions. It was found that total airborne endotoxin concentrations exceeded 20 ng/m^3 (0.02 μg/m^3) in half the stables examined, and that even healthy horses in these environments may be exposed to sufficient endotoxin to cause airway inflammation and bronchial hyper-responsiveness.

N Kannegieter

However, as normal horses do not develop detectable pulmonary inflammation or hyper-responsiveness when housed in conventional stables (Derksen et al, 1985; McGorum et al, 1993), it is likely that that the minimal total airborne endotoxin concentration causing LRT disease in normal horses exceeds 20 ng/m^3 (0.02 µg/m^3).

Malikides and Hodgson (2003) reported that under normal outdoor circumstances low concentrations of endotoxin are inhaled. However, the respiratory tract has efficient defence mechanisms to counteract this airborne endotoxin. It is only when high concentrations of dusts containing endotoxin are inhaled and deposited within the airways that inflammation develops.

3.3 COMPARATIVE AIR QUALITY IN RACING AND BREEDING LOCATIONS WORLDWIDE

In all environments that horses are raised or raced there is a background level of dust. This is inevitable due to the agricultural nature of raising horse stock as well as the necessity to provide food, bedding and protection from the elements for horses in race training. Information on background dust levels experienced by horses as part of their normal environment was documented in the literature review.

To date there has been no comprehensive evaluation of dust levels at different geographic locations around the world. Therefore an evaluation of dust levels at various established and well recognised racing and breeding locations was undertaken and the report entitled "Comparative review of air quality backgrounds near horse breeding and racing areas" prepared by PAEHolmes, 2012, is attached as Appendix 2. It was considered important to determine dust levels from other horse racing and breeding sites to enable comparison on a wider basis and to understand how they compared to the existing climate of the Hunter Valley and that of the Project being assessed.

The review evaluated established and well recognised racing and breeding locations within Australia and internationally. Locations identified as fitting the criteria included:

- Australia;
  - Hunter Valley, NSW;
  - Randwick, NSW; and
  - Flemington, Victoria.
- United States of America;
  - Louisville, Kentucky; and
  - Lexington, Kentucky.
- Ireland;
  - Kildare;
  - Tipperary; and

- o Meath.

- United Kingdom;
  - o Newmarket.

- Saudi Arabia;

- Hong Kong.
  - o Sha Tin; and
  - o Happy Valley.

Based on this information, background data for particulate matter finer than 10 microns (PM_{10}) was determined at monitoring stations close to these sites and are reproduced in the **Table 2**.

Table 2
PM_{10} Annual Average Concentrations

Location		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
		PM^{10} Annual Average Concentration (µg/m^3)										
Saudi Arabia	Saudi Arabia	148	143	137	128	118	112	107	104	-	-	-
Hong Kong	Sha Tin	-	-	-	-	53	53	51	50	45	45	-
	Eastern	-	-	-	-	49	47	49	46	43	43	-
Australia	Footscray	-	-	-	-	21	22	20	21	22	-	-
	Singleton	-	-	-	-	-	-	-	-	-	20	19
	Muswellbrook	-	-	-	-	-	-	-	-	-	20	19
	Tamworth	-	-	-	-	-	18	16	16	18	12	13
	Randwick	-	-	-	-	-	22	18	17	20	16	15
United States of America	Louisville	-	-	25	23	26	23	25	22	-	-	-
	Louisville 2	-	-	23	21	24	22	24	21	-	-	-
	Lexington-Fayette	-	-	23	21	24	21	23	19	-	-	-
	Elizabethtown	-	-	19	18	21	17	-	-	-	-	-
	Richmond	-	-	20	18	21	18	-	-	-	-	-
Ireland	Cork, Old Station Road	-	24	26	22	19	16	15	16	18	22	-
	Cork, Heatherton Park	-	21	20	19	17	18	17	15	15	18	-
	Dublin, Dun Laoghraine	-	-	-	-	-	-	-	15	15	15	-
	Tipperary, Clonmel	-	-	-	20	19	-	-	-	-	-	-
	Kildare, Naas	-	-	17	17	-	-	-	-	-	-	-
	Kildare, Newbridge	-	-	-	-	-	-	-	-	14	20	-
	Meath, Navan	-	-	-	-	-	-	23	-	-	-	-
United Kingdom	Newmarket Racecourse	-	-	-	21	20	17	17	16	16	16	16

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This review concluded that the majority of horse breeding and racing businesses within Australia and internationally operate in similar and comparable PM_{10} air quality backgrounds, typically ranging between 15 and 26 μg/m³. As might be expected, Saudi Arabia and Hong Kong, had higher concentrations of between 104 and 148 μg/m³ and 53 and 43 μg/m³, respectively.

This data is important in that it provides a background level of dust that horses currently experience during racing and breeding. As noted in the literature review, the level of endotoxins attached to the dust is of more importance to equine health. To date, there is no data on endotoxin levels from soil in association with potential mining projects. Research in this regard is presented in **Section 4.2** below.

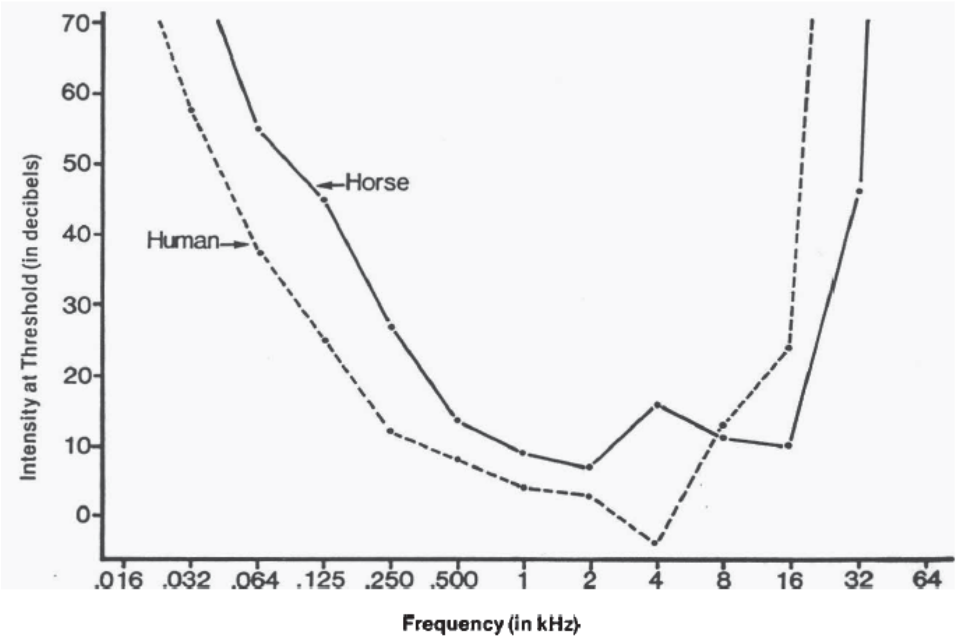
3.4 NOISE LITERATURE REVIEW

There are a small number of articles that have evaluated the hearing ability of horses and their response to noise. Anecdotal information suggest that horses are very adverse to sudden increases in noise and that they are prone to a “flight” response which may result in injury to themselves.

One of the more comprehensive studies on hearing in horses was undertaken by Heffner and Heffner (1983). These authors found that when comparing horses with humans, it can be seen that while most sounds audible to horses are also audible to humans and vice versa, several differences do exist (see **Figure 3** below).

Firstly, horses are less sensitive to low-frequency sounds than humans, whose low-frequency range extends down to 29 Hz (at 60 dBA). Secondly, this difference extends into the mid-frequency range from 500 Hz to 8 kHz where it can be seen that humans' lowest threshold of -4 dBA at 4 kHz is significantly lower than the horse's lowest threshold of 7dBA at 2 kHz. However, above 8 kHz horses are clearly more sensitive than humans, whose 60 dBA high-frequency hearing limit is 19 kHz.

Figure 3
Comparative hearing ability of horses and humans



A review of research into the relative hearing ability of a wide variety of animals (in *Comparative Psychology: A Handbook* by Greenberg and Haraway) found that the hearing threshold of horses was 5-15 dBA higher than humans – that is, horses are somewhat deaf compared to humans.

A more detailed report of hearing in cattle and horses was documented by Heffner and Heffner (1983a). They found that horses' hearing ranged from 55 Hz to 33.5 kHz, with a region of best sensitivity from 1 kHz to 16 kHz, with a lowest threshold of 7 dBA. Audiograms showed a gradual increase in sensitivity as frequency increased to about 500 Hz. At this point the audiogram levels off, with a range of best sensitivity extending from 1 kHz to 16 kHz, with a dip in sensitivity at 4 kHz. Above 16 kHz, sensitivity decreases rapidly until the upper limit of audibility is reached. Overall, at an intensity of 60 dB, the horses' range of hearing extends from 55 Hz to 33.5 kHz.

In low-frequency hearing ability, horses are more sensitive than most other mammals. At an intensity of 60 dBA, the highest frequencies audible were 30 kHz, 38 kHz, and 32 kHz for Horses A, B, and C, respectively. These results give the horse an average high-frequency hearing limit of 33.5 kHz, a value lower than that of most other mammals.

In summary, horses, cattle, and sheep are more sensitive to low frequencies and less sensitive to high frequencies than most other mammals. However, unlike the horse, both cattle and sheep possess well-defined best frequencies at which points they are 13-18 dBA more sensitive than the horse.

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Whether these differences are related to the fact that horses are members of a different mammalian order remains to be determined. Both horses and cattle do not hear as high as most mammals, an observation that coincides with their larger than average interaural distance.

The response of horses to loud, unexpected noise, as well as sights and smell, was investigated by Christensen et al (2005). During the auditory test, a novel sound (white noise, 10–20,000 Hz, 60 dBA) was played as horses approached the feeder. Exposure to the novel visual and auditory stimuli elicited significantly increased heart rate (HR) responses in the horses, whereas there was no increase in HR to the olfactory stimulus. The average HR responses to the different test situations gave a similar picture (control: 52+/-2, visual: 57+/-2, olfactory: 51+/-2.72, auditory: 62+/- 2.28)

Time spent eating was negatively correlated with all other variables, i.e. the more time a horse spent eating, the less it responded to the test stimulus.

The test stimuli elicited different behavioural responses in the horses and the heart rate increased in response to the visual and auditory stimuli, but not to the olfactory stimulus. Apart from a significantly reduced eating time in all test situations compared to the control situation, it is noteworthy that the behavioural responses to the novel visual and auditory stimuli were similar, whereas the responses towards the novel olfactory stimulus differed. The horses in the study showed very little adverse locomotion activity in the tests.

The United States National Park Service's 2004 Sheep Report provides a comprehensive review of the likely effects of aircraft fly-over noise on animals, with particular emphasis on wildlife. The report differentiates between chronic exposure, for which the major concerns are related to the animals' energy conservation, and acute exposure, such as startle and panic behaviour. The report advised that "acute responses… occur in most wildlife species evaluated at noise levels greater than 95 dBA."

One other factor to consider is habituation. If the noise is familiar and not associated with danger, the animals' response will become moderated. This is most evident in the (often ineffectual) use of scare guns to remove pest species such as cockatoos from crops or seagulls from airports. Habituation in horses is commonly seen, for example in horses used in large scale performance events and shows as well as police horses. One of the best examples was the use of army and cavalry horses in many wars up until the early part of last century where horses became accustomed to explosions.

When Flemington Racecourse became the proposed venue for Australia's largest music festival – the Big Day Out – there was concern expressed by the owners of the thoroughbred race horses stabled at the racecourse that the horses may react badly to the potentially excessive music noise. In order to evaluate the potential impact of this concert on horses stabled at the site, a comprehensive noise impact study was undertaken (Huybregts 2008).

The noise exposure (LAeq,15 minutes) of horses during major race events was measured at 58-62 dBA in the stables (rising to 66-68 dBA during helicopter flyovers), and 65-70 dBA in the stalls. The Clerk of the Course's horse was exposed to 76 dBA LAeq, 6h at Randwick Racecourse during the New Easter Carnival and 85 dBA LAeq, 6h at Flemington during the Melbourne Cup. Results of the noise monitoring near the stables showed that on non-race days, the LAeq, 15 minutes noise levels were in the range 50-65 dBA during the day. On race days, noise levels were about 51-68 dBA.

During the Big Day Out, the noise exposure (LAeq, 15 minutes) of horses in the stables was measured at 54-70 dBA. The horses generally showed little response to the music noise except when the noise was associated with visible stimuli, or when the noise was of an alarming character such as short bursts of high-pitched singing.

The figures below show the noise levels experienced in the stalls and by the clerk of the course horse. These show many sudden changes in noise levels, peaking at 100 dBA.

Figure 4 (below) shows the measured noise levels in the stalls. Noise levels (LAeq, 15 minutes) were in the range 64-70 dBA. **Figure 5** (below) shows the noise exposure of Yotis, the Clerk of Course's horse, moving between stalls, the pre-mounting yard, the mounting yard and the race track for the whole event. Noise levels (LAeq,15 minutes) were in the range 69-84 dBA. The LAeq,6h noise level for the whole of the measurement period was 76 dBA.

Figure 4
Measured Noise Levels in the Stalls

(Huybregts 2008)

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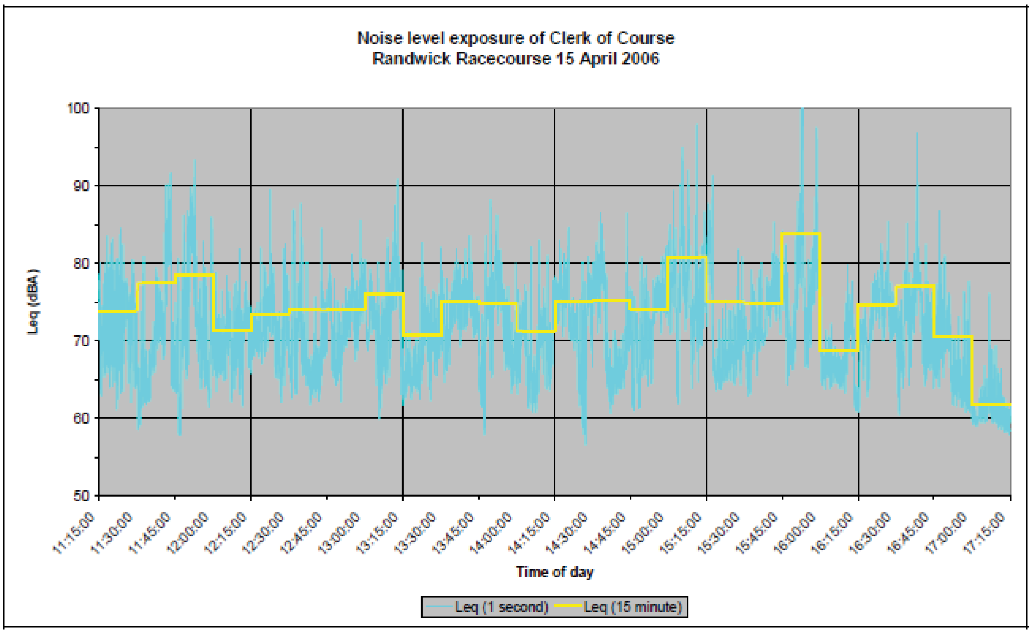
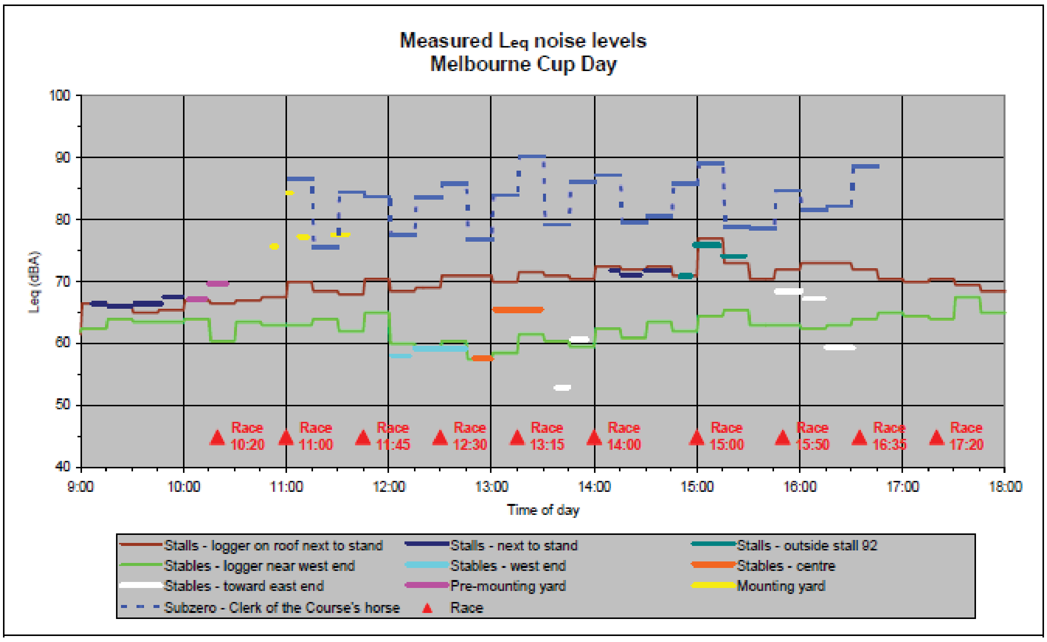
Figure 5
Noise Exposure of Yotis, a Clerk of the Course horse



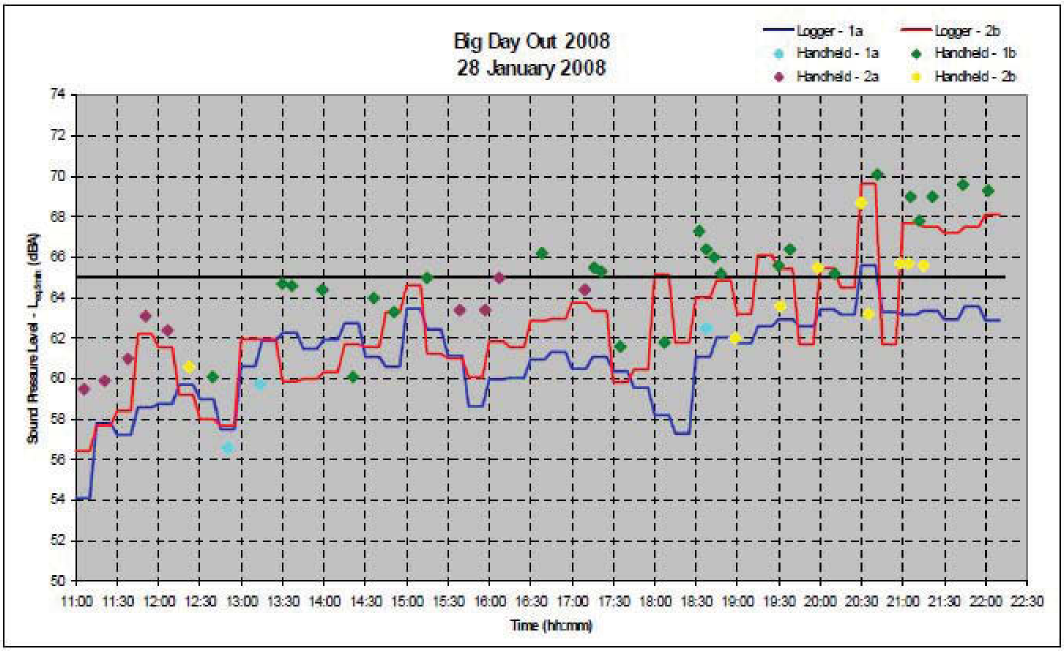
(Huybregts 2008)

Figure 6
Measured Noise Levels Melbourne Cup Day



(Huybregts 2008)

Figure 7
Noise levels in stables during the Big Day Out



(Huybregts 2008)

The Huybregts report (2008) considered:

- That the circumstances of the exposure to concert noise would be somewhat unfamiliar;
- That the noise would not be associated with any danger and if there is any initial startle responses, habituation may occur quickly; and
- That the horses at the two race events investigated were exposed to “average” noise levels of 65-70 dBA in the stalls and 70-90 dBA when moving in and out of the stalls.

They concluded that while definite recommendations regarding criteria for the exposure of thoroughbred horses to noise could not be provided, it was felt that some kind of threshold level would be useful. In summary they advised … “it appears that use of Flemington Racecourse as a concert venue would be acceptable provided that the LAeq noise level in the stables did not exceed 65 dBA.”

This was combined with recommendations that:

- Fireworks or other activities causing loud bangs should not be permitted; and
- Noise levels should be monitored in the stables to confirm that the LAeq noise levels do not generally exceed 65 dBA.

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3.4.1 Environmental Noise in Other Horse Populations

Horses are subject to high noise levels in a number of different environments. One of the most extreme locations is at Clarendon, NSW which is the location of a large RAAF base. Planes land and take off at irregular intervals and the noise and vibration is excessive, The Hawkesbury Equine Veterinary Centre is directly across the road from the base. Despite the loud noise and vibration even horses that are recently introduced to the clinic environment display no ill effects. Hawkesbury race course is also in very close proximity and horses stabled in this location, and those arriving to race at the track, also cope without any adverse effects.

3.5 VIBRATION LITERATURE REVIEW

There is little scientific data regarding the effect of ground vibration on equine health. While little is known of the effects of ground vibration, there have been many studies evaluating the effects of whole body vibration (WBV) in people and animal models (Crewther et al 2004). There is a growing body of evidence, both anecdotal and scientific, which suggests that WBV can be used as a performance-enhancing tool. In most studies it has been found that vibration can positively influence maximal strength, force output, power output and vertical jump height. The use of WBV has been found effective in the prevention of bone loss and/or increasing bone density within various animal models. Considering its stimulatory effect upon bone, this tool may be a potential treatment for osteoporosis and other related bone disorders.

Using animal models it has been shown that low level vibration can double bone formation rates, inhibit disuse osteoporosis and increase the strength of trabecular bone by 25% (Rubin et al 2001). Considering that the magnitude of these mechanical signals are several orders of magnitude below those which cause damage to the bone tissue, it was proposed that this modality could be useful in the treatment of metabolic bone diseases.

A comprehensive review of investigations in the literature provided evidence of the effectiveness of WBV in enhancing skeletal mass in the elderly, in individuals with low-bone mineral density, and adolescents (Prisby et al 2008).

More recently Mikhael et al (2010) in an extensive review of the literature regarding WBV felt there was some indication that high-frequency mechanical strain elicited by WBV may have an anabolic effect on bone and muscle. They reported that the inconsistency in study design made comparison of results difficult but that further research was warranted.

The amount of vibration associated with intermittent blasting that might be experienced by horses close to the Project would seem to be less than that which may be experienced by animals during transport. Unfortunately, little objective work has been done to evaluate any potential effects (Randall, 1992). Given that horses are regularly transported long distances in both planes and motor vehicles without any ill effects or concern, it is likely that vibration from blasting will also cause no concern to horses in the area.

The potential advantages documented in human and animal models have prompted a number of companies to promote the benefits of vibration therapy for horses. One such company is “Vitafloor” (http://dutchdreamhorses.com/products/vitafloor) which produces vibrating surfaces for horses to stand on. The company advises as follows:

RESEARCH & TESTING
The Swedish University of Agricultural Sciences (SLU) in Uppsala has carried out extensive research and testing programs on the Vitafloor ® since 2003, with close consideration towards possible negative side-effects, of which none were found.

Since becoming commercially available in 2005, some 100 Vitafloors® have been used in barns to help horses reach their full potential and to assist in the recovery from acute or chronic problems. In all cases, the rehabilitation time was quicker, and more successful than usual. Remarkably, with horses in absolute box rest no colic occurred (always consult your vet in case of colic).

FACTS
1.Vibration training is the only treatment with a documented positive effect on osteoporosis (brittle bones).
2.Increase of bone density by up to 20% (human study).
3.Vibration training stimulates the entire blood circulation.
4.Vibration training demands energy and burns fat.
5.Vibration training raises production of testosterone.
6.Significant muscle power increases after only 10 minutes per day for 10 days on the Vitafloor®.
7.Horses trained on the Vitafloor® can accelerate faster from standstill and develop more spring in their stride. With trotters, elasticity and flexibility increase.
8.Tests show it has a positive effect on the warming up, as well causing less energy to be needed for the transition into trot.
9.The Vitafloor® benefits and shortens the recovery processes, especially of tendon injuries.

ADVANTAGES
1.Shorter training duration and less man hours necessary for power, flexibility and stamina training.
2.Less risk for rip and fracture of muscles, tendons and bone.
3.Competitive advantage of gaining greater acceleration with less energy consumption.
4.Helps in the prevention and treatment of colic
5.Helps in the treatment of laminitis and silicosis
6.Stimulates more rapid hoof growth – important for horses with hoof problems
7.Enables horses to continue rehabilitation without supervision, thus less labor-intensive.
8.Suitable and effective for rehabilitating horses on box-rest or too difficult in hand.

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Unfortunately, the work undertaken by the Swedish University quoted does not appear to have been published in the scientific literature. However there may be beneficial effects from low grade whole body vibration which makes it even more unlikely that the vibration from blasting will have any detrimental effect on equine health.

Due to its irregular and intermittent nature, it is not suggested that the vibrations from mine blasting will have any measurable positive benefits to nearby horses. However, provided that the impacts of mine blasting are carefully managed so that blasting does not startle grazing or stabled horses, it is not envisaged that any detrimental effects should be encountered.

4 ASSESSMENT AGAINST PREDICTED PROJECT DUST

4.1 EA AIR QUALITY ASSESSMENT

As part of the Environmental Assessment for the Project, PAEHolmes completed an Air Quality and Greenhouse Gas Impact Assessment (the EA Air Quality Assessment) which characterised the existing baseline air quality for the project site and surrounding areas and undertook regulatory dispersion modelling to predict future ambient air quality during the Project life. As part of the equine health impact assessment, the findings of the EA Air Assessment have been reviewed and included in this report for discussion where relevant.

4.1.1 Existing Baseline Air Quality

As part of the EA Air Quality Assessment, background air monitoring data collected over the last 10 years was reviewed and incorporated into the report for the purpose of characterising the existing baseline air quality for the area. The locations of the monitoring sites are shown on **Figure 8** with the results presented in **Table 3**. From the results in **Table 3** it can be seen that the Llanillo site (HV2a), which is closest to the horse studs, recorded an average PM_{10} concentration of 25 µg/m^3 over the monitoring period from 2000 to 2011. During this time the measured level ranged from 12 to 42 µg/m^3.

Figure 8
Monitoring Locations (Figure 4-1 from EA Air Quality Assessment)

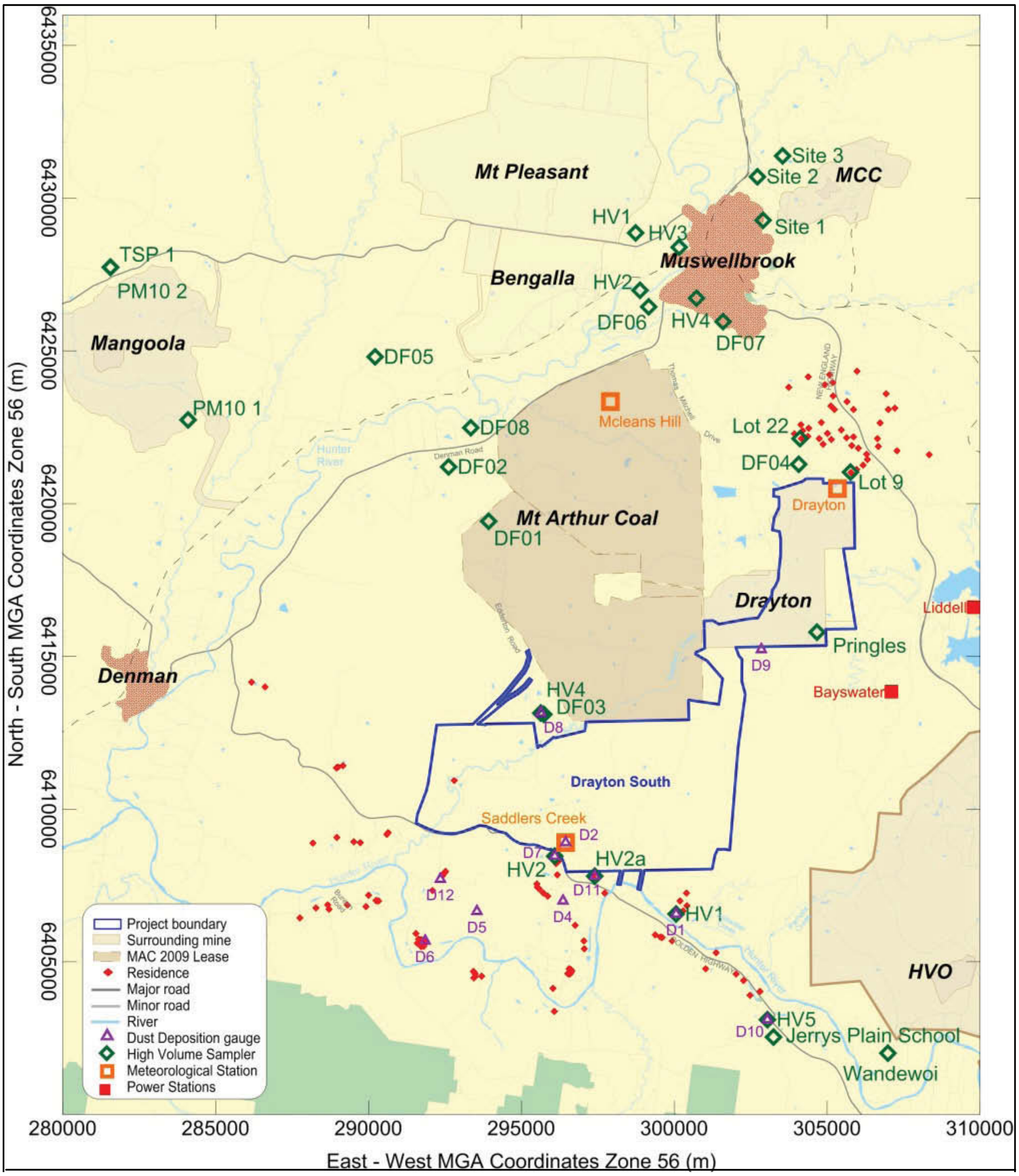

Table 3
TSP and PM_{10} annual average concentrations (µg/m^3) (Table 4.2 from EA Air Quality Assessment)

Year	Edderton (HV4)	Llanillo (HV2a)		Jerrys Plains School (HV5)		LOT 9	LOT 9
	TSP	TSP	PM_{10}	TSP	PM_{10}	PM_{10}	TSP
1998	31	-	-	-	-	-	-
1999	32	-	-	-	-	-	-
2000	30	38	17	-	-	-	-
2001	35	44	15	32	19	-	-
2002	44	53	**39**	49	22	-	-
2003	46	58	**31**	42	**31**	-	-
2004	42	43	**32**	38	25	-	-
2005	45	46	**37**	42	14	21	50
2006	61	59	**42**	52	15	27	-
2007	43	51	20	49	18	31	68
2008	50	43	16	58	17	23	52
2009	45	49	24	55	15	26	63
2010	37	35	14	42	15	-	50
2011	35	32	12	38	13	-	44
Average all data	**41**	**46**	**25**	**45**	**17**	**26**	**54**

Of interest are **Figure 9** and **Figure 10** which show PM_{10} 24hr values from 2001 to 2012 at Llanillo and Jerrys Plains School, both of which are relevant to the studs. There are many readings that were above 100 µg/m^3 with a maximum of approximately 165 µg/m^3 in 2003 at Llanillo and 190 µg/m^3 at Jerrys Plains School. It is noted in this report that the data suggests that higher 24-hour concentrations are generally recorded at HV2a. The Llanillo (HV2a) monitor is located near a cultivated site and is being moved to a more representative location. Even if this is the case, the PM_{10} levels are probably representative of the conditions currently experienced by horses in the district.

The background PM_{10} data highlights the high levels of dust exposure that can be experienced in this area.

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Figure 9
PM_{10} Concentration at Llanillo (HV2a), 2000-2011 (Figure 4-6 from EA Air Quality Assessment)

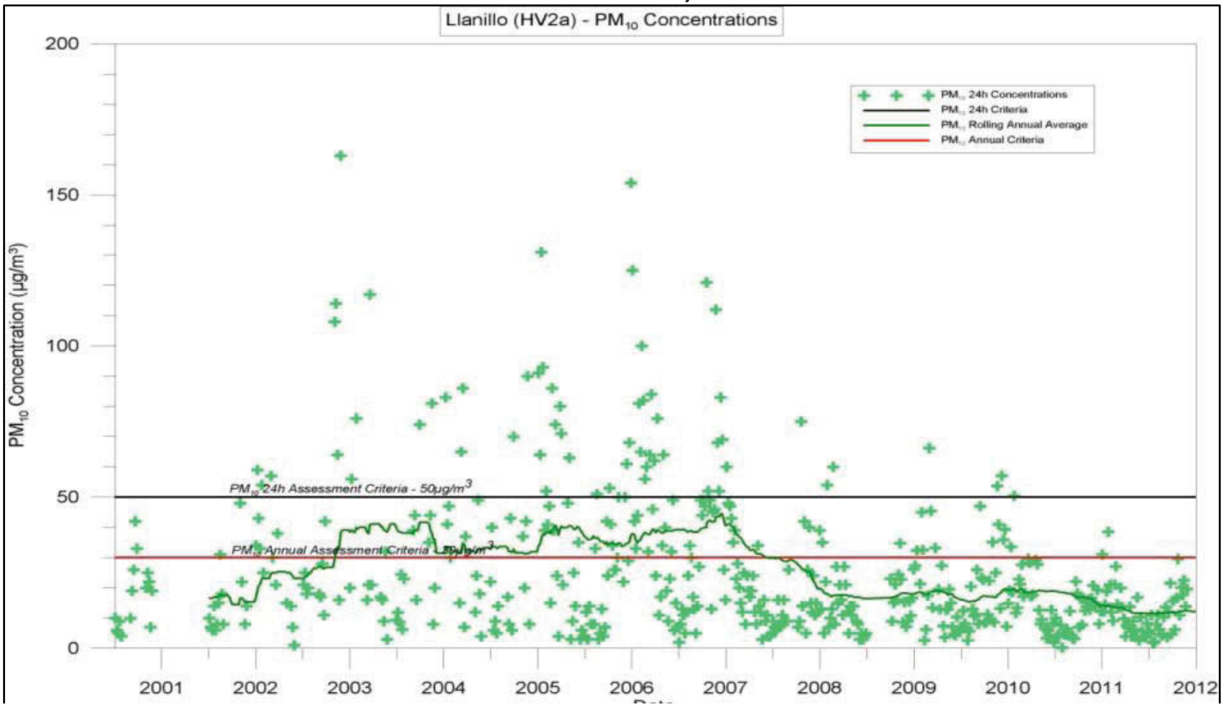
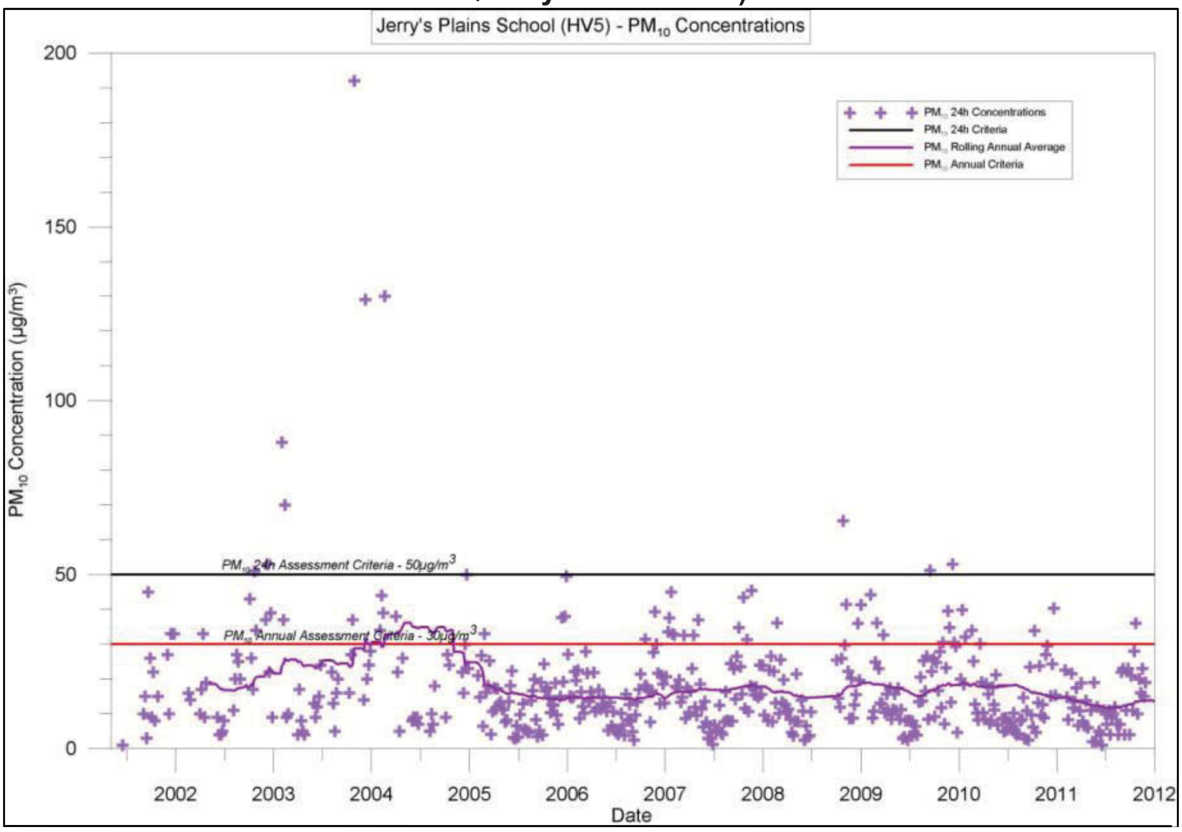


Figure 10
PM_{10} Concentration at Jerrys Plains School (HV5), 2001-2011 (Figure 4-7 from EA Air Quality Assessment)



4.1.2 EA Air Assessment Predictions

From the EA Air Quality Assessment (Table 8.5) it is predicted that the Project will result in small increases in the annual average PM_{10} levels of less than 1 μg/m^3 at Darley receivers and generally less than 3 μg/m^3 at Coolmore receivers (apart from one location (227F), that will experience increased PM_{10} of up to 10 μg/m^3). Representative receivers and monitoring locations are shown on **Figure 12**.

Table 4 provides a summary of the days predicted to exceed 50 μg/m³ during the worst case year of year 10 for Project alone and cumulative. The worst affected residence in the location of the studs is property 226B on Arrowfield Estate. From the equine literature review it is noted that a maximum recommended level of respirable dust in stables should be 230 μg/m³, while levels of 80-170 μg/m³ are considered normal for a paddock. Based on the worst case scenario on Coolmore at site 227F, the EA Air Quality Assessment predicts that as a result of the Project alone the maximum 24hr PM_{10} concentration from the Project will be 52 μg/m³. This is only predicted to be exceeded for 1 day during the year. Cumulative levels above 50 μg/m³ are predicted to occur 53 days of the year taking into account other sources. No exceedance of the cumulative criteria of 150 μg/m³ are predicted. Based on this there should be no risk to pastured horses from combined mine and background PM_{10} even if the worst case scenario occurred on a regular or even daily basis. The annual average results show that the dust levels will generally be below 30 μg/m³ at all locations on Coolmore and Darley.

Table 4
Summary of days exceeding 50 μg/m3 – Year 10 project alone and cumulative (Table 8.4 from EA Air Quality Assessment)

Receptor ID	Maximum predicted PM_{10} 24-hour concentrations	Predicted number of days exceeding 50 μg/m^3 cumulative criteria		Predicted number of days exceeding 150 μg/m^3 Acquisition criteria
	Project Alone	Project Alone	Cumulative	Cumulative
Units	μg/m^3	Number of days	Number of days	Number of days
57	69	4	44	0
58A	79	11	92	0
145A	31	0	37	0
226B	106	23	102	0
226D	72	3	50	0
227A	43	0	30	0
227F	52	1	53	0
240A	26	0	26	0
250A	30	0	27	0
209	21	0	11	0
217	27	0	12	0
410	23	0	12	0
411	23	0	12	0

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The worst case scenario for the paddocks at Coolmore and Darley would seem most likely to occur around Year 10 of the Project, as seen in **Figure 11** below. This indicates that there may be increases of between 10 and 25 μg/m³ on some days of the year and up to 50 μg/m³ in the northern most paddocks for one day of the modelled year. It is noted in the EA Air Quality Assessment that these occurrences are only predicted for 1 day in the modelled year and that it is proposed that the worst case impacts would be managed on a day to day basis using a network of real-time monitoring stations, which will enable mine personnel to respond to high dust levels prior to reaching worst case predicted levels by modifying activities and / or increasing controls as required.

Figure 11
Predicted 24-hour average PM_{10} concentrations due to emissions from Drayton South only - Year 10 (Figure 8-4 from EA Air Quality Assessment)

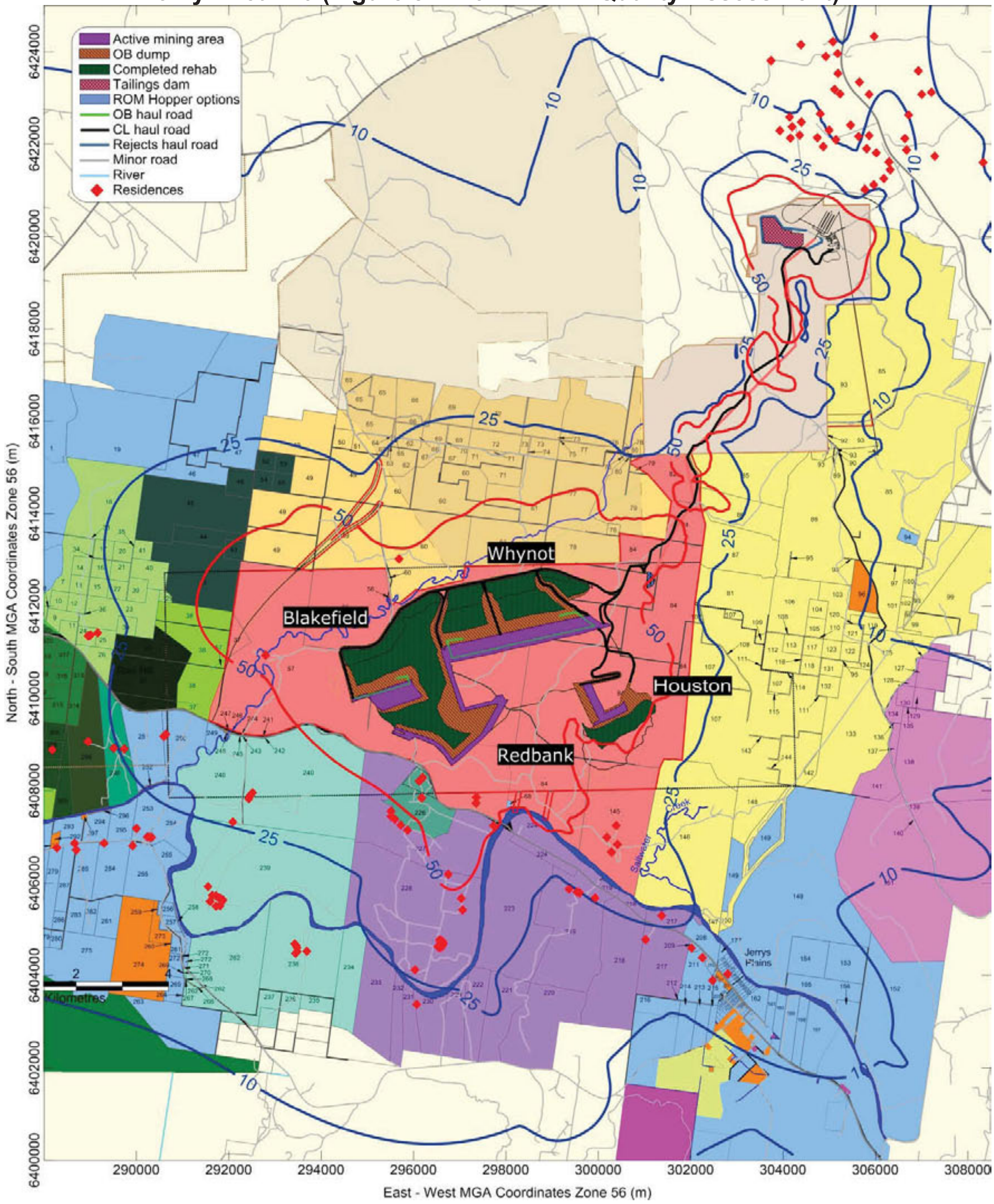
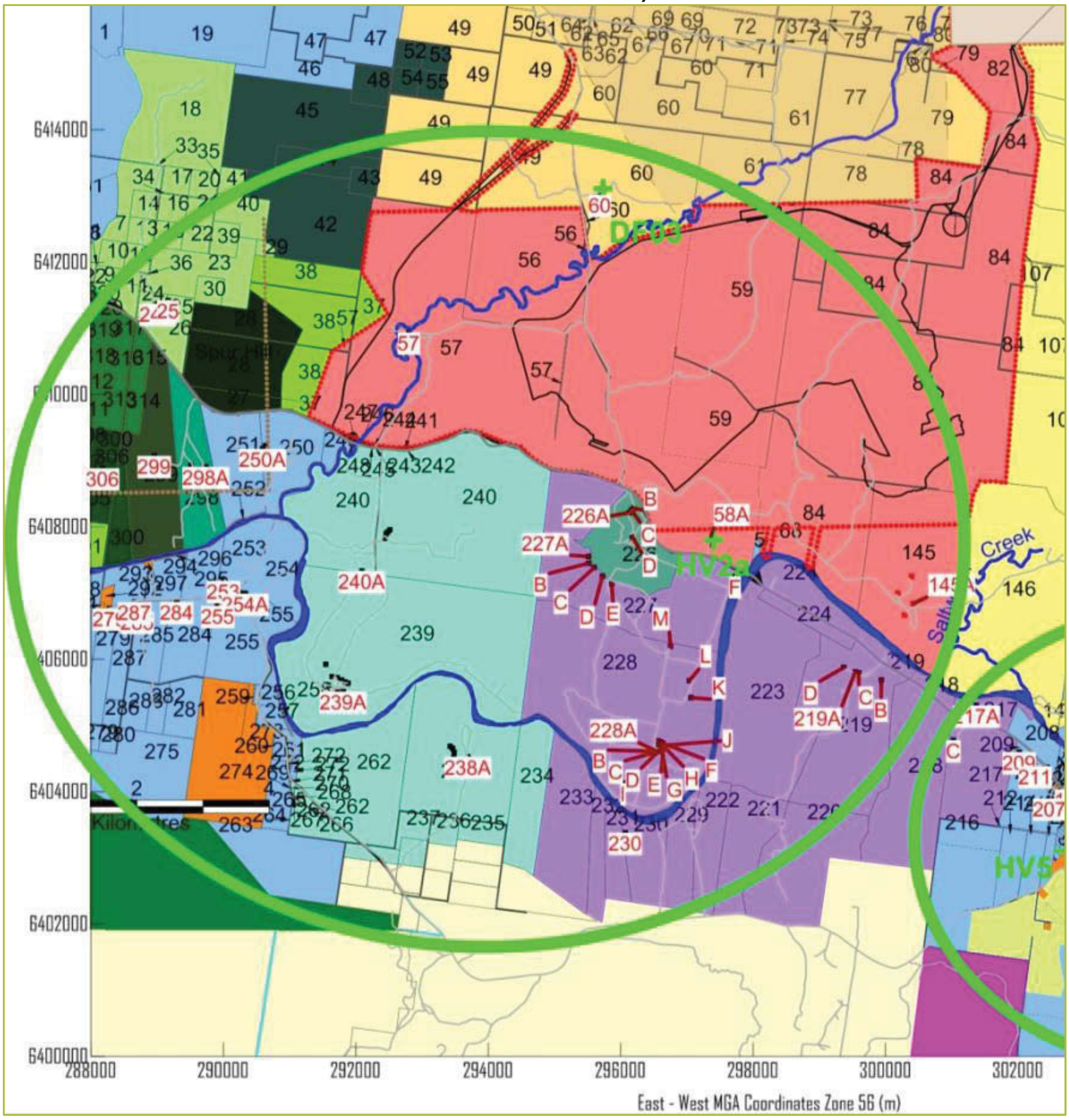


Figure 12
Representative receivers and monitoring locations (Figure 8-8 from EA Air Quality Assessment)



4.1.3 Comparative Dust Levels from Literature Review, Worldwide and the Project

Based on the findings from the literature review, a recommended maximum value for inhalable dust in a stable is thought to be in the order of 2.5 to 3.0 mg/m^3 (2500-3000 µg/m³). Concentrations of respirable dust in stables range from 0.15 to 9.28 mg/m^3 (150–9280 µg/m³) with a recommended maximum value of 0.23 mg/m^3 (230 µg/m³) (Cargill 1999). Total dust concentration in normal pasture environment for horses has been measured at 0.17 mg/m^3 (170 µg/m³), respirable dust at 80 µg/m³ and endotoxin levels of 0.00129 µg/m³ in total airborne dust (McGorum et al, 1998).

Airborne concentrations of dusts in looseboxes, mostly small fungal and actinomycete spores, regardless of quality of ventilation, can be 2-50 times higher when bedding in looseboxes is being changed and cleaned.

A summary of dust levels from the dust literature review and the EA Air Quality Assessment is provided in **Table 5**. This comparison shows that at all stages of the Project and in all locations on both Coolmore and Darley Studs, the levels of dust predicted to be experienced by horses will be less than that which has been recommended for stable horses. This is less than that found in some other studies at pasture and is far less than would be experienced in a normal stable setting. This includes stables in a training environment as well as those that are located on the studs.

Table 5
Comparative Data from Literature Review and Air Quality Review

Activity	Inhalable dust µg/m³	Respirable dust µg/m³	Author
Preparing feed	14970	1080	Reed et al 2006 (Australia)
Conventional stable **In pasture**	2190-5480 80-170	440-2200 80-170	McGorum et al 1998
Stables sawdust **Straw bedding**	397 606		Banhazi et al 2002 (Aust)
Stables	1130		Banhazi et al 2002 (Aust)
Stable Cleaning	1200-2810		Davidson 2004 (Aust)
Stables	410-20,000		Ghio et al 2006
Breathing zone, stabled horse	17510	9280	Woods et al 1993
Stable	200-17200	150-9280	Cargill 1999
Maximum recommended level stables, Europe	2500-3000	230	Cargill 1999
Coolmore, Darley existing residences, PM_{10}		Annual average - 25 (12-42) 24hr – max approx. 190	PAE Holmes 2012
Projected annual average increase Project only, worst case Yr 15		1 (Darley residences) 3 (Coolmore residences except 227F) 10 (Residence 227F) 0-5 generally pasture 5-10 (small area north Coolmore)	PAE Holmes 2012
Predicted cumulative PM_{10} (Project and background)		28 (Annual average PM_{10}) at 227F	PAE Holmes 2012
Hong Kong		PM_{10} 43-53	PAE Holmes 2012
Saudi Arabia		PM_{10} 104-148	PAE Holmes 2012
Randwick		PM_{10} 15-22	PAE Holmes 2012
Lexington USA		PM_{10} 19-24	PAE Holmes 2012

The issue of the possibility of an increased risk of transmission of disease due to higher dust levels has been addressed more fully in the equine literature review. In the Hunter Valley, Rattles (*Rhodococcus equi*) would be considered the most serious potential problem. As manure from “carrier” mares is the primary source of Rattles and no horses have been on the land associated with the Project for some considerable time it is considered there should be no risk of an increased incidence of Rattles in foals arising from the Project.

4.1.4 Conclusions on Predicted Dust

The additional dust levels predicted to be generated by the Project will be a small increase on the current levels and are still well below levels experienced by horses, including adults and foals, in a stable environment (both at the studs and while in race training). The increased levels are still approximately equivalent to those found in Lexington, Kentucky, USA and far less than those which occur in Hong Kong and Saudi Arabia.

Short term increases in dust levels well above those predicted would be well handled by the equine population on the studs and any dust that is inhaled should be rapidly cleared with no adverse effects. This would apply to horses permanently residing on the properties and those visiting temporarily.

There is no apparent increased risk of transmission of diseases such as Rattles as there will not be any of the infecting bacteria in the soil of the Project. Foals and yearlings on the properties are routinely stabled either as a result of illness, for management purposes or for training and are therefore exposed to high dust levels on a regular basis. There will be no increase in risk to foals or yearlings from disease or from the physical impact of dust inhalation as a result of the Project.

Based on the equine health literature review dust levels experienced by horses are likely to be much higher in a stable environment. All horses produced by Darley and Coolmore are intended for a racing career so that the dust levels they will be exposed to during their career will be very high. The increased dust levels should result in no additional health or production problems for any of the horses on these studs.

4.2 ENDOTOXIN ANALYSIS

Endotoxins are bacterial structural components that are released when such a cell is lysed. These components are toxic if administered to humans and/or animals, causing a pyrogenic response (rise in body temperature). One of the important findings from the literature review was that the major cause of adverse effects from dust exposure in any environment is not the particulate matter as such but the endotoxins, bacteria and fungi that is attached to the particulate matter. While dust alone is relatively inert and rapidly expelled by the horse, endotoxins trigger an immediate inflammatory reaction that can result in varying severities of lower respiratory tract disease.

There are high levels of endotoxin present in stables so it was considered important to determine endotoxin levels in the dust which horses might be exposed to as a result of the Project.

4.2.1 Sampling protocol

In order to determine the levels of endotoxin that are present in the soils that will be disturbed by the Project a range of samples were collected and analysed for endotoxin levels.

Sampling for endotoxin analysis involved the following:

- Topsoil – Collection of at least 10g of soil (free of manure or organic matter) from representative areas within the Project Boundary;
- Dust Deposition – Collection of available dust from gauges
- High Volume Air Sampler (HVAS) – Provide filter papers for lab to analyse PM_{10}.

The following samples were collected:

Topsoil samples:

- Site 1 - Plashett Ridge
- Site 2 – HVAS Ridge
- Site 3 – Stockyards Ridge
- Site 4 – Plashett HVAS site

Dust samples

- D8 (Dust Deposition gauge)
- D11 (Dust Deposition gauge)
- D12 (Dust Deposition gauge)
- Plashett HVAS paper

By knowing the predicted dust levels generated by the Project (as discussed in **Section 4.1.2**) an estimate could be made of potential exposure to endotoxins by horses in the paddocks near to the Project.

4.2.2 Methodology

Background

There are several methods available for conducting the endotoxin test, which include the in vivo rabbit pyrogen test and several in vitro alternatives that utilize the Limulus Amebocyte Lysate (LAL) system. The latter has become the method of choice, which can be accomplished by various options including gel clot, kinetic chromogenic and kinetic turbidetric assays. This is done by extracting the test product with pyrogen-free water (PFW) and testing for the presence of endotoxin in the extracts. The method used for this assessment was the kinetic chromogenic method.

Kinetic Chromogenic Method

The kinetic chromogenic method involves an enzymatic reaction between the endotoxin and lysate which results in the production of a yellow colour in the presence of endotoxin. The intensity of the colour production is directly linked to the quantity of endotoxin present in the sample. The laboratory analysis was undertaken by AMS Laboratories.

The method used for the current testing is shown below:

EXAMINATION: LAL VALIDATION

METHOD: Kinetic Chromogenic Method TM125

VALIDATION PROFILE:

- **Pretreatment:** Dissolve 1g in 10mL PFW to make initial 1/10 dilution.
- **Dilution:** Further dilutions were made in PFW to investigate the inhibition and enhancement effect by spiking a known amount of endotoxin and testing for recovery.
- Dilution of 1/1000 in PFW was shown to have a satisfactory recovery, indicating an adequate prevention of inhibition and enhancement by the product.

VALIDATED PROTOCOL: Dilute 1/1000 in PFW.

4.2.3 Results of Endotoxin Testing

The results of the endotoxin analysis for soil and dust samples collected from the Project site are presented in **Table 6**.

Table 6
Results of Endotoxin Testing from Project Site

Location	EU/g	EU/µg	Approx. ng/mg	Approx. ng/µg
Site 1 - Plashett Ridge	189.337	0.000189	0.0189	0.0000189
Site 2 – HVAS Ridge	167.591	0.000168	0.0168	0.0000168
Site 3 – Stockyards Ridge	403.321	0.000403	0.0403	0.0000403
Site 4 – HVAS	353.152	0.000353	0.0353	0.0000353
Average	278.35	0.000278	0.0278	0.0000278
HVAS filter paper Plashett PM 10 27/3/12	<300 EU/filter paper*			
Plashett TSP 27/3/12	3102 EU /filter paper#			
Dust residue sample D3B (D11)	14.691 EU/mg 14691 EU/ gram	0.014691	1.4691	0.0014691
Dust Residue sample D12	<5 EU/mg*			
Dust Residue sample D12	<5 EU/mg*			

*Indicates levels less than limit of detection
Insufficient dust on filter paper to weigh

It is difficult to directly compare results as different studies report findings as either EU/m^3 or as ng/ m^3. Depending on the source of the endotoxin, the conversion from endotoxin units to nanograms will vary. The United States Food and Drug Administration (FDA) initially defined the Endotoxin Unit (EU) as the endotoxin activity of 0.2 ng of Reference Endotoxin Standard (RSE), EC-2 or 5 EU/ng. To convert the current FDA RSE, EC-6 from EU's into ng, the conversion is 10 EU/ng. McGorum et al (1998) used a conversion rate of 12 EU per ng. In this report a conversion rate on 10EU/ng was used.

Based on the levels of endotoxin in soil and the amount of dust that horses will potentially be exposed to, an estimate can be made as to the amount of endotoxin exposure that might occur. Using the worst case 24hr PM_{10} level of 52 µg/m^3 from the Project at receiver 227F (on Coolmore) in year 10 and using the average endotoxin level of 0.0000278 ng/µg derived from the initial four sampling sites (see **Table 6**) this would equate to endotoxin levels of approximately 0.00145 ng/m^3 in the PM_{10} fraction of dust. Assuming the highest annual average PM_{10} level predicted at 227F (28 µg/m^3 in year 10) the annual average endotoxin levels would be approximately 0.00078 ng/m^3.

At site D3B (D11) there was considerably more endotoxin in the dust samples than at other sites. The reasons for this are unclear and repeat sampling over a period of time would be required to provide greater accuracy. However even at this higher level, the endotoxin levels of dust would still only be approximately 0.076 ng/m^3 in a worst case scenario at site 227F on Coolmore in year 10. The levels of exposure in the remaining properties for the duration of the Project will be far less than this upper level.

These results are lower than those obtained by McGorum et al (1998) who reported levels of airborne endotoxin levels in respirable dust at pasture of 0.04 to 0.16 ng/m^3.

The levels are far lower than those reported by Reed et al (2006) in other environments (horse feed sheds had 66 EU/m^3 for example) while Eduard et al (2001) reported levels of 13 x 10^3 EU/m^3.

Berndt et al (2010) found that inflammatory airway disease was common in stabled horses, with a prevalence of 17.3% in Michigan pleasure horses. This was a result of stable dust that was rich in endotoxin and which may induce neutrophilic airway inflammation. In this study endotoxin exposure was about 8 times higher in stables than on pasture. Mean values obtained of endotoxin concentrations in the breathing zone of stabled horses was 7.08 x 10^3 EU/m^3 and for horses on pasture was 0.85 x 10^3 EU/m^3. On pasture, endotoxin varied widely, despite constant climatological conditions. It was suggested that manure was a primary source of endotoxin in both stables and at pasture.

Levels of endotoxin in horse stables were found to be generally less than 0.1 µg/m^3 (Dutkiewicz et al., 1994), the "cut-off" for OH&S purposes, but in other studies, levels were reported ranging from 7.52 to 60.53 ng/m^3 (0.00752 - 0.06053 µg/m^3) in total dust and 1.25 to 11.27 ng/m^3 (0.00125 – 0.01127 µg/m^3) in respirable dust (McGorum et al, 1998).

In the latter studies, concentrations of airborne endotoxins were considerably higher in conventional stables than in "low dust stables", which are hay and straw free environments. In the conventional stables levels in total dust samples ranged from 7.52 to 60.53 ng/m^3, (0.00752 - 0.06053 µg/m^3) compared with 2.12 to 17.41 ng/m^3 (0.00212 - 0.01741 µg/m^3) in low dust stables. The concentrations in respirable dust were 1.25 to 11.27 ng/m^3 (0.00125 – 0.01127 µg/m^3) and 0.09 to 0.56 ng/m^3 (0.00009 – 0.00056 µg/m^3) respectively.

By comparison, concentrations of endotoxins in a pasture paddock at least 50 metres from the nearest hay ranged from 0.25 to 1.57ng/m^3 (0.00025 - 0.00157 μg/m³) and 0.04 to 0.16 ng/m^3 (0.00004 - 0.00016 μg/m³) respectively.

Table 7
Endotoxin levels in equine environment and from the Project

Location	Endotoxin level ng/m^3	Reference
Paddock	0.04 to 0.16	McGorum et al (1998)
Total dust	7.5 to 60.5	McGorum et al (1998)
Respirable dust	1.25 to 11.27	McGorum et al (1998)
Feed shed	6.6 (approx.)	Reed et al (2006)
	1300 (approx.)	Eduard et al (2001)
Breathing zone stable	708	Berndt et al (2010)
Breathing zone pasture	85	Berndt et al (2010)
Cleaning stables	5-8.2	Davidson 2004
Stables	20–9846	Samidi et al 2009
Min level likely to cause respiratory disease in horses	>20	McGorum et al (1998)
Drayton South Project **Worst case** **Average**	 0.076 0.00078	AMS/Kannegieter 2012

4.2.4 Conclusion Regarding Endotoxin

Given that the minimal total airborne endotoxin concentration likely to cause lower respiratory tract disease in normal horses most likely exceeds 20 ng/m^3 (0.02 μg/m³) (McGorum et al, 1998) and the worst case scenario as a result of this Project would be approximately 0.076 ng/m^3 then it seems highly unlikely that there will be any effect on horse health as a result of inhalation of endotoxin associated with dust that might arise from the Project. This would apply to horses of all ages as well as those both permanently on the properties and those visiting temporarily.

The more likely worst case exposure to horses on Coolmore and Darley horse studs is exposure to approximately 30 µg/m^3 of PM_{10} dust in any 24 hr period (from Table 8.1 of the EA Air Quality Assessment) which would equate to approximately 0.001 ng/m^3 using the highest level of endotoxin or 0.0008 ng/m^3 using average endotoxin levels. Both values are well below the 20 ng/m^3 threshold suggested by McGorum et al (1998).

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5 ASSESSMENT AGAINST PREDICTED PROJECT NOISE

As part of the Environmental Assessment for the Project Bridges Acoustics completed an Acoustics Impact Assessment (the EA Acoustics Assessment) which characterised the existing baseline noise environment for the Project site and surrounding areas and undertook modelling to predict future ambient noise during the mines development. This report also included an assessment of blasting and vibration impacts as a result of the Project. As part of the equine health impact assessment, the findings of the EA Acoustics Assessment have been reviewed and included in this report for discussion where relevant.

5.1 EXISTING BASELINE NOISE LEVELS

As part of the EA Acoustics Assessment background noise monitoring was undertaken at key locations surrounding the Project for the purpose of characterising the existing baseline noise levels for the area. From the results of the background noise monitoring, the adopted rating background levels were able to be determined for each of the key receiver areas surrounding the Project. The adopted rating background levels as presented in the EA Acoustics Assessment are provided in **Table 8**. It should be noted that Coolmore Stud is covered by the results for Receiver group C whereas Darley's Woodlands Stud is covered by receiver group D.

Table 8
Adopted Rating Background Levels (Table 7 from EA Acoustics Assessment)

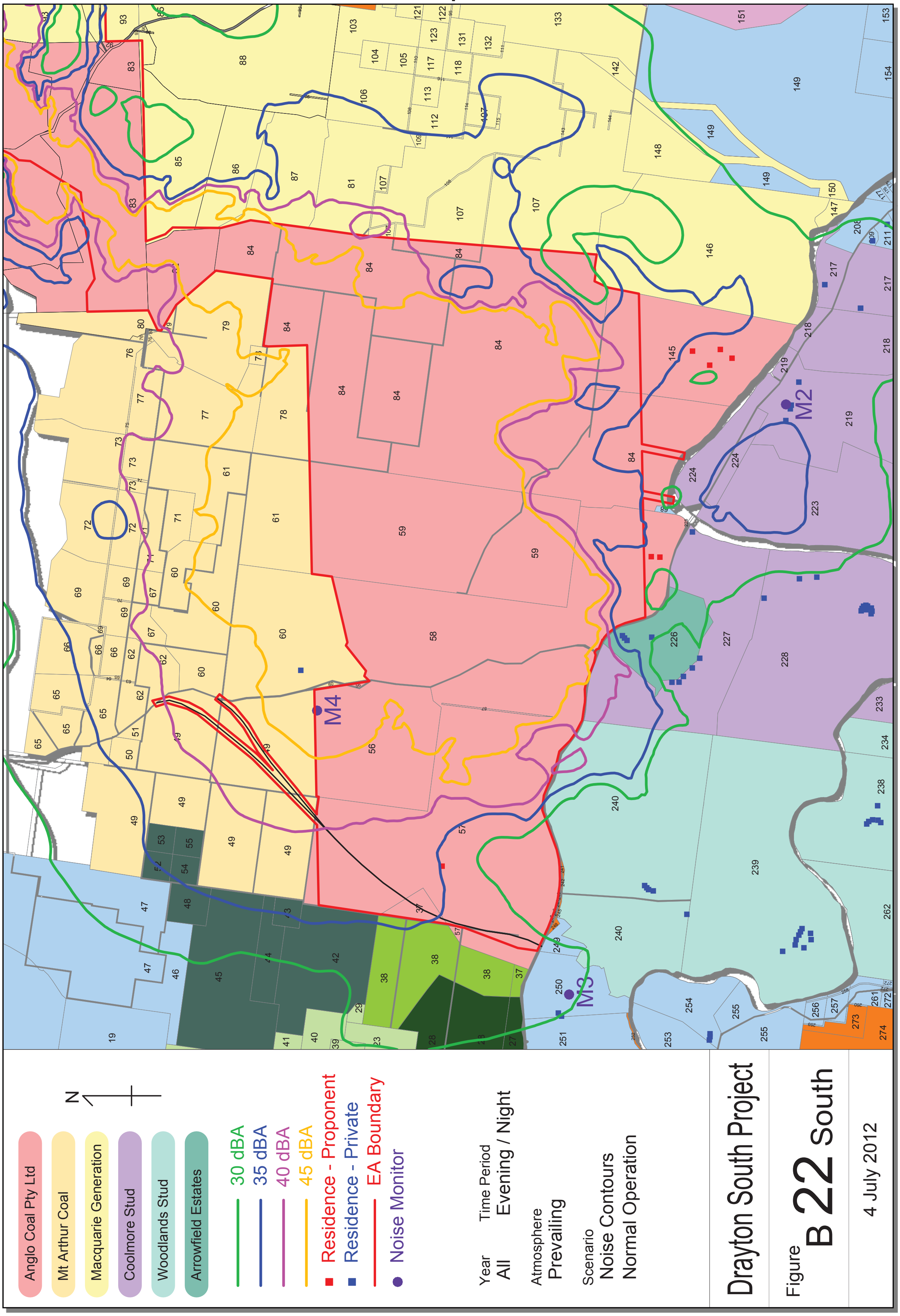
Receiver Area	Rating Background Level, LA90,15min		
	Day	Evening	Night
Receiver Group C M1 – Jerrys Plains and surrounds M2 – Coolmore Stud	35	33	33
Receiver Group D M3 – Woodlands Stud, private properties M4 – private properties	30	30	30

5.2 EA ACOUSTIC ASSESSMENT PREDICTIONS

From the EA Acoustics Assessment, it is predicted that there will be increases in ambient noise levels as a result of the Project (between 5 – 10 dBA above background levels) over relatively small parts of Coolmore Stud and the Darley owned Woodlands Stud. The areas where these increased noise levels have been predicted are the higher slopes and back country located to the north-west on Coolmore Stud land and north east on Woodlands Stud land. **Figure 13** shows the worst case noise envelope for all modelled years for prevailing meteorological conditions during the evening/night.

As can be seen on **Figure 13**, for the vast majority of land associated with these two properties, there will be no noticeable increases in background noise levels as a result of the Project.

Figure 13
Worse Case Noise Envelope for all Modelled Years



N Kannegieter

5.3 CONCLUSIONS REGARDING NOISE

Equine hearing is similar to humans although less sensitive. As a guide it is probable that horses are slightly deafer, with hearing approximately 15 dBA less sensitive than humans. In light of this, human guidelines regarding noise will have some relevance; however horse reaction to noise in terms of rest and productivity is unknown.

Given that the noise levels are not predicted to exceed 40 dBA at any locations on Coolmore or Darley owned land, it is not expected that noise levels will have any impact on the equine population. For the most part at both Coolmore and Darley there will be no noticeable increases in background noise levels as a result of the Project, with only small areas on the northern borders increasing to 35 dBA and even smaller areas to 40 dBA for a short period of the Project's duration (around year 5 and 10).

As such the anticipated noise levels over the vast majority of both properties will remain as they are currently (between 30-33 dBA) with no noticeable increases in background noise as a result of the Project.

The anticipated noise levels predicted from the Project in any case are far less than those experienced by horses in a stable environment and much less than that on race day when noise levels can approach 90 dBA in stalls close to major grandstands during the running of each race. This peaking of noise at high levels would generally be considered the most unsettling for horses. However in the majority of cases horses can tolerate sudden increases in noise well, particularly once they have become accustomed to it.

Foals born during the duration of the Project will be accustomed to any noise from the Project as they mature. Mares and foals visiting the properties temporarily will have been exposed in transit to noise levels much higher than are predicted to arise from the Project and should not be affected by any slight increase in noise.

As the resultant overpressure (or noise from blasting) will gradually (over 27 years) advance towards the horse receptors and blast over pressure is well regulated and limited to human amenity levels it is considered that horses will assimilate and not be stressed by this activity.

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6 ASSESSMENT AGAINST PREDICTED VIBRATION

The EA Acoustics Assessment also included an assessment of blasting and vibration impacts as a result of the Project. As part of the equine health impact assessment the findings of the EA Acoustics Assessment have been reviewed and included in this report for discussion where relevant.

Table 9 shows calculated ground vibration and overpressure levels for closest blast events for two representative receiver locations on both Coolmore and Darley, taking into account topographical or other shielding between the blast site and the receiver where relevant. Results have been calculated assuming blasting in the closest part of the mine to each location in the absence of mitigation measures, and are therefore representative of worst case impacts.

Table 9
Predicted Blast Effects for Locations on Coolmore and Darley, No Mitigation

MIC, kg	500	1000	1500	2000	500	1000	1500	2000	Criteria
Receiver (closest distance)	**Ground Vibration, mm/s**				**Overpressure, dBL**				**mm/s, dBL**
Coolmore Stud									
227 Coolmore Office, 1610m *	1.2	2.1	2.9	3.7	103	106	108	109	5, 115
Strowan Homestead, 3550m	0.3	0.6	0.8	1.0	98	101	103	104	5, 115
Woodlands Stud									
Woodlands Homestead, 5400m	0.2	0.3	0.4	0.5	93	96	97	99	5, 115
Randwick Homestead, 3130m	0.4	0.7	1.0	1.3	100	102	104	105	5, 115

* Overpressure level has been reduced by 5 dBL due to significant topographical shielding.

Results in **Table 9** indicate blast effects should be acceptable at all residential receivers on the Coolmore and Darley properties with a Maximum Instantaneous Charge of up to 2000kg. Anecdotal evidence including that from the Muswellbrook Racecourse and its stable facilities (located adjacent to the Bengalla Mine) would suggest that these levels of impact should not startle either grazing or stabled racehorses of any age.

7 RECOMMENDATIONS

As it is anticipated that the Project will have no adverse impact on equine health, it is considered that few specific recommendations are required. Ensuring that dust levels correlate with projected outcomes throughout the duration of the Project would be considered important as well as assessing the response to the effects of blasting. Specific recommendations are as follows:

7.1 DUST

- Real-time monitoring of dust levels and regular consultation with Darley and Coolmore throughout the duration of the Project;
- The real-time monitoring and proactive dust management system approach would enable Anglo American to pro-actively manage the short-term impacts of the Project (24hr PM_{10}) and minimise dust impacts at sensitive residences to the greatest practical extent.

7.2 NOISE

- No recommendations specific to horse health are considered necessary.

7.3 VIBRATION

- An agreed regime of representative blasts should be monitored, at representative locations on both Coolmore and Woodlands Stud over time to determine if or when horses begin to respond to overpressure or vibration. Additional and agreed visual monitoring should then occur to confirm if reactions are ongoing or they dissipate. In the event that there becomes a threshold level where horses are startled, this should become a limiting factor in blast design.

A Blast Management Plan recommended for the Project should include relevant criteria, management measures and monitoring strategies to ensure ongoing compliance with adopted blast criteria and minimise reaction from horses to blast events based on the above recommendations.

N Kannegieter

8 CONCLUSIONS

8.1 DUST

8.1.1 EA Air Assessment

The additional dust levels expected to be generated by Project will be a small increase on the current levels and still well below levels experienced by horses in a stable environment (both at the studs and while in race training). The increased levels are still approximately equivalent to those found in Lexington, USA and far less than those which occur in Hong Kong and Saudi Arabia.

Short term increases in dust levels well above those predicted would be well handled by the equine population of all ages on the studs and any dust that is inhaled should be rapidly cleared with no adverse effects. This would apply to horses permanently residing on the properties and those visiting temporarily. There is no apparent increase risk of transmission of diseases such as Rattles as there will not be any of the infecting bacteria in the soil of the Project. There will be no increase in risk to foals or yearlings from either disease or the physical impact of dust inhalation as a result of the Project.
Based on the equine health literature review, dust levels experienced by horses are likely to be much higher in a stable environment. All horses produced by Darley and Coolmore are intended for a racing career so the dust levels that they will be exposed to over their life will be very high.

The minor increase in dust levels should not result in any additional health or production problems for the horses on these studs.

8.1.2 Endotoxin Analysis

Given that the minimal total airborne endotoxin concentration likely to cause lower respiratory tract disease in normal horses most likely exceeds 20 ng/m^3 (McGorum et al, 1998) and the extreme worst case scenario (considered unlikely to actually occur) as a result of this Project would be approximately 0.076 ng/m^3 then it seems highly unlikely that there will be any effect on horse health as a result of inhalation of endotoxin associated with dust that might arise from the Project. This would apply to horses of all ages as well as those both permanently living on the properties and those visiting temporarily.

8.2 NOISE

Equine hearing is similar to human hearing although less sensitive. As a guide, it is probable that horses are slightly deafer, with hearing approximately 15 dBA less sensitive than humans. In light of this, human guidelines regarding noise will have some relevance; however horse reaction to noise in terms of rest and productivity is unknown.

Given that the noise levels are not predicted to exceed 40 dBA over any part of the horse studs it is not expected that noise levels will have any impact on the equine population. For the vast majority of land associated with these two properties, there will be no noticeable increases in background noise levels as a result of the Project.

Foals born during the duration of the Project will be accustomed to any noise from the Project as they mature. Mares and foals visiting the properties temporarily will have been exposed in transit to noise levels much higher than are predicted to arise from the Project and should not be affected by any slight increase in noise.

8.3 VIBRATION

The ground vibration arising from the Project is expected to be intermittent and minimal across the Coolmore and Darley properties and is very unlikely to have any adverse effects on equine health. Any vibration experienced is likely to be far less than that experienced by horses during road or plane transport.

Similarly, overpressure from blasting will remain far below that of the resultant frequent helicopter landings at both horse studs. Horses should continue to assimilate to mine blasting impacts as blasting slowly moves closer to the horse studs so long as they remain within human amenity levels.

9 REFERENCES

Berndt A, Derksen FJ, Edward Robinson N. (2010) Endotoxin concentrations within the breathing zone of horses are higher in stables than on pasture. Vet J Jan; 183(1):54-7.

Cargill, C. (1999) Reducing dust in horse stables and transporters: A report for the Rural Industries Research and Development Corporation. South Australian Research and Development Institute. RIRDC Publication No. 99/44.

Christensen, JW, Keeling, LJ and Nielsen BL. (2005) Responses of horses to novel visual, olfactory and auditory stimuli. Applied Animal Behaviour Science 93:53–65

Crewther B, Cronin J, and Keogh J (2004) Gravitational forces and whole body vibration: implications for prescription of vibratory stimulation. Physical Therapy in Sport 5: 37–43

Davidson M 2004, 'Evaluation of a Method for Monitoring Airborne Endotoxin in Australian Animal Handling Environments', BAppSc(Hons) Thesis, University of Western Sydney

SydneyDECOS (Dutch Expert Committee on Occupational Standards). (1998) *Endotoxins: health based recommended occupational exposure limit*. Rijswijk: Health Council of the Netherlands., pp. 1–79. (cited by Reed et al 2006)

Derksen, F. J. & Woods, P. S. A. (1994) Chronic lung disease in the horse: role of aeroallergens and irritants and methods of evaluation. Equine Practice 16, 11-13.

Dutkiewicz, J.; Pomorski, Z. J. H.; Sitkowsaka, J.; Krysinska, T. E.; Wojtowicz, H. (1994) Airborne microorganisms and endotoxin in animal houses. Grana, 33, 85-90.

Eduard W D, Mehl R, Heederik D & Melbostad E (2001), Short term exposure to airborne microbial agents during farm work: exposure-response relations with eye and respiratory symptoms', *Journal of Occupational Environmental Medicine*, vol. 58, pp 113-118.

Heffner, H.E and Heffner, RS (1983) The Hearing Ability of Horses
EQUINE PRACTICE Vol. 5. No. 3 March Page 27-32

Heffner, H.E and Heffner, RS (1983a) Hearing in Large Mammals: Horses (Equus caballus) and Cattle (Bos taurus) Behavioral Neuroscience. Vol 97, No 2. 299-.109

Huybregts. C. (2008) Protecting horses from excessive music noise – a case study.
9th International Congress on Noise as a Public Health Problem (ICBEN), Foxwoods, CT 2008

McGorum, B.C., Ellison, J., Cullen, R.T., (1998) Total and respirable airborne dust endotoxin concentrations in three equine management systems. Equine Vet. J. 30, 430–434.

Malakides N and Hodgson J (2003) Inflammatory Airway Disease in Young Thoroughbred Racehorses RIRDC Publication No. 03/089

Mikhaela,M , Orra R, Fiatarone Singha M. (2010) The effect of whole body vibration exposure on muscle or bone morphology and function in older adults: A systematic review of the literature. Maturitas 66:150–157

Muscatello et al (2006) Rattles in Horses. Effects of stud management on ecology of virulent Rhodococcus equi. RIRDC Publication No 06/097

Odberg, F. 0 A study of the hearing ability of horses, Equine Veterinary Journal, 1978, 10, 82-84

Prisby R et al (2008) Effects of whole body vibration on the skeleton and other organ systems in man and animal models: What we know and what we need to know. Ageing Research Reviews 7:319–329

Randall, JM (1992) Human Subjective Response to Lorry Vibration: Implications for Farm Animal Transport J. agric. Engng Res. 52, 295-307

Reed et al (2006) Respiratory Illness in Farmers -Dust and bioaerosols exposures in animal handling facilities, RIRDC Publication No 06/1071289

Rubin, C et al (2001) Inhibition of osteopenia by low magnitude, high-frequency mechanical stimuli DDT Vol. 6, No. 16:848-858

Samadi S, Wouters I M, Houben R, Jamshidifa A, Van Eerdenburg F, Heederik D J (2009) Exposure to Inhalable Dust, Endotoxins, b(1/3)-Glucans, and Airborne Microorganisms in Horse Stables Ann. Occup. Hyg., Vol. 53, No. 6, pp. 595–603

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APPENDIX 1

Horse Health Literature Review – The effects of dust on the equine lower respiratory tract

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Drayton South Coal Project – Literature Review

The effects of dust on the equine lower respiratory tract

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Summary and Key Points

a) Based on the literature search it was concluded that there is likely to be a poor correlation between humans and horses in regards to the adverse effects of dust pollution on health.

b) Horses are exposed to a large amount of dust in their lives particularly when performing as athletes. The primary sources of dust are bedding, hay and feed.

c) The major causes of adverse effects from dust exposure in any environment is not the particulate matter as such but the endotoxins, bacteria and fungi etc. that is attached to the particulate matter.

d) Horses have a highly refined respiratory tract that greatly protects against contamination of the upper and lower respiratory tracts.

e) Despite exposure to high levels of dust horses can compete to the best of their ability

f) Dust that does not have high levels of endotoxin associated with it (e.g. nuisance or crustal dust) does not appear to increase the incidence of Inflammatory Airway Disease in horses.

Conclusion: The amount of dust horses are exposed to as an "occupational hazard" both as a result of being fed hay and in particular being kept in a stabled environment is very high. There are undoubtedly effects of this "dust" on the respiratory tract, particularly Inflammatory Airway Disease. However it is well documented that the effects of dust are primarily a result of endotoxins attached to the dust particle, rather than the inorganic dust component itself.

Key Points

a) The minimal total airborne endotoxin concentration likely to cause lower respiratory tract disease in normal horses most likely exceeds 20 ng/m^3 (0.02μg/m^3) (McGorum et al, 1998)

b) Total dust concentration in normal pasture environment for horses has been measured as 0.17mg/m^3 (170 μg/m^3), respirable dust at 80 μg/m^3 and endotoxin levels of 0.00129 μg/m^3 in total airborne dust (McGorum et al, 1998).

c) The recommended maximum value for inhalable dust in a stable is probably in the order of 2.5 to 3.0 mg/m^3 (2500-3000μg/m^3) Concentrations of respirable dust in stables range from 0.15 to 9.28 mg/m^3 ,(150–9280 μg/m^3) with a recommended maximum value of 0.23 mg/m^3 (230 μg/m^3). (Cargill 1999)

d) Racehorses, who spend most of their time (up to 22 hours) in looseboxes, are exposed to aerosolised foreign dusts and gases almost continuously and any effects these agents have would likely be cumulative. There can be peaks of dust exposure up to 50 times greater than background levels depending on type of bedding, feed, activity of the horse and stable management. (Malakides and Hodgson 2003)

e) Forty percent of horses develop Inflammatory Airway Disease within the first 2 weeks of entering racetrack stables for training. This represents a very high percentage of affected horses that are clinically well and preparing for the high stresses of racing. (Malakides and Hodgson 2003)

f) More than a t hird of horses entering racetrack stables had some form of airway inflammation prior to transport to racetrack stables but these horses were not necessarily at greater risk of continuation of inflammation or further respiratory disease . (Malakides and Hodgson 2003)

g) Contrary to expectation, many dust-generating activities and sources are not associated with Inflammatory Airway Disease (Malakides and Hodgson 2003)

Table of Contents

1 Introduction

The author has been engaged by Hansen Bailey Environmental Consultants (Hansen Bailey) on behalf of Anglo American Metallurgical Coal Pty Ltd (Anglo American) to complete a horse health literature review for the Drayton South Coal Project (the Project). The Project involves the continuation of the existing Drayton Mine by the development of open cut and highwall mining operations within the Drayton South area (Drayton South) while continuing to utilise the existing infrastructure and equipment from Drayton Mine.

The Project is located approximately 10 km north-west of the village of Jerrys Plains and approximately 13 km south of the township of Muswellbrook in the Upper Hunter Valley of New South Wales (NSW). Two major thoroughbred horse studs are situated to the immediate south of the Project. The purpose of the review is to gain an understanding of the available information that exists with regard to the potential impacts of dust on horse health. This literature review will form part of an Environmental Assessment (EA) being prepared by Hansen Bailey to support an application for Project Approval under Part 3A of the Environmental Planning and Assessment Act 1979 (EP&A Act).

1.1 Overview

There is very little published scientific information which evaluates the effects of dust of topsoil or crustal origin on the health of pastured horses. In contrast there is a large amount of data that examines the effects of dust from bedding, feed and the environment on stabled horses. This review evaluates the available data in both areas. There is also a vast amount of information regarding the effects of dust and pollution on human health. As this is not my area of expertise and there would appear to be sufficient differences between equine and human responses to dust exposure, few reliable conclusions can be made.

The primary aim of this review is to provide information on known sources of dust as well as the composition and effect of this dust on the equine lower respiratory tract (LRT). Considerable data has been presented in regards to dust in a stabled environment even though this may not seem totally applicable to the situation in pastured horses as generally exists in the horse studs immediately adjacent the Project. This has been done for two reasons. Firstly to provide data on “occupational” background dust levels experienced by horses that undertake any athletic career and secondly to examine the effects that different levels and types of dust may have on the LRT. Unless otherwise stated all information presented relates to horses.

Definition of dust:

There is considerable imprecision in use of the term (dust) in both the human and veterinary literature. Many articles describe studies evaluating “dust” however the composition of this dust can vary widely. This makes comparison between reports and evaluation of data very difficult. In many papers the composition of the dust is not defined at all.
The report of Reed et al (2006) provides full details of regulatory standards in relation to human health and dust in agricultural industries from an Australian perspective and can be referred to if human health guidelines are required.

Inspirable Dust - Inspirable (inhalable) dust is defined as a material that may be deposited anywhere along the respiratory tract, where the aerodynamic diameter of the dust may range from 0 to 100 µm.(ACGIH, 2005)

Respirable Dust - Respirable dust is defined as the proportion of airborne dust levels that when inhaled may penetrates to the unciliated airways of the lung. The median diameter of the dust particles is 4.25 µm. Respirable dust fraction is defined by ISO 7708 (AS2985, 2004) (Reed et al 2006)

Particulate matter is derived from several sources:

- *Background crustal dust from local and distant areas.*
- *Biologically derived;*
    - *'hay dust' at the horse stud infrastructure, and*
    - *pollen, and plant and insect fragments in ambient air blown from local and distant areas.*
- *Combustion derived particulates (e.g. automobile exhaust, emissions from industrial boilers and other processes, smoke from domestic and grass/bush fires).*
- *Dust associated with the proposed mine (e.g. over burden and coal dust).*

Dust may often be a combination of several of the above sources.

1.2 Data Bases Searched

The following data bases were searched in compiling this report.

a) CAB abstracts (1990 to present)
b) PUBMED
c) Science Direct
d) Wiley Online Library.
e) Medline (1950 to present)
f) Personal data base N Kannegieter
g) Web of Science
h) Cambridge Journal Online

2 Literature Search results

2.1 Effects of Dust or Pollution on animals in an open environment

There are few studies evaluating this effect.

Newman (1979) documents a large number of outbreaks of pollution causing problems in many species, including horses. Despite a long list of documented pollution associated incidents in a variety of species, all were associated with specific pollutants rather than the general effects of dust. They concluded that some of the major effects of industrial air pollution on wildlife included direct mortality, debilitating industrial-related injury and disease, physiological stress, anaemia, and bioaccumulation.

An example is given below.

TABLE 1
EARLY INCIDENTS INVOLVING THE ADVERSE EFFECTS OF INDUSTRIAL AIR POLLUTANTS ON DOMESTIC ANIMALS

Date	*Location*	*Pollutant(s)*	*Effects*	*Reference*
1873	England	Sulphur dioxide	Death of cattle	Schwabe (1969)
1878	England	Smoke	Blinding of cattle near copper works	Royal Commission (1878)
1908	Montana, USA	Arsenic	Widespread sickness and death to cattle and horses	Formad (1908); Harkins & Swain (1908)
1914	England	Industrial smoke	Respiratory problems in cattle and reduced wool production in sheep	Anon. (1914)
1915	California, USA	Lead	Widespread respiratory problems in horses near smelter	Haring & Meyer (1915)
1930	Belgium	Smoke and fog	Death of cattle from respiratory failure	Alexander (1931); Rubay (1932)
1931	Austria	Iron-containing flue gases	Stomach and intestinal disorders in cattle	Henneman (1931)
1935	Italy	Fluroide	Death of cattle and goats	Bardelli & Menzani (1935)
1939	Germany	Arsenic	Widespread sickness in cattle, sheep, horses and poultry	Bischoff (1939); Wiemann (1939)

From Wildlife, Biol. Conserv. 15:181-190

Crichlow et al (1980) found that the concentration of airborne particles in stables was more than ten times greater than that of outdoor air.

A report from India (Dogra et al, 1984) examined the effects of pollution from mining on the lungs of bovines. These authors collected lungs from bovines slaughtered in areas identified as being in high density mining areas and c ompared them to those from remote regions in which no mining was conducted. The report documented there was considerable gross microscopic lung pathology in cattle from mining areas. This was mostly and possible exclusively due to the presence of heavy metals in the lungs. They identified the presence of as many as 20 trace metals, in particular mercury, iron, zinc and lead. They concluded that the main finding in the lungs of animals inhabiting mining and industrial areas were deposits of particulate dust. The implication was that animals living in mining and industrial areas, but not actually associated with mining and industrial operations, can accumulate large amounts of particulate matter with adsorbed heavy metals in their lungs.

Unfortunately the paper does not provide sufficient detail on levels of pollution, nor the condition under which the cattle were kept. Given that there is a much closer interaction between cattle and pollution in India, where stock frequently live and graze on major highways and roads, it is difficult to differentiate effects of mining from general industrial pollution, particularly from motor vehicles. This paper seems to evaluate the effects of heavy metal pollution rather than dust on the LRT.

As a result of the rapid industrial development in the Hunter Region in New South Wales, which was considered to be a result of its vast coal reserves, James et al (1985) explored the use of applied input output analysis for air quality assessment and management in the region. The technique linked economic activities to pollutant emissions and yields total emissions, which are spatially allocated across the region for use with air pollutant dispersion models. These authors considered this a useful model to address the issue of air quality in relation to human health which they considered had emerged as a major public issue and a possible constraint on development.

Work by McGorum et al (1998) gives a good indication of base line levels for dust in a pasture management system for horses although the pasture setting was in Edinburgh (UK) so dust levels may be low compared with general Australian conditions. It was found that as total airborne endotoxin concentrations exceeded 20 ng/m^3 (0.02 µg/m^3) in half the stables examined that even healthy horses in these environments may be exposed to sufficient endotoxin to cause airway inflammation and bronchial hyperresponsiveness. However, as normal horses do not develop detectable pulmonary inflammation or hyper-responsiveness when housed in conventional stables (Derksen et al, 1985; McGorum et al, 1993), it is likely that that the minimal total airborne endotoxin concentration causing LRT disease in normal horses exceeds 20 ng/m^3 (0.02 µg/m^3).

B. C. McGorum *et al.* 431

TABLE 1: Airborne dust concentration (mg/m^3), airborne endotoxin concentration (ng/m^3), and endotoxin content of dust (ng/mg) in 3 equine management systems (median and range)

Management system	Sample type	Airborne dust concentration (mg/m^3)	Airborne endotoxin concentration (ng/m^3)	Endotoxin content of dust (ng/mg)
Conventional	Total dust	2.74 (2.19–5.48)	19.76 (7.52–60.53)	7.57 (3.43–11.04)
(Hay + straw)	Respirable dust	1.10 (0.44–2.20)	1.67 (1.25–11.27)	1.52 (0.73–25.70)
Low dust	Total dust	0.80 (0.29–2.78)	3.91 (2.12–17.41)	6.90 (3.07–12.40)
(Shavings + silage)	Respirable dust	0.22 (0.15–0.29)	0.11 (0.09–0.56)	0.70 (0.30–1.90)
Pasture	Total dust	0.17 (0.08–0.17)	1.29 (0.25–1.57)	4.85 (0.59–9.40)
	Respirable dust	0.08 (0.08–0.17)	0.11 (0.04–0.16)	1.10 (0.10–1.70)

From Equine Vet. J 30:431

According to Mazan et al (2001) horses from urban areas did not have increased airway inflammation or bronchoconstriction compared with horses from rural locations.

Malikides and Hodgson (2003) reported that under normal outdoor circumstances low concentrations of endotoxin are inhaled however the respiratory tract has efficient defence mechanisms to counteract this airborne endotoxin. It is only when high concentrations of dusts containing endotoxin is inhaled and deposited within the airways that inflammation develops.

Deaton and Marlin (2004) undertook a r eview of the potential effects of general industrial pollution on equine respiratory health but provided no actual data. This report is speculative and based mainly on experiences in people. They summarised that despite numerous publications on the effects of dust from the housing environment in humans that the effects of chemical pollutants on equine lung function were largely unknown. The concentration and type of pollutants to which horses are exposed to in any situation is affected by factors such as time of day of exercise, wind direction and other environmental factors, including sunlight, temperature and cloud cover. Also the responses between horses to airborne pollutants are likely to be highly variable. They concluded that the effects of airborne pollutants on a irway inflammation and performance, if they occurred at all, were likely to be sub-clinical and impossible to differentiate from other causes of airway inflammation such as transport, heat or pre-existing airway inflammation.

Reed et al (2006) reviewed dust levels in several industries from a human exposure perspective, as documented in the table below.

Table 1 Summary of Worker Exposures to Dust and Bioaerosols in Animal Handling Facilities

Animal Industry	Inspirable dusts mg/m^3	Respirable dusts mg/m^3	Endotoxins EU/m^3	Fungi CFU/m^3	Bacteria CFU/m^3	Sources
Pig	10.04	0.81	841.7		2.08×10^5	Rhyder 1993, Holyoake 2002, Chinivasagam & Blackall 2005
Poultry	9.95	0.48				McGarry & Ivin 2002
Sheep Shearing	0.74			3.43×10^3	2.84×10^3	Kift et al 2004b
Horse feedsheds	8.49	1.08	66	1.49×10^3	0.86×10^3	Reed et 2003b & Davidson 2004
Cow feedlots	0.20	2.72		1.80×10^3	1.42×10^3	Reed et 2003a
Deer	2.74	1.64		0.91×10^3	2.53×10^3	Kift et al 2002a

From RIRDC Publication No 06/1071289

Reed at al (2006) also documented average dust levels associated with mixed farming, which included a range of animal types.

Table 3.15 Average Dust and Bioaerosol Exposures Reported in Mixed Animal Farming

Activity	Dust		Bioaerosols		Endotoxins		Source
	Inhalable Dust (mg/m^3)	Respirable Dust (mg/m^3)	Fungi (CFU/m^3)	Bacteria (CFU/m^3)	Inhalable Endotoxin (EU/m^3)	Respirable Endotoxin (EU/m^3)	
Mixed Framing	0.84		1.2 x 10^6	2.5 x 10^6	13 x 10^3		Eduard et al. 2001

From RIRDC Publication No 06/1071289

A study on the general effects of pollution on racing TBs in US was undertaken by Gates (2007). It was concluded that the impact of pollution on the Thoroughbred racing industry was minimal and that only under the most hazardous ozone levels were race times markedly slower, and such conditions were rarely encountered on race day.

Berndt et al (2010) found that inflammatory airway disease was common in stabled horses, with a prevalence of 17.3% in Michigan pleasure horses. This was a result of stable dust that was rich in endotoxin and which may induce neutrophilic airway inflammation. In this study endotoxin exposure was about 8 t imes higher in stables than on pasture. Mean values obtained of endotoxin concentrations in the breathing zone of stabled horses was 7.08 x 10^3 EU/m^3 and for horses on pasture was 0.85 x10^3 EU/m^3. On pasture, endotoxin varied widely, despite constant climatological conditions. It was suggested that a pr imary source of endotoxin in both stables and at pasture was manure.

2.2 Respiratory Defence mechanisms and Comparative Physiology between human, equine and other species

2.2.1 General comments

Species difference in histamine release from lungs in response to cotton dust were found both between species as well as intra-species by Evans and Nicholls (1974). In this study horses were less refractory to dust while human and pig lungs were most sensitive. Reactivity was also found to increase with age. These authors cited earlier works by Nicholls et al (1967) and. Douglas et al (1970) that failed to detect any histamine release in response to dust in cattle, sheep and horses. (Douglas et al (1970) Histamine release and bronchoconstriction due to textile dusts and their components , Proc. 2nd Int. Conf. Respiratory Diseases in Textile Workers, Alicante, Spain, 1968 pp. 148-155): NICHOLLS, P. J., NICHOLLS, G. R., & BOUHUYS, A.(I967) , ' Histamine release by compound 48/8o and textile dusts from lung tissue in vitro ', in Inhaled Particles and Vapours. II (ed. Davies), pp. 69-74. Oxford: Pergamon.)

Sweeney et al (1989) confirmed there was good tracheal mucus clearance rates in horses with a tracheal mucus velocity of approximately 20mm/min, as had been shown in many studies. These authors considered there were considerable differences between species in structure and function of the respiratory tract.

Malakides and Hodgson (2003) in a major review article cited other authors and advised that endotoxin concentrations in airborne dust measured over an 8-hour/5-day week must generally be above 4.5 to 10 ng/m^3 (0.0045 – 0.01 µg/m³) for detectable airway inflammation to occur in humans and be substantially higher, approximately 100 to 200 ng/m^3 (0.1 -0.2 μg/m³) to induce general respiratory symptoms and disease (Rylander R. Evaluation of the risk of endotoxin exposure. International Journal of Occupationaland Environmental Health 1997;3:S32-S36 ; Douwes J, Heederik D. Health-based Recommended Occupational Exposure Limit for Endotoxin.Wageningen, The Netherlands: Agricultural University of Wageningen, 1995;1-3) . Although these "no-effect levels" frequently are found in human occupational settings as well as in horse stable environments (Pirie RS, Dixon PM, Collie DDS, McGorum BC. Pulmonary and systemic effects of inhaled endotoxin in control and heaves horses. Equine Veterinary Journal 2001;33:311-318) they cannot be used as guidelines for horses due to extreme differences in duration and types of exposure.

Ghio et al (2006) provided information that suggests there is a close correlation between inhaled particulate matter lung injury in humans and both RAO and COPD in horses. The observations are very general in in many respects and reflect more the similar nature of a chronic inflammatory response rather than a response to any specific particulate matter.

Purdy et al (2008) undertook an experiment in goats which were exposed 7 times to sterilized fine feedyard dust (mean+/-SD particle diameter, <7.72+/-0.69 micron) for 4 hours in a specially constructed tent. Half had been inoculated intra-tracheally with fungal spores prior to dust exposure. They concluded that fine dust inhalation appeared to decrease the ability of goats to successfully clear fungal spores from the lungs following intratracheal inoculation.

2.2.2 What size particles reach the lungs

O'Callaghan et al (1987) reported that in humans the distribution of aerosol particles in the lung is dependent on two principal factors; particle size and the character of the respiration. Larger particles (2 to 10 μm) tend to deposit on the larger airway surfaces while moderate sized particles (0.5 to 2.0 μm are more easily able to follow the direction of air flow and are deposited by sedimentation when air flow velocity slows sufficiently, allowing then to gravitate out in the smallest airways or alveoli. Initially, particles smaller than 0.1 to 0.5 μm distribute in the same way but may be further affected by humidity in the airways causing significant increases in droplet size. Nearly all deposition of particles less than 2 μm in size is by sedimentation. Furthermore, the quantity of particles deposited by sedimentation increases with increasing bronchial number. Based on studies using radioaerosols in the horse similar behaviour seems probable since no significant central deposition occurred to indicate impaction of large particles in the major airways, and uniform distribution of the radioaerosol in the lung periphery was consistently observed.

Clements and Pirie (2007a) reported that the respirable dust concentration (RDC) (defined in this report as the portion that is of a sufficiently small aerodynamic size, usually with a diameter of <5 μ m, to allow penetration of the peripheral, smaller airways) is considered a good index of the health hazard posed by airborne dust inhalation in humans and horses (Derksen and Woods, 1994). The respirable dust particles have been measured in stables (Dunlea and Dodd, 1994, 1996) and are generally accepted to have an aerodynamic diameter of 0.5–5 μm.

Fleming et al (2008b) stated that particle fractions are subdivided into (a) an inhalable fraction being particles less than 100 μm (the proportion by mass of all suspended particles that are inhaled through the nose and mouth); (b) the extrathoracic proportion, being 100 to 10 μm (proportion by mass of the inhaled particles that can reach as far as the larynx but not further in the respiratory tract); (c) tracheobronchial fraction, being less than 10 µm (proportion by mass of the inhaled particles which can reach beyond the larynx, but not in the non-cilia region of the lungs [alveoli]); (d) the fraction that can reach the alveoli, less than <5 µm (percentage by mass of the inhaled particles that can reach right into the non-cilia region of the lungs).

Votion et al (1997) reported only particles under having a diameter less than 5 µm potentially reach the lower respiratory tract. Factors affecting the deposition of droplets into the lungs include droplet size and respiratory conditions, such as the inhalation and exhalation flow rates and the tidal volume, and anatomical peculiarities. Since characteristics relating to physiological and anatomical factors have a significant influence on aerodynamic behaviour and hence the deposition of the inhaled particles, extrapolation of results obtained in human medicine to horses is not appropriate. These authors documented that the majority (92.6%) of the particles produced by nebulisation of a 0. 9% saline solution will have a M MAD smaller than 5 µm. Aerosol deposition in the lungs expressed as percentage of the activity released from the nebuliser were mean +/- s.d. 5.09 +/- 0.66% and 7.35+/- 1.96%, respectively, meaning that only a fraction of that released at the nostrils actually reaches the lungs. The percentage of aerosol reaching the lungs was smaller than the average 10% of aerosol deposition achieved in human medicine.

Malikides and Hodgsen (2003) provided a very detailed report regarding dust and IAD in horses. They reported that *During normal breathing more than 95% of inhaled particles greater than 5 µm (e.g. small hay and straw fibres, fine wood shaving fibres, sand, pollens, plant spores, and larger bacteria, such as Streptococci) are filtered in the nasal passages, pharynx and tracheal bifurcation as a result of collision and impaction between high velocity particles within the airflow and changing airway anatomy. In the nasal passages, soluble noxious gases are concomitantly removed and neutralised via buffering by fluid and protein found in nasal mucus, and the air is humidified and warmed before entering the smaller lower airways. Once a particle impacts upon the moist nasal respiratory epithelium, it is trapped by mucus and removed by ciliary transport. It is important to note that even though few particles larger than 5 to 15 µm enter the trachea and more distal airways, those travelling with high velocity and volume of air can reach these airways and induce an inflammatory response. This response may be cleared or worsen depending on the agents' toxicity and concentration, particularly if particles contain or have adherent toxins such as bacterial endotoxin. As air reaches the small bronchi and terminal bronchioles of the LRT, total airway cross-section increases and air velocity drops. Deposition of smaller particles, between 0.5 to 5 µm (e.g. large viruses, fungal spores and small to medium sized bacteria, fine feed or bedding particle, smoke and motorised pollution) subsequently occurs by sedimentation onto airway surfaces under the force of gravity where, under certain circumstances, they may induce pulmonary inflammation.*

In general, slow, deep breathing (at rest) enhances sedimentation and leads to relatively uniform deposition of particles throughout the LRT. However, rapid breathing with increased airflow as occurs in horses during exercise increases impaction in the larger lower airways producing high local particle concentrations (100 times more) around the bifurcation carinas of these airway compared to airway walls. This pattern and nature of deposition of potentially harmful particles may therefore be an important determinant of distribution of inflammatory responses in the LRT of racehorses (e.g. diffuse or localised to airways with major branches).

When air reaches the level of the respiratory bronchioles and alveoli, most particles < 0.5 µm do not contact the respiratory epithelium and are expelled in exhaled air. However, because air velocity is very low in the gas exchanging structures, particles with a diameter < 0.1 µm (e.g. gas molecules, endotoxin molecules, viruses, proteins, combustion nuclei, ultra-fine particles) are subject to random thermal kinetic buffeting (Brownian motion/diffusion) and have time to diffuse to the walls of surrounding air surfaces.

Except perhaps for ozone and ammonia, there are no scientific studies demonstrating that exposure to noxious gases in stables or outdoor air pollution plays an equivalent role in equine airway inflammation, and TLVs (threshold limiting values) for any noxious gas for horses are unknown.

2.2.3 Equine respiratory tract defence mechanisms

Due to the absence of information specifically addressing the effects of dust on equine respiratory health many authors look to the results from human studies. Unfortunately the differences in anatomy, physiology, environment and lifestyle make such comparisons unreliable. While the data from human studies, in particular that relating to acceptable exposure levels, should not be ignored, it needs to be evaluated carefully in the correct context.

There are considerable anatomical and physiologically differences between horses and humans. In humans the nostrils, which serve as an e ntry and exit point for PM lies directly above the lungs so deposition of PM into the lungs would be considered much easier, while removal would be much harder. In contrast the nostrils of horses are much lower than the lungs for most of the time, which means it is very difficult for particulate matter to enter the lungs. It is also easier to excrete, particularly in grazing horses and those fed from ground level. Also the trachea in horses is considerably longer and has a very efficient muco-cilary clearance mechanism.

Riihimäki et al (2008) found that the innate immunity in the airways of stabled horses improved in response to mild elevations in respirable dust, 1,3-beta-glucan, and/or cold ambient air.

2.3 Potential Influence of Dust on known Equine Lower Respiratory Tract diseases

There are a large number of potential causes of lower respiratory tract disease in horses.

The following table form Spendlove et al (2008) summarises these causes.

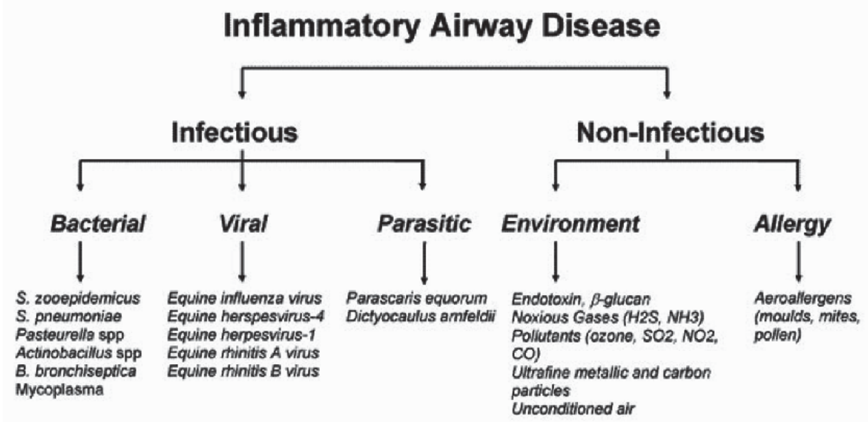


Figure 2: *Potential causes of lower airway inflammation in horses.*

From RIRDC Publication No 08/051

Buechner-Maxwell (1993 a,b) found that airway hyper responsiveness in horses was due to many factors, but dust alone not cited as a major cause. In a later report the same author (Buechner-Maxwell et al, 1996) found that short term exposure (4 weeks) to a stabled environment did not increase any inflammatory mediators in normal horses, although there were some limitations to this study.

Hoffman et al (1993) examined the incidence of distal respiratory tract disease in TB foals in breeding farms in Canada. The study found that 82% (+/- 5%) of foals were affected at one time by lower respiratory tract disease over a 7 mth period with bacterial infection the most common inciting factor.

Hoffman et al (1993b) considered that respiratory tract infection in young foals was regarded as enzootic on horse breeding farms. Bacteria, in particular strep zooepidemicus, were primarily responsible. Dust may also be involved in the spread of infections within stables, and it was suggested that stables need to be at least 100 to 150 metres apart to prevent dust-driven disease spread between buildings (Collins and Algers, 1986).

2.3.1 COPD (Heaves)

Chronic Obstructive Pulmonary Disease (COPD) often referred to as heaves is a common LRT disease in horses in the Northern Hemisphere. It was sometimes referred to as recurrent airway obstruction (RAO). It is uncommon, even in stabled horses, in the Southern Hemisphere.

The disease is seen virtually exclusively in stabled horses and is considered to be an allergic reaction to environmental contamination, in particular "dust". It is not the dust particles alone, rather the material attached to them. In any stable environment there is considerable dust, and attached to that are endotoxins and fungi which are considered the most likely trigger of COPD. While it is beyond the scope of this review to evaluate all the data on COPD in horses, it is clear that COPD would not be considered a potential problem in pastured horses as a result of exposure to dust. For COPD to develop it would be likely the affected horses would need to have a predisposition to the disease (which if they did would manifest in a far more severe form once the horse entered stables for race training); there would have to be prolonged levels of exposure to high levels of dust for repeated, prolonged periods (e.g. days to weeks); and most importantly the specific allergenic trigger (e.g. fungi) would need to be on the dust. It is considered extremely unlikely that such a scenario would exist in the Lower Hunter Region in pastured horses.

McGorum et al (1993) reported that the absence of pulmonary disease in control horses after fungal challenge suggests that COPD is a pulmonary hypersensitivity rather than a non-specific toxic response.

Robinson et al (1996) concluded that as natural and specific antigen challenge affects only COPD-susceptible horses that COPD is a pulmonary hypersensitivity to specific antigens rather than a non-specific response to dusts and irritants in the stable environment.

Art and Lekeux (2005) reported that several equine respiratory disorders are directly caused by, or exacerbated by, inhalation of agents in airborne organic dust, including moulds and bacterial endotoxins. Keeping horses permanently on pasture, with a shelter against inclement weather and no supplementary hay feeding, is the ideal dust-free regime. Unfortunately, for numerous practical reasons, sport horses are often stabled indoors.

Deaton et al (2006) found that healthy horses demonstrated a mild but significant early phase (within 20 minutes) response to inhaled organic dust. This response may serve to decrease the subsequent dose of dust inhaled and as such provide a protective mechanism, which may be compromised in horses with RAO. The duration of organic dust exposure required before RAO affected horses develop clinical evidence of airway obstruction varies from hours to days, and clinical evidence of an early/immediate obstructive response is not apparent.

2.3.2 Exercise Induced Pulmonary Haemorrhage (EIPH)

EIPH or “Bleeding” affects over 95% of racing horses at one time or another. Most horses bleed into their lungs only, while a small percentage show evidence of bleeding at their nostrils. Horses that show external evidence of bleeding (i.e. From both nostrils) after a race or trial face bans from racing including a permanent ban for repeat offences.

EIPH occurs primarily as a result of extremely high physiological blood pressures achieved in the equine lungs during maximal exercise. While it is generally regarded as a physiological event there is speculation that other factors, including lower respiratory tract disease, may increase the incidence or severity of bleeding. As a result of the adverse effects on human RTD from inhaling pollution some work has looked at the possible effect of respirable pollution on the incidence of EIPH.

Mason et al (1984) carried out an investigation into the incidence of exercise induced pulmonary haemorrhage (EIPH) in thoroughbreds in Hong Kong between the 1981 and 1983 racing seasons. As it had been postulated that dust, especially from straw, could be implicated in lung haemorrhage a proportion of horses were bedded on paper in the 1982-83 season. No significant differences could be demonstrated in the incidence of EIPH resulting from the use of paper bedding in preference to straw.

A more recent follow up study (Hurley, 2011) was undertaken in an attempt to evaluate if the increasing levels of pollution in Hong Kong had caused an increase in the incidence EIPH. Despite considerable deterioration in air quality there was no change in incidence or severity of EIPH compared to the earlier study.

It is concluded that increased dust levels or other forms of pollution is extremely unlikely to have any effect on the incidence or severity of EIPH in horses raised on the horse studs immediately adjacent the Project.

2.3.3 Inflammatory Airway Disease (IAD)

There is a r elatively recently categorised condition that covers most LRT diseases of horses apart from COPD. It is recognised in Australia and well over 20-55% of horses in any form of athletic endeavour may have IAD. The incidence in pastured horses is much lower.

IAD is a very general response to a wide variety of stimuli. Causes include viruses, bacteria, fungi, stable dust, etc. The effects that IAD have on horse health and performance vary greatly from no effect through to death in cases that become complicated. IAD is primarily a disease of stabled horses. IAD is considered to be more a response to the presence of irritant material in the lower airways, rather than the more severe allergic response seen in COPD. No doubt the precise definition of both these diseases will evolve with future research and as the links between IAD and COPD, if any, are further evaluated.

As IAD is a non-specific response to general irritants this is potentially a disease that can have an increased incidence directly as a result of increased exposure to dust. The concentration and composition of dust the horses might be exposed to in a paddock situation needs to be evaluated before any conclusion can be drawn as to the potential risk of IAD in pastured horses.

The report of Malakides and Hodgsen (2003) provides comprehensive information on IAD in horses. They found that endotoxin was primarily responsible for IAD. The source of endotoxin is from degenerate g*ram-negative bacteria and as such endotoxin is ubiquitous in nature and is commonly present on surfaces of plants and animals. Variable concentrations of endotoxins are found in agricultural environments such as housing for pigs, chickens, cows and horses, as well as sources such as bedding, hay, grains, straw, and bird droppings. Endotoxins also are present in industrial processing and storage of organic materials such as cotton and flax, wood chips, timber, fibreglass, wastes, potatoes and paper, as well as in animal slaughter and processing facilities, domestic water, house dust contaminated by pets and vermin, and cigarette smoke. Most environments in which horses have contact potentially can be contaminated with dusts with adherent bacteria and their cell-wall fragments, which subsequently may become aerosolised and inhaled. In addition, endotoxins are present in the oral, pharyngeal and nasal cavities as well as the intestinal tracts of humans and animals. Aspiration of secretions from the mouth and nasopharynx may lead to bronchial contamination by endotoxins although horses are more likely to be exposed to endotoxin-contaminated dusts via inhalation.*

It was found that a significant linear (or exposure-response) relationship existed between the average percentage of neutrophils in lower airways and exposure to high (>4-5ng/m^3) (0.004-0.005 µg/m³) respirable endotoxin concentrations in breathing zone dust. In addition, young racehorses that became cases (as defined) and developed neutrophilic IAD were approximately 4 times more likely to have been exposed to either low (<1.2ng/m^3) or high (>4.2ng/m^3) (0.0042 µg/m³) concentrations of endotoxin. These associations controlled for the possible confounding effects of many other variables, highlighting the importance of endotoxin alone in the genesis of neutrophilic lower airway inflammation.

In conclusion, results of this study indicate that endotoxin from dust sources (such as feed, bedding and sources outside boxes) in stables is associated with neutrophilic IAD in 2 and 3 year old thoroughbred racehorses in Australia. As in humans, the severity of this airway inflammation is related to the endotoxin concentration of the inhaled dust. Ventilation quality and meteorological conditions, particularly evaporation level, in looseboxes and stables also were significant risk factors for IAD.

Malikides and Hodgsen (2003) also performed a nu mber of experiments and concluded that 40.3% (range 30-50%) developed IAD within the first 2 weeks of entering racetrack stables for training. This represents a very high percentage of affected horses that are clinically well and preparing for the high stresses of racing. In half these horses the problem persists and m ay develop into clinical disease as a result of training and management stresses. More than a third of horses entering racetrack stables for training had some form of airway inflammation prior to transport to racetrack stables. The conditions young racehorses are placed in and their health management (particularly with regard to regular anthelmintic treatment) prior to transport to racetrack stables may influence whether or not horses arrive with some form of IAD. However, the high proportion of young racehorses that develop IAD prior to arrival at racetrack stables are not necessarily at greater risk of continuation of inflammation or further respiratory disease.

A further key conclusion was that Contrary to expectation, many dust-generating activities and sources were not associated with IAD.

These studies provided the strong evidence that inhaled dust endotoxin is associated with and may cause neutrophilic IAD in young thoroughbred racehorses housed in racetrack stables. In addition, the higher the concentration of endotoxin inhaled by racehorses, the higher the proportions of neutrophils in lower airways. Although it is difficult to predict what dust sources contain higher endotoxin content, reducing overall airborne particle burdens in horse looseboxes and stables clearly is of paramount importance to health and welfare of racehorses.

Millerick-May et al (2008) found that IAD had an overall prevalence of 67%. They confirmed an association with PM<10 µm in diameter (PM10; odds ratio [OR] = 5.8, 95% CI = 1.64 – 20.56, p<0.0064) and PM 2.5 µm in diameter (PM2.5; OR = 4.5, CI=1.35 – 14.90, p<0.0151). The prevalence of tracheal mucus was highest in the stables and months with highest overall PM. The prevalence of tracheal mucus was least in the open-sided stable with low PM and after a period of wet weather when PM was low. Significant improvement can be seen by just opening a window in a stable.

Hughes et al (2011) undertook a study that indicated IAD of horses is associated with increased mRNA expression of proinflammatory cytokines in BALF cells, which may reflect stimulation of the innate immune responses to inhaled antigens. There was no evidence of a polarised T-cell cytokine response suggesting hypersensitivity responses may not be involved in the aetiopathogenesis of IAD. The prevalence of IAD in racehorses has been reported to be between 11.2% and 50% (McNamara et al., 1990; Burrell et al., 1996; Wood et al., 2005).

Laan et al. (2006a) found increased expression of mRNA of these cytokines in isolated alveolar macrophages from RAO horses compared to healthy horses after inhalational challenges with LPS, hay dust suspension (HDS) or Aspergillus fumigatus extract (AF), suggesting differences in innate immune responses exist between healthy horses and those susceptible to RAO.

2.3.4 Rhodococcus equi (Rattles)

Rattles is a common LRT disease in foals and is particularly prevalent in the Hunter Valley of NSW. There is an increased incidence of the disease associated with dry dusty conditions and dust particles may carry the R Equi bacteria considerable distances. The most common source of R Equi is considered to be the manure from “carrier” mares, which is then compacted into soil and in dry dust conditions can be inhaled by foals. As the foals immune system is poorly developed they are susceptible to the disease.

A comprehensive review of this disease, which has included studs in the Hunter Valley, has been published by Muscatello et al (2006). They found the most dangerous areas on studs for foals are likely to be laneways and holding pens and that control may be aided by minimising the time that foals spend in these environments. In addition areas on farms that had low pasture cover, sandy, dry and acidic soils seemed to be a greater risk.

While there is a potential risk to foals as a result of short term increased exposure to dust in reality it is extremely unlikely to be a problem unless R Equi bacteria is present in considerable numbers in the topsoil over the areas proposed to be mined by the Project.

2.3.5 Other diseases

Diseases such as Hendra virus, Herpes virus etc are not known to be transmitted by dust over long distances. Such diseases should not be considered a potential problem.

2.4 Dust exposure in the normal horse population

Horses are exposed to large amounts of dust from many sources. These are well reviewed in the report of Malikides and Hodgson (2003) as follows.

> *Factors Contributing to Dust Exposure - In order to be inhaled and cause an effect, dusts must be aerosolised such that solid particles (dusts or smoke) or liquid droplets (mists) of sufficiently small diameter maintain stability as a suspension in air. In racehorse environments this is achieved by machinery (eg., mechanical walkers), ventilation air, or movement of humans and horses in and around stables. Particles that are aerosolised (eg., large viruses, fungal spores and small to medium sized bacteria, fine feed or bedding dust,*

smoke and motorised pollution) usually range between 0.1 to 10 μm in diameter. They are cleared from air via direct removal by ventilatory airflow (particularly small fungal spores), removal of pathogenic potential while airborne (eg., death of infectious agent) or by reaching an equilibrium and subsequently falling out of the air. Although aerosolised bacteria and viruses may die within seconds, viruses being particularly sensitive to changes in relative humidity, many species still maintain their pathogenicity, antigenicity and ability to induce airway inflammation. This is an important mechanism for certain bacteria, which after death release endotoxin and (1→3)-β-D-glucan, potent pro-inflammatory agents when inhaled into airways. In general, clearance of airborne dusts takes many hours whereas gases such as ammonia or ozone diffuse through air and can remain airborne in still air for much longer. Racehorses, who spend most of their time (up to 22 hours) in looseboxes, are therefore exposed to aerosolised foreign dusts and gases almost continuously and any effects these agents have would likely be cumulative. However, it is important to note that these "steady state" dust levels do not reflect the true levels to which racehorses are exposed due to large variation induced by horse behaviour in loose boxes, horse and human activity in and outside looseboxes, and stable ventilation, bedding type and management. This large variation in concentrations of dusts can occur within horse looseboxes, between looseboxes in the same stable and particularly around the horse's head. Airborne concentrations of dusts in looseboxes, (mostly small fungal and actinomycete spores), regardless of quality of ventilation, can be 2-50 times higher when bedding in looseboxes is being changed and cleaned. On average, this increase in concentration is higher when horses are bedded on straw rather than wood shavings or paper. As well, short periods lying and resting on dust rich bedding exposes horses to massive quantities of infectious and non-infectious particles. Sweeping laneways or stable corridors, catching and moving horses, delivery of feed and bedding to stables, and proximity to roads and urban environments also contribute to increasing exposure of horses to airborne dusts. In addition, airborne concentration of dusts is significantly higher during the day, due to increased stable activity, than at night. During the night, aerosolisation of larger dust particles is reduced and equilibration and settling of these airborne dusts occurs. Although this results in decreased concentration of large size dusts, concentrations of very small dust particles remains similar or greater than values during the day, which ensures that racehorses in looseboxes continue to be exposed to pathogenic dusts. Finally, although not well studied in horses, ambient temperature, relative humidity and wind may alter airborne dust concentrations with hot, dry and/or windy conditions increasing exposure to dusts. Low temperature and humidity (<60%) can significantly reduce viability of most microorganisms. Conversely, increased humidity promotes fungal and bacterial growth in bedding and on walls, with subsequent spore and endotoxin elaboration, and may also increase moisture content of airborne dusts and increase their settling rate.

2.4.1 Stable environment

Horses are exposed to large amounts of dust in a stable and this has been well documented in a number of sophisticated studies. Stabled horses can be exposed to very high levels of organic dusts that contain a variety of moulds and other components capable of inducing airway inflammation (McGorum et al, 1998).

Woods et al. (1993) measured mean total and respirable dust concentrations of 17.51 mg/m^3 (17510 µg/m³) and 9.28 mg/m^3 (9280 µg/m³) respectively, in the breathing zone of a h orse managed in a conventional hay and straw stable.

Cargill (1999) reported that dust is mostly measured as mg (airborne dust) per cubic metre of airspace (mg/m^3) but can also be measured in terms of particles per cubic metre or particles/ml. The size of the particles varies from less than 0.1 µm to over 100 µm. The important fractions are inhalable dust (less than 10 µm) and respirable dust (less than 5.0 µm). The levels of dust in Australian stables have received little study, but concentrations of inhalable dust in European stables range from 0.2 to 17.2 mg/m^3 (200-17200 µg/m³). The recommended maximum value is probably in the order of 2.5 to 3.0 mg/m^3 (2500-3000 µg/m³) Concentrations of respirable dust in stables range from 0.15 to 9.28 mg/m^3 (150 – 9280 µg/m³) with a recommended maximum value of 0.23 mg/m^3 (230 µg/m³). The important airborne micro-organisms present in stables and horse boxes appear to be a combination of Actinomycete and fungal spores, although mesophilic bacteria such as Corynebacterium spp and Arthrobacter sp, and gram positive organisms such as Micrococci spp, have been isolated in significant amounts.

Levels of endotoxin in horse stables were found to be generally less than 0.1 µg/m3 (Dutkiewicz et al., 1994), the “cut-off” for OH&S purposes, but in other studies levels were reported ranging from 7.52 to 60.53 ng/m^3 (0.00752- 0.06053 µg/m³) in total dust and 0.1.25 to 11.27 ng/m^3 (0.00125 – 0.01127 µg/m³) in respirable dust (McGorum et al., 1998). In the latter studies concentrations of airborne endotoxins were considerably higher in conventional stables than in “low dust stables”, which are hay and straw free environments. In the conventional stables levels in total dust samples ranged from 7.52 to 60.53 ng/m^3, (0.00752- 0.06053 µg/m³) compared with 2.12 to 17.41 ng/m^3 (0.00212- 0.01741 µg/m³) in low dust stables. The concentrations in respirable dust were 1.25 to 11.27 ng/m^3 (0.00125 – 0.01127 µg/m³) and 0.09 to 0.56 ng/m^3 (0.00009 – 0.00056 µg/m³) respectively. By comparison, concentrations of endotoxins in a pasture paddock at least 50 metres from the nearest hay ranged from 0.25 to 1.57ng/m^3 (0.00025- 0.00157 µg/m³) and 0.04 to 0.16 ng/m^3 (0.00004- 0.00016 µg/m³) respectively.

Reed et al (2006) in a review of Australian facilities reported dust levels as follows.

Table 3.13 Average Dust and Bioaerosol Exposures Reported in Australia in Horse Facilities

Activity	Dust		Bioaerosols		Endotoxins		Source
	Inhalable Dust (mg/m³)	Respirable Dust (mg/m³)	Fungi (CFU/m³)	Bacteria (CFU/m³)	Inhalable Endotoxin (EU/m³)	Respirable Endotoxin (EU/m³)	
Preparing feed for horses	14.97	1.08	1.494×10^3	8.64×10^2			Reed et al. 2003b
In stall	0.70-2.55	0.20-0.44					Cargill 1999
In barn	0.20-0.95						
Conventional stable	2.19-5.48	0.44-2.20					
In pasture	0.08-0.17	0.08-0.17					
Stables: sawdust	0.397						Banhazi et al. 2002a
Straw bedding	0.606						
Horse Nappy	0.287						
Stables	1.13	0.35					Banhazi et al. 2002a
Stable cleaning	1.20-2.81				50-82		Davidson 2004

From RIRDC Publication No 06/1071289

Ghio et al (2006) reported that particle concentration in the stables ranges from 410–20,000 µg/m³ (Crichlow et al. 1980; Woods et al 1993) and are biological in composition originating from feed and bedding.

Fleming et al (2008a) reported that means of gaseous ammonia were found to be 178.0 mg/m³ for wheat straw, 155.2 mg/m³ for wood shavings, 144.6 mg/m³ for hemp, 133.7 mg/m³ for linen, 60.3 mg/m³ for straw pellets, and 162.6 mg/m³ for paper cuttings. Horses exposed to high levels of ammonia in stables A further important factor linked to climate in the stable is the concentration of air particle impurities. In addition to the mechanical irritation caused by particles, there is an allergenic, infectious, and toxic effect that can damage respiratory health. Stable air contains, in addition to gases, animate and inanimate particulate pollutants (citing Art et al 2002). Microorganisms such as bacteria, yeasts, fungi, viruses, mites, or protozoa are classified as animate particles; inanimate particles are referred to as dust. However, the latter are also able to carry other substances, such as microorganisms and endotoxins. (citing Pearson et al 1995).

(Art T, McGorum BC, Lekeux P. Environmental control of respiratory disease. In: Lekeux P, ed. Equine respiratory diseases. Ithaca, New York: International Veterinary Information Service (www.ivis.org); 2002. ; Pearson CC, Sharples TJ. Airborne dust concentrations in livestock buildings and the effect of feed. J Agr Eng Res 1995;60: 145–154)

Dunlea and Dod (1994, 1996) stated that respirable dust particles have been measured in stables and are generally accepted to have an aerodynamic diameter of 0.5–5 um.

Wålinder et al (2011) measured carbon dioxide (CO2), ammonia, particles, horse allergen, microorganisms and endotoxin in a riding-school stable after placement of ventilation devices. Levels of CO2 were nearly halved and airborne horse allergen levels were markedly reduced (5-0.8 kU/m³) after the intervention. A decreased level of ultrafine particles was observed (8000-

5400 particles/cm^3) after the intervention, while total and respirable dust levels were mainly unchanged (200 and 130 μg/m^3). Levels of microorganisms in surface samples decreased following the intervention, whereas airborne microorganisms and endotoxin increased. In horses, the mean score of lower airway mucus and inflammation was significantly reduced. The installation of a mechanical ventilation system resulted in an increased air exchange rate, as demonstrated by reduced levels of CO2, ammonia, ultrafine particles and horse allergen. There was no significant clinical effect on human airways, but there was a tendency for reduced inflammation markers. The results on the horses may have indicated less impact on their airways after the intervention.

Clements and Pirie (2007a, b) examined dust levels in stables under various management systems. The results are shown below.

Table 1b
Maximum RDC (mg/m^3; mean and standard deviation; *represents geometric mean and geometric standard deviation) in a pony's breathing zone when managed in four different stable environments ($n = 6$)

	Bedding	
	Wood shavings	Straw
Feed		
Hay	0.9185 (0.9155) **0.6614 (2.3416)*	4.0758 (2.6693) **3.4800 (1.8308)*
Haylage	0.2182 (0.1331) **0.1829 (1.9837)*	0.2667 (0.1284) **0.2434 (1.5838)*

From Research in Veterinary Science 83: 256–262

Table 2
Mean and maximum RDCs (mg/m^3; median and ranges) obtained from stables 1 and 2, when the horse in stable 1 was either bedded on straw and fed hay or bedded on wood shavings and fed haylage ($n = 8$)

Feed bedding	Stable	Mean RDC median (range)	Maximum RDC median (range)
Hay and straw window closed	Stable 1	0.096^{b} (0.031–0.11)	1.416^{a} (0.262–4.931)
	Stable 2	0.0575^{a} (0.016–0.118)	0.333^{c} (0.117–2.673)
Haylage and shavings window open	Stable 1	0.0175^{b} (0.01–0.033)	0.191^{ab} (0.148–0.457)
	Stable 2	0.0165^{a} (0.011–0.046)	0.1065^{bc} (0.053–0.137)

Identical superscript letters indicate highly significant differences ($P < 0.01$).

From Research in Veterinary Science 83: 263–268

Vandenput et al. (1997) reported higher numbers of respirable dust particles to be liberated from dusty or good quality hay (60.88 and 6.30 x10^4 particles/litre air, respectively) than from silages of 78% and 50 % dry matter and alfalfa pellets (0.88, 0.45 and 0.95 x10^4 particles/litre air, respectively), using a Rion particle counter. Airborne dust liberated from a variety of bedding materials has also been documented (Clarke, 1987) including wood shavings, good quality straw and flax straw (3.15, 1.16 and 0.93 x10^4 respirable particles per litre air, respectively) (Vandenput et al., 1997).

Cargill (1999) considered that dust in animal housing is a mixture of organic material, including bacteria, bacterial and fungal toxins and spores, urine, dung, pollen, and other feed and animal components. (When horses eat feed out of a trough, they agitate it in such a way that dust is released. Under such conditions, the dust concentration in the breathing zone can be 30 to 40 times higher than that measured a few feet away (Cargill 1999).

Gerber et al (2003) determined that all horses housed in a stable environment examined in their study showed evidence of inflammatory airway disease.

Hessel et al (2009) reported that stabled horses are constantly exposed to high concentrations of airborne particles (ie, dust), because they spend up to 23 hours per day in the stable. This study demonstrated that feed also can generate a high dust load, which is situated directly in the breathing zone of horses.. Results are shown in tables below.

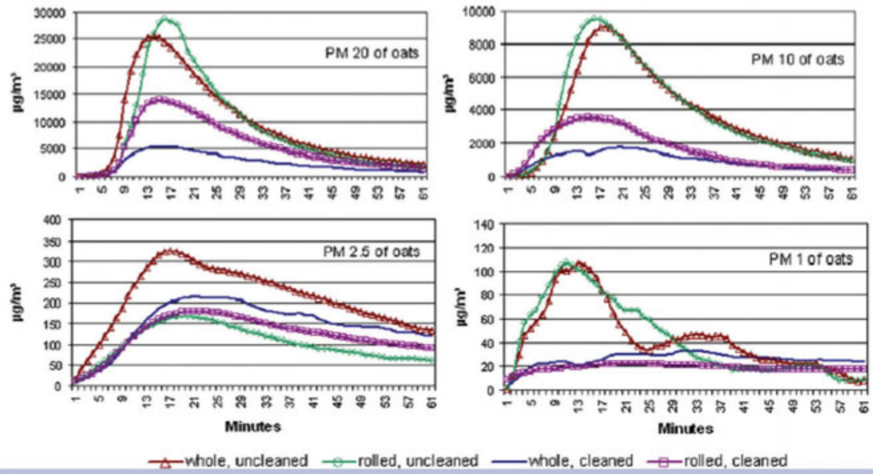


Fig. 2. Generation of airborne particles (PM 20, PM 10, PM 2.5, and PM 1) of oats as a function of time and processing (per 2-kg feed, n = 3 repetitions per fraction size and processing).

From Journal of Equine Veterinary Science 29: 665-674

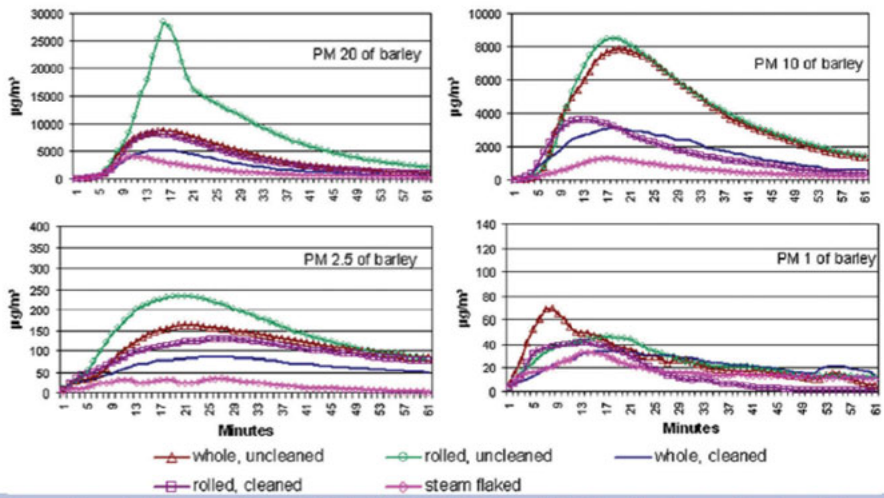


Fig. 3. Generation of airborne particles (PM 20, PM 10, PM 2.5, and PM 1) of barley as a function of time and processing (per 2-kg feed, n = 3 repetitions per fraction size and processing).

From Journal of Equine Veterinary Science 29: 665-674

Garlipp et al (2010) undertook a study that looked at the effects of four different treatment procedures on the levels of dust in hay. Particle separation techniques (involving use of highspeed airflows) resulted in a reduction in the airborne particle (PM_{20}) generation in all materials: hay 49.16 to 22.79 mg/m^3 (49160 to 22790 µg/m^3) (53.6% reduction), haylage 28.57 to 25.04 mg/m^3 (12.3%), wood shavings 141.68 to 15.04 mg/m^3 (141680 to 15040 µg/m^3) (89.4%), wheat straw 143.08 to 22.97 mg/m^3 (83.9%), flax 135.11 to 53.31 mg/m^3 (60.5%), and hemp 63.67 to 17.64 mg/m^3 (72.3%). The 8-week storage of the treated materials as compressed materials led to a renewed significant increase in the airborne particle (PM_{10}) concentration in the haylage (+29.9%), wheat straw (+104.0%), wood shavings (+40.4%), and hemp shives (+30.7%). Storage of the incoherent materials caused a significant increase in these particles only in the wheat straw (+44.2%). Detailed results are shown in two tables below.

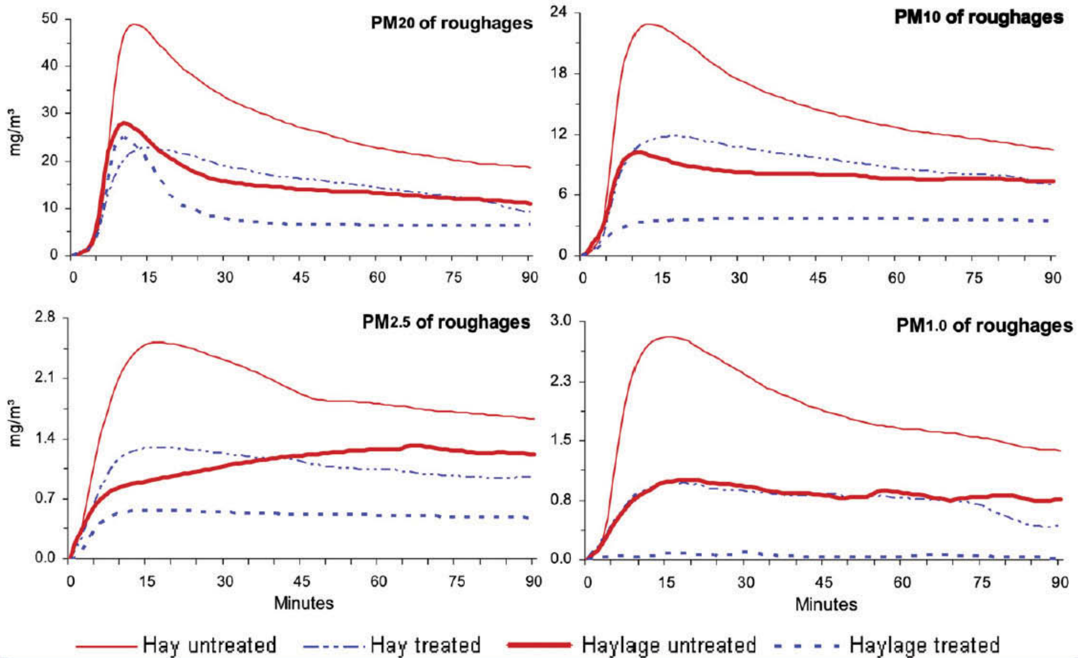


Figure 3. Mean generation of airborne particles (PM_{20}, PM_{10}, $PM_{2.5}$ and $PM_{1.0}$) of hay and haylage (untreated/treated) as a function of time (per 1.5 kg; n = 3 repetitions per fraction size and material).

From Journal of Equine Veterinary Science 30: 545-559

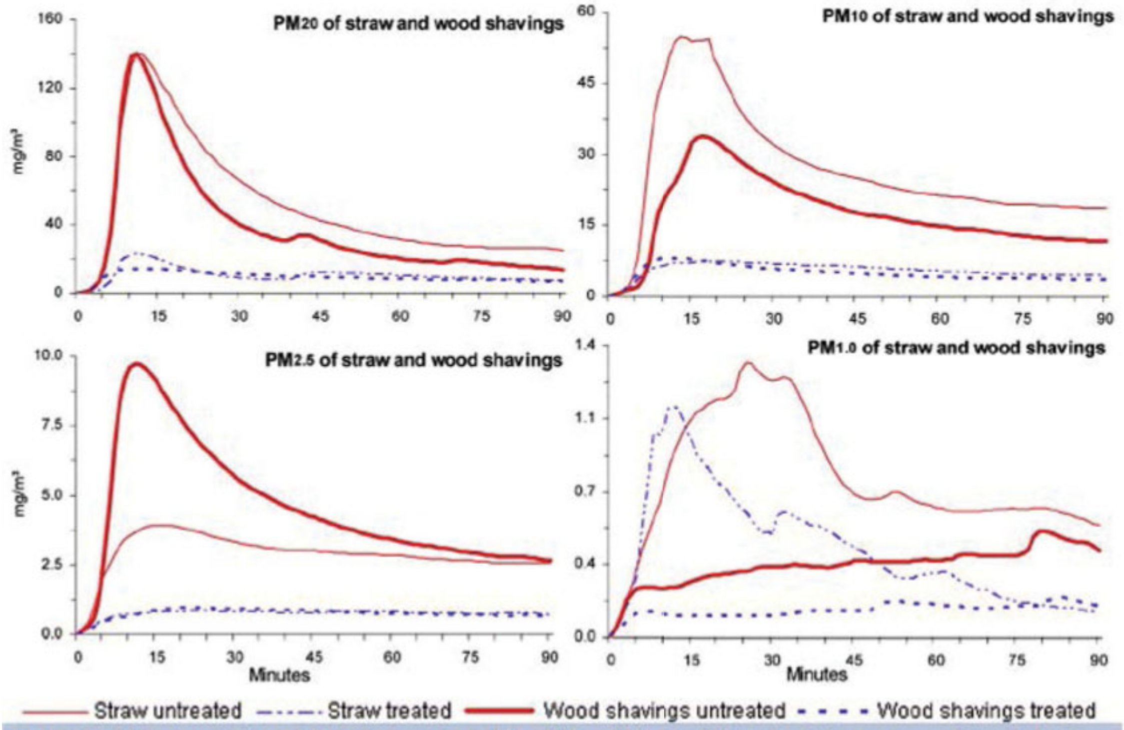
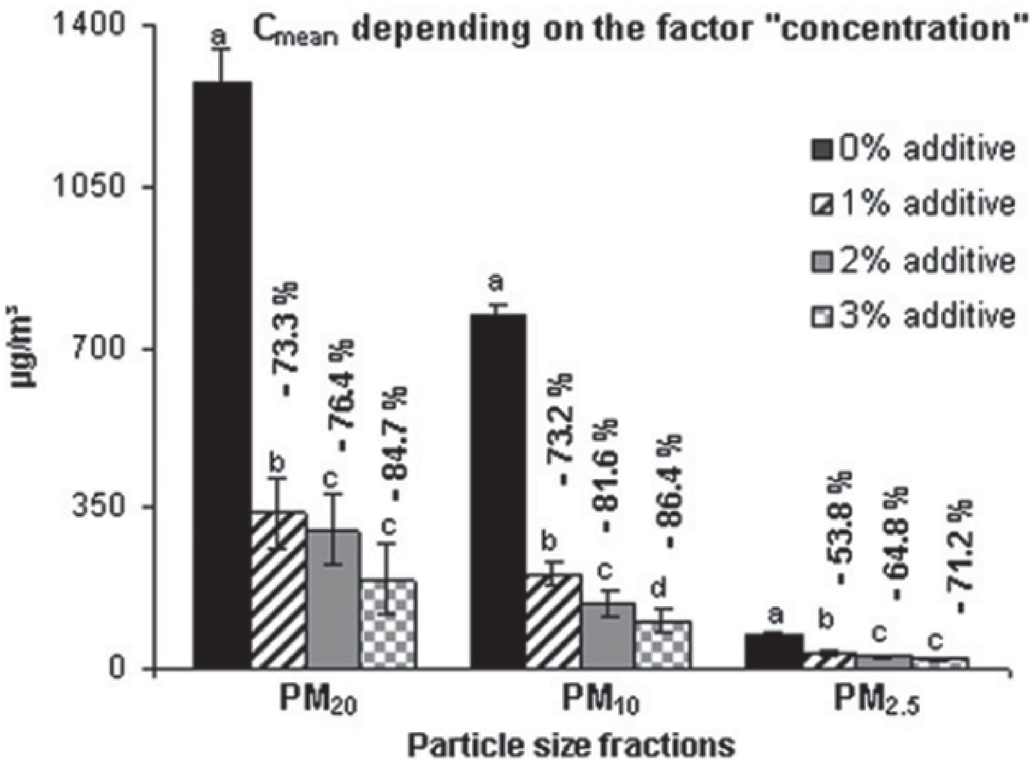


Figure 4. Mean generation of airborne particles (PM_{20}, PM_{10}, $PM_{2.5}$ and $PM_{1.0}$) of wheat straw and wood shavings (untreated/treated) as a function of time (per 1.5 kg; n = 3 repetitions per fraction size and material).

From Journal of Equine Veterinary Science 30: 545-559

Garlipp et al (2011) stated that in many reports respiratory diseases are considered to be “occupational diseases” in horses. As large numbers of horses are housed in stables there is a direct correlation between the stable climate (including permanent mechanical irritation by particles) and the occurrence of respiratory diseases. They found that although horses are usually provided with small amount of oats per day (<2 kg), which they consume within a few minutes, a critical concentration of airborne particles can also occur from oats.

The figure below shows the percentage reduction in airborne particle generation as a consequence of adding different concentrations of the liquid additive with respect to the airborne particle generation of the controls (0% additive).



From Journal of Equine Veterinary Science 31 630-639

Samidi et al (2009) and Whitaker et al (2009) looked at human dust exposure levels in horse stables. Means of personal exposure to dust, endotoxin, and b-glucan were 1.4 mg/m^3 (1400 µg/m³) (range 0.2–9.5, 200-9500), 608 EU/m^3 (20–9846), and 9.5 mg/m^3 (9500 µg/m³) (0.4–631 mg/m^3), respectively. The mean and r ange of culturable bacteria and f ungi were 3.1 x 10^3 colony-forming unit (CFU) perm^3 (6.7 x 10 to 1.9 x 10^4) and 1.9 x 10^3 CFU/m^3 (7.4 x 10 to 2.4 x 10^4), respectively. It was found that the predominant task explaining exposure levels of dust, endotoxin, and b(1/3)-glucan was sweeping the floor. For b(1/3)-glucan, feeding the horse was also an i mportant determinant. It was concluded that dust, endotoxin, and b(1/3)-glucan exposure are considerable in horse stables.

Millerick-May et al (2011) used Direct reading instruments to determine the mass concentration and numbers of particles 3 t imes daily (early morning, midday and late afternoon) in July, September and November (northern hemisphere), in 3 different racing stables. The results are presented below.

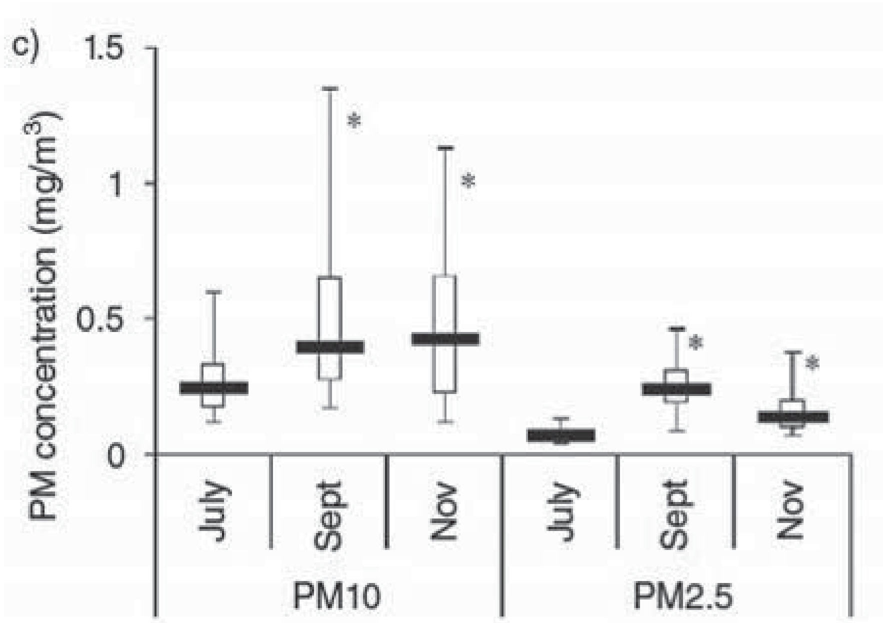


*Fig 1: Effect of stable (a), time of day (b) and month (c) on concentrations of PM10 and PM2.5. Median, interquartile range and 5th and 95th percentiles are shown for 3 racing stables. *Significantly different from referents, which were* Stable 3, *afternoon and July.*

From Equine Vet. J. 43 (5) 599-607

2.4.2 From bedding

Currently the most popular forms of bedding in stables in Australia would be straw and saw dust. These both produce large amounts of dust. As a result of both the production process and the stable environment, whereby horses manure regularly into the bedding, dust in from the stable bedding is very high in endotoxins, bacteria and fungi. These can be very detrimental to equine health.

Kirschvink et al (2002) determined the respirable dust fraction (0.5-5 μ m diameter), determined as dust particles per litre of air, and the concentration of viable spores of Aspergillus fumigatus, Faenia rectivirgula and Thermoactinomyces vulgaris in shredded cardboard bedding compared to values for other forms of bedding, the results of which are shown below.

Table I

Respirable dust particles per litre of air and concentration of viables spores of *Aspergillus fumigatus*, *Faenia rectivirgula* and *Thermoactinomyces vulgaris* in shredded cardboard bedding in comparison to values for other forms of bedding (the latter taken from Vandenput *et al.*, 1997). Data are presented as mean ± standard deviation

	Respirable dust *Particles/L of air*	A. fumigatus *CFU/42.45 L of air*	F. rectivirgula *CFU/42.45 L of air*	T. vulgaris *CFU/42.45 L of air*
Cardboard	5670 ± 1597^{a}	1.1 ± 1.9^{a}	0 ± 0^{a}	0.1 ± 0.4^{a}
Wood shavings	31492 ± 12910^{b}	710 ± 124^{b}	53 ± 29^{b}	79 ± 59^{b}
Wheat Straw	11571 ± 4897^{c}	402 ± 214^{b}	18 ± 17^{bc}	33 ± 17^{c}
Flax Straw	9251 ± 1776^{c}	104 ± 23^{c}	10 ± 9^{c}	60 ± 13^{b}

[a,b,c] Values with no common designations are significantly different (Mann–Whitney test, $P < 0.05$).

From The Veterinary Journal, 163, 319-325

Spendlove et al (2008) reported that endotoxin is a recognised cause of lower airway inflammation in both humans and horses. They found that bedding type was a significant factor (p=0.001) in contributing to aerosolised stable endotoxin concentrations, and different bedding types generated different concentrations of endotoxin in the horse's breathing zone.

Fleming et al (2008b) found that particle generation from straw pellets (average of 111.2 +/- 149.2 µ g/m^3) was significantly lower than that from wheat straw (227.5+/- 280.8 µ g/m^3). The particle generation of wood shavings had an average of 140.9+/- 141.9 µ g/m^3.

2.4.3 From feed

Dusty feed presents a big problem in that the nostrils of a horse are placed immediately above the feed and the inhalation of dust from fed can be extremely high. Even though this may be for only short periods during a day it represents a further source of "occupational" dust exposure for horses. One of the worst feeds for dust generation is hay. This is not only for stabled horses but is also a potential source of dust for paddock horses that receive supplemental feeding. Hay dust is well recognised as containing large amounts of endotoxins and fungi etc. and has the potential to cause IAD and COPD.

Malakides and Hodgson (2003) reported that the dust concentrations in the breathing zone (i.e. a hemisphere of 300 mm radius extending around the nostrils) of horses housed in conventional looseboxes (i.e. hay feed, straw bedding with adequate ventilation) can be 7 to 21 times higher respectively than background loosebox concentrations as a result of considerable amounts of time eating with their muzzles in close contact with feed and sometimes bedding

Art and Lekeux (2005) found that some degree of fungal contamination is present in all batches of hay, regardless of their quality. Hay that has heated during production and is very dusty may contain very high levels of many different pro-inflammatory agents, including mould spores, bacteria, endotoxins, proteinases, and forage mites (Clarke and Madelin, 1987; Woods et al., 1993). A horse consuming heated hay may inhale 10^{10} dust particles per breath (Clarke and Madelin, 1987).

Clements and Pirie (2007b) measured respirable dust concentrations (RDC) in association with different feeding conditions.

Table 1
Mean and maximum RDC (mg/m^3; mean and standard deviation) in a pony's breathing zone when fed dry (D), immersed (I) and soaked (S) hay ($n = 6$)

	D	I	S
Mean RDC	0.0428 (±0.0221)	0.0170 (±0.0037)	0.0122 (±0.0041)
Max RDC	1.0620 (±0.5164)	0.4968 (±0.2050)	0.7005 (±0.4754)

From Research in Veterinary Science 83: 263–268

Table 2
Mean and maximum RDCs (mg/m^3; median and ranges) obtained from stables 1 and 2, when the horse in stable 1 was either bedded on straw and fed hay or bedded on wood shavings and fed haylage ($n = 8$)

Feed bedding	Stable	Mean RDC median (range)	Maximum RDC median (range)
Hay and straw window closed	Stable 1	0.096[b] (0.031–0.11)	1.416[a] (0.262–4.931)
	Stable 2	0.0575[a] (0.016–0.118)	0.333[c] (0.117–2.673)
Haylage and shavings window open	Stable 1	0.0175[b] (0.01–0.033)	0.191[ab] (0.148–0.457)
	Stable 2	0.0165[a] (0.011–0.046)	0.1065[bc] (0.053–0.137)

Identical superscript letters indicate highly significant differences ($P < 0.01$).

From Research in Veterinary Science 83: 263–268

Seguin et al (2010) confirmed there was a large number of fungi in all hay. The highest fungal contamination in airborne particles and dust contamination occurred during late harvest, when hay moisture remained high during (rainfall after cut) or after the making process. (Eurotium amstelodami and Eurotium repens were mainly found in all hays, while Aspergillus fumigatus was mostly found in hays showing the highest colony forming units (CFUs).

3 Bibliography and Additional References

Amis, T.C., Pascoe, J.R. and H omof. W. (1984) Topographical distribution of pulmonary ventilation and perfusion in the horse. Am. J. vet. Res. 45, 1597-1601

Art T and Lekeux P (2005) Exercise-induced physiological adjustments to stressful conditionsin sports horses Livestock Production Science 92: 101 – 111

Berndt A, Derksen FJ, Edward Robinson N. (2010) Endotoxin concentrations within the breathing zone of horses are higher in stables than on pasture. Vet J Jan;183(1):54-7.

Buechner-Maxwell V, (1993a) Airway Hyperresponsiveness, Compendium on continuing education for the practicing veterinarian, 15(10), 1379-1389

Buechner-Maxwell V, (1993b), Normal Respiratory Epithelial Structure and Function, Compendium on continuing education for the practicing veterinarian, 15(4), 618-625

Buechner-Maxwell V, Murray M, Crisman M, Ley W, Saunders G, Walton A, (1996) Relationship of equine housing to large airway inflammation, Journal of Equine Veterinary Science, 16(11), 502-506

Burrell, M.H., (1985) Endoscopic and virological observations on respiratory disease in a group of young Thoroughbred horses in training. Equine Vet. J. 17, 99 –103.

Cargill, C. (1999) Reducing dust in horse stables and t ransporters: A report for the Rural Industries Research and Development Corporation. South Australian Research and Development Institute. RIRDC Publication No. 99/44.

Clarke, A.F. and Madelin, T.M., (1987) Technique for assessing respiratory health hazards from hay and other source materials. Equine Vet. J. 19, 442 – 447

Clarke, A.F., Madelin, T.M., Allpress, R.G., (1987) 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.

Clements JM and P irie RS (2007) Respirable dust concentrations in equine stables. Part 1: Validation of equipment and e ffect of various management systems. Research in Veterinary Science 83: 256–262

Clements JM and Pirie RS (2007b) Respirable dust concentrations in equine stables. Part 2: The benefits of soaking hay and optimising the environment in a neighbouring stable. Research in Veterinary Science 83: 263–268

Collins, M and Algers, B. (1986) Effects of stable dust on farm animals. Veterinary research communications, 10, 415-428.

Crane S A, Ziemer E L, Sweeny C R, (1989) Cytologic and bacteriologic evaluation of tracheobronchial aspirates from clinically normal foals, American Journal of Veterinary Research, 50(12), 2042-2048

Crichlow, E. C.; Yoshida, K.; Wallace, K. (1980) Dust levels in riding stables. Equine veterinary Journal, 12, 185-188.

Dacre KJ, McGorum BC, Marlin DJ, Bartner LR, Brown JK, Shaw DJ, Robinson NE, Deaton C, Pemberton AD.. (2007) Organic dust exposure increases mast cell tryptase in bronchoalveolar lavage fluid and airway epithelium of heaves horses Clin Exp Allergy Dec;37(12):1809-18.

Davis MS, Foster WM. (2002) Inhalation toxicology in the equine respiratory tract. In: Lekeux P, ed. Equine respiratory diseases. Ithaca, NY: International Veterinary Information Service

Deaton, C. M. and Marlin, D. J. (2004) A review of the effects of environmental pollution on the equine respiratory tract: considerations for the 2004 Olympic games. Equine and Comparative Exercise Physiology. 1: 171-176

Deaton CM, Deaton L, Jose-Cunilleras E, Vincent TL, Baird AW, Dacre K, Marlin DJ. (2007) Early onset airway obstruction in response to organic dust in the horse.J Appl Physiol. Mar;102(3):1071-7

Derksen, F.J.. Robinson, N.E., Armstrong, P.J.. Stick, J.A. and S locomhe. R.F. (1985) Airway reactivity in ponies with recurrent airway obstruction (heaves). J. appl. Physiol. 58.598-604.

Derksen, F. J. & Woods, P. S. A. (1994) Chronic lung disease in the horse: role of aeroallergens and irritants and methods of evaluation. Equine Practice 16, 11-13.

Dixon P M, 1992, Respiratory mucociliary clearance in the horse in health and disease, and its pharmaceutical modification, The Veterinary Record, 131, 229-235

Dogra R. K. S., R. Shanker, A. K. Saxena, S. Khanna S. N. Srivastava, L. J. Shukla & S. H. Zaidi (1984) Air Pollution: Significance of Pulmonary Dust Deposits in Bovine Species Environmental Pollution (Series A) 36: 109-120

Douglas, J. S, A., Ridoway, P, A. (1968) Histamine release and bronchocoustriction due to textile dusts and their components, Proc. 2nd Int. Conf. Respiratory Diseases in Textile Workers, Alicante, Spain, pp. 148-155

Dunlea, A.P., Dodd, V.A., (1994) Measurement of respirable dust levels in horse stables. Can. Agric. Eng. 37, 205–209.

Dunlea, A.P., Dodd, V.A., (1996) Respirable dust control in a s cale-model horse stable using filtration and mechanical ventilation. Can. Agric. Eng. 38, 215–221

Dutkiewicz, J.; Pomorski, Z. J. H.; Sitkowsaka, J.; Krysinska, T. E.; Wojtowicz, H. (1994) Airborne microorganisms and endotoxin in animal houses. Grana, 33, 85-90.

Einbrodt H J, Klosterkoetter W, Metze H, (1963) Comparative studies one the size of granules of dusts retained in the lungs of man and animal [Article in German] . Grundfragen Silikoseforsch.;6:491-5.

Einbrodt HJ, Fitzek J. (1967) The chemical composition of accumulated dust in the lungs and lymph node regions of mining horses. Beitr Silikoseforsch. 1967;91:29-35. German. No abstract available.

Einbrodt, H. J. & Weller, W. (1966). Dust retention in lungs and lymph nodes of large animals. Beitr. Silikose-Forsch., 90, 13-18.

Evans E and Nicholls P J, (1974) Comparative Study of Histamine Release by Cotton Dust from the Lung of Several Species Comp. Gen. Pharmac., Vol. 5, PP. 87 to 90

Fleming, K, E.F. Hessel, PD Dr. and H.F.A. Van den Weghe, Prof. Dr. Ir. (2008) Evaluation of Factors Influencing the Generation of Ammonia in Different Bedding Materials Used for Horse Keeping Journal of Equine Veterinary Science, Vol 28, No 4 223-231

Fleming,K, E.F. Hessel, PD Dr. and H.F.A. Van den Weghe, Prof. Dr. Ir. (2008b) Generation of Airborne Particles from Different Bedding Materials Used for Horse Keeping. Journal of Equine Veterinary Science Vol 28, No 7 408-418

Garlipp F , Hessel, PD, van den Hurk, M , Timmerman, M Van den Weghe, H (2010) The Influence of a Particle Separation Technology on the Generation of Airborne Particles from Different Roughages and Bedding Materials Used for Horses, Journal of Equine Veterinary Science Vol 30, No 10 545-559

Garlipp F, Engel F. Hessel PD, Herman F.A. Van den Weghe Prof. Dr. Ir. (2011) Effects of Three Different Liquid Additives Mixed with Whole Oats or Rolled Oats on the Generation of Airborne Particles from an Experimental Simulating Horse Feeding. Journal of Equine Veterinary Science 31: 630-639

Gates, M. C. (2007). The influence of air pollution on Thoroughbred race performance. Equine and Comparative Exercise Physiology. 4 (02): 79-88.

Gerber V, Robinson NE, Luethi S, Marti E, Wampfler B, Straub R. (2003) Airway inflammation and mucus in two age groups of asymptomatic well-performing sport horses. Equine Vet J 35:491-5.

Ghio, A. J., Mazan, M. R., Hoffman, A. M. and Robinson, N. E. (2006b). Correlates between human lung injury after particle exposure and recurrent airway obstruction in the horse. Equine Veterinary Journal. 38 (4): 362-367.

Hagen T (2011) Literature Review for effects of dust from Drayton South proposed coal project on health and performance of horses . Toxikos report prepared for Hansen Bailey

Hessel, PD, F Garlipp, and Van den Weghe, (2009) Generation of Airborne Particles from Horse Feeds Depending on Type and Processing. Journal of Equine Veterinary Science Vol 29, No 9 665-674

Hedenstierna G, Nyman G, Kvart C, Funkquist B, (1987) Ventilation-perfusion relationship in the standing horse: An inert gas elimination study, Equine Veterinary Journal, 19(6), 514-519

Hodgson, J. L., Malikides, N., Spendlove, P. J., Christley, R. M. and Hodgson, D. R. (2005). Environmental factors and airway inflammation in Australian racehorses. In Third World Equine Airways Symposium. International Veterinary Information Service, Ithaca NY.

Hoffman A M, Viel L, Prescott J F, Rosendal S, Thorsen J, (1993) Association of microbiologic flora with clinical, endoscopic, and pulmonary cytologic findings in foals with distal respiratory tract infection, American Journal Veterinary Research, 54(10), 1615-1622

Hoffman A M, Viel L, Juniper E, Prescott J F, (1993) Clinical and endoscopic study to estimate the incidence of distal respiratory tract infection in thoroughbred foals on Ontario breeding farms, American journal of veterinary research, 54(10), 1602-1607

Hughes K et al (2011) Evaluation of cytokine mRNA expression in bronchoalveolar lavage cells from horses with inflammatory airway disease. Veterinary Immunology and Immunopathology 140 82–89

Hurley MJ (2011) Observations on Post-race Endoscopic Examinations. WEVA, India Abstract 58.

James D E, Chambers J A, Kalma J D, Bridgman H A, (1985) Air Quality Prediction in Urban and Semi-urban regions with generalised input – Output analysis: The Hunter Region, Australia, Urban Ecology, 9: 25--44

Kirschvink N, DiSilvestro F, Sbai I E, Vandenput S, Art T, Roberts C, Lekeux P, (2002) The Use of Cardboard Bedding Material as Part of an Environmental Control Regime for Heaves-affected Horses: In Vitro Assessment of Airborne Dust and Aeroallergen Concentration and I n Vivo Effects on Lung Function, The Veterinary Journal, 163, 319-325

Lapointe J, Lavoie J, Vrins A, (1993), Effects of triamcinolone acetonide on pulmonary function and bronchoalveolar lavage cytologic features in horses with chronic obstructive pulmonary disease, 54(8), 1310-1316

Malakides N and Hodgsen J (2003) Inflammatory Airway Disease in Young Thoroughbred Racehorses RIRDC Publication No. 03/089

Mason DK, Collins EA, Watkins KL. (1984) Effect of bedding on the incidence of exercise induced pulmonary haemorrhage in racehorses in Hong Kong. Vet Rec. Sep 15;115(11): 268-9.

Mazan MR, Ghio AJ and Hoffman AM (2001). Airway reactivity, inflammation, iron and i ron-binding proteins in horses from urban versus rural environments. In: World Equine Airways Symposium, Edinburgh. Abstract p. 31.

McGorum B C, Dixon P M, (1993) Evaluation of local endobronchial antigen challenges in the investigation of equine chronic obstructive pulmonary disease, Equine Veterinary Journal, 25(4), 269-272

McGorum B C, Dixon P M, Halliwell R E W, (1993) Responses of horses affected with chronic obstructive pulmonary disease to inhalation challenges with mould antigens, Equine Veterinary Journal, 25(4), 261-267

McGorum, B.C., Ellison, J., Cullen, R.T., (1998) Total and respirable airborne dust endotoxin concentrations in three equine management systems. Equine Vet. J. 30, 430–434.

Millerick-May M.L., W. Karmaus, F. J. Derksen, B. Berthold, S. J. Holcombe and N. E. Robinson (2008) Horse-Environment Interaction: The Relationship Between Environmental Particulate Matter and Airway Inflammation in Thoroughbred Racehorses 54th Annual Convention of the American Association of Equine Practitioners - AAEP, 2008 - San Diego, CA, USA

Millerick-May M L, Karmaus W, Derksen F J, Berthold B, Holcombe S J, Robinson N E (2011) Particle mapping in stables at an American Thoroughbred racetrackevj_331 599..60 Equine vet. J. 43 (5) 599-607

Mills PC, Roberts CA, Smith NC. (1996) Effects of ozone and airway inflammation on glutathione status and iron homeostasis in the lungs of horses. Am J Vet Res 57:1359-1363

Muscatello et al (2006) Rattles in Horses. Effects of stud management on ecology of virulent Rhodococcus equi. RIRDC Publication No 06/097

Newman J, 1979, Effects of Industrial Air pollution on Wildlife, Biol. Conserv. 15:181-190

Nicholls P J, Nicholls G R, Bouhuys A (I967) Histamine release by compound 48/8o and textile dusts from lung tissue in vitro. In I nhaled Particles and Vapours. II (ed. Davies), pp. 69-74. Oxford: Pergamon.

O'Callaghan, M.W. (1991) Nuclear imaging techniques for equine respiratory disease. Vet. Clin. N. Am.: Equine Pract. 7,417-33.

O'Callaghan, M.W., Hornof, W.J., Fisher, P.E. and Rabbe, O.G. (1987) Ventilation imaging in horse with 99m-Technetium-DTFP`A radioaerosol. Equine vet. J. 19, 19-24.

Pirie RS, McLachlan G, McGorum BC(2002) Evaluation of nebulised hay dust suspensions (HDS) for the diagnosis and investigation of heaves. 1.Preparation and composition of HDS. Equine Vet J 34: 332–336

Purdy CW, Layton RC, Straus DC, Ayers JR. (2008) Effects of inhaled fine dust on lung tissue changes and antibody response induced by spores of opportunistic fungi in goats. Am J Vet Res Apr;69(4):501-11.

Reed et al (2006) Respiratory Illness in Farmers -Dust and bioaerosols exposures in animal handling facilities, RIRDC Publication No 06/1071289

Riihimäki M, Raine A, Elfman L, Pringle J. (2008) Markers of respiratory inflammation in horses in relation to seasonal changes in air quality in a conventional racing stable. Can J Vet Res. Oct;72(5):432-9

Robinson N E, Dereksen F J, Olszeweski M A, Buechner-Maxwell V A, (1996) Review the pathogenesis of Chronic obstructive pulmonary disease of horses Br vet. Journal 152, 283

Samadi S, Wouters I M, Houben R, Jamshidifa A, Van Eerdenburg F, Heederik D J (2009) Exposure to Inhalable Dust, Endotoxins, b(1/3)-Glucans, and Airborne Microorganisms in Horse Stables Ann. Occup. Hyg., Vol. 53, No. 6, pp. 595–603

Seahorn T L, Beadle R E, (1994) Managing summer-pasture-associated obstructive pulmonary disease in horses, Veterinary Medicine

Se´guin V et al (2010) Effect of agricultural and environmental factors on the hay characteristics involved in equine respiratory disease. Agriculture, Ecosystems and Environment 135 : 206–215

Séguin V, Garon D, Lemauviel-Lavenant S, Lanier C, Bouchart V, Gallard Y, Blanchet B, Diquélou S, Personeni E, Ourry A. (2011) How to improve the hygienic quality of forages for horse feeding. J Sci Food Agric. 2011 Oct 17. doi: 10.1002/jsfa.4680.

Spendlove, P, Jennifer L. Hodgson and David R. Hodgson (2008) Prevention and Control of Lower Airway Inflammation in Horses. RIRDC Publication No 08/051

Sweeny C R, Leary H J, Ziemer E L, Spencer P A, 1989, Effect of water vapour-saturated air therapy of bronchoalveolar lavage and tracheal mucus transport rate in clinically normal horses, American Journal of Veterinary Research, 50(2), 276-279

Traub-Dargatz J L et al, (1988) Effect of transportation stress on bronchoalveolar lavage fluid analysis in female horses, American Journal of Veterinary Research, 49(7), 1026-1029

Tulamo R, Maisi P, (1997) Hyaluronate concentration in tracheal lavage fluid from clinically normal horses and horses with chronic obstructive pulmonary disease, American Journal of Veterinary Research, 58(7), 729-732

Vandenput, S., Istasse, L., Nicks, B., Lekeux, P., (1997) Airborne dust and aeroallergen concentrations in different sources of feed and bedding for horses. Vet. Quart. 19, 154–158.

Votion D et al (1997) Aerosol deposition in equine lungs following ultrasonic nebulisation versus jet aerosol delivery system. Equine vet. J. 29 (5) 388-393

Votion, D., Vandenput, S., Duvivier, D.H., Art. T. and Lek eux, P. (1997) Analysis of equine scintigraphical lung images. The Vet. J. 153,49-61.

Wålinder R, Riihimäki M, Bohlin S, Hogstedt C, Nordquist T, Raine A, Pringle J, Elfman L.(2011), Installation of mechanical ventilation in a horse stable: effects on air quality and human and equine airways. Environ Health Prev Med. Jul;16(4):264-72.

Whittaker AG, Hughes KJ, Parkin TD, Love S.. (2009) Concentrations of dust and endotoxin in equine stabling. Vet Rec Sep 5; 165(10):293-5.

Woods, P.S.A., Robinson, N.E., Swanson, M.C., Reed, C.E., Broadstone, R.V., Derksen, F.J., (1993) Airborne dust and aeroallergen concentration in a hor se stable under two different management systems. Equine Vet. J. 25, 208 –213.

Zeitler-Feicht MH. (1993) Minimal requirements for lighting and air quality in horse stables with respect to animal welfare. Tierarztliche Umschau; 48: 311–317.

4 Further Reading (references reviewed but not cited)

Airaksinen S et al. 2005, Variety in dustiness and hygiene quality of peat bedding, Ann Agric Envriron Med, 12, 53-59

Angulo L C , Huertas J I, Restrepo G M, 2010, Characterization of Total Suspended Particles (TSP) and I nhalable Particulate Matter (PM10) generated in Open Pit Coal Mining Areas, Informacion Tecnologica 22(4), 23-34

Connor L, Freeman S, Higginbotham N, 2009, Not Just a Coalmine: Shifting Grounds of Community Opposition to Coal Mining in Southeastern Australia, Ethnos, 74(4), 490-513

Fink U. Vera ¨nderungen der Lungenfunktion nach Inhalation eines Futter-Pellet-Staubes bei klinisch lungengesunden Pferden [Changes in the pulmonary function of clinically healthy horses after the inhalation of pellet dust]. PhD Thesis. Hanover, Germany: University of Veterinary Medicine; 1998.

Higginbotham N, Freeman S, Connor L, Albrecht G, 2010, Environmental injustice and air pollution in coal affected communities, Hunter Valley, Australia, Health and Place, 16, 259-266

Kalina W V, Pettigrew H D, Gershwin L J, 2003, IgE ELISA using antisera derived from epsilon chain antigenic peptides detects allergen-specific IgE in allergic horses, Veterinary Immunology and Immunopathology, 92, 137-147

Korthals M et al. 2008, Application of PCR-SSCP for molecular epidemiological studies on the exposure of farm children to bacteria in environmental dust, Journal of Microbiological methods, 73, 49-56

Marti E et al. 2008, Report of the 3rd Havemeyer workshop on a llergic diseases of the Horse, Holar, Iceland, June 2007, Veterinary Immunology and Immunopathology, 126, 351-361

Mazan M, 2010, Inflammatory Airway Disease in the Horse, Proceedings of the AAEP Focus on Upper and Lower Respiratory Diseases, 100-106

McManus P, 2008, Their grass is greener but ours is sweeter – Thoroughbred breeding and water management in the upper hunter region of New South Wales, Australia, Geoforum, 39, 1296-1307

Olenchock S A, Murphy S A, Mull J C, Lewis D M, 1992, Endotoxin and complement activation in an analysis of environmental dusts from a horse barn, Scandinavian Journal of Work, Environment and Health, 18(2), 58-59

Palacios H, Iribarren I, Ollalla M J, Cala V, 2002, Lead poisoning of horses in the vicinity of a battery recycling plant, The Science of the Total Environment, 290, 81-89

Prescott J F, 1987, Epidemiology of Rhodococcus equi Infection in Horses, Veterinary

Microbiology, 14, 211-214

Rietmeijer F, Janeczek J, 1997, An Analytical Electron Microscope Study of Airborne Industrial Particles in Sosnowiec, Poland, Atmospheric Environment, 31(13), 1941-1951

Riihimaki M, Raine A, Elfman L, Pringle J, 2008, Markers of respiratory inflammation in horses in relation to seasonal changes in air quality in a conventional racing stable, The Canadian Journal of Veterinary Research, 72, 432-439

Rittinghausen S, Mohr U, Dungworth D L, 1997, Pulmonary cystic keratinizing squamous cell lesions of rats after inhalation/instillation of different particles, Experimental and Toxicologic Pathology, 49, 433-446

Singer J W, Bobsin N, Bamka W J, Kluchinshi D, 1999, Horse Pasture Management, Journal of Equine Veterinary Science, 19(9), 540-592

Simonen-Jokinen T et al. 2006, Direct activation of gelatinase B (MMP-9) by hay dust suspension and different components of organic dust, Veterinary Immunology and Immunopathology, 109, 289-295

Simonson R, 1995, Airborne dust and its significance to soils, Geoderma, 65, 1-43

Takai S, Fujimori T, Katsuzaki K, Tsubaki S, 1987, Ecology of Rhodococcus equi in Horses and their environment on horse-breeding farms, Veterinary Microbiology, 14, 233-239

Tanner M K et al. 1997, Effect of Phone Book Paper versus Sawdust and Straw Bedding on the Presence of Airborne Gram-Negative Bacteria, Fungi and Endotoxin in Horse Stalls, Journal of Equine Veterinary Science, 18(7), 457-461

Tanner M K et al. 1997, Respiratory and Environmental Effects of Recycled Phone Book Paper versus Sawdust as Bedding for Horses, Journal of Equine Veterinary Science, 18(7), 468-476

Werhahn H, Hessel E, Bachausen I, Van den Weghe H, 2010, Effects of Different Bedding Materials on the Behaviour of Horses Housed in Single Stalls, Journal of Equine Veterinary Science, 30(8), 425-431

Wilkins P A, 2003, Lower airway diseases of the adult horse, The Veterinary Clinics Equine Practice, 19, 101-121

Williamson K, Davis M, 2007, Evidence-Based Respiratory Medicine in Horses, Veterinary Clinics Equine Practice, 23, 215-227

APPENDIX 2

Comparative review of air quality backgrounds
near horse breeding and racing areas

DRAYTON SOUTH COAL PROJECT
COMPARATIVE REVIEW OF AIR QUALITY BACKGROUNDS NEAR HORSE BREEDING AND RACING LOCATIONS

for
Hansen Bailey on behalf of Anglo American Metallurgical Coal Pty Ltd

Job No: 3617c

18 April 2012

A PEL Company

PROJECT TITLE:	Drayton South Coal Project: Comparative Review of Air Quality Backgrounds Near Horse Breeding and Racing Locations
JOB NUMBER:	**3617C**
PREPARED FOR:	Daniel Sullivan Hansen Bailey on behalf of Anglo American Metallurgical Coal Pty Ltd
PREPARED BY:	Chris Marsh
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

PAEHolmes was commissioned by Hansen Bailey Environmental Consultants on behalf of Anglo American Metallurgical Coal Pty Ltd to complete a desktop review of air quality backgrounds near horse breeding and racing areas within Australia and internationally.

2 METHODOLOGY

The review targeted key horse breeding and racing locations within Australia and internationally. Locations identified included:

- Australia:
  - Hunter Valley, NSW.
  - Randwick, NSW.
  - Flemington, Victoria.
- United States of America:
  - Louisville, Kentucky.
  - Lexington, Kentucky.
- Ireland:
  - Kildare.
  - Tipperary.
  - Meath.
- United Kingdom:
  - Newmarket.
- Saudi Arabia.
- Hong Kong:
  - Sha Tin.
  - Happy Valley.

Background data for particulate matter less than 10 micron (PM_{10}) was obtained from monitoring stations closest to each horse breeding and racing location, including:

- Australia:
  - Singleton, NSW.
  - Muswellbrook, NSW.
  - Tamworth, NSW.
  - Randwick, NSW.
  - Footscray, Victoria.
- United States of America:
  - Louisville, Kentucky.
  - Lexington-Fayette, Kentucky.
  - Elizabethtown, Kentucky.
  - Richmond, Kentucky.
- Ireland:
  - Tipperary.
  - Kildare.
  - Meath.
  - Cork.
  - Dublin.

* United Kingdom:
    * Newmarket.
* Saudi Arabia.
* Hong Kong:
    * Sha Tin.
    * Eastern.

Data was sourced between August and October 2011 from various government departments and international agencies associated with each monitoring station, including:

* NSW Office of Environment and Heritage (OEH).
* Victorian Environmental Protection Agency (EPA).
* United States EPA.
* Ireland EPA.
* United Kingdom Department of Environment, Food and Rural Affairs (DEFRA).
* World Bank.
* Hong Kong Environmental Protection Department (EPD).

3 RESULTS

Available background concentrations for PM_{10} were collated from 2001 to 2011 for each of the monitoring stations closest to each horse breeding and racing location (see **Table 1**). The concentrations are illustrated in **Figure 1**.

Table 1 PM_{10} Annual Average Concentrations (μg/m³)

Location		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
		PM_{10} Annual Average Concentration (μg/m³)										
Saudi Arabia	Saudi Arabia	148	143	137	128	118	112	107	104	-	-	-
Hong Kong	Sha Tin	-	-	-	-	53	53	51	50	45	45	-
	Eastern	-	-	-	-	49	47	49	46	43	43	-
Australia	Footscray	-	-	-	-	21	22	20	21	22	-	-
	Singleton	-	-	-	-	-	-	-	-	-	20	19
	Muswellbrook	-	-	-	-	-	-	-	-	-	20	19
	Tamworth	-	-	-	-	-	18	16	16	18	12	13
	Randwick	-	-	-	-	-	22	18	17	20	16	15
United States of America	Louisville	-	-	25	23	26	23	25	22	-	-	-
	Louisville 2	-	-	23	21	24	22	24	21	-	-	-
	Lexington-Fayette	-	-	23	21	24	21	23	19	-	-	-
	Elizabethtown	-	-	19	18	21	17	-	-	-	-	-
	Richmond	-	-	20	18	21	18	-	-	-	-	-
Ireland	Cork, Old Station Road	-	24	26	22	19	16	15	16	18	22	-
	Cork, Heatherton Park	-	21	20	19	17	18	17	15	15	18	-
	Dublin, Dun Laoghraine	-	-	-	-	-	-	-	15	15	15	-
	Tipperary, Clonmel	-	-	-	20	19	-	-	-	-	-	-
	Kildare, Naas	-	-	17	17	-	-	-	-	-	-	-
	Kildare, Newbridge	-	-	-	-	-	-	-	-	14	20	-
	Meath, Navan	-	-	-	-	-	-	23	-	-	-	-
United Kingdom	Newmarket Racecourse	-	-	-	21	20	17	17	16	16	16	16

The results indicate that Saudi Arabia represents the highest PM_{10} concentrations ranging between 104 and 148 μg/m³ followed by Hong Kong (Sha Tin and Eastern). All other locations maintain a similar PM_{10} concentration range typically between 15 and 26 μg/m³.

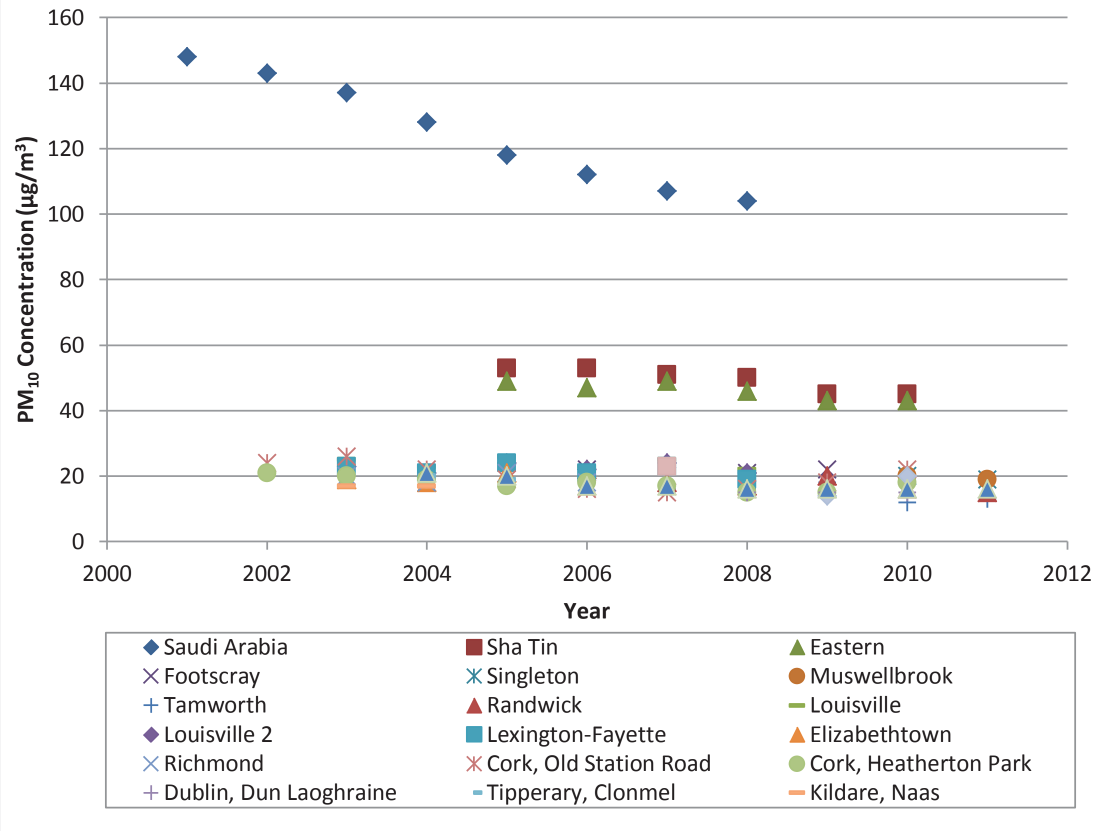

Figure 1 PM_{10} Annual Average Concentrations

4 CONCLUSION

From the review, it can be concluded that the majority of horse breeding and racing enterprises within Australia and internationally operate in similar and comparable PM_{10} air quality backgrounds, typically ranging between 15 and 26 µg/m³. The outliers include Saudi Arabia and Hong Kong, which have significantly higher concentrations between 104 and 148 µg/m³ and 53 and 43 µg/m³, respectively.

5 REFERENCES

Department of Environment, Food and Rural Affairs, United Kingdom (2011) Background Maps, Viewed October 2011, *http://laqm.defra.gov.uk/review-and-assessment/tools/background-maps.html*

Environmental Protection Agency, Ireland (2011) *Archive of PM10 Monitoring Data*, Viewed 10 October 2011, *http://erc.epa.ie/safer/resource?id=154a12f7-76e4-102b-aa08-55a7497570d3*

Environmental Protection Agency, United States (2011) AirData, Viewed 30 August 2011, *http://www.epa.gov/air/data/reports.html*

Environmental Protection Agency, Victoria (2011) Air Monitoring Reports, Viewed 31 August 2011, *http://www.epa.vic.gov.au/air/monitoring/monitoring_reports.asp*

Hong Kong Environmental Protection Department (2011) Past Air Quality Monitoring Data, Viewed 8 September 2011, *http://epic.epd.gov.hk/ca/uid/airdata/p/1*

Office of Environment and Heritage, NSW (2011) Search Air Quality Data, Viewed 31 August 2011, *http://www.environment.nsw.gov.au/AQMS/search.htm*

World Bank (2011) PM_{10} Country Level (Micrograms per Cubic Meter), Viewed 30 August 2011, *http://data.worldbank.org/indicator/EN.ATM.PM10.MC.M3*

APPENDIX 3

Results of Endotoxin Analysis

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Address: 8 Rachael Close
Silverwater NSW 2128
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Phone: 02 9704 2300
Fax: 02 9737 9425
Website: www.amslabs.com.au
Email: info@amslabs.com.au

Certificate of Analysis

Dated: 4/05/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069

ATTN: Nicholas Kannegieter

OUR REFERENCE: 1205184

ORDER NO: Not Given

DATE RECEIVED: 11/04/2012

SAMPLE DESCRIPTIONS:
1 x Soil Sample - Site 1
Plashett Ridge
Anglo Coal - Drayton South Project

DATE COMMENCED: 1/05/2012

Samples tested as received

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Dissolve 1g in 10mL PFW to make initial 1/10 dilution. Further dilute to 1/1000 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/g
Soil Sample - Site 1 Plashett Ridge Anglo Coal - Drayton South Project	5.0	**189.337**	**189.337**

< = less than   EU = Endotoxin Units   PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.

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Email: info@amslabs.com.au

Certificate of Analysis

Dated: 4/05/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069
ATTN: Nicholas Kannegieter

OUR REFERENCE: 1205185

ORDER NO: Not Given

DATE RECEIVED: 11/04/2012

SAMPLE DESCRIPTIONS:
1 x Soil Sample - Site 2
HVAS Ridge
Anglo Coal - Drayton South Project
Samples tested as received

DATE COMMENCED: 1/05/2012

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Dissolve 1g in 10mL PFW to make initial 1/10 dilution. Further dilute to 1/1000 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/g
Soil Sample - Site 2 HVAS Ridge Anglo Coal - Drayton South Project	5.0	**167.591**	**167.591**

< = less than EU = Endotoxin Units PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.

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Address: 8 Rachael Close
Silverwater NSW 2128
Australia
Phone: 02 9704 2300
Fax: 02 9737 9425
Website: www.amslabs.com.au
Email: info@amslabs.com.au

Certificate of Analysis

Dated: 4/05/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069

ATTN: Nicholas Kannegieter

OUR REFERENCE: 1205186

ORDER NO: Not Given

DATE RECEIVED: 11/04/2012

SAMPLE DESCRIPTIONS:
1 x Soil Sample - Site 3
Stockyards Ridge
Anglo Coal - Drayton South Project
Samples tested as received

DATE COMMENCED: 1/05/2012

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Dissolve 1g in 10mL PFW to make initial 1/10 dilution. Further dilute to 1/1000 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/g
Soil Sample - Site 3 Stockyards Ridge Anglo Coal - Drayton South Project	5.0	**403.321**	**403.321**

< = less than   EU = Endotoxin Units   PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.

THIS REPORT MUST NOT BE REPRODUCED EXCEPT IN FULL
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Certificate of Analysis

Dated: 4/05/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069

ATTN: Nicholas Kannegieter

OUR REFERENCE: 1205187

ORDER NO: Not Given

DATE RECEIVED: 11/04/2012

SAMPLE DESCRIPTIONS:
1 x Soil Sample - Site 4
HVAS
Anglo Coal - Drayton South Project
Samples tested as received

DATE COMMENCED: 1/05/2012

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Dissolve 1g in 10mL PFW to make initial 1/10 dilution. Further dilute to 1/1000 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/g
Soil Sample - Site 4 HVAS Anglo Coal - Drayton South Project	5.0	**353.152**	**353.152**

< = less than EU = Endotoxin Units PFW = Pyrogen Free Water

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Elizabeth Georgievska BSc.

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Certificate of Analysis

Dated: 18/06/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069
ATTN: Nicholas Kannegieter

OUR REFERENCE: 1206073

ORDER NO: Not Given

DATE RECEIVED: 26/04/2012

SAMPLE DESCRIPTIONS:
1 x HVAS Filter Paper
Plashett PM10 - 27/3/12 - 612727

DATE COMMENCED: 24/05/2012

Samples tested as received

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Extracted one HVAS filter paper sample into 600mLs PFW at 37°C for 30 minutes. Further diluted to 1/100 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/device
HVAS Filter Paper Plashett PM10 - 27/3/12 - 612727	0.5	**<0.500**	**<300.000**

< = less than EU = Endotoxin Units PFW = Pyrogen Free Water

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Certificate of Analysis

Dated: 18/06/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069
ATTN: Nicholas Kannegieter

OUR REFERENCE: 1206072

ORDER NO: Not Given

DATE RECEIVED: 26/04/2012

SAMPLE DESCRIPTIONS:
1 x HVAS Filter Paper
Plashett TSP - 27/3/12 - 612726

DATE COMMENCED: 6/06/2012

Samples tested as received

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Extracted one HVAS filter paper sample into 600mLs PFW at 37°C for 30 minutes. Further diluted to 1/100 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/device
HVAS Filter Paper Plashett TSP - 27/3/12 - 612726	0.5	**5.170**	**3,102.000**

< = less than EU = Endotoxin Units PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.

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VALIDATION REPORT
Dated: 18/06/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069
ATTN: Nicholas Kannegieter

OUR REFERENCE: 1206072/1

ORDER NO: Not Given

DATE RECEIVED: 26/04/2012

SAMPLE DESCRIPTIONS:
1 x HVAS Filter Paper
Plashett TSP - 27/3/12 - 612726

DATE COMMENCED: 10/05/2012

Samples tested as received

EXAMINATION: LAL VALIDATION

METHOD: Kinetic Chromogenic Method TM125

VALIDATION PROFILE:

- **Pretreatment**: 1 x HVAS filter paper sample was extracted in 600mLs of PFW.
- **Extraction:** The sample was extracted at 37°C for 30 minutes.
- **Dilution:** Further dilutions were made in PFW to investigate the inhibition and enhancement effect by spiking a known amount of endotoxin and testing for recovery.
- Further dilution to 1/100 in PFW was shown to have a satisfactory recovery.

VALIDATED PROTOCOL: Dilute 1/100 in PFW after extraction.

PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.

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Certificate of Analysis

Dated: 18/06/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069
ATTN: Nicholas Kannegieter

OUR REFERENCE: 1206069

ORDER NO: Not Given

DATE RECEIVED: 26/04/2012

SAMPLE DESCRIPTIONS:
1 x Dust Residue Sample
D3B (D11)
Anglo Coal - Drayton South Project
Samples tested as received

DATE COMMENCED: 5/06/2012

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Scrape dust residue from filter paper & make a 1mg/mL solution. Further dilute to 1/1000 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/mg
Dust Residue Sample D3B (D11) Anglo Coal - Drayton South Project	5.0	**14.691**	**14.691**

< = less than EU = Endotoxin Units PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.

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Certificate of Analysis

Dated: 18/06/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069

ATTN: Nicholas Kannegieter

OUR REFERENCE: 1206071

ORDER NO: Not Given

DATE RECEIVED: 26/04/2012

SAMPLE DESCRIPTIONS:
1 x Dust Residue Sample
D12
Anglo Coal - Drayton South Project
Samples tested as received

DATE COMMENCED: 31/05/2012

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Scrape dust residue from filter paper & make a 1mg/mL solution. Further dilute to 1/1000 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/mg
Dust Residue Sample D12 Anglo Coal - Drayton South Project	5.0	**<5.000**	**<5.000**

< = less than   EU = Endotoxin Units   PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.

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Certificate of Analysis

Dated: 18/06/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069
ATTN: Nicholas Kannegieter

OUR REFERENCE: 1206070

ORDER NO: Not Given

DATE RECEIVED: 26/04/2012

SAMPLE DESCRIPTIONS:
1 x Dust Residue Sample
D8
Anglo Coal - Drayton South Project
Samples tested as received

DATE COMMENCED: 31/05/2012

EXAMINATION: Kinetic Chromogenic LAL Test

METHOD: TM125

PRETREATMENT: Scrape dust residue from filter paper & make a 1mg/mL solution. Further dilute to 1/1000 in PFW.

RESULTS:

SAMPLE REFERENCE	LAL ASSAY DETECTION LIMIT EU/mL	LAL RESULT EU/mL	LAL RESULT EU/mg
Dust Residue Sample D8 Anglo Coal - Drayton South Project	5.0	**<5.000**	**<5.000**

< = less than EU = Endotoxin Units PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.

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VALIDATION REPORT

Dated: 18/06/2012

CLIENT: Nicholas Kannegieter
8 Roseville Avenue,
ROSEVILLE NSW 2069
ATTN: Nicholas Kannegieter

OUR REFERENCE: 1206069/1

ORDER NO: Not Given

DATE RECEIVED: 26/04/2012

SAMPLE DESCRIPTIONS:
1 x Dust Residue Sample
D3B (D11)
Anglo Coal - Drayton South Project
Samples tested as received

DATE COMMENCED: 10/05/2012

EXAMINATION: LAL VALIDATION

METHOD: Kinetic Chromogenic Method TM125

VALIDATION PROFILE:

- **Pretreatment**: Scrape dust residue from filter paper and make a 1mg/mL solution.
- **Dilution**: Further dilutions were made in PFW to investigate the inhibition and enhancement effect by spiking a known amount of endotoxin and testing for recovery.
- Further dilution to 1/1000 in PFW was shown to have a satisfactory recovery.

VALIDATED PROTOCOL: Dilute 1/1000 in PFW.

PFW = Pyrogen Free Water

Signed:
Elizabeth Georgievska BSc.