# Identification of Risk Factors for Sub-Optimal Housing Conditions in Australian Piggeries: Part 2. Airborne Pollutants

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**ABSTRACT.** The concentrations of total airborne bacteria, respirable endotoxins, ammonia, and respirable and inhalable particles were monitored in 160 piggery buildings in Australia between autumn 1997 and autumn 1999. The overall mean airborne bacteria, respirable endotoxins, ammonia  $(NH_3)$ , and inhalable and respirable particle concentrations measured were  $1.17 \times 10^{5}$  cfu m<sup>-3</sup>, 33.1 EU m<sup>-3</sup>, 3.7 ppm, 1.74 mg m<sup>-3</sup>, and 0.26 mg m<sup>-3</sup>, respectively. The characteristics of the buildings and management systems used were documented at the time of sampling. A multifactorial general linear model (GLM) statistical procedure was used to analyze the effects of housing and management factors on the concentrations of the airborne pollutants. Both airborne bacteria and respirable endotoxin concentrations were affected by building classification (type), and respirable endotoxin concentrations were positively correlated with increasing humidity. The concentrations of airborne bacteria increased as the level of pen hygiene (cleanliness) decreased. The NH<sub>3</sub> concentrations were primarily affected by level of pen hygiene, building volume, pig flow management, and season. Building classification, pig flow management, season, building volume, ventilation rates, and temperature affected inhalable particle concentrations. Respirable particle concentrations were primarily affected by building classification, pen hygiene, pig flow management, season, ventilation rates, temperature, and humidity. These findings suggest that environmental improvement strategies (such as improved cleaning, ventilation, and temperature control) are likelv to reduce airborne pollutant concentrations in pig buildings and in the environment, thus improving the health and welfare of both pigs and farm staff.

Keywords. Air quality, Ammonia, Dust, Endotoxin, Farm building, Microorganisms, Statistical models.

The main airborne pollutants found in piggery buildings are airborne bacteria, bacterial products such as endotoxins, ammonia (NH<sub>3</sub>), and airborne particles (Wathes et al., 1998). Airborne bacteria are usually attached to airborne particles and are often referred to as "viable" particles (Seedorf et al., 1998). Endotoxins are the cell-wall components of gram-negative bacteria, and these compounds are released after the death of the bacteria. NH<sub>3</sub> present in livestock buildings is produced as a result of chemical and biological breakdown of waste material (Groot Koerkamp et al., 1998).

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Airborne particles in piggery buildings originate from animal skin, hair, dried urine, feces, bedding material, microorganisms, grain, pollen, and other particles (Takai et al., 1998). Airborne particles usually act as carriers for pathogenic bacteria, viruses, endotoxins, gases, and liquid substances, and odorous material can also be adsorbed by the airborne particles (Bottcher, 2001; Takai et al., 2002). A brief literature review conducted as part of the study (reported in this series) confirmed that high concentrations of these airborne pollutants could affect the external environment, production efficiency, and human and animal health and welfare (Banhazi et al., 2008b).

To solve the identified problems associated with airborne pollution, a national project was undertaken in Australia. As a first step, the housing and management factors that have a significant influence on concentrations of major airborne pollutants have been reviewed (Banhazi et al., 2008b). The review identified a number of different management, environmental, and housing factors that could affect concentrations of airborne particles within piggery buildings. However, because these factors had not been evaluated simultaneously, a comprehensive field study of air quality in piggery buildings was designed (Banhazi et al., 2008a; Banhazi et al., 2008b; Banhazi et al., 2008c). The ultimate aim was to determine the key piggery design and management factors that affect the internal concentrations of the five major airborne pollutants in piggery buildings. We envisage that manipulation of the factors identified will ultimately lead to a reduction in the concentrations and emissions of these airborne pollutants.

## Materials and Methods

As an initial step in the study, an instrumentation kit was developed so that the concentrations and emissions of the airborne pollutants and other important environmental parameters could be monitored in the study buildings. Data collection procedures and databases were also designed to enable the characteristics of the buildings and management systems used to be documented systematically at the time of sampling and taken into account during the subsequent statistical modeling (Banhazi et al., 2008b). Other researchers have used sophisticated and relatively complicated instrumentation during previous air quality surveys (Phillips et al., 1998). However, we purposely simplified the instrumentation kit to ensure that the equipment used during the survey could be applied routinely on farms after the study had been concluded (Banhazi, 2005).

Details of the study design have been published as part of this series (Banhazi et al., 2008b). Briefly, 160 piggery buildings from 40 farms distributed widely across Australia's pork production zones were included in the study of effects of housing and management factors during autumn 1997 to autumn 1999. Table 1 presents the main measurement techniques used, and figure 1 shows the typical placement of sampling instruments used for the detection of different environmental pollutants during the study.

#### **Particle Measurement**

Concentrations of airborne particles were determined gravimetrically using cyclone samplers for respirable ( $<5 \mu m$ ) particles and seven-hole samplers for inhalable particles (Casella, Ltd., Kempston, U.K.), respectively. These types of samplers are used routinely in studies of personal particle exposure (Li et al., 2000; Vaughan et al., 1990). The sampling rate was controlled at 1.90 L min<sup>-1</sup> for respirable particles and at 2.00 L min<sup>-1</sup> for inhalable particles, which were the standard sampling rates at the time of the study (Takai et al., 1998). The samplers were connected to GilAir air pumps (Gilian Instrument Corp., West Caldwell, N.J.) and were usually placed above the walkways. An 8 h sampling time was standardized throughout the project, starting between 0800 and

Variable	Technique/Equipment	Data Collection Frequency
Airborne particle concentration	Standard gravimetric filtration	8 h
Airborne particle concentration	Light-scattering instrument	6 min
Respirable endotoxin concentration	Limulus amoebocyte lysate technique	Single sample
Viable bacteria concentration	Andersen samplers and horse-blood agar plates	Single sample
Air temperature	Self-contained dataloggers	1.5 min
Air humidity	Self-contained dataloggers	1.5 min
Ammonia concentration	Electrochemical sensors	30 readings h <sup>-1</sup>
Carbon dioxide concentration	Infrared sensors	30 readings h <sup>-1</sup>
Air velocity	Hot-wired instrument	7.5 min
Ventilation rate	Mass balance of CO <sub>2</sub>	Over 60 h

Table 1. Measurement techniques used during the Australian air quality and housing survey (based on Wathes et al., 1998).



Figure 1. Placement of instruments used for the survey: 1 = external temperature, 2 = external humidity, 3 = internal temperature, 4 = internal humidity, 5 = continuous dust monitoring, 6 = inhalable dust, 7 = respirable dust, 8 = Andersen bacterial samplers, 9 = MGM machine, and 10 = internal air velocity measurement.

0900 h. To allow the animals to settle, the equipment was placed in the building the day before measurement commenced. A built-in timer automatically started the sampling routine. In light of the results of previous studies, sampling was selected to occur when particle concentrations were likely to peak (Pedersen and Takai, 1999). After the field measurements were complete, the concentrations of airborne particles were determined by weighing the particle mass collected on filters, to the nearest 0.001 mg, in a controlled environment room. The filters were conditioned appropriately by being kept in the laboratory for approximately 24 h before and after deployment. Gilian field calibration instrumentation (Gilian Instrument Corp.) was used to recalibrate the flow rates of the sampling pumps before installation in the buildings. In some piggery buildings, an OSIRIS light-scattering instrument (Turnkey Instruments, Ltd., Northwich, U.K.) was used to monitor airborne particle distribution over the monitoring periods. Lightscattering instruments have been used previously in air quality studies (Kerker, 1997). The OSIRIS instruments were supplied with the factory calibration and were recalibrated annually by the supplier. Both the OSIRIS dust monitors and the gravimetric filters were attached by wire cables to the ceiling or a beam, and lowered to pig level (approx. 1.1 to 1.3 m height).

#### **Endotoxin Measurement**

To estimate the endotoxin concentrations in respirable particles, the contents of the exposed respirable filters were extracted with sterile and pyrogen-free water at room temperature after the weights of the filters were determined gravimetrically. The commercially available test kit (BioWhittaker, Inc., Walkersville, Md.) used was based on the limulus amoebocyte lysate (LAL) test (Seedorf et al., 1998). This test utilizes the initial part of the LAL endotoxin reaction, which produces a vellow color reaction. Endotoxin concentrations were measured by a microplate method, which involved reading the absorbency of each microplate well at a wavelength of 405 nm. Distilled water was used to adjust the photometer to zero. All disposable products used were pyrogen-free (Sarstedt Australia Pty. Ltd., Adelaide, South Australia). Each filter was diluted with pyrogen-free water (Delta West Pty. Ltd., Perth, Australia) at 25 mL per filter. The optimum pH was 7, and the pH was adjusted by the addition of NaOH or HCl. The water-dust suspension was vortexed for 20 s, shaken for 2 h at room temperature, and then centrifuged at 2000 rpm for 10 min. A 500 µL aliquot was taken for subsequent analysis. For calibration, six standard solutions were made with the endotoxin of *Escherichia coli* (BioWhittaker, Inc.) with a control standard endotoxin (CSE) potency equivalent to 10 EU ng-1. A multipoint calibration with 50 uL solutions with concentrations of 20, 5, 1, 0.5, 0.25, or 0.10 EU mL<sup>-1</sup> (endotoxin units per milliliter) was used, and the magnitude of the color intensity was measured by photometry. All data were analyzed by linear regression and compared with a standard curve from the reference endotoxin. Solutions of endotoxin-free water and lysate served as controls. The measurements were made using a QCL-1000 Chromogenic LAL test kit (BioWhittaker, Inc.) with a Kinetic-OCL Reader (BioWhittaker, Inc.).

#### **Bacterial Measurement**

Airborne microorganisms were sampled with a standard Andersen sampler or six-stage bacterial impactor (Seedorf et al., 1998). Horse-blood agar (HBA) plates were used (Medvet Science Pty. Ltd., Stepney, Australia) to determine the total number of bacterial colonies (colony forming units, or cfu). Samples were taken at about midday (1100 h to 1500 h), usually in the center of the animal building and above the pens. The flow rate during sampling was 1.9 L min<sup>-1</sup>, and the sampling time was 5 min. The exposed HBA plates were incubated at 37° C under aerobic conditions, and bacterial colonies were counted after 24 h.

#### **Gas Concentration Measurements**

Gases such as NH<sub>3</sub> and CO<sub>2</sub> were monitored continuously using a multi-gas monitoring (MGM) machine developed in-house. An electrochemical gas monitoring head (Bionics TX-FM/FN, Bionics Instrument Co., Tokyo, Japan) was used to detect internal concentrations of NH<sub>3</sub>, and an infrared sensor (GMM12, Vaisala Oy, Helsinki, Finland) was used to detect CO<sub>2</sub> concentrations. The gas monitoring heads and the supporting electrical components were enclosed in a shock-resistant electrical box. An air delivery system was also built into the MGM machine, which delivered air samples from the sampling points within and outside the buildings to the actual gas monitoring heads. Air was drawn at a nominal rate of 0.5 to 0.8 L min<sup>-1</sup> from the sampling points. After each sampling point had been monitored for 15 min, the system was purged for 15 min with fresh air drawn from outside the buildings to flush out the sampling lines and zero the NH<sub>3</sub> monitoring head. Electronic (voltage) tags corresponding to the internal and external sampling sites were logged, and this enabled automatic separation of the data. A computer program was developed to facilitate the automatic separation and graphing of data. The program also contained algorithms for calculating the amount of time spent above and below the relevant recommended levels. At the end of each data collection period, the raw data were assessed by the data collectors. If drift had occurred in the raw dataset (i.e., if during the purge periods the data did not demonstrate a dramatic decline towards zero in the case of  $NH_3$ , or to the expected ambient levels in the case of  $CO_2$ ), the data were discarded from the dataset designated for analysis. The MGM machine was frequently calibrated using a custom-made 2500 ppm CO<sub>2</sub> mixture and a standard 50 ppm NH<sub>3</sub> calibration gas mixture (Calgaz, Air Liquide Australia, Ltd., Melbourne, Australia). For most monitoring events, the enclosure containing the gas monitoring heads was deployed as close to the actual sampling locations as possible to minimize the length of sampling tube used. Sampling tubes were not heated, as condensation was unlikely to occur under typical Australian climatic conditions (Banhazi et al., 2008c). A filter was attached to the end of each intake tube to prevent the deposition of particles in the sampling line. The sampling lines were frequently cleaned both internally and externally using anti-viral disinfectant to avoid cross-contamination between farms. Sampling inlets were usually placed at a height of about 1.1 to 1.3 m.

#### Additional Measurements and Considerations

At the time of data recording, the level of pen hygiene was assessed visually by estimating the percentage of manure-covered solid area in the representative pen and then classifying the hygiene into three levels (poor = more than 50% manure cover on pen floors; fair = between 10% and 50% manure cover on pen floors; good = less than 10%manure cover on pen floors) (Randall et al., 1983). Similar hygiene assessment methods were used during previous studies (Aarnink et al., 2006; Aarnink et al., 1997). Seasons were defined as "summer" from November to April and "winter" from May to October. Ventilation rates were calculated using the CO<sub>2</sub> balance method, and temperature and humidity readings were recorded in all buildings using Tinytalk temperature and humidity dataloggers (Tinytalk-2, Hastings Dataloggers Pty. Ltd., Port Macquarie, Australia) as detailed in a companion article of this series (Banhazi et al., 2008c). The data collected were forwarded to a central location where they were checked for errors prior to analysis. The distribution of all airborne pollutants was skewed and thus required log-transformation prior to analysis. The transformed data were analyzed by a general linear model procedure (PROC GLM; SAS, 1989), which allows simultaneous adjustment of the data for multiple effects (using the method of least squares) (Banhazi et al., 2008b). Thus, this effectively dealt with the unbalanced nature (unequal numbers in all factor classes) of the data obtained under field conditions. The specific factors and covariates included in the analysis are outlined in detail in the first part of this series (Banhazi et al., 2008b). Significance was defined as either P < 0.05 or P < 0.01 depending on the level of stringency required. Results presented are based on back-transformed least squares mean or median values on a natural scale ( $\pm 95\%$  confidence intervals) of fixed effects and best-fit slopes of covariates, where relevant.

## Results

Summary statistics of the pollutant concentrations measured in the study buildings are presented in table 2. Unfortunately, but not surprisingly, high-quality data were not available for all buildings, especially for bacteria. The analysis of variance is presented in table 3, and the significance of each effect associated with airborne pollutant

			No. of		
Pollutant	Mean	Median	Buildings	Min.	Max.
Airborne bacteria concentration (10 <sup>5</sup> cfu m <sup>-3</sup> ) <sup>[a]</sup>	1.2	0.8	122	0.2	6.1
Endotoxin concentration (EU m <sup>-3</sup> ) <sup>[b]</sup>	33.1	17.0	153	0.0 <sup>[c]</sup>	238.0
Ammonia (ppm)	3.7	1.1	141	0.0 <sup>[c]</sup>	29.4
Respirable particles (mg m <sup>-3</sup> )	0.26	0.19	159	0.01	2.13
Inhalable particles (mg m <sup>-3</sup> )	1.74	1.27	159	0.12	10.07

Table 2. Concentrations of airborne pollutants across all study buildings (based on building averages).

[a] 100,000 colony-forming units per cubic meter of air.

<sup>[b]</sup> Endotoxin units per cubic meter of air.

<sup>[c]</sup> It is likely that concentrations were below detectable levels of the instrumentations used.

	Airborne Bacteria	Respirable Endotoxins	Ammonia	Inhalable Particles	Respirable Particles
Model degrees of freedom	6	5	7	18	26
Corrected total degrees of freedom	111	93	98	95	89
Total sum of sum of squares	53.137	61.285	133.117	68.204	67.791
Model $R^2$ (%)	50.6	24.9	21.4	72.6	68.9

Table 3. Analysis of variance for airborne pollutant concentrations.

concentrations is summarized in table 4. The most significant results are shown in figures 2 through 8 and in table 5.

The models developed explained significant portions of the variation in concentrations for all pollutants, except for ammonia, where approximately 75% of the variation in concentrations remained unexplained (table 3). A relatively small number of degrees of freedom was used in the final models compared to the available degrees of freedoms within the dataset, indicating the robustness of the models developed.

The models developed at the 99% confidence level included only two major factors for predicting endotoxin and two for airborne bacteria concentrations. For airborne bacteria concentrations, the two main effects identified were building classification and level of hygiene. For endotoxin concentrations, humidity level and the building classification were shown to be significant (table 4).

For NH<sub>3</sub> concentration, four significant effects (management, level of hygiene, season, and building volume) were identified, together with two significant interactions at the 95% confidence level (table 4). Inhalable particle concentrations were affected at the 99% confidence level by the building classification, management, season, building volume, ventilation rate, temperature, number of sows (farm size), and four interactions (table 4). For respirable particle concentrations, the building classification, level of hygiene, management, season, ventilation rate, temperature, humidity, number of sows (farm size), and five interactions were identified as being significant at the 99% confidence level (table 4).

The highest concentrations of total airborne bacteria  $(2.17 \times 10^5 \text{ cfu m}^{-3})$  and endotoxins (76.26 EU m<sup>-3</sup>) were detected in deep-bedded shelters (DBS) (figs. 2 and 3), while the concentrations in all other buildings were not statistically different for the same pollutant.

A significant effect of manure contamination of the pen floor was demonstrated, as NH<sub>3</sub> concentrations were highest (2.5 ppm) in pens with sub-optimal pen hygiene/cleanliness (fig. 4). The NH<sub>3</sub> concentrations in buildings classified as having "good" or "fair" hygiene levels were not statistically different.

Airborne bacterial concentrations rose significantly with a decrease in pen hygiene, as determined by the amount of manure covering the pen floor (fig. 5).

Table 4. Tests of significance for effects associated with airborne pollutant concentrations. <sup>141</sup>					
	Airborne	Respirable		Inhalable	Respirable
Effects And Interactions	Bacteria	Endotoxins	Ammonia	Particles	Particles
Building classification (type)	**	**	ns	**	**
Hygiene/cleanliness	**	ns	*	*	* *
Management	*	ns	*	**	* *
Season	ns	ns	*	**	* *
Pig weight per building	ns	*	ns	ns	*
Building volume	ns	ns	*	**	*
Airflow	*	*	ns	ns	ns
Airflow <sup>2[b]</sup>	*	ns	ns	**	**
Temperature	ns	ns	ns	**	**
Humidity	ns	**	ns	ns	**
Number of sows (farm size)	ns	ns	ns	**	**
Building classification × hygiene	ns	ns	ns	ns	**
Building classification × management	*	ns	ns	ns	ns
Building classification × temperature	ns	ns	ns	**	ns
Building classification × humidity	ns	ns	ns	ns	* *
Building classification × pig weight per building	ns	ns	ns	ns	*
Building classification × number of sows	ns	ns	ns	ns	*
Building classification × season	ns	ns	ns	ns	*
Building classification $\times$ airflow <sup>2</sup>	ns	ns	ns	ns	*
Management $\times$ airflow <sup>2</sup>	*	ns	ns	**	* *
Management × season	ns	ns	*	ns	ns
Management × temperature	ns	ns	ns	ns	* *
Management × humidity	ns	ns	ns	ns	* *
Management × building volume	ns	ns	ns	**	ns
Season × building volume	ns	ns	*	ns	ns
Season × temperature	ns	ns	ns	*	ns
Season × number of sows	ns	ns	ns	ns	* *
Hygiene × temperature	ns	ns	ns	*	ns

Table 4.	Tests of	significance	for effects	associated	with airborn	e pollutant	concentrations. <sup>[a]</sup>	
		Significance				• • • • • • • • • • • • • • • • • • • •	· · · · · · · · · · · · · · · · · · ·	

[a] \* = P < 0.05; \*\* = P < 0.01; and ns = not significant.

[b] If a quadratic term was retained in the model, so was the linear term.



Figure 2. Effect of building classification on concentrations of airborne bacteria concentrations (cfu m<sup>-3</sup>) in the study buildings (median values with 95% confidence intervals). Different letters indicate significant difference. DBS = deep bedded shelters.



Figure 3. Effect of building classification on respirable endotoxin concentrations (EU m<sup>-3</sup>) in the study buildings (median values with 95% confidence intervals). Different letters indicate significant difference. DBS = deep bedded shelters.



Figure 4. Effects of pen hygiene classification on ammonia concentrations (ppm) in the study buildings (medians with 95% confidence intervals). Different letters indicate significant difference.



Figure 5. Effect of hygiene classification on concentrations of airborne bacteria (cfu m<sup>-3</sup>) in the study buildings (median values with 95% confidence intervals). Different letters indicate significant difference.



Figure 6. Effect of hygiene (cleanliness) and building classification interaction on respirable particle concentrations (mg m<sup>-3</sup>) in the study buildings (medians with 95% confidence intervals). Different letters indicate significant differences within any given building type. DBS = deep bedded shelters.

Decreasing pen hygiene resulted in increasing respirable particle concentrations in weaner, grower/finisher, and farrowing buildings. In deep-bedded shelters (DBS), lower respirable particle concentrations were associated with poorer pen hygiene classification (fig. 6). Respirable particle concentrations in dry sow buildings with different hygiene classifications were not statistically different.

Higher (3.5 ppm)  $NH_3$  concentrations were recorded in summer than in winter in continuous-flow (CF) buildings, whereas concentrations were stable across seasons in all-in/all-out (AIAO) buildings (fig. 7).

Significantly higher inhalable particle concentrations were measured in winter  $(2.95 \text{ mg m}^{-3})$  in piggery buildings (fig. 8) than in summer  $(1.68 \text{ mg m}^{-3})$ .

Temperature and humidity were positively correlated with respirable particle concentration in all-in/all-out (AIAO) buildings (table 5). However, humidity in interaction with building type had the effect of reducing respirable particle concentration in DBS (table 5). Number of sows, which was an indicator of farm size, was positively



Figure 7. Effect of management and season interaction on ammonia concentration (ppm) in the study buildings (medians with 95% confidence intervals). Different letters indicate significant difference. AIAO = all-in/all-out; CF = continuous flow.



Figure 8. Effect of season on inhalable particle concentrations (mg m<sup>-3</sup>) in the study buildings (medians with 95% confidence intervals).

Pollutant	Unit	Covariate	Interaction <sup>[a]</sup>	Slope
Respirable	mg m <sup>-3</sup>	Temperature	AIAO management	Positive
Respirable	mg m <sup>-3</sup>	Humidity	AIAO management	Positive
Respirable	mg m <sup>-3</sup>	Humidity	DBS building type	Negative
Respirable	mg m <sup>-3</sup>	Number of sows (farm size)	Summer	Positive
Respirable	mg m <sup>-3</sup>	Ventilation airflow	AIAO management	Positive
Respirable	mg m <sup>-3</sup>	Ventilation airflow	CF management	Negative
Inhalable	mg m <sup>-3</sup>	Temperature	Farrowing building	Negative
Inhalable	mg m <sup>-3</sup>	Temperature	Weaner building	Positive
Inhalable	mg m <sup>-3</sup>	Building volume	AIAO management	Positive
Inhalable	mg m <sup>-3</sup>	Building volume	CF management	Negative
Inhalable	mg m <sup>-3</sup>	Ventilation airflow	AIAO management	Quadratic effect
Inhalable	mg m <sup>-3</sup>	Number of sows (farm size)	N/A	Positive
Ammonia	ppm	Building volume	Summer	Negative <sup>[b]</sup>
Ammonia	ppm	Building volume	Winter	Positive <sup>[b]</sup>
Endotoxins	EU m <sup>-3</sup>	Humidity	N/A	Positive

Table 5. Effects of various covariates on the concentration of airborne pollutants.

[a] AIAO = all in/all out, DBS = deep-bedded shelter, and CF = continuous flow.

[b] Slopes were not significantly different from zero, but the difference between the two slopes was significant.

correlated with respirable particle concentration in summer (table 5). Increased ventilation airflow was negatively correlated with respirable particle concentration in continuous flow (CF) buildings, but was positively correlated with respirable particle concentration in AIAO buildings (table 5). Temperature had a positive correlation with inhalable particle concentrations in weaner, DBS, and grower/finisher buildings, but had a negative correlation with particle concentration in dry sow, DBS, and grower/finisher buildings. The slopes describing particle concentration in dry sow, DBS, and grower/finisher buildings were not significantly different from zero (data not shown). Inhalable particle concentrations increased with building volume (size) in AIAO piggery buildings and decreased in CF buildings (table 5). Increased airflow had a significant effect on inhalable dust concentration in the study buildings. The results suggested that ventilation airflow has a quadratic effect in AIAO piggery buildings. Inhalable particle concentrations increased with high some mumbers (table 5). The effect of building size (volume) on NH<sub>3</sub> concentration interacted with the season and affected NH<sub>3</sub> concentration differently

in summer than in winter (table 5). The model developed for respirable endotoxins demonstrated a positive correlation between endotoxin concentration and humidity measured inside the building (table 5).

# Discussion

#### **Measurement Methods**

Instruments were developed that are simpler in design and thus more user friendly for use in piggery buildings compared to those used previously (Phillips et al., 1998). The standard air pumps used in conjunction with the filter heads worked well and proved to be quite practical. The OSIRIS light-scattering instrument did not provide consistently reliable concentration data, but it did provide good information in relation to the relative distribution of particles over time. The test kit used for endotoxin measurements was sold as a ready-to-use commercial kit. However, it still required a considerable amount of additional work and fine-tuning. Nevertheless, as one operator conducted all the analyses, the comparative concentrations of samples were consistent, ensuring reliable interpretation of data. The bacterial measurements used were relatively straightforward and, although labor intensive, easy to apply. However, the relevance of a 5 min spot check to the overall bacterial concentration found in animal buildings has been questioned by some authors (Agranovski et al., 2002; Agranovski et al., 2004). A more representative method has to be found for the assessment of bacterial concentrations in animal buildings (Agranovski et al., 2004). The assessment of hygiene was purposely kept simple to ensure that the classification of manure cover could be reliably reproduced by the operators.

The MGM machine performed well, but the  $NH_3$  sensors required frequent calibration. At high concentrations, the electrochemical ammonia sensors proved to be reliable. However, low  $NH_3$  concentrations might have been slightly underestimated, as electrochemical sensors are generally unpredictable in measuring very low  $NH_3$ concentrations (Hoy, 1995). Nevertheless, it was reassuring that high concentrations were correctly recorded, because these are the important parameters in terms of impact on both animal health and welfare. The long measuring periods (60 h) also ensured that reliable average concentrations were obtained from each building. The infrared sensors used to measure  $CO_2$  concentrations proved to be stable, robust, and reliable. Transportation of the enclosure was sometimes problematic because of the large size and weight of the equipment. The dimensions and weight of the gas measurement unit have been intentionally reduced as part of the subsequent developments to make it readily available for routine farm building assessments (Banhazi, 2005).

#### Concentrations

The current recommendations for acceptable airborne bacteria, respirable endotoxins, NH<sub>3</sub>, and inhalable and respirable particle concentrations in livestock buildings in Australia are  $1 \times 10^5$  cfu m<sup>-3</sup>, 50 EU m<sup>-3</sup>, 10 ppm, 2.4 mg m<sup>-3</sup>, and 0.23 mg m<sup>-3</sup>, respectively, as discussed in the first article of this series (Banhazi et al., 2008b). These recommendations are not enforced, but producers accept that maintaining lower concentrations of viable airborne bacteria and endotoxins in livestock buildings is the best practice. The research presented here demonstrates that the average concentrations of respirable endotoxins, airborne particle, and to a lesser extent, viable airborne bacteria, in Australian piggery buildings are generally below, or near, the recommended limits. The relatively low concentrations of airborne bacteria and endotoxins could be related to climate. In southern Australia, temperatures are high and humidity levels are low, and these conditions do not sustain high populations of airborne bacteria (Zucker et al., 2000).

In 91% of Australian piggery buildings tested, the measured NH<sub>3</sub> concentrations were below the 10 ppm limit suggested (data not shown), on the basis of building averages. Our survey generally recorded lower concentrations of NH<sub>3</sub> than in European buildings (Groot Koerkamp et al., 1998). The low concentrations of both gases are related to the fact that, in southern Australia, where these measurements were taken, the temperatures are higher than in Europe, and therefore ventilation levels are also higher. However, in some traditional buildings and in most of the DBS, very high concentrations of airborne bacteria, respirable endotoxin, and airborne particles were measured (table 2), which provide reasons for concern. The fact that the measurements were highly variable, and in some buildings very low concentrations were measured, also confirmed that there is room for further improvements in the management and engineering of these buildings.

#### **Identified Risk Factors**

The main outcome of the study was the identification of the key factors influencing the concentration of airborne pollutants inside piggery buildings, and these effects are discussed below.

#### **Building Effects**

In contrast with the generally low concentrations measured in traditional buildings, the least squares median concentration of endotoxins in DBS exceeded the recommended limits by a factor of 1.5, and similarly the total bacterial concentrations were exceeded by a factor of 2 (figs. 2 and 3). Farm workers attending pigs housed in DBS are potentially exposed to higher concentrations of these airborne pollutants when compared to traditional buildings. It has been demonstrated by previous studies that even short-term exposure to high concentrations of airborne particles can cause a respiratory reaction in naïve subjects (Larsson et al., 1994; Zhiping et al., 1996). The presence of bedding materials was probably the main reason for the high concentrations of airborne bacteria and endotoxins measured in DBS. Under the dry Australian climate (especially in southern Australia), the top layer of bedding material tends to dry quickly, creating opportunities for airborne particle formation. Further studies on the relationship between the quality of straw and concentrations of endotoxins/airborne bacteria are required. Examination of the effects of treating bedding materials in various ways could help to develop methods of reducing the production of viable particles and endotoxins in DBS. Incorporating vegetable oil or a plant extract that reduces bacterial growth in the bedding might be a practical option for reducing concentrations of viable and non-viable particles (Ellen et al., 2000; Pedersen et al., 2001). The introduction of alternative bedding materials with anti-bacterial properties could also be considered.

One would expect that pig production in DBS would be affected by the high concentrations of total bacteria and respirable endotoxins measured, as our brief literature review (conducted as part of this study) demonstrated that high concentrations of airborne pollutants could negatively affect production efficiency of pigs housed in traditional (pens with slats and no bedding material) buildings (Banhazi et al., 2008b). However, research conducted in Western Australia has demonstrated that DBS have acceptable levels of production efficiency when compared with traditional buildings (Payne, 1995), and the very high concentrations of airborne bacteria and respirable endotoxins typically measured in DBS do not appear to dramatically interfere with the production efficiency of pigs. Therefore, we hypothesize that the effect of high concentrations of these pollutants on the health of animals is complex and is perhaps affected by a number of stress-related and/or physiological factors. An understanding of the underlying physiological reasons why pigs in DBS are able to tolerate higher concentrations of airborne bacteria in the provements in

management of both conventional housing and DBS. In the light of previous research demonstrating negative effects of high dust concentrations in unexposed subjects after very short exposure (Larsson et al., 1994; Zhiping et al., 1996), there is also a need to establish maximum safe limits for exposure of piggery workers in DBS to endotoxins, airborne particles, and airborne bacteria, as the concentrations of these pollutants are typically high in DBS. Lastly, emission rates of these pollutants are typically very high from DBS and should be minimized (Banhazi et al., 2008a).

#### Hygiene Effects

The effect of pen floor hygiene (essentially pen surface cleanliness) on ammonia, bacterial, and respirable particle concentrations was a significant finding of this study (table 4 and figs. 4, 5, and 6). The effects of manure contamination of pen floors have been demonstrated in previous studies on ammonia concentrations (Aarnink et al., 1996). However, this study also demonstrated a direct link between the hygiene level of pen floors and bacterial and respirable particle concentrations in the air in livestock buildings. The association between pen hygiene and air quality arises from the ready generation of ammonia, respirable, and viable airborne particles from dried feces on the pen floors. Fecal material on solid pen floors dries out in the low-humidity southern Australian climate, generating ammonia and fine particles that are dispersed by the animals. Dried feces on the animals' skins can also become a major source of dust and bacteria (Takai et al., 1998). However, in DBS, the effect of pen soiling appeared to be reversed (fig. 5), as a result of increased adhesiveness of bedding material owing to fecal contamination. Such soiled bedding material would retain particles within the fibers of the straw more effectively (thus preventing the particles from becoming airborne) than clean and dry bedding material. The respirable particle concentrations of dry sow buildings with different hygiene classification were not significantly different. This is probably due to the fact that all dry sow buildings included in the study had dry sow stalls, ensuring a relatively efficient separation and removal of waste material from the surroundings of the sows. However, it is evident that sub-optimal hygiene in traditional buildings is one of the main causes of high bacterial concentrations; therefore, improvement of pen hygiene could make a marked contribution to healthier conditions in piggery buildings and in the external environment. No significant effect of manure cover of the pen floor on inhalable particle concentration was demonstrated. This is also an interesting finding because it appears to support the claim that the main source of inhalable particles is feed (Aarnink et al., 1999).

The interactive effect of pig management and season on NH<sub>3</sub> concentration (fig. 7) confirms the results of previous Australian studies demonstrating the positive effects of AIAO management on hygiene maintained in piggery buildings and therefore on air quality (Cargill and Banhazi, 1998; Cargill et al., 1998). In AIAO buildings, the effect of season is minimal and the NH<sub>3</sub> concentration stable, as increased opportunities for cleaning enable better control of the level of hygiene throughout the seasons. However, in CF buildings in summer, increased NH<sub>3</sub> concentrations were detected. It is likely that pen surface hygiene is poorer in CF buildings than in AIAO buildings, and therefore in summer more NH<sub>3</sub> evaporates from the contaminated pen floors (fig. 7) (Groot Koerkamp et al., 1998). Pigs tend to soil their pens more readily at higher temperatures (Huynh et al., 2005). Therefore, summer appears to be a high-risk period for elevated NH<sub>3</sub> concentrations in CF buildings.

#### Effects of Season, Airflow, and Building Size

In piggery buildings, winter ventilation rates are usually lower than in summer, thus leading to higher inhalable particle concentrations in winter than in summer (fig. 8). Although no significant seasonality of ventilation rates has been demonstrated in

southern Australia, ventilation rates are numerically lower in winter than in summer (Banhazi et al., 2008c). It has been suggested that ventilation airflow is more capable of removing larger (inhalable) particles than smaller (respirable) particles, because the air turbulence associated with ventilation air movement agitates (and therefore randomly dissipates) the smaller particles in the air (Pearson and Sharples, 1995). Thus, it is not surprising that there were higher concentrations of inhalable particles in winter than in summer. The reduced ventilation rate typically used during winter leads to higher inhalable particle concentrations. The effect of season on respirable particles was more complex, as it interacted with number of sows (farm size), while the ventilation airflow rates interacted with the type of pig flow management used in the buildings (table 5). It appeared that on larger farms in summer, the concentration of respirable particles is likely to increase, probably due to reduced hygiene levels.

Increasing building size (volume) had opposite effects on NH<sub>3</sub> concentrations in summer and winter (table 5). In summer, NH<sub>3</sub> concentrations appeared to decrease in larger buildings, whereas in winter NH<sub>3</sub> concentrations appeared to increase. However, while the interaction was significant (table 4), the slopes of both graphs did not differ significantly from zero. We can hypothesize that in summer the increased ventilation rates clear the NH<sub>3</sub> from larger buildings. An interaction could therefore occur between building size and season, as absolute ventilation throughput is greater in larger buildings (Banhazi et al., 2008c) and differs in different seasons. In winter in larger buildings, perhaps there are more opportunities for NH<sub>3</sub> generation from contaminated floors, without the benefits of correspondingly increased ventilation rates. However, more data are needed to draw any firm conclusions about the effect of building volume on ammonia concentration.

#### Effects of Humidity and Temperature

Generally, increasing temperature had a positive relationship with the concentrations of both inhalable and respirable particles (table 5). As temperature increases, piggery buildings tend to become drier environments, creating greater opportunities for particle generation (Pedersen et al., 2001). As a result of increased temperatures, respirable particle concentrations increased in AIAO buildings. Inhalable particle concentrations were also significantly affected by temperature, but the relationship was more complex owing to an interaction with building classification. In weaner, grower/finisher, and DBS buildings, inhalable particle concentrations increased with temperature, although this relationship was not significant in the case of DBS and grower/finisher buildings (data not shown). However, in farrowing buildings, temperature increase had an opposite effect on inhalable particle concentrations. A similar trend was observed in dry sow buildings, although the effect of temperature could not be demonstrated at a significant level in this type of building (data not shown). The fact that this negative correlation was observed in buildings housing large animals indicates that temperature might affect large animals differently. It is very likely that large sows that are housed in either farrowing or dry sow buildings and become affected by the heat will become less active and therefore eat less as the temperature rises. As a consequence of the reduced animal activity, a reduction in feeding frequency and therefore reduced feed disturbance would lead to a reduction in inhalable particle concentrations. It is also interesting that no building/temperature interaction effect was observed for respirable particles. Respirable particle concentrations might not be affected by animal activity to the same extent as inhalable particle concentrations, owing to their different aerodynamic behavior in the air (Pearson and Sharples, 1995). Respirable particles, being smaller, would be suspended in the air for longer after animal activity, so reduced animal activity would have less impact on their concentrations. The settling rate for larger (inhalable) particles is greater, so a reduction in animal activity would have a more immediate effect on airborne concentrations of these larger particles; larger particles tend to settle out of the air as soon as animal activity decreases (Pearson and Sharples, 1995; Pedersen and Takai, 1999). It would have been advantageous for the project to have measured animal activity in addition to the other parameters, but at the time the project was designed, low-cost instrumentation was not available in Australia for measuring animal activity.

The increased humidity in the air had a mixed effect on respirable particle concentrations. Humidity had a positive relationship with respirable particle concentrations in AIAO buildings, whereas in CF buildings no significant relationship was found. It is possible that this association is a causal relationship. It could be the result of the drying of manure on the pen floors, creating opportunities for increased respirable particle concentrations and higher humidity levels. However, a reduction effect of increasing humidity was found for DBS. Normally, the presence of bedding materials is a risk factor for high viable particle concentrations under the dry Australian climate, especially in southern Australia. The demonstrated reduction effect of increased humidity on respirable particle concentrations in DBS would have resulted from the increased "stickiness" of the bedding materials under high-humidity conditions. Bedding in such a condition would retain a larger percentage of particles, preventing them from becoming airborne. Further studies on the relationship between potential treatments of bedding materials, increased humidity, and particle concentrations would be useful (Breum et al., 1999).

It is likely that the higher endotoxin concentrations (table 5) with increased humidity were due to prolonged bacterial survival times (Butera et al., 1991; Zucker et al., 2000). Generally, the natural half-life of airborne gram-negative bacteria ranges between a few minutes and perhaps an hour. After this time, airborne bacteria die naturally and release increased amounts of endotoxins into the air. Previous studies have reported that water remaining on the pen floors provides an ideal environment for the multiplication of microbes (Chang et al., 2001) and that concentrations of microbes are higher in winter (when humidity levels are usually high) in Northern Hemisphere piggery buildings than in summer (Curtis et al., 1975). This finding has implications for dust-reduction methods such as the spraying of oil-water mixtures. Spraying the floors of pig pens with a mixture of oil and water has been demonstrated by a number of authors to be an effective way of reducing dust (Takai et al., 1995; Takai and Pedersen, 2000). However, this technique could generate higher endotoxin concentrations because of the possible effects of elevated humidity on endotoxins (table 5). This result might also explain the lack of positive effects of particle reduction on production efficiency in experiments using oil spraying (Banhazi et al., 1999; Takai et al., 1995). Additional experiments under controlled conditions are required to give a better understanding of these effects.

#### Effects of Farm Size

Larger farms (number of sows) had higher inhalable and higher respirable particle concentrations (table 3). However, the effect of sow numbers on respirable dust was more complex. In summer, respirable particle concentrations were positively correlated with sow numbers, whereas no significant effect was observed in winter. It has long been suspected that farm size has some effect on air quality. It is quite possible that on larger farms, because of work pressures, less time is available for cleaning and general maintenance of the pigs' environment. The reduced hygiene and increased intervals between cleaning episodes creates an ideal environment for higher dust concentrations in piggery buildings. Perhaps the difference between the types of workers employed on large corporate farms to carry out cleaning duties and on smaller family-operated farms might also explain this difference. While workers on family farms tend to be owners who

live on site, on larger farms the majority of the work force is hired and might not have the same level of availability and dedication as owner-operators. Summer is also a heightened risk period for high respirable particle concentrations, as hygiene levels tend to decrease in summer, creating more opportunities for respirable particle generation (Huynh et al., 2005). This is so, as manure smeared on pen floors dries easily in summer and is stirred up by the movement of pigs and by air currents.

#### Other Considerations

The complex interaction between season, airflow, and temperature requires further investigation and analysis. This study used a linear model and least square means to "separate" the important effects and therefore allow them to be considered and explained individually. However, in reality, season, temperature, and airflow have a more complex interaction in piggery buildings. When temperature increases in piggery buildings, the ventilation rate would also normally be increased. So, for example, whereas increased temperature and the related drier environment would increase the opportunities for inhalable particle generation, the higher ventilation rate would decrease inhalable particle concentrations inside the piggery building. Because of these complexities, it is important to create a combined model for airborne pollutants, based on the effects identified for individual pollutants. This work is currently being undertaken at the South Australian Research and Development Institute.

Although a relationship between stocking density and airborne pollution has been demonstrated by a number of authors (Donham, 1991; Gustafsson, 1999), this relationship was not confirmed herein. The effects of various factors combined in complex interactions to determine the final concentrations of airborne bacteria. A general linear modeling technique was used to consider all effects simultaneously, but most previous studies have examined simple relationships between management or housing parameters and air quality, rather than considering all variables together. In addition, a number of factors were included in addition to those in previous studies. For example, in other studies, the effect of stocking density might have been demonstrated as a substitute for the effect of actual hygiene classification. It has been reported that overstocking can negatively affect dunging behavior in pigs, creating opportunities for reduced pen hygiene (Aarnink et al., 1996; Hacker et al., 1994; Wechsler and Bachmann, 1998). The reduced pen hygiene can, in turn, be manifested in high bacterial concentrations. However, this study found that when a number of other effects, including pen hygiene, were included in models, the effect of stocking density itself was not significant.

## Conclusion

Building type had a significant effect on the concentrations of some airborne pollutants (table 4). However, we consider improvement of pen hygiene to be the most practical recommendation for decreasing concentrations of ammonia, respirable particles, and bacteria, as demonstrated herein that dried fecal material deposited on pen floors is an important source of these pollutants. Therefore, the source of airborne pollution (unhygienic conditions) should be minimized by controlling dunging patterns and improving pen hygiene. In this study, improvement in pen hygiene is the most practically beneficial recommendation for reducing concentrations of airborne pollutants.

Airflow increase would have a beneficial effect on inhalable particle concentrations, but there is an upper limit above which airflow cannot be increased, as it would interfere with thermal comfort. In the same way, temperatures cannot be reduced, as the negative economic impact would be far greater than the air quality improvement achieved. Reduced pen cleanliness and increased humidity decreased the concentrations of respirable particles in DBS. Nonetheless, reduction of hygiene in DBS cannot be recommended, as it could compromise the health of the pigs. On the other hand, the economic viability of using dust-reduction methods that would have particle-bonding effects in DBS and in traditional buildings (such as spraying or incorporation of oil into bedding material) should be further investigated. However, care must be taken to ensure that humidity levels are not adversely affected during the treatment of bedding materials, as the increased humidity could affect endotoxin concentrations, as demonstrated by this study.

Farm size cannot be manipulated, as the general trend toward larger farm size is driven mainly by economic considerations, and the likely economic benefits of scale would most probably outweigh the benefits gained from air quality improvements. However, farm managers on larger farms need to be aware of the potential air quality risk associated with deteriorating hygiene on farms. In the same way, seasonal effects have to be accepted, but producers must be aware of the increased risks of high particle concentrations associated with certain seasons and take appropriate preventive actions.

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