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Original article

Chronic exposure of pigs to airborne dust and endotoxins in an environmental chamber: technical note

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Summary — A new experimental setup was developed to expose pigs to dust and airborne endotoxins in an environmental chamber, at levels liable to be encountered in pig farm buildings. The following parameters were evaluated in a chamber containing two pigs of 10 kg body-weight: inhalable and respirable dust gravimetric concentrations were measured using area samplers and expressed as mg/m^3. The respirable dust concentration was also measured using a 'TM digital μP respirable dust-measuring instrument', which has been shown to give similar results to the gravimetric method. The endotoxin concentration was evaluated using the *Limulus*-assay and expressed as ng/m^3 of air containing the inhalable or respirable dust or as ng/mg of inhalable and respirable dust. Feed flour dust was introduced into the chamber to obtain different concentrations of inhalable and respirable dust ranging from 3.62 to 76.66 mg/m^3 and from 0.24 to 1.40 mg/m^3, respectively. The endotoxin concentration was modulated by mixing the feed flour with *Escherichia coli* endotoxins before blowing it into the chamber. The endotoxin concentrations in the air containing inhalable or respirable dust ranged from 28.9 to 270.0 ng/m^3 and from 2.22 to 36.38 ng/m^3, respectively, depending on the amount of endotoxins added to the dust. Data were also obtained in a piggery. The experimental setup detailed in this paper could be used to study the significance of air contaminants in the development of pig respiratory diseases.

airborne dust / endotoxin / environmental chamber / air pollution

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Résumé — Exposition chronique de porcs à des poussières et des endotoxines aéroportées dans une chambre d'inhalation : aspects techniques. *Une chambre d'inhalation a été développée afin d'exposer des porcs à une atmosphère contenant des poussières et des endotoxines. Les concentrations gravimétriques en poussières inhalables et respirables ont été mesurées à l'aide d'échantillonneurs de poussières, et exprimées en mg/m³ d'air. Les concentrations en poussières respirables ont aussi été mesurées en utilisant un appareil de mesure optique de poussière fine, qui s'est avéré donner des résultats similaires à ceux de la méthode gravimétrique. Les concentrations en endotoxines ont été évaluées par le test de la Limule et exprimées en ng/m³ d'air contenant les poussières inhalables et respirables ou par ng/mg de poussières inhalables et respirables. Une poussière de farine alimentaire a été introduite dans l'enceinte afin d'atteindre plusieurs niveaux de poussières inhalables et respirables à des concentrations variant de 3,62 à 76,66 mg/m³ et de 0,24 à 1,40 mg/m³ respectivement. Les concentrations en endotoxines ont été modulées en mélangeant des lipopolysaccharides d'* Escherichia coli *avec la farine avant de la pulvériser dans l'enceinte. Les concentrations en endotoxines dans l'air contenant les poussières inhalables et respirables variaient respectivement de 28,9 à 270,0 ng/m³ et de 2,22 à 36,38 mg/m³, en fonction du taux d'endotoxines ajoutées à la farine. Ce modèle expérimental permet de reproduire des niveaux de concentration en poussières et en endotoxines comparables à ceux rencontrés dans les porcheries. Son utilisation pourrait permettre l'étude de l'importance des contaminants de l'air dans le développement des pathologies respiratoires chez le porc.*

pollution de l'air / poussière / endotoxine / chambre d'inhalation

INTRODUCTION

There is growing interest in the influence of piggery air pollution in the pathogenesis of respiratory diseases in pigs and farmers. Epidemiological studies have shown that the prevalence of respiratory disorders in pig farmers and animals correlates well with the concentrations of some indoor pollutants such as dust, endotoxins, and ammonia (Bongers et al, 1987; Donham, 1991). These studies suggest that the pollutants have a possible role in triggering or aggravating respiratory diseases but they do not establish the precise contribution of each pollutant. Complementary experimental studies are required on exposure to pollutants, in order to assess their toxicity for the respiratory system, their mechanism of action, and to establish dose–response curves that would indicate acceptable levels of pollution in pig farming. Such studies necessitate the use of environmental chambers where the microclimate and contaminant concentrations can be controlled.

In the present work, a new experimental setup was developed. Its purpose was to expose pigs to dust and airborne endotoxins at levels liable to be encountered in pig buildings. Basal concentrations of dust and endotoxins were measured both in a piggery and in a chamber housing two piglets. Afterwards, dust was introduced into the chamber to obtain different levels of dust concentrations. A method for enriching the introduced air with endotoxins is also described.

MATERIALS AND METHODS

Air-pollutant exposure chambers

The environmental chambers used to expose pigs to controlled aerial ammonia concentrations have been previously described in detail (Urbain et al, 1993). Briefly, closed stainless-steel and plastic chambers (1.9 m^3) were constructed to house pigs weighing up to 35 kg. Outside air was passed through fiberglass filters to remove dust and aerial bacteria. The air flow rate was 10 m^3/h. The mean concentration of bacterial colony-forming particles (BCFP), measured in the presence of the pigs with an Andersen sampler containing

L-broth agar Petri dishes, was 28 385 ± 9 665 BCFP/m^3; the percentage of respirable bacteria was 55% (BCFP < 5 µm). The pigs were set on a grating located 15 cm above the floor. There was no litter and the manure was removed twice daily by cleaning the floor with water to maintain the ammonia concentration below 5 ppm. Instantaneous and mean ammonia concentrations were measured respectively with Gastec tubes or by titration of the scavenged gas with a specific electrode in an acidic solution. Pigs were fed with pellets ad libitum and water was supplied by an automatic valve. In the present experiment, two pigs with a body weight of 10 kg were placed in the chamber in order to measure the background of dust and endotoxin concentrations in the presence of animals.

Air chamber enrichment with dust

A commercial pig feed flour (11111 Schyns, Battice, Belgium) was used as the dust source. The flour was filtered on a sieve through a 450 µm mesh screen (VEL 3130006, Liège, Belgium), to eliminate the largest particles. The flour was then homogenized in a commercial food mixer for 4 h (Kenwood KM 201) and dried at 50 °C for 72 h. One kilogram of this flour was introduced into a 0.03 m^3 closed plexiglass cylindrical tank (fig 1) (150 cm height; 16 cm internal diameter (ID)). A tube (ID: 1.8 cm), extending from the top of the tank to 20 cm above the floor inside the tank, was connected to a source of compressed air to produce turbulence and create a dust cloud. Inflow pressure measured at the air source and air flow were controlled, respectively, with a manometer (B4593BD Technofluid, Liège, Belgium) and a flow-meter (R101 Technofluid, Liège, Belgium). The inflow pressure in the tank was maintained constant throughout all experiments (1 bar). Air with suspended particles was introduced into the chamber by connecting one of the nine exit tubings, located at different heights on the tank, to the chamber ventilation circuit (fig 1).

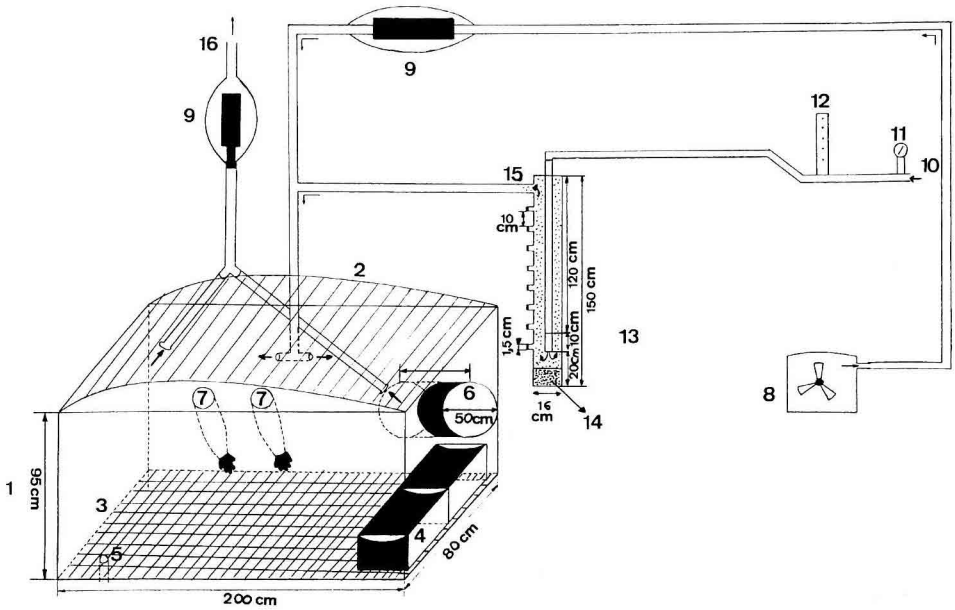

Fig 1. Environmental chamber and dust generator system. 1. Stainless steel tank (200 x 80 x 95 cm). 2. Plastic cape. 3. Grating. 4. Trough. 5. U-bend. 6. Airlock. 7. Rubber gloves. 8. Turbine. 9. Air filter. 10. Source of compressed air. 11. Manometer. 12. Flow-meter. 13. Dust tank. 14. Flour. 15. Dust exhaust. 16. Air exhaust.

Measurements of dust concentration in the chamber

All air samples were taken within the normal breathing zone of the pigs.

'Inhalable dust' is defined as the fraction of airborne material which enters the nose and mouth during breathing and is thus available for deposition anywhere in the respiratory tract (Health and Safety Executive, 1986). The weight of the inhalable dust per cubic meter of air can be estimated using an air sampler composed of a 'seven-hole' sampling head (SK-225-70, DEHA, Belgium) holding a 25 mm polycarbonate filter (Millipore ATTP02500) and connected to a pump unit which maintains a constant air flow rate of 2 L/min through this filter for 2 or 3 h (SK-224-43-EXKB, DEHA, Belgium). The air flow of the pump was controlled with a precision rotameter (Bioblock A29443, Illkirch, France). Filters were weighed on a Mettler balance immediately before and after the pumping period. Several blanks were retained as reference filters. The results were expressed in mg/m^3.

'Respirable dust' designates the fraction of material which penetrates to the gas exchange regions of the lung. Since it could not be measured directly, we used one of two methods to simulate the deposition of dust in the respiratory tract. One method involved the use of a personal sampling head (SK-225-3, DEHA, Belgium) containing the same filter as mentioned above, equipped with an aluminium cyclone preselector (SK-225-01-01, DEHA, Belgium) connected to a pump unit (SK-224-43-EXKB, DEHA, Belgium). This cyclone head has a 50% cut-off effectiveness value of 5.3 µm at a flow rate of 1.9 L/min. This method, however, lacks precision when the dust concentration is low, due to the slight changes in the weight of the filter during the sampling period. For this reason, the filters were used only for measuring endotoxin concentrations expressed in nanograms of endotoxin in respirable dust per cubic meter of air (see below).

A second method involved use of the 'TM digital µP respirable dust-measuring instrument' (TMµP) (Drägger ballings, Brussels, Belgium), which operates using the principle of stray light measurement. The intensity of stray light is directly proportional to the concentration of dust particles ranging in size from 0 to 8 µm. The apparatus is calibrated by the manufacturer who determines the dust concentrations in milligrams per cubic meter (mg/m^3), using wheat flour particles, by comparing the values displayed by the TMµP with the gravimetrically measured values in milligrams per cubic meter (mg/m^3). The apparatus displays a dust concentration in milligrams per cubic meter (mg/m^3) with a sensitivity of 0.01 mg/m^3. Measurements were generally performed every 20 s and averaged over a period of 2–3 h. However, to assess this method, the values were compared with those recorded with the gravimetric method performed over a longer period of 6 h.

The particles size was measured by optical examination. Dust samples were taken using the method described to measure the weight of the inhalable dust. The pump unit operated at 2 L/min for 2 min. Then the filter was examined using an Olympus microscope connected to a frame analyser (Vidas 21). The size of 100 particles ranging in size from 10 to 100 µm was measured on each filter, taking into account the higher dimension of each particle.

Enriching dust in endotoxins and measuring air endotoxin concentrations

The method consisted of mixing freeze-dried *Escherichia coli* endotoxins (55 or 100 mg of serotype O127:B8, Sigma) with 250 g of sifted flour in a Kenwood feed mixer for 1 h. Next, 250 g flour was added at hourly intervals in order to obtain 1 kg of mixed flour. The air endotoxin concentrations were measured on the filters used for gravimetric measurements of inhalable and respirable dust. After sampling, each filter was placed in a sterile polypropylene tube (Corning 25330-50, VEL Belgium) and stored at –20 °C. Endotoxin-free phosphate-buffered saline was introduced into the tube and the *Limulus* assay was used to measure the endotoxin content of the dust (Michel et al, 1991). The results are expressed in nanograms of endotoxin in the inhalable or respirable dust per cubic meter of air, or in nanograms per milligram of inhalable or respirable dust.

Experimental protocol

Two pigs were introduced into the chamber. Sampling and measurements were performed over periods ranging from 2 to 3 h. After basal value

measurements, dust with or without added endotoxins was introduced into the chamber. In order to obtain different dust concentrations in the chamber, the air flow inside the tank was adjusted (1.5 or 2 m^3/h) while maintaining a constant inflow pressure. A second method consisted of connecting one of several outflow tubes, located at different heights on the tank (65, 95, 115, and 135 cm from the floor of the tank), to the air ventilation circuit of the chamber, taking advantage of the fact that the higher the tank exit, the lower the amount of dust expelled from the tank. The weight of flour in the tank was measured before and after each experimental period. All measurements were also performed in the grower facility of a piggery in order to compare the values obtained in the chamber with those measured in commercial units.

RESULTS

The amount of dust blown from the tank decreased exponentially with increasing exit tube height (table I). The equations linking y, the amount of dust blown per hour (g/h), and x, the height of the tube (cm), were respectively $y = 176 \times 10^{-0.0102x}$ ($R = 0.912$) and $y' = 9\,793 \times 10^{-0.0205x}$ ($R = 0.950$) for air flow rates of 1.5 and 2 m^3/h inside the tank. The highest concentrations of blown dust were considered to be too high (98 and 535 g/h) for use in our experiments.

The recorded base-line values for the inhalable and respirable dust concentrations in the chamber were respectively 0.70 ± 0.43 ($n = 5$) and 0.05 ± 0.01 ($n = 5$) mg/m^3 (table I). These values were lower than the values recorded in a pigpen: 3.80 ± 0.27 ($n = 4$) and 0.32 ± 0.05 ($n = 3$) mg/m^3, respectively. The percentage of respirable dust was about the same in the chamber (7.14%) as in the pigpen (9.30%). The inhalable dust concentrations inside the chamber were closely related to the height of the exit tube connecting the tank to the ventilation circuit of the isolator (table I). However, the percentage of respirable particles measured when high amounts of dust were suspended in the air chamber was smaller (1.22%) than the value obtained at a low level of enrichment (6.63%). The data shown in table I also revealed that increasing the dust concentration by adjusting the air flow in the tank led to a higher percentage of respirable particles than that which was recorded at a similar dust concentration obtained with a

Table I. Inhalable and respirable dust concentrations and respirable/inhalable ratio (R) in the chamber with and without dust enrichment.

Air flow rate in the tank (m^3/h)	*Height of the outflow tube (cm)*	*Inhalable dust (mg/m^3)*		*Respirable dust (mg/m^3)*		R (%)	*Added dust (g/h)*	
0	–	0.70 ± 0.43	(5)	0.05 ± 0.01	(5)	7.14	–	
1.5	135	3.62 ± 1.58	(16)	0.24 ± 0010	(15)	6.63	8.12 ± 1.83	(5)
1.5	115	4.73 ± 0.46	(3)	0.36 ± 0.02	(3)	7.61	12	(1)
1.5	95	14.40 ± 4.13	(9)	0.36 ± 0.26	(9)	2.5	13.6 ± 5.78	(3)
1.5	65	76.66 ± 14.15	(3)	0.94 ± 0.22	(4)	1.22	45	(1)
2	135	14.75 ± 4.14	(4)	1.09 ± 0.04	(4)	7.39	22.7	(1)
2	115	18.99 ± 3.17	(4)	1.40 ± 0.06	(4)	7.37	28.03	(1)

The latter was obtained by changing the flow rate inside the tank (0, 1.5 and 2 m^3/h) and the height of the outflow tube. The amount of dust chased from the tank per hour is also indicated. Number in brackets are number of samples. Inflow air pressure was 1 bar in the tank in all experiments. Values are means ± SD.

lower air flow in the tank, respectively, 7.39 versus 2.5%.

The optical examination of the dust present on the filters has shown that the mean size of particles ranging from 10 to 100 µm was 26.3 µm. The mean class size distribution from 22 examined filters is illustrated in figure 2.

Figure 3 shows the respirable dust concentration values measured with the TMµP compared with those obtained by the gravimetric method. The values obtained with both methods were similar as illustrated by the good linear correlation between these values and the absence of significant difference ($P < 0.05$) of the calculated slope value from 1.

The base-line endotoxin concentrations in the chamber were 16.16 ± 12.49 ng/m^3 ($n = 2$) (air containing all inhalable particles) and < 1.17 ng/m^3 ($n = 2$) (air containing only the respirable particles). The corresponding values measured in a piggery were 190.5 ± 77.5 ng/m^3 ($n = 4$) and 2.6 ± 0.5 ng/m^3 ($n = 3$). These concentrations expressed in nanograms per milligram of inhalable dust were 41.7 ± 36.5 ng/mg ($n = 2$) in the chamber and 49.9 ± 19.4 ng/mg ($n = 4$) in the piggery. The respirable endotoxin concentration in the piggery, estimated on the basis of the weight of the dust measured with the TMµP instrument, was 7.30 ± 0.46 ng/mg. By enriching the air inside the chamber with dust containing endotoxins we were able to increase the endotoxin concentration in the air containing inhalable dust 9.3-fold, from 28.9 to 270.0 ng/m^3. In air containing respirable dust, the increase factor was 16.4 (from 2.22 to 36.38 ng/m^3) (table II). These changes were due to an increase in the endotoxin concentration in the dust, as shown in the table II.

DISCUSSION

In the present study, pig feed flour was used to create a dusty cloud in an environmental chamber. The dust present in pig buildings comes partly from feed. Other constituents are fecal material and pig scales (Donham, 1986). Fecal material is classically considered a source of small particles and Gram-negative bacteria containing endotoxins. Feed particles are bigger and constitute the bulk of the dust. Inhalable dust concentra-

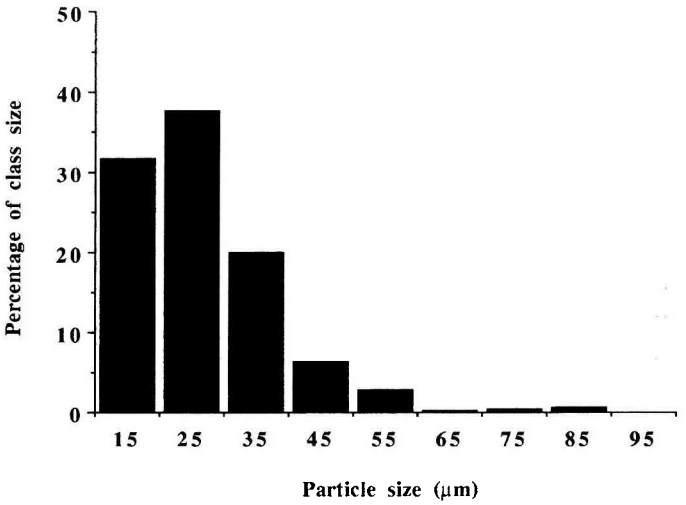

Fig 2. Class size distribution of inhalable particles of a size ranging from 10 to 100 µm measured on filters held in an inhalable dust sampler.

Table II. Dust and endotoxin concentrations in air containing inhalable and respirable dust, measured in the chamber before and after air enrichment with flour containing different concentrations of endotoxins.

	Inhalable fraction			*Respirable fraction*		
	Dust	*Endotoxin*		*Dust*	*Endotoxin*	
	(mg/m³)	*(ng/m³)*	*(ng/mg)*	*(mg/m³)*	*(ng/m³)*	*(ng/mg)*
Base-line values	0.41 ± 0.06 (2)	16.16 ± 12.49 (2)	41.7 ± 36.5 (2)	0.05 ± 0.01 (2)	< 1.17 (2)	ND
Values obtained after addition of flour without endotoxin enrichment						
	3.28 ± 1.45 (7)	28.90 ± 34.20 (7)	18.0 ± 35.9 (7)	0.25 ± 0.09 (5)	2.22 ± 1.50 (5)	10.66 ± 9.56 (5)
Values obtained after addition of flour enriched with endotoxins						
55 ng/mg endotoxins[a]	3.76 ± 1.69 (5)	187.0 ± 123.0 (5)	54.1 ± 35.5 (5)	0.36 ± 0.04 (5)	8.46 ± 10.48 (5)	23.59 ± 30.0 (5)
100 ng/mg endotoxins[a]	2.65 ± 1.17 (7)	270.0 ± 225.5 (7)	98.3 ± 54.2 (7)	0.27 ± 0.08 (5)	36.38 ± 42.10 (5)	117.96 ± 124.03 (5)

Number in brackets are the number of samples. [a] Concentration of added endotoxins in the flour. Values are means ± SD. ND: value not determined.

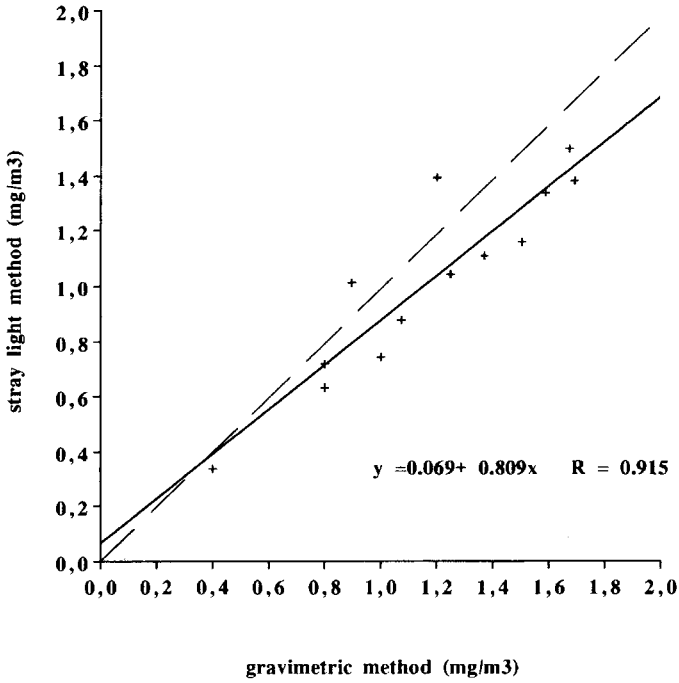

Fig 3. Comparison of individual values (+) of respirable dust concentrations measured by *stray light method* (TMµP digital instrument) and by the gravimetric method. — — —: line of identity; ———: linear regression line.

tions in pig buildings generally range from 2 to 20 mg/m^3 and the respirable fraction, measured by the gravimetric method, varies from 0.2 to 1.0 mg/m^3 (Donham et al, 1986; Donham, 1991). The highest values are recorded during the winter period and in fattening houses. Inhalable dust concentrations recorded during feeding (up to 27.3 mg/m^3) or weighing (up to 24.0 mg/m^3) are higher than during normal maintenance periods (up to 13.7 mg/m^3) (Larsson et al, 1992, 1994), suggesting that pig activity causes considerable agitation of settled dust. These values are in agreement with our values recorded in the piggery. However, values as high as 183 ± 85 and 4.4 ± 2.3 mg/m^3 (for inhalable and respirable dust respectively) have been recorded during the winter period in grower facilities (Pickrell et al, 1993).

Inhalable dust levels in our study were measured using a 'seven-hole' head for technical reasons. If the IOM (Institute of Occupational Medicine) open-phase cassette system is regarded as an evolving standard for inhalable dust sampling, it is inconvenient for determining endotoxin concentrations in dust. Indeed, the endotoxin-contaminated dust is collected on both the filter and the internal walls of the IOM head. This makes it necessary to store the whole cassette holding the filter. The endotoxin concentrations can be determined later. Moreover, these cassettes are expensive. Using the seven-hole head, only the filter needs to be stored for further endotoxin determination. However, in our experimental conditions, the dust concentrations measured with the IOM head were 43 ± 33% higher than with the seven-hole head. The dust concentrations measured with the latter are therefore probably underestimated.

Base-line values recorded in the chamber were lower than those obtained under field conditions. However, the data presented in

table I show that dust concentrations as high as those measured in the field can be obtained using the method detailed in the present study. The best way to adjust the dust concentrations inside the chamber was to select the highest exit tubes and to adjust the air flow rate. The ratio of respirable to inhalable dust (7–9%) recorded under these conditions in the chamber was comparable to that recorded in our piggery and also comparable to the values recorded by Donham et al (1986) in pig buildings. Choosing the lower exit tubes increased the dust concentration, but the percentage of respirable particles was lower than under field conditions. Isolation chambers with dust enrichment have been previously used with pigs, but without refering to any deposition in the respiratory system. Doig and Willoughby (1971) exposed animals to corn-starch dust and to ground corn dust at 200 and 10 mg/m^3 respectively. Curtis et al (1975) exposed piglets to dust collected from convection tubes in a swine-finishing house, at concentrations of 10 and 300 mg/m^3. Respirable dust concentrations were not measured by these authors. Moreover, lower dust concentrations have not yet been tested in pigs.

At low dust concentrations, the lack of sensitivity of the gravimetric method (see *Materials and methods*) did not allow precise measurement of the respirable dust concentration. This is why another method (a TMμP respirable dust-measuring instrument) was used to determine this parameter. The gravimetric method is based on the principle that small particles are scavenged on the filter according to a statistical distribution (Health and Safety Executive, 1986). For example, the cyclone head used in this study selects 50% of all 5.3 μm particles, the selected particles being finally scavenged on the filter. This distribution is theoretically similar to that observed in the human respiratory system. The TMμP apparatus measures the concentration of small particles and converts the measured values to milligrams per cubic meter taking into account a correction factor calculated by the manufacturer. A good correlation (R = 0.915) was obtained between the values measured by the two methods, and the absence of significant difference between the values obtained with both methods (fig 3) shows the equivalence of these two techniques. Note that the manufactor gives a correlation of 0.99 for values up to 10 mg/m^3.

Endotoxin concentrations in pig buildings range from 4 to 445 ng/m^3 in air containing inhalable dust (Attwood et al, 1987; Donham et al, 1986; Donham 1991; Heederick et al, 1991; Pickrell et al, 1993). Values as high as 716 and 1 300 ng/m^3 have been recorded during feeding and weighing respectively (Larsson et al, 1992, 1994). In respirable dust, the mean endotoxin concentration ranges from 8 to 300 ng/m^3 (Donham 1991; Larsson et al, 1992; Pickrell et al, 1993). Under our experimental conditions, endotoxin concentrations were in the range of the values recorded in pig buildings. Expressed in nanograms per milligram of inhalable dust, the concentrations range from 0.4 to 113 ng/mg (Donham et al, 1986; Attwood et al, 1987; Crook et al, 1991; Pickrell et al, 1993). Pickrell et al (1993) found that respirable dust was four times richer in endotoxins than inhalable dust. Under our experimental conditions, the respirable/inhalable ratio for this factor ranged from 0.43 to 1.20. The ratio obtained in our piggery was 0.15.

In conclusion, inhalable and respirable dust concentrations in our environmental chamber can be measured and reproduced at levels similar to those encountered in pig buildings. Furthermore, it is possible to modulate endotoxin concentrations, while maintaining a given dust concentration. We have developed a new tool for exposing piglets to common air pollutants. This model could be used to determine the importance of

these contaminants in the development of respiratory diseases.

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