

Animal and worker exposure to dust and biological particles in animal care houses

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Abstract

An aerobiological study was performed to evaluate the potential exposure of animals and workers to dust constituents generated during routine animal house work. Different rooms of air conditioned (A, control) and passively ventilated (B, non-air conditioned) animal facilities were sampled, in order to evaluate total airborne culturable fungi and bacteria, fungal spore concentrations and particle levels. Airborne room particles were analyzed gravimetrically and for endotoxin content. All parameters, except for culturable fungi, were higher in facility B and statistically significant, with respect to those from the control facility A. Median values for airborne particle concentration, endotoxin and fungal spores in facility B were: $115 \mu\text{g m}^{-3}$, 25 EU m^{-3} , and $2173 \text{ spores m}^{-3}$, respectively. Median values for facility A were: $66 \mu\text{g m}^{-3}$, 9 EU m^{-3} , and $248 \text{ fungal spores m}^{-3}$. Bronchoalveolar lavage from rats kept in the rat room of B, presented median concentrations of total cells and lactate dehydrogenase, higher than those found in the control facility (4.4×10^5 vs. 1.1×10^5 and 2.7 U mL^{-1} vs. 0.39 U mL^{-1} , respectively). Values of total and biological particles of both facilities, as well as the time spent in different rooms, showed that worker exposure was higher during cage washing. It was especially high in the passively ventilated facility (airborne particles $686 \mu\text{g m}^{-3} 3.5 \text{ h}^{-1}$ vs. $976 \mu\text{g m}^{-3} 3.5 \text{ h}^{-1}$, endotoxin $70 \text{ EU m}^{-3} 3.5 \text{ h}^{-1}$ vs. $108 \text{ EU m}^{-3} 3.5 \text{ h}^{-1}$). The type of basidiospores and ascospores found, as well as the significant correlation between particle levels and endotoxin contents suggests that wood chip bedding disturbance during cage washing is an important source for airborne biological particles. The changes in bronchoalveolar lavage components found in rats from these facilities and previously reported changes in pro-inflammatory cellular responses found in workers, indicate that these relatively low levels of exposure are enough to induce a biological response. Studies considering the composition of mixed organic dusts, would be needed to reevaluate current occupational standards.

1. Introduction

Epidemiological and experimental evidence supports the relationship between occupational exposure to organic dust and lung disease, which is also associated to immunological and non-immunological responses. These health problems have been reported in environments contaminated by organic dust generated from

cotton, grain, wood chips, compost, animal food, household waste, and house dust, among others (Thedell et al., 1980; Michel et al., 1991; Rylander and Holt, 1998; Burrell, 1995; Wintermeyer et al., 1997; Kullman et al., 1998).

However, due to the complex composition of organic dust, including antigens and toxins, it has been difficult to regulate for the component or components

responsible of causing pulmonary health problems. At present, only dust and endotoxin concentrations have been considered for occupational safe levels (Heederik et al., 1991; Smid et al., 1992; Herbert and Breum, 1995). Based on these results, exposure of workers and animals to organic dust in animal care houses has been reduced in developed countries over the past 25 years (Baldwin et al., 1976; Lipman et al., 1992; Sebesteny et al., 1992; Quimby, 1993; Clough et al., 1995).

Recent reports have shown that most animal care facilities in Mexico present odors and dust that contain food and fecal particles, skin debris, bacteria, and fungi. Consequently, experimental animals do not reach optimal quality and workers are subjected to high risk exposure (Contreras, 1979; Martínez, 1980; Villalobos, 1980; Hernández-González y Hernández-Espinosa, 1994). Considering this, some animal care houses in Mexico have been redesigned and built adopting the proposed environmental control measures (ABPI, 1984; Anderson et al., 1990; ESAC, 1990; Hunskaar and Fosse, 1993), nevertheless the air and animal quality have not been evaluated since. This includes the animal facilities with no air conditioning. The aim of this study is to evaluate both worker and animal exposure to microbial and endotoxin levels associated with airborne particles in both types of animal care facilities.

2. Materials and methods

2.1 Animal care houses

The aerobiology of both, air conditioned (control, A) and non-air conditioned (B) animal care houses that operate in the southern area of Mexico City was studied. The rats in Facility A are kept in a 20 m² room where 150 – 200 animals are located in 50 cages (3 – 4 animals/cage). This room has 15 changes of air per hour and the air is supplied through a one-way airflow system equipped with HEPA filter. The rat room in the other animal house, has an area of 14 m² where approximately 1250 animals are located in 324 cages (3 – 4 animals/cage). This room is passively ventilated through open doors.

Viable and non-viable particle samples were simultaneously collected in different areas (rat room, cage wash room, office and outdoors) of each animal house on ten occasions. The number of rats and the type of bedding (wood shavings) used in each house remained constant through this investigation. Samplers and

sampling conditions to collect viable and non-viable particles are indicated in Table 1.

2.2 Airborne particles

Airborne particles were collected in different rooms and outside the animal houses, during 8h (8:00–16:00 h) using personal samplers (SKC, model 224-PCXR8) with 37 mm diameter Teflon filters (Millipore Corp., Bedford, MA, USA). Different sizes of particulate matter and the endotoxin associated with them, were monitored in the cage wash rooms and outdoors during the same sampling time, using a seven stage Andersen sampler (Graseby Andersen, Atlanta, GA, USA).

The filters for gravimetric analysis, were weighed before and after sample collection using an analytical balance (Sartorius A 200S). The blank filter for gravimetric analysis presented an average concentration of 12.3 $\mu\text{g m}^{-3}$ (Vol. sampler of 1.9 m³).

2.3 Sampling and microbiological analyses

Airborne culturable bacteria and fungi were isolated with a six-stage microbial sampler (Graseby Andersen, Atlanta, Ga). The sampler was loaded with Petri dishes containing trypticase soy agar (TSA) amended with cycloheximide (50 $\mu\text{g/mL}$), for culturable bacteria; eosin methylene blue agar (EMB) for gram-negative bacteria; and malt extract agar (MEA) for culturable fungi. The colonies from exposed TSA and EMB plates were biochemically analyzed for identification of enteric bacteria (API 20-E; bio-Merieux Vitek, Inc., Hazelwood, MO, USA.). Fungal colonies from MEA plates were counted daily during incubation, before colonies became overgrown. Morphological characteristics were observed microscopically. Results for each stage of the sampler were expressed as colony forming units (cfu) per cubic meter (m³) of air. Total concentrations were obtained by adding the cfu m⁻³ from each stage. The non-respirable counts (stages 1 and 2, aerodynamic diameter > 4.5 μm) and those from respirable fractions (stages 3 – 6, 4.5 μm) were expressed as percentages of the total.

2.4 Endotoxin analyses

The sampled filters for endotoxin extraction, including 15 laboratory and field filters, were kept at 2–8 °C for a period not longer than 48 h. The extraction was performed using new glassware sterilized at 270 °C for 2 h, at least 30 min before use. Endotoxin-free standard

Table 1. Viable and non-viable particles sampling conditions in different animal house rooms

Particles	Sampler	Sampling time	Flow rate (l/min)	Culture media/filter	Incubation time/temp
Biological particles:					
– Total bacteria	6-stage Andersen *	15 min	28	TSA (Difco)	24–48 h/35 °C
– Gram negative bacteria		15 min		EMB (Difco)	24–48 h/35 °C
– Fungal propagules		15 min		EMA (Oxoid)	1 week/27 °C
– Fungal spores	Allergenco	24 h/10 min/2 h	15		
Endotoxin:					
– Fixed monitors	Personal monitor (SKC)	8 h	4	Polycarbonate 37 mm	
	7-stage Andersen	8 h	28	76 mm	
Total Particles:					
Gravimetric analyses					
– Fixed monitors	Personal monitor (SKC)	8 h	4	Teflon 37 mm	
Distributed by size	7-stage Andersen	8 h	28	76 mm	

* Aerodynamic diameter of $> 0.68 \mu\text{m}$.
50% cut off for stage 6.

buffer (0.05 M potassium phosphate, 0.01% triethylamine, pH 7.5) was used for the endotoxin extraction, serial dilutions and endotoxin standard reconstitution. Five mL of buffer were added to each 37 mm sampled filter and 10 mL for 76 mm filters, and then sonicated (Bransonic 5210. Branson Ultrasonic Co. USA) for 60 min at 22 °C. Five 1:2 serial dilutions of each extract were prepared. Endotoxin concentration was evaluated by the kinetic Limulus amoebocyte lysate (LAL) kit (BioWhittaker, Inc., Walkersville, MD, USA), following the kit instructions. Reactions were monitored in the automated Kinetic-QCL reader (BioWhittaker, Inc.), at 37 °C. Absorbance was recorded at 405 nm every 150 sec. Results were determined by the kinetic-QCL software using a linear regression analysis. Endotoxin concentrations were expressed as EU m^{-3} . Endotoxin concentrations associated with particles collected in the respirable and non-respirable stages were expressed as percentages of the total concentration. The filters for gravimetrical analysis, were weighed before and after sample collection using an analytical balance (Sartorius A 200S). The blank filter for endotoxin analysis presented an average concentration of 1.7 EU m^{-3} (Vol. sampler of 1.9 m^3).

2.5 Airborne fungal spores

Fungal spores were collected with an Allergenco Air sampler MK-2 (Allergenco/Blewstone Press, USA), which was programmed to obtain 10 min samples every 2 hours, over a period of 24 h. Spore density was expressed as spores m^{-3} of air.

2.6 Evaluation of exposure

Microbial and non-microbial particle concentrations in the rat rooms were considered as an indirect index of rat exposure. Worker exposure was calculated using the model $E_{\text{tot}} = \sum_i f_i C_i$, where: E_{tot} = total exposure, f_i = fraction of time in the microenvironment, C_i = concentration in microenvironment (Ryan, 1989). Total articles and biological particles were evaluated by fixed monitors in each microenvironment. The time that the worker spent in each room was evaluated by a time activity questionnaire.

2.7 Rat bronchoalveolar lavages

Ten male Wistar rats (140–160 g) from the studied animal facilities were sacrificed by intraperitoneal Nembutal overdose (50 mg/100 g of weight). Before the animals stopped breathing, the abdomen was dissected to sever the aorta. 50 mL of lung lavage were

Table 2. Median and (min and max) worker's and rat's exposure to dust and biological particles in different animal houses

Variable N=10	Rat's Exposure 24 h		Worker's Exposure 8 h	
	Animal House A	Animal House B	Animal House A	Animal House B
Particles ($\mu\text{g m}^{-3}$)	66 (46–84)	115 (45–290)	870 (643–1125)	1209 (623–216)
Endotoxin (EU m^{-3})	9 (5–15)	25 (2–43)	78 (43–85)	174 (95–863)
Bacteria (cfu m^{-3})	80 (18–178)	3339 (113–6427)	1999 (510–3265)	33306 (1675–45577)
Gram negative (cfu m^{-3})	7 (0–9)	135 (6–1158)	155 (9–446)	2182 (19–7843)
Fungi (cfu m^{-3})	346 (113–848)	514 (72–1212)	3117 (751–6082)	3072 (632–9058)
Fungal spores (spores m^{-3})	248 (35–311)	2173 (1026–3422)	6762 (1715–8453)	54609 (3470–68345)

collected from each animal and centrifuged at 1500 rpm/15 min (Beckman CPR). The concentration of total proteins (Bradford method) and lactate dehydrogenase (LDH detection kit, Sigma Chemical Co., St. Louis, MO, USA.) were measured in the supernatant. Cell numbers were evaluated using a hemocytometer, and the percentage of macrophages was determined in cell preparations (smears) stained with Giemsa.

2.8 Statistical methods

The non-parametric Mann-Whitney test was applied to test the differences between non-microbial particles, microbial particles and inflammatory biomarkers from both animal houses. Spearman's rank correlation coefficients were calculated for correlations between gravimetric analysis of dust and endotoxin, bacteria, and fungi. Statistical significance was considered when $p < 0.05$.

3. Results

Exposure of workers and rats to microbial and total particles found in the studied animal houses is shown in Table 2. In general, exposure was higher in the non air-conditioned animal facility B.

3.1 Rat exposure

The rat rooms of animal house B, had a median air-borne particle concentration of $115 \mu\text{g m}^{-3}$ 24 h^{-1}

and a median endotoxin concentration of 25 EU m^{-3} 24 h^{-1} . These values were significantly higher than the ones found in facility A ($66 \mu\text{g m}^{-3}$ 24 h^{-1} of particles and 9 EU m^{-3} 24 h^{-1} of endotoxin) ($p < 0.05$) (Table 2).

Bacteria and fungal propagules were detected in the rat rooms of both animal facilities, however, statistically significant higher values were observed in facility B, except for culturable fungi (Figure 1). Table 3 presents the different types of bacteria and fungal propagules found in both animal houses. Non-fermenting bacteria were always the most abundant type isolated. *Enterobacter* spp, *Hafnia* and *Serratia* were common, particularly in facility B.

The most common fungal spores found in the rat rooms of both facilities were *Cladosporium*, *Aspergillus/Penicillium*, *Ustilago* and hyaline ascospores (Table 4).

The inflammatory markers of rat bronchoalveolar lavages are shown in Table 5, significant differences were observed between lactate dehydrogenase levels in rats from facilities A and B (0.39 vs. 2.7 U mL^{-1} , respectively) ($p < 0.05$).

3.2 Worker exposure

A similar exposure pattern was observed for the workers, with higher exposures in facility B (Table 2), mainly to fungal spores (median of 54609 vs. 6762 spores m^{-3} 8 h^{-1}) and gram negative bacteria (median 2182 vs. 155 cfu m^{-3} 8 h^{-1}). In order to

Table 3. Culturable bacteria and fungi isolated from rooms of different animal houses

Abundance (%)	Animal house A		Animal house B	
	Rat Room	Cage Wash	Rat Room	Cage Wash
Bacteria (cfu m ⁻³)				
Non-Fermenting	80	67.7	46.5	46.2
<i>Enterobacter</i> spp.	–	17.7	12.0	18.2
<i>E. cloacae</i>	–	–	3.8	0.8
<i>E. agglomerans</i>	–	–	10.4	13.4
<i>Citrobacter</i>	–	–	2.2	0.8
<i>Escherichia coli</i>	–	–	3.3	4.8
<i>Klebsiella</i>	–	–	–	2.4
<i>Hafnia</i>	–	–	19.1	11.3
<i>Serratia</i>	20	14.5	2.7	2.0
Fungi (cfu m ⁻³)				
<i>Alternaria</i>	0.2	0.4	15.2	1.1
<i>Aspergillus</i>	2.7	6.5	10.5	3.3
<i>Aureobasidium</i>	0.4	0.5	1.0	0.3
<i>Cladosporium</i>	9.6	6.1	34.6	55.7
<i>Epicoccum</i>	–	–	0.4	0.7
<i>Fusarium</i>	–	0.3	0.2	0.1
<i>Monilia</i>	0.6	–	–	0.02
<i>Paecilomyces</i>	0.2	–	0.2	–
<i>Penicillium</i>	64.3	74.5	6.6	5.7
<i>Rhizopus</i>	0.2	3.2	–	0.1
<i>Trichothecium</i>	13.5	2.8	0.2	–
Non-sporulating mycelia	8.3	5.7	12.8	17.0
Others	–	–	15.0	16.0

A: rat room with air-conditioned.

B: without air-conditioned.

–: Not detected.

determine which activity represented higher exposure for the workers, the concentration of particles, endotoxin and biological particles were determined in different rooms (Figure 1), as well as the time they spent in each room. Results of the time activity questionnaire indicated that workers spend approximately 1 h outdoor, 1.5 h in the office, 2 h per day in the rat rooms and about 3.5 h per day in the cage wash room. This was the room where the highest concentrations of all evaluated parameters were observed.

A clear tendency in both facilities to have higher levels in the cage wash room was observed (Figures 1 A–F). The median concentrations of particles were 196 $\mu\text{g m}^{-3}$ and 279 $\mu\text{g m}^{-3}$ for house A and B, respectively (Figure 1A). Median endotoxin levels were 20 EU m^{-3} in facility A and 31 EU m^{-3} in

facility B (Figure 1B). A correlation of $r = 0.68$ was observed for particles and endotoxin ($p < 0.05$, slope 17.5) in both facilities (Figure 2A). No correlation was observed for outdoors results (Figure 2B).

Levels of bacteria were also higher in the cage wash room of both facilities. Facility B had the highest values (427 bacteria m^{-3} vs. 13341 bacteria m^{-3} , $p < 0.05$) (Figure 1C). Gram negative bacteria followed the same pattern (Figure 1D). No main differences were observed among animal facilities for fungi (Figure 1E). In/out ratios > 1 indicate that an indoor source is present.

The evaluation of size distribution of airborne particles and biological particles (larger and smaller than 4.5 μm) in the cage wash room of both facilities, showed that about half of the particles were in the non-respirable fraction ($> 4.5 \mu\text{m}$), whereas the larger

Table 4. Relative abundance of fungal spores collected from different animal houses

Fungi	Animal house A		Animal house B	
	Rat room (%)	Cage wash (%)	Rat room (%)	Cage wash (%)
DEUTEROMYCETES:	53.5	85.8 ^a	42.6	73.5 ^a
<i>Alternaria</i>	–	7.2	1.5	0.1
<i>Arthrimum</i>	–	0.4	–	0.3
<i>Aspergillus/Penicillium</i>	13.3	17.6	77.1	39.7
<i>Botrytis</i>	–	–	–	0.09
<i>Cercospora</i>	–	0.3	–	–
<i>Cladosporium</i>	24.5	56.2	18.3	45.4
<i>Curvularia</i>	–	0.3	–	0.06
<i>Drechslera/Helminthosporium</i>	–	0.4	–	9.4
<i>Epicoccum</i>	24.5	0.7	3.1	3.1
<i>Helicoma</i>	–	–	–	0.05
<i>Nigrospora</i>	37.7	0.3	–	0.09
<i>Periconia</i>	–	0.3	–	0.23
<i>Stemphylium</i>	–	–	–	7.7
<i>Torula</i>	–	0.5	–	0.3
BASIDIOMYCETES:	12.2	11.4 ^a	56.6	25.5 ^a
<i>Agrocybe</i>	–	5.5	–	1.6
<i>Coprinus</i>	25.0	4.5	0.6	3.9
<i>Ganoderma</i>	25.0	1.9	–	0.42
<i>Inocybe</i>	–	–	–	0.43
<i>Puccinia</i>	–	1.9	–	0.41
<i>Tilletia</i>	–	–	–	3.4
<i>Ustilago</i>	25.0	6.1	31.0	0.7
Other smuts	–	37.6	68.4	85.7
Rusts	25.0	9.3	–	1.94
Hyaline basidiospores	–	–33.2	–	1.5
ASCOMYCETES:	34.2	2.8 ^a	0.8	1.0 ^a
<i>Chaetomium</i>	79.4	10.9	–	13.0
<i>Leptosphaeria</i>	–	52.4	–	23.8
<i>Leptosphaerulina</i>	–	–	–	3.6
<i>Pleospora</i>	–	10.9	–	5.2
<i>Sporormiella</i>	–	–	–	6.9
<i>Venturia</i>	–	–	–	10.6
Hyaline ascospores	20.6	10.9	100	17.9
Ascospores (Sordaria type)	–	14.9	–	19.0

^a Abundance: % with respect to total spores; – : Not isolated

proportion of the particles carrying gram-negative bacteria and fungi were collected in the respirable fraction ($\leq 4.5 \mu\text{m}$), especially in facility B (Figure 3).

The relative abundance of bacteria and fungi in the cage wash rooms of both facilities is presented in Table 3. Bacteria of the non-fermenting type were mainly isolated in facility A. More heterogeneity was

observed in facility B and fecal bacteria *Escherichia coli* were found. In the case of fungi, *Penicillium* was the predominant type found in facility A, whereas, *Cladosporium* and *Aspergillus* were predominant in facility B. The pattern of species observed in house B resembled that observed in outdoor air. Spore diversity was higher in the cage wash room of facility B, with a

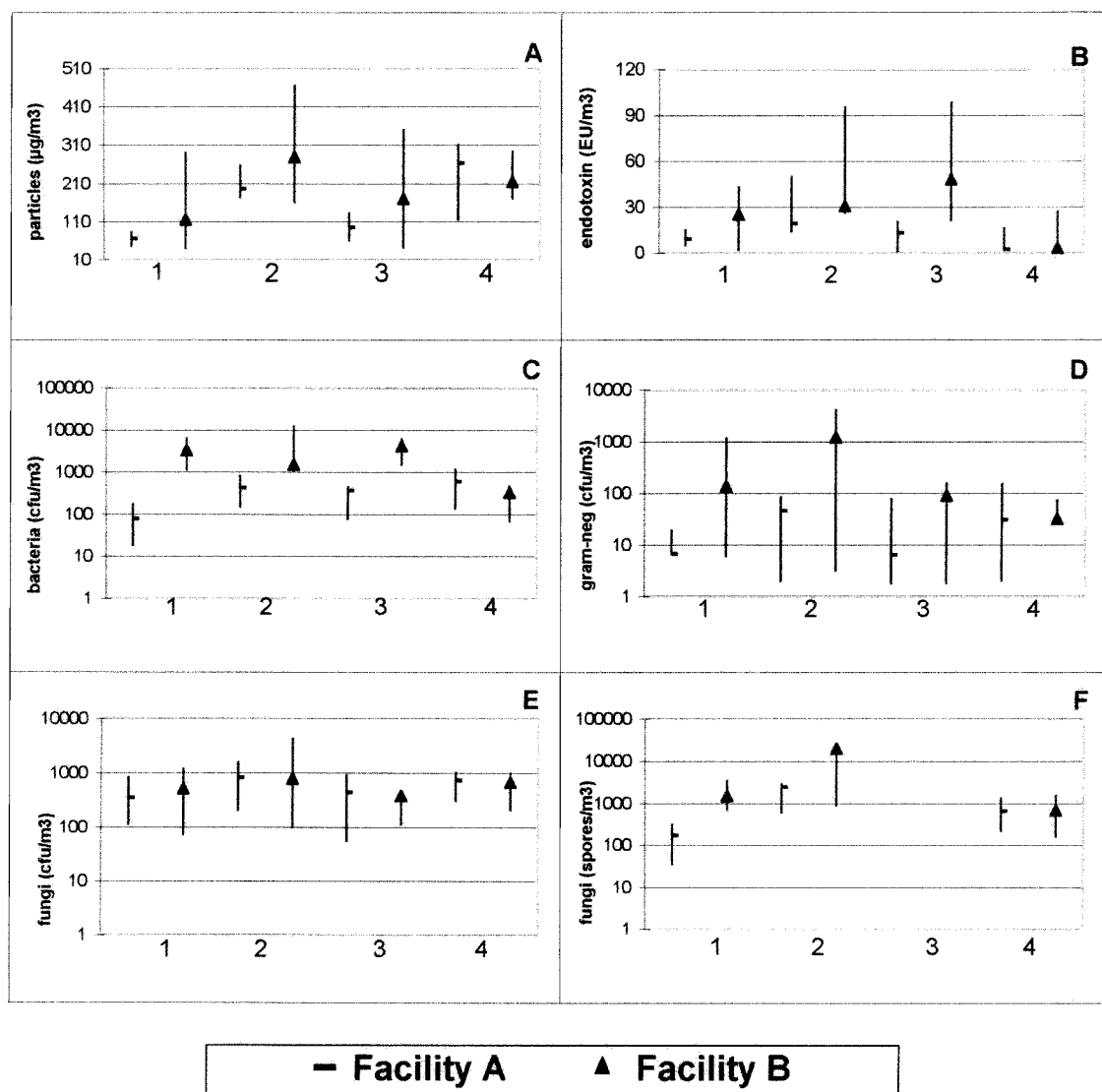


Figure 1. Concentrations of airborne particles, endotoxin, culturable bacteria and fungi from different rooms of the studied animal houses: 1, rat room; 2, cage wash room; 3, office; and 4, outdoors. Results are mean and range.

high proportion of Basidiomycetes and Ascomycetes, not common in indoor environments (Table 4).

4. Discussion

In Mexico the number of animal housing facilities has increased in the last few years, due to the increasing demand for experimental animals. However, most of those facilities do not meet the international criteria for animal room design and construction (Quimby, 1993),

or the criteria for animal housing (ILAR/CLS/NRC, 1999). Results of the present study show that the activities carried out in the studied animal houses generate an organic dust that contaminates the rooms, albeit less importantly in the rat room of the air conditioned animal facility. There, the number of animals meets the guide for care and use of laboratory animals (ILAR/CLS/NRC, 1999). However, in other rooms, workers and laboratory animals are exposed to microorganisms or their products in association with airborne particles. Similar types of exposure have

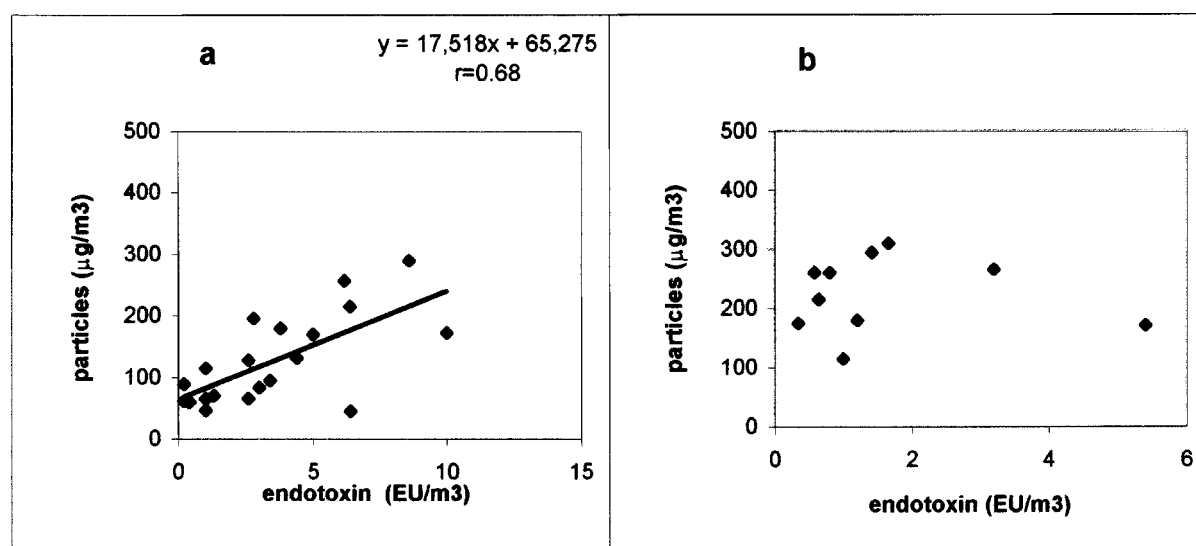


Figure 2. Correlation between airborne particles and endotoxin from different rooms of the studied animal houses (a) and outdoor (b).

Table 5. Comparison of inflammatory markers in rat bronchoalveolar lavage from different animal care facilities

Animal care facility/ Type of ventilation	Median	Minimum	Maximum
Air-conditioned (A)			
Total cells $\times 10^5/\text{mL}$ of bronchoalveolar lavage	1.1	0.076	1.3
Differential cell count (%)			
Polymorphonuclear			
Neutrophils	1	0	8
Macrophages	98	92	100
Proteins ($\mu\text{g mL}^{-1}$)	9.8	8.3	23.3
Lactate dehydrogenase (U mL^{-1})	0.39	0.05	0.45
Passive ventilation (B)			
Total cells $\times 10^5/\text{mL}$ of bronchoalveolar lavage	4.4	3.0	7.1
Differential cell count (%)			
Polymorphonuclear			
Neutrophils	2	1	3
Macrophages	98	92	100
Proteins ($\mu\text{g mL}^{-1}$)	10.8	5.7	14.9
Lactate dehydrogenase (U mL^{-1})	2.7	0.05	3.7

Ten 150g male Wistar rats from each facility.

been related to allergic diseases (Botham et al., 1995; Hollander et al., 1997; Bush et al., 1998) and inflammatory processes (Wang et al., 1996; Borm et al., 1996; Adisesh et al., 1998).

Bronchoalveolar lavage of rats from the non air-conditioned facility had in significantly higher levels of total cells and lactic dehydrogenase (LDH) in comparison with the rats from the facility with an efficient ventilation system to remove airborne particles (Burnett, 1969; Honey and McQuitty, 1979). The rat rooms equipped with one-way-airflow system had a maximum total culturable bacteria concentration of 178 cfu m^{-3} , and 19 cfu m^{-3} of gram-negative bacteria, 20% were enteric. In contrast, those from the rat rooms with passive ventilation, had a maximum of 6427 cfu m^{-3} culturable bacteria, 1158 cfu m^{-3} gram-negative. 43.5% were enteric bacteria, represented by *Enterobacter* spp, *Hafnia* spp, *Escherichia coli* and *Klebsiella*.

Not only biological particles were isolated from the rat rooms, but also bacterial endotoxin was identified. The median concentration was around 25 EU m^{-3} in the non air-conditioned facility. This is low for the 50 EU m^{-3} accepted in the industrial occupational environment (Heederik et al., 1991) and the 10 ng m^{-3} standard, used in the poultry industry in the USA (Herbert and Breum, 1995). However, it suggests that the inhalation of a complex mixture of particles and substances like those in organic dust, even at low

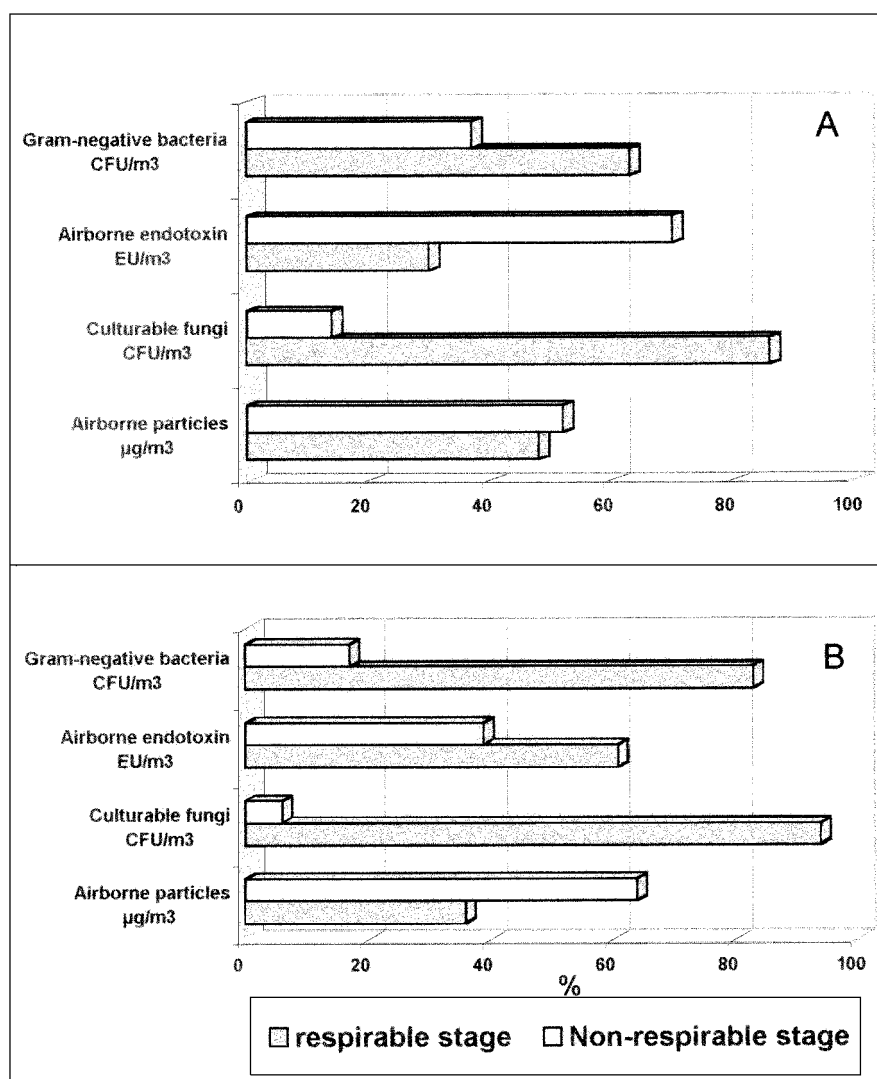


Figure 3. Distribution of microbial and non-microbial particles according to respirable and non-respirable size distribution.

concentration, could produce inflammatory responses in animals.

On the other hand, despite the significant difference between exposure levels of workers in facility A with respect to facility B (median particles exposure of 870 vs. 1209 $\mu\text{g m}^{-3} \text{ h}^{-1}$), did not exceed the Danish occupational exposure limit of 3 mg m^{-3} (Herbert and Breum, 1995) or the Dutch maximum allowed concentration of 10 mg m^{-3} (Smid et al., 1992). However, previously published results show that workers from both facilities had increased cellular cytokine production and increased blood levels of bactericidal permeability-increasing protein, different from unexposed workers (Borm et al., 1999). This suggests that

worker exposure to a mixture of biologically active substances during washing activities is high in both cases (e.g., median endotoxin exposure of 70 vs. 109 $\text{EU m}^{-3}/8 \text{ h}$, respectively). On the other hand, during cage washing not only high concentrations of bacteria were detected, but also fungal spores (2450 spores m^{-3} facility A, and 20906 spores m^{-3} facility B) were liberated into the air. Therefore, (1–3)- β -D-glucan could be present, although this compound was not measured in the present study. It is well known that glucan forms part of the fungal spore wall and is very active in inducing inflammatory responses (Rylander and Holt, 1998). It has been reported that levels of glucan ranged from 0.43 to 2.3 ng m^{-3} and fungal pro-

pagules in average of 100 cfu m^{-3} , can be related to the presence of respiratory symptoms (Alwis, 1998). In our study, culturable fungal concentrations of up to 400 cfu m^{-3} were found.

Since the type of fungal spores found in the cage wash rooms, basidiospores and ascospores have been reported as typical of green areas (Calderón et al., 1995), it could be that the wood chips bring this type of contaminants into the animal facilities. Not only that, but since wood chips trap animal waste (Tejero, 1994), disturbance of animal bedding during cage washing can represent an important source of airborne bioparticles.

Results of this investigation indicate that aerobiological studies are important to evaluate the conditions before and during environmental control of animal facilities. Gravimetric analysis of airborne dust seems to be a good indicator of other biological contaminants, based on the statistically significant correlation that we have found with biological products such as endotoxin. Moreover, a specific organic dust safe level needs to be established for research animal houses, based on the potential risk associated with the exposure to inhalation of a complex mixture of organic dust and its effects at immunogenic or non-immunogenic level (Norn, 1994; Hansen et al., 1997; Roepstorff and Sigsgaard, 1997; Wintermeyer et al., 1997; Milanowski, 1998).

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