

Characterization of Endotoxin and Mouse Allergen Exposures in Mouse Facilities and Research Laboratories

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Objectives: Researchers and technicians who use mice in research are exposed to complex mixtures containing mouse allergen, endotoxin and particulates from animals, bedding and feed. The particle characteristics of these different exposures, and whether they are encountered together or separately, are important to better understand their adjuvant and allergic effects. Endotoxin and mouse allergen are derived from the same animal source, but have different physicochemical attributes. It is not known if airborne exposures to these agents are correlated in the laboratory animal workplace.

Methods: Side-by-side personal and area samples for airborne endotoxin (52), mouse allergen (46) and total particulates (43) were obtained in the animal facility and laboratories of a medical research institution. Animal handlers and researchers reported time spent on work tasks with mice, symptoms upon exposure to mice and mouse sensitization was determined by skin test or RAST.

Results: Mean airborne endotoxin exposure was highest during mouse experiments in the animal facility at 960 pg m⁻³, peaked at 3125 pg m⁻³, and ranged from 46 to 678 pg m⁻³ with work in mouse rooms and research labs. Mouse allergen concentrations were highest during direct mouse work and background in research labs (mean 63–68 ng m⁻³, range 41–271 ng m⁻³), but were undetectable during mouse research performed under a hood. Endotoxin and mouse allergen concentrations were correlated during direct research with mice and mouse care activities. Particle counts were low, typically <1 cm⁻³, varied widely, and exhibited peaks and valleys during different work tasks. From 80–90% of particles were <1 µm in aerodynamic diameter during background measurements. The contribution of respirable particles 1–5 µm in size increased to 25–30% during mouse care and mouse research activities, but we found no association between any particle size and endotoxin or mouse allergen concentrations. Animal handlers and researchers in the mouse facility were exposed to the highest daily endotoxin concentrations, whereas researchers working with mice in the mouse facility and in laboratories were exposed to the highest daily mouse allergen concentrations.

Conclusions: These findings suggest that endotoxin and mouse allergen are co-exposures during mouse handling and research, and that control of exposure peaks may be necessary to limit allergic disease in the laboratory animal workplace.

Keywords: bioaerosols; endotoxin; laboratory animal allergy; mouse allergen; occupational asthma

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INTRODUCTION

Research scientists and technicians who work with laboratory animals are exposed to both animal allergens and endotoxin in the workplace, as we and others have shown (Milton *et al.*, 1990; Lieutier-Colas *et al.*, 2001; Pacheco *et al.*, 2003). Typical rat and mouse allergen concentrations in lab animal environments range from 10 to 200 ng m⁻³. Average endotoxin exposures for laboratory animal workers range from 20 to 1500 pg m⁻³, lower than the range of 60 to >1000 ng m⁻³ in grain handlers, hog confinement workers, and poultry farmers (Schwartz *et al.*, 1995; Simpson *et al.*, 1998; Larsson *et al.*, 1999). However, since most reported concentrations are averaged over time, it is not clear whether these results represent a continuous, relatively even exposure or the average of peak exposures. High exposure peaks may induce different biological effects than do lower, more constant exposures, and may trigger symptoms and/or immunologic sensitization to lab animals more readily (Karol, 1983; Aoyama *et al.*, 1994).

Up to 30% of exposed animal handlers and research scientists develop allergic sensitization and disease to lab animals. These rates are higher than those reported for other jobs with exposure to allergenic proteins such as bakers or dental technicians (Gautrin *et al.*, 2000). However, the risk for lab animal sensitization does not clearly follow an exposure gradient, in that sensitized workers are not necessarily those in the highest allergen exposure categories (Heederik *et al.*, 1999; Nieuwenhuijsen *et al.*, 2003). A healthy worker effect, in which those least affected stay in the job, may in part explain these findings. They also suggest that other exposures in addition to animal allergens play a role in the allergic process. We previously identified airborne endotoxin, and not mouse allergen, as a major risk factor for nasal, chest and skin symptoms when working with mice in a subgroup of non-mouse sensitized researchers (Pacheco *et al.*, 2003). Together, these findings support an important role for endotoxin as well as mouse allergen in risk for symptoms and sensitization in the laboratory animal workplace.

Earlier reports detected rat and mouse allergens on particles >5–10 µm aerodynamic diameter in animal facilities and research laboratories. Platts-Mills showed that <1/3 of *Rat n 1* antigen was present on particles <5 µm (Platts-Mills *et al.*, 1986). Swanson also found only 33% of total rat urinary protein on particles <4.5 µm. Similar to rat allergen, from 50 to 100% of airborne *Mus m 1* was detected on particles 3.3–10 µm in diameter in high density mouse rooms (Ohman *et al.*, 1994). In undisturbed mouse rooms (Sakaguchi *et al.*, 1989), only 6% of mouse urine allergens and 11% of mouse pelt allergens were measured on particles <3.3 µm in diameter, although with

activity, this proportion increased to 16%. The particle size distribution for endotoxin, and whether it is present on the same particles as mouse allergen in these settings, is unknown. Because endotoxin and mouse allergen have different sizes and charges (Petsch and Anspach, 2000), they may not be associated with the same size particles. However, because both emanate from mice and mouse droppings, endotoxin and mouse allergen are likely to be detected together.

To characterize these complex exposures, we performed task-specific air sampling for endotoxin, mouse allergen and particulates in the breathing zone of animal handlers and researchers. We investigated the temporal pattern of airborne particles during the course of each task. We then calculated a mean daily exposure to endotoxin and to mouse allergen for each research job title to better define risk for occupational sensitization and disease in these workers.

METHODS

Description of animal and research facilities and processes

This study was conducted in an academic biomedical research institution where the majority of animal research utilizes mice. Animals are housed in an animal facility where experiments are also conducted. Mice may be transported from that facility to individual research laboratories throughout the campus. The animal care facility includes 20 animal holding rooms, a separate facility of nine rooms for immunocompromised animals, four rooms for animal quarantine, five experimental rooms for animal research, a dirty and a clean cage wash area. The facility contains 10 000 mice, with 100–1000 mice per room. The animal facility has a ventilation system separate from the rest of the institution, with 12–25 fresh air changes per hour. Personal protective equipment (PPE) is required for all animal handlers and researchers working in the animal facility, and includes surgical bonnet, mask, nitrile gloves, booties and lab coat provided by the facility. Workers in the dirty cage wash also wear a face shield. Respirators are generally not used. Two main research buildings provide laboratory space for 310 research scientists and technicians, where mice in cages are stored in ventilated hoods as well as stacked next to research benches. Mouse research is performed in a variety of settings, including in hoods, in dedicated mouse rooms and directly atop research benches. No PPE is typically used to work with lab animals in the research buildings, although most researchers wear latex gloves.

Sampling protocol

We identified major tasks for laboratory animal research based on a walk-through of the animal facility and research labs. Animal facility tasks consisted of cage changing, cage washing and animal experiments. Research laboratory tasks included direct experimental work with mice, research performed adjacent to but not directly with lab animals and non-animal research. We obtained simultaneous lapel samples for mouse allergen and endotoxin. Particle size and count were measured immediately adjacent to the worker during each of these work tasks. We compared these measurements with area samples obtained during quiescent periods without human activity. For area samples, equipment was placed in the middle of each task area, at an approximate height of four feet. We collected a total of 52 samples, including 35 lapel samples and 17 area samples. A total of 20 workers were sampled. Most workers were sampled more than once. Our sampling times ranged from 30 up to 189 min based on task duration and from 120 to 1035 min for background measurements.

Endotoxin sampling and analysis

Endotoxin was sampled at 3 l min^{-1} with an SKC AIRCheck pump, onto $5 \mu\text{m}$ pore size 37 mm PVC filters (Pacheco *et al.*, 2003). Endotoxin was eluted from filters in 10 ml of sterile, pyrogen-free water with 0.05% Tween-20 at room temperature for 60 min. The eluate was then assayed by the endpoint chromogenic Limulus Amoebocyte Lysate (LAL) test (Bio-Whittaker, QCL 1000) according to kit directions, with a limit of detection (LOD) of $0.00625 \text{ EU ml}^{-1}$ or 6 pg filter^{-1} . Results are reported as Endotoxin Units (EU) converted to picograms on the basis of $1 \text{ EU} = 100 \text{ pg}$. Airborne task concentrations in pg m^{-3} reflect the quantity of endotoxin detected during a work task, divided by the volume of air sampled. For the analysis reported in 'Recalculated endotoxin and mouse allergen by peak particulate counts', endotoxin concentrations were adjusted by reducing the sampling volume to that collected during which detectable particle concentrations were $>1 \text{ cm}^{-3}$ as measured by the aerodynamic particle sizer.

Mouse allergen sampling and analysis

Mouse allergen was obtained on 37 mm Teflon (PTFE) filters with $2 \mu\text{m}$ pore size, using a vacuum pump sampling at 15 l min^{-1} during the same time periods as for endotoxin sampling. Filters were shipped overnight to the University of Iowa, where they were assayed for mouse allergen using a polyclonal mouse urinary protein antibody (Greer, Lenoir, NC) RAST inhibition assay. Briefly, filters were eluted into 2 ml of PBS with 0.01% TWEEN-20.

Assay plates (Immulon 2 HB, Dynex Technologies, Chantilly, VA) were coated overnight with $200 \mu\text{l}$ of $2 \mu\text{g}$ mouse urine protein per millilitre PBS and then blocked with PBS with 0.01% TWEEN-20 and 1% gelatin. One hundred microlitre of each eluate was added to appropriate wells followed by $100 \mu\text{l}$ at $4.5 \mu\text{g ml}^{-1}$ polyclonal mouse urinary protein antibody produced in rabbit (Greer Laboratories, Inc) so that the antigen in the sample competed with the coated antigen for binding to the antibody in the fluid phase. Following incubation and multiple washes, a goat anti-rabbit Ig (Biosource Inter., Camarillo, CA) was added, in a horseradish peroxidase detection system. The amount of mouse allergen was measured in nanograms by comparison to a known amount of control allergen. The LOD of the assay was 3 ng ml^{-1} or 6 ng per filter . In settings where no mouse allergen was detected, results are presented as the LOD/2 and adjusted for sampling volume. Airborne task concentrations in ng m^{-3} reflect the quantity of mouse allergen detected during a work task, divided by the volume of air sampled. For the analysis reported in 'Recalculated endotoxin and mouse allergen by peak particulate counts', mouse allergen concentrations were adjusted by reducing the sampling volume to that collected during which detectable particle concentrations were $>1 \text{ cm}^{-3}$ as measured by the aerodynamic particle sizer.

Particle size analysis

Particle counts were measured in real time using an aerodynamic particle sizer (APS, Model 3320, TSI, Shoreview, MN). The APS measures light scattering intensity to get particle counts and time-of-flight spectrometry to measure aerodynamic diameter. The two independent measuring approaches allow for accurate particle counts and sizes. Counts were acquired over 1–5 min intervals, for a total of 60–120 min during specified tasks or background periods of no human activity. Counts were separated into three size groups: $0.523\text{--}1$, $1\text{--}5$ and $>5 \mu\text{m}$. Particle counts $<0.523 \mu\text{m}$ were excluded, as counts below this size are not reliably quantified by the APS. Particle results were analyzed as geometric mean counts cm^{-3} in the three size ranges over the sampling period, as particle counts over time, as well as a summary statistic of count median aerodynamic diameter. Since overall particle counts were $\leq 3 \text{ cm}^{-3}$, results are presented as the percent distribution of the three size ranges.

Exposure evaluation of the laboratory animal workforce

This study was approved by the institutional review board, and all subjects provided written informed consent prior to enrolling in the study. We then

administered a questionnaire to 269 of 310 (87%) researchers and animal technicians in our research institution. We ascertained the number of hours per week spent working in proximity to mice and in different job tasks, and reported symptoms when working with mice. Mouse-related symptoms included itchy, runny or stuffy nose, sneezing, cough, chest tightness or wheeze, shortness of breath and skin rash. Sensitization to mice was based on a positive skin test, defined as a wheal ≥ 3 mm and a flare ≥ 10 mm, or RAST, defined as specific IgE binding of kUA/l ≥ 0.35 .

We divided the workforce into four job categories based on decreasing likelihood of exposure: (i) animal handlers and ancillary workers in the animal facility; (ii) research scientists and technicians performing research with mice in the animal facility and in their labs; (iii) researchers working with mice only in their own labs and (iv) laboratory researchers who performed no animal-based research. We compared hours per week spent in mouse research with symptoms and sensitization to mice across all four exposure categories. We then combined task-specific endotoxin and mouse allergen values from air sampling with questionnaire responses on task frequency to calculate a mean daily exposure to endotoxin and mouse allergen for each exposure category. The mean daily exposure was derived by multiplying sampling means for each task by the reported hours spent per week on each task, to develop a task-specific exposure for each subject. The sum of all task-specific exposures ($E \text{ ng m}^{-3} \times h \text{ week}^{-1}$) was divided by 5 days week^{-1} , and adjusted for an estimated volume of air breathed per hour to derive an estimate of exposure in pg or ng day^{-1} . We used estimates of ventilation rates in $\text{m}^3 \text{ h}^{-1}$ from tables 5 and 6 in the U. S. EPA Exposure Factors Handbook (U. S. EPA, 1997). We categorized research and most laboratory animal work as light exertion, and attributed ventilation rates of $1.45 \text{ m}^3 \text{ h}^{-1}$ for men, and $1.33 \text{ m}^3 \text{ h}^{-1}$ for women.

Statistical analysis

We compared differences in airborne concentrations of mouse allergen and endotoxin for different job titles, for different tasks, and compared with background using ANOVA or least squares analysis where appropriate. The distribution of hours per week spent in mouse research, symptoms and sensitization to mice were similarly compared. Mouse allergen and endotoxin concentrations were log transformed, and their association evaluated using Pearson correlation, or Spearman's Rho where the data were not normally distributed. Associations with sized particle counts were also analyzed by Pearson correlation or Spearman's Rho as appropriate. Endotoxin and mouse allergen values above and below the median, and above and below the 75th percentile, were compared using a Chi-square statistic. Significance

was set at a conventional $P < 0.05$. Significance set at $P \leq 0.15$ was considered suggestive of an association, but was not considered to be significant. Analyses were performed using SAS statistical software (version 9.1; SAS Institute, Cary, NC) and JMP 5.1.1 (also from the SAS Institute, Cary, NC).

RESULTS

Airborne endotoxin concentrations

We detected airborne endotoxin throughout the animal facility and in all sampled research laboratories (Table 1). Peak (3125 pg m^{-3}) and mean airborne endotoxin concentrations (960 pg m^{-3}) were highest during direct mouse research work in animal facility research areas and were significantly higher than background measurements made when the areas were unused by workers (background). In the research laboratories, endotoxin concentrations were similar during mouse experiments on the bench, in the hood and in background measurements, but were significantly lower in laboratories that did not conduct mouse research. This suggests that the source of endotoxin was from mice and cages kept in the labs. Endotoxin values were then combined for all mouse care activities (cage change and wash) and all direct mouse research work at the bench, and concentrations during different activities were compared. Airborne endotoxin was higher during mouse care compared with background in the animal facility (292 pg m^{-3} versus 152 pg m^{-3} , $P = 0.10$), although the practical differences in health effects between these values may be negligible. Airborne endotoxin was also higher during any mouse experiments on the bench compared with background (600 pg m^{-3} versus 84 pg m^{-3} , $P = 0.06$) and in non-mouse labs (600 pg m^{-3} versus 69 pg m^{-3} , $P = 0.15$). The small number of samples limits our ability to detect statistically significant differences. In several settings, airborne endotoxin was detected when mouse allergen was not.

Airborne mouse allergen concentrations

Concentrations of mouse allergen ranged from undetectable to a peak of 271 ng m^{-3} (Table 1). In the animal facility, mean mouse allergen concentrations were highest in the dirty cage wash area at 92 ng m^{-3} , and during cage changing (65 ng m^{-3}). Airborne mouse allergen was lower during background sampling in mouse rooms and the dirty cage wash area compared with those areas with activity, and undetectable in clean cage wash without activity. In the research labs, the highest mean concentration of mouse allergen, 68 ng m^{-3} , was measured during direct mouse work on a bench, though none was detected when mouse work was performed in a hood. Interestingly, mean background mouse allergen

Table 1. Endotoxin and mouse allergen concentrations in the animal facility and in laboratories based on tasks

Location	(1a) Endotoxin (pg m ⁻³)			(1b) Mouse allergen (ng m ⁻³)		
	<i>n</i>	Arithmetic mean (range)	Geometric mean (95% CI)	<i>n</i>	Arithmetic mean (range)	Geometric mean (95% CI)
Animal facility						
Cage changing	4	315 (164, 678)	264 (96, 731)	2	65 (51, 78) ^c	63 (4, 907) ^c
background	2	266 (31, 500)	126 (n.d.)	2	37 (35, 38) ^c	37 (22, 60) ^c
Cage wash/dirty	4	305 (110, 471)	264 (92, 760)	3	92 (36, 179)	73 (9, 561)
background	2	113 (37, 189)	83 (n.d.)	2	18 (0.5, 36)	4 (n.d.)
Cage wash/clean	2	219 (24, 414)	99 (n.d.)	3	44 (2, 93)	13 (n.d.) ^d
background	2	70 (25, 114)	53 (n.d.)	2	0.6 (0.5, 0.6)	0.6 (0.3, 1) ^d
Administrative office	3	157 (70, 233)	140 (30, 661)	3	13 (0.5, 39)	2 (n.d.)
Mouse expt-research rms	7	960 (60, 3125) ^a	493 (137, >900) ^a	5	31 (3, 113)	12 (2, 80)
background	3	35 (12, 56) ^a	29 (4, 209) ^a	3	18 (0.2, 54)	1.3 (n.d.)
Research buildings						
Mouse expt: bench	6	176 (46, 363) ^b	132 (53, 327)	5	68 (41, 121) ^{e,f}	64 (39, 105) ^{e,f}
Mouse expt: hood	3	102 (10, 432) ^b	188 (57, 625)	3	8 (5, 12) ^{e,f}	8 (3, 20) ^{e,f}
Background mouse lab	8	203 (114, 300) ^b	38 (11, 132)	9	63 (24, 271) ^e	44 (25, 78) ^e
Expt: nonmouse lab	6	69 (10, 194) ^b	43 (13, 137)	5	28 (19, 37) ^e	27 (19, 39) ^e

n.d. = Not determined as too few measurements.

^a*P* < 0.05 airborne endotoxin in animal facility mouse research compared with background, arithmetic (AM) and geometric means (GM).

^b*P* < 0.10 airborne endotoxin during bench mouse research in research labs compared with non-mouse labs, AM.

^c*P* < 0.15 airborne mouse allergen during cage changing in animal facility compared with background, both AM and GM.

^d*P* < 0.10 airborne mouse allergen during clean cage wash compared with background, GM.

^e*P* < 0.05 Airborne mouse allergen during bench mouse research in research labs compared with nonmouse lab, AM and GM.

^f*P* < 0.05 Airborne mouse allergen during mouse research on bench compared with research in hood, AM and GM.

concentrations in mouse research labs were 63 ng m⁻³, similar to concentrations during direct mouse work at the bench, suggesting allergen persistence even when no current animal work is being done. Airborne mouse allergen was detected, but significantly lower, in labs reporting no work with mice.

We then similarly aggregated mouse allergen concentrations for mouse-based tasks, which included mouse care (cage changing and washing), any bench mouse research and background concentrations in mouse research areas. The mean airborne mouse allergen during mouse care activity in the animal facility was 67 ng m⁻³, significantly higher than the average background concentration of 17 ng m⁻³ (*P* = 0.02). Allergen concentrations during any direct mouse experiments performed on a bench were higher than for mouse experiments performed in a hood (50 ng m⁻³ versus 8 ng m⁻³ or non-detect, *P* < 0.05) but similar to background in all mouse research areas (52 ng m⁻³).

Particle counts and mean mass concentration

Mean particle counts were in general low, typically ≤1 cm⁻³ for each size range, and total counts did not vary much with activity. From 80 to 90% of background particles were <1 μm in aerodynamic diameter (Fig. 1), and in all settings the count mean aerodynamic diameter was <1 μm. The relative

contribution of particles 1–5 μm rose to 25–30% of the total during mouse care (cage change, cage wash), and mouse research activities. These particles were lowest, 7% of the total, in non-mouse labs. In most samples, particle counts in all three size ranges varied together (Fig. 2), suggesting a common source.

Association of endotoxin, mouse allergen and particle counts

Airborne endotoxin was detected in 100% of the samples taken, while mouse allergen was detectable in only 70% of the samples, likely because there are sources of endotoxin other than mice. When both endotoxin and mouse allergen were detected, there was evidence suggestive of an association as reflected by Spearman's rank sum, Rho, for the correlation 0.30 (*P* = 0.09). When a single outlier endotoxin measurement was removed (6424 pg m⁻³), this correlation increased to 0.41 (*P* = 0.01). Endotoxin and mouse allergen correlated most strongly at higher concentrations of both. We found no association when endotoxin and mouse allergen concentrations were compared when dichotomized into above and below median values. However, the highest concentrations of airborne endotoxin and mouse allergen (>75th percentile value, 304 pg m⁻³ for endotoxin and 54 ng m⁻³ for mouse allergen) were significantly associated (*P* = 0.006). When airborne endotoxin and mouse allergen were compared based on task and

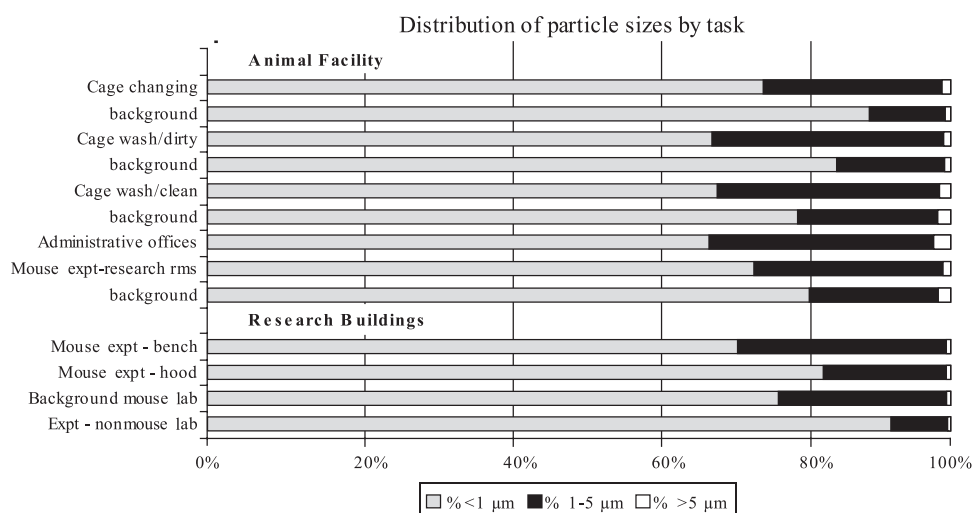


Fig. 1. Distribution of particle sizes during different animal care and research tasks.

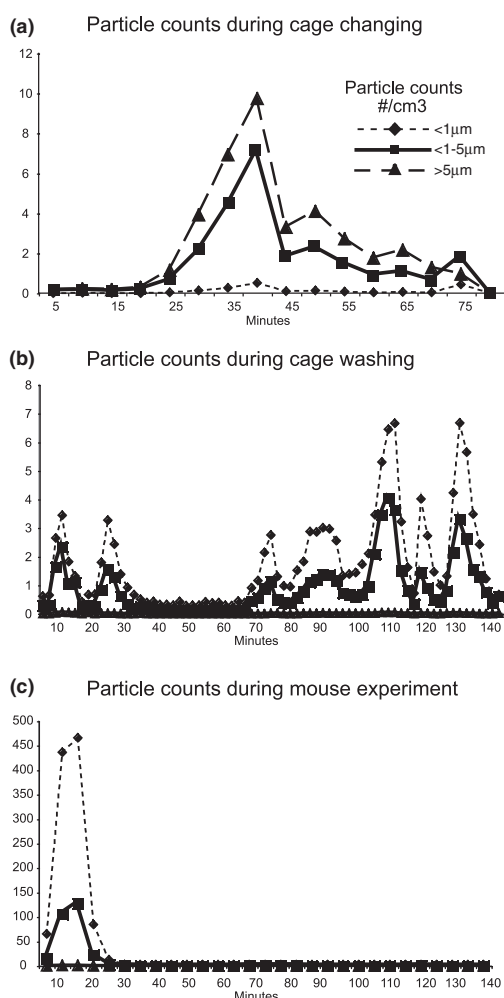


Fig. 2. Representative plots of particle counts over time grouped by size: <1, 1–5 and >5 μm, obtained during cage changing (a), cage washing (b) and during a mouse experiment (c). Counts for particles < 0.523 μm were not included.

Table 2. Associations between airborne endotoxin and mouse allergen

Job task	Samples	Correlation (r^2)	P
Animal facility			
Mouse care (cage change, cage wash)	7	0.68	0.05
Mouse care background	9	0.22	ns
Mouse experiment	5	0.46	ns
Mouse experiment background	3	−0.96	ns
Research buildings			
Mouse experiment	5	0.60	0.13
Mouse experiment background	8	0.74	0.03
Mouse experiment in hood	3	0.98	0.08
Non-mouse experiment in non-mouse lab	5	0.50	ns

The data are log transformed, and compared using pairwise correlation, or Spearman's Rho where appropriate.

location, they correlated significantly during direct mouse handling, including cage change/cage wash, mouse experiments in the animal facility and in a hood, with a trend toward significance during mouse experiments in research labs (Table 2). We found no associations between any sized particles and endotoxin or mouse allergen concentration.

Distribution of exposures, symptoms and sensitization to mice in research scientists and technicians

Since the preponderance of direct work with mice in this institution involves mouse research, we determined the mean hours per week spent in mouse experiments for each exposure category (Table 3). Researchers who worked with mice in both the animal facility and their own labs had, not surprisingly,

Table 3. Comparison of weekly hours spent in animal research, symptoms during work with mice and sensitization rates to mice between exposure categories

Exposure groups	Animal Handler research w/mice in animal facility only (<i>n</i> = 20)	Researcher w/mice in animal facility and labs (<i>n</i> = 89)	Researcher w/mice in labs only (<i>n</i> = 40)	Non-mouse researcher (<i>n</i> = 120)
Mean hours per week in mouse experiments	4.25	6.40 $\xleftrightarrow{P=0.02}$	3.27	*0
Nose, chest, and/or skin symptoms to mice	4/20 (20%)	33/89 (37%)	10/40 (25%) $\xleftrightarrow{P=0.03}$	13/120 (11%)
		$P < 0.0001$		
Sensitization to mice	3/20 (15%)	21/89 (24%)	6/40 (15%)	27/120 (22%)

* $P < 0.0001$ compared to all other exposure groups.

the highest weekly hours of exposure to mice. This was similar to those who did mouse research solely in the animal facility, but significantly higher than those who did mouse research in labs only. The group with the highest weekly hours of mouse exposure also reported the greatest frequency of symptoms to mice. Overall, researchers who reported symptoms to mice spent longer hours per week working with mice, than did mouse researchers asymptomatic to mice (5.3 h week⁻¹ versus 2.2 h week⁻¹, $P = 0.0004$). Researchers working with animals in any setting reported significantly more symptoms to mice than those who did not currently work with mice. In contrast, sensitization to mice was distributed equally across all four job exposure categories. This may in part be due to the movement of sensitized symptomatic workers out of direct contact with mice. Of the 120 workers who did not currently work with mice, 81 or 76% had previously worked with lab animals, and 55 or 46% had previously worked with mice.

Mean calculated exposures to endotoxin and mouse allergen by job exposure category

We calculated the arithmetic mean daily exposures to endotoxin and mouse allergen for the four job exposure categories (Fig. 3). Endotoxin exposures varied by the hours of direct mouse care and research, and differed significantly between each job category. Animal handlers had the highest mean daily exposure to endotoxin, significantly higher than other mouse researchers in the animal facility. Endotoxin exposures were also significantly higher for animal handlers than for researchers performing mouse or non-mouse research outside the animal facility ($P < 0.0001$). Endotoxin exposures were also significantly higher for researchers directly working with mice in a research lab compared with those working in a non-mouse lab. In contrast, mouse allergen exposures were highest for mouse researchers working either inside or outside the animal facility. Similar and significantly lower exposures to mouse allergen ($P < 0.0001$) were recorded for animal handlers, likely because they did most of their work in

a hood, and in a facility with a ventilation system designed with more frequent air changes per hour than the research buildings. Not surprisingly, non-mouse researchers also had some of the lowest mouse allergen exposures. Results were similar when the geometric means for endotoxin and mouse allergen were compared across the same job categories.

Particulate counts vary over time

An APS sampler records particle counts over time, and results can be graphed to demonstrate the pattern during each task. Plots of the timed data demonstrate that particle counts were rarely stable during specific job tasks, but instead showed peaks of increased counts separated by valleys and plateaus. Figure 2 demonstrates representative plots for particle concentrations during cage changing, dirty cage washing and mouse research in an animal research room. Typically, all three particle size counts tracked together, although the highest counts were seen in only one or two size categories. During cage changing (Fig. 2a), peak counts occurred in particles $>1 \mu\text{m}$, with the highest counts in the $>5 \mu\text{m}$ size range that may indicate aerosolized sawdust bedding. Dirty cage washing (Fig. 2b) demonstrated cyclical peaks in particles $\leq 5 \mu\text{m}$ that may reflect water droplet aerosols. A sizeable, 20 min peak of $5 \mu\text{m}$ and smaller sized particles was measured during a mouse experiment (Fig. 2c), although total sampling continued for 145 min. These results demonstrate that particle-associated exposures to endotoxin and mouse allergen may occur during several short-term peaks. Exposure measurements that are averaged over the sampling period may thus not reflect much higher peaks of exposure that may be most relevant to workers.

Recalculated endotoxin and mouse allergen by peak particulate counts

We recalculated airborne concentrations of endotoxin and mouse allergen based on the time of peak particle counts, to better characterize the potential impact of brief exposures on the worker. For cage

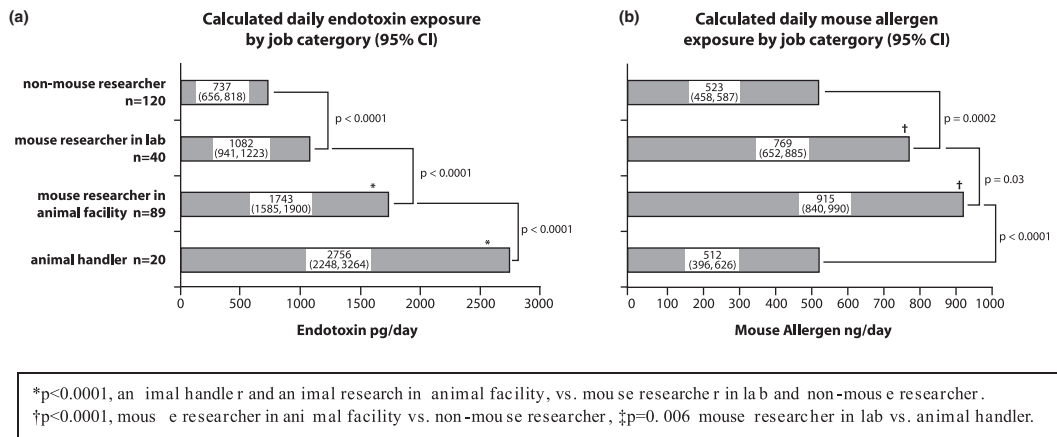


Fig. 3. Daily endotoxin (a) and mouse allergen (b) exposures were estimated based on the sum of the mean airborne concentrations for all tasks performed by the different job titles, multiplied by the number of hours spent daily in each task and adjusted for the calculated volume of air breathed in a day.

changing in Fig. 2a, peak particle counts lasted 35 min and then rapidly subsided, although sampling time was 110 min for endotoxin, and 198 min for mouse allergen. The highest counts were in the 1–5 and >5 μm size particles. Recalculated peak exposure to endotoxin was 1600 pg m^{-3} (1.6 ng m^{-3}) and 215 ng m^{-3} of mouse allergen. Figure 2b from the dirty cage wash area demonstrates rhythmic particle peaks, primarily in the <5 μm range. Samples were obtained over 2 h, however, conservatively estimated peak exposures > 1 count cm^{-3} covered only 104 min. Recalculated endotoxin exposure rose from 225 to 260 pg m^{-3} , and peak mouse allergen increased from 179 to 206 ng m^{-3} . Figure 2c shows particle counts obtained over 145 min during a mouse experiment, although counts > 1 cm^{-3} were observed only for 25 min. The endotoxin concentration recalculated to reflect peak particle counts was 9000 pg m^{-3} or 9 ng m^{-3} .

DISCUSSION

There remains uncertainty regarding the nature of complex aerosols generated in the laboratory animal environment. We previously found that the allergic and non-allergic respiratory diseases that develop in laboratory animal workers are differentially associated with the workers' exposures to mouse allergen versus endotoxin (Pacheco *et al.*, 2003). This observation prompted a more detailed investigation of the particulates generated in this work environment. Our data suggest that conventional measures of total airborne endotoxin and mouse allergen mass underestimate both exposures encountered by these workers. By performing real time analysis of particle size and count, we found that actual exposures in these environments are significantly higher, and more variable, than previously thought, and that there are sizeable bursts of exposures produced during cage

changing and direct mouse work. Importantly, exposures to endotoxin and to mouse allergen correlated most strongly in the setting of direct mouse care and experiments. However, airborne endotoxin was detected in settings where mouse allergen was not, whereas mouse allergen was always identified with endotoxin. This suggests that endotoxin may be encountered both with, and without mouse allergen, although part of this effect might be attributable to differential sensitivities of the two assays, and part due to sources of endotoxin separate from mice, such as the researchers themselves. In this study, neither endotoxin nor mouse allergen was strongly associated with any one particle size, although our samples were limited in number.

Other authors have also reported great variability in particle counts associated with mouse work (Ohman *et al.*, 1994; Gordon *et al.*, 1997). When we reanalyzed endotoxin and mouse allergen concentrations to reflect the timing of the peaks, exposures were substantial. Averaging exposures over the sampling time, for example with an 8 h mean, is thus likely to underestimate peak exposures. As has been shown in animal models for isocyanate induced respiratory allergy (Karol, 1983; Aoyama *et al.*, 1994), peak exposures to endotoxin and to mouse allergen may also be more important than mean exposures in triggering both symptoms and immunologic sensitization. It is possible that distinct peak exposures to endotoxin and mouse allergen, present on different particles and in different time periods, are responsible for the high rates of lab animal symptoms and sensitization in this workforce. We interpret our findings with caution, however, since we found no correlation between overall particle counts and air concentrations of endotoxin or mouse allergen. Combining real-time particle counts with more traditional and quantitative measures of particles, allergen and endotoxin

concentrations may be a more effective sampling strategy to determine workplace exposures relevant to disease.

Particle counts in all size ranges were low, and most were submicron in size. Particle counts in the 1–5 μm fraction rose during mouse care and research tasks, compared with the same areas without activity. Although endotoxin and mouse allergen concentrations correlated during direct mouse work, peak endotoxin and mouse allergen concentrations followed different patterns, and endotoxin was detected during all mouse research tasks even when mouse allergen was undetectable. Neither endotoxin nor mouse allergen was associated with any one particle size. Together, these data suggest that endotoxin and mouse allergen may be associated with particles of different size, charge or aerodynamic properties, which may require different control strategies.

The highest concentrations of endotoxin were detected during mouse experiments in both the animal facility and in research laboratories. The source of endotoxin in the research labs is most likely the mice, as some mice can spend up to a week sitting in a lab awaiting an experiment. Clean bedding and animal chow were likely not the source of endotoxin in these labs, as has been suggested for animal facilities (Lieutier-Colas *et al.*, 2001), as none are kept in the labs. In contrast to our findings, Lieutier-Colas found the highest concentrations of endotoxin associated with cage cleaning and feeding, and the lowest levels during handling rats in the experimental rooms. These differences in results may reflect different ventilation or work practices between the two institutions.

Mouse allergen concentrations were increased during mouse care activities and in all areas of mouse research labs. Unlike endotoxin, background concentrations fell sharply in the animal facility when mouse care activities were completed. Our results are analogous to the work of Gordon *et al.* who found high mouse allergen exposures during mouse handling and with indirect mouse lab work (Gordon *et al.*, 1994). Other studies have reported the highest concentrations of mouse allergen during cage cleaning (Eggleston *et al.*, 1989; Nieuwenhuijsen *et al.*, 1995; Hollander *et al.*, 1997; Hollander *et al.*, 1998). The high ambient mouse allergen in research labs may be due to performing research with mice on benches without engineering controls, and our results are comparable to those from other animal labs without specific ventilation (Thulin *et al.*, 2002). Mouse allergen detected in non-mouse labs was probably due to common air handling systems between floors.

Overall particle counts were highest in the submicron size range, typically considered to include products of combustion not associated with animal fur or bedding. However, in one study (Swanson *et al.*, 1990) 23% of rat allergen sampled in an animal

facility was found on particles $<1 \mu\text{m}$ in size. Another found cat, dog and birch allergen on submicron particles in homes (Ormstad, 2000). We speculate that these submicron particles in our facilities may also be important reservoirs of both mouse allergen and endotoxin.

We recognize there are limitations to our study. The real-time aerodynamic particle sizer does not efficiently measure particles $<1 \mu\text{m}$ in size, and may have mischaracterized those exposures. Our associations between particle size, endotoxin and mouse allergen concentrations were assessed by correlation, rather than by direct measurement using a cascade impactor. The use of the APS, however, had the advantage of demonstrating changes in particle counts over time during a task, which more accurately characterizes the changing exposures to a worker. The endpoint chromogenic LAL test may underestimate endotoxin concentrations, and the recovery of endotoxin from the PVC filters we used may have been lower than had we used glass fiber filters or polycarbonate filters. Our detected endotoxin exposures may thus be inaccurately low. They are, nonetheless, similar to airborne concentrations reported by other researchers (Milton *et al.*, 1990; Borm *et al.*, 1999; Lieutier-Colas *et al.*, 2001).

These findings have potentially important implications for our understanding of how endotoxin and allergens interact to produce immunologic responses and workplace symptoms. Variable endotoxin and mouse allergen particle mixtures may permit endotoxin to have different effects on the immune response to mouse allergen, depending on the pattern of exposure and other host susceptibility factors (Wan *et al.*, 2000). When taken in context with our previous study of the workers from this same environment, our current results suggest that in some workers, preferential exposure to endotoxin might trigger upper and lower respiratory symptoms, while dampening or ablating their allergic response to mice. In others, predominant early exposure to high concentrations of mouse allergen with low concentrations of endotoxin could accentuate the allergic or inflammatory response. It will be important to study these interactions in future research, attentive to the observation that the ‘laboratory animal worker environment’ is not a single entity, but a highly variable, complex mixture of particles that differ in size and in associated immunogenicity.

CONCLUSIONS

Airborne exposures in laboratory animal work environments are complex and highly variable mixtures of particulates, animal allergen and endotoxin. Particle counts vary greatly during work tasks, and show sizeable valleys and peaks that suggest similar

peak exposures to adsorbed allergen and/or endotoxin. Endotoxin and mouse allergen concentrations correlate during mouse-based experiments and mouse care activities, but are not associated with any one sized particle. Understanding the exposure characteristics of allergen and endotoxin, the temporal patterns of exposure, as well as different work practices in animal facilities and research labs, will guide more effective environmental control of these workplace hazards.

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