

# Airborne Endotoxin Predicts Symptoms in Non-Mouse-sensitized Technicians and Research Scientists Exposed to Laboratory Mice

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Research scientists, laboratory technicians, and animal handlers who work with animals frequently report respiratory and skin symptoms from exposure to laboratory animals (LA). However, on the basis of prick skin tests or RASTs, only half are sensitized to LA. We hypothesized that aerosolized endotoxin from mouse work is responsible for symptoms in nonsensitized workers. We performed a cross-sectional study of 269/310 (87%) workers at a research institution. Subjects completed a questionnaire and underwent prick skin tests ( $n = 254$ ) or RASTs ( $n = 16$ ) for environmental and LA allergens. We measured airborne mouse allergen and endotoxin in the animal facility and in research laboratories. Of 212 workers not sensitized to mice, 34 (16%) reported symptoms compared with 26 (46%) of mouse-sensitized workers ( $p < 0.001$ ). Symptomatic workers were more likely to be atopic, regardless of mouse sensitization status. Symptomatic non-mouse-sensitized workers spent more time performing animal experiments in the animal facility ( $p = 0.0001$ ) and in their own laboratories ( $p < 0.0001$ ) and had higher daily endotoxin exposure ( $p = 0.008$ ) compared with asymptomatic coworkers. In a multivariate model, daily endotoxin exposure most strongly predicted symptoms to mice in non-mouse-sensitized workers (odds ratio = 30.8,  $p = 0.003$ ). We conclude that airborne endotoxin is associated with respiratory symptoms to mice in non-mouse-sensitized scientists and technicians.

**Keywords:** endotoxin; respiratory allergy; asthma etiology; laboratory animals; laboratory personnel

More than 2 million workers in the United States, including research scientists, laboratory technicians, veterinarians and animal handlers, are exposed to laboratory animals (LA) in the course of their jobs (1). LA are used in the development of disease models and in the production of antibodies and other factors for research and treatment of human illness. Between 20 and 50% of workers exposed to LA report symptoms related to animal exposure. Nearly one-third of them have lost workdays due to workplace symptoms or were permanently removed from their jobs because of work-related animal symptoms (2–8). However, not all animal-related symptoms are due to allergy. In fact, between 38 and 67% of symptomatic workers do not demonstrate LA allergy

by either prick skin testing (PST) or RASTs to animal allergens (4, 7, 9–11). Although some animal-related eye, chest, and skin symptoms have been associated with higher allergen exposure and atopy in the host (3, 7), nasal symptoms often have not (12–14). These findings suggest that there may be environmental triggers for work-related symptoms other than animal allergens.

LA workers are exposed to a complex mixture of allergens and irritants, including endotoxin, volatile organic compounds, ammonia, and fecally contaminated particulates, among others. Past evidence suggests that volatile organic compounds and ammonia levels are generally below levels that should trigger health effects in animal facilities (15). Airborne endotoxin has been shown to induce respiratory symptoms (16–19), and in at least three prior studies of a LA workplace, airborne endotoxin was detected (20–22). We hypothesized that aerosolized endotoxin triggers nasal and other symptoms in technicians and research scientists during experimental work with and care of LA, particularly in those workers not sensitized to LA. This study addresses the discrepancy between symptoms and LA allergy and explores the role of endotoxin as well as mouse allergen in causing work-related symptoms.

## METHODS

### Subjects

All 310 animal handlers, laboratory-based research scientists and technicians employed at an academic research center, were eligible to participate. Job classifications included animal handlers, cage washers, administrators, laboratory technicians, clinical research co-ordinators, and research scientists, including pre- and postdoctoral students, associates, and principal investigators, regardless of duration of employment. The study was approved by the Institutional Review Board, and informed written consent was obtained from all subjects.

### Questionnaire

All 269 participants (87%) completed a 15-minute self-administered questionnaire that covered job titles, current and previous exposure to mice, hours per week in different work tasks, and nose, chest, and skin symptoms associated with mouse exposure. Work tasks in the animal facility included changing cages and feeding LA, washing dirty cages and setting up clean cages, conducting experimental work with animals in research rooms, and administrative tasks. Research building work tasks included animal and nonanimal research. The questionnaire elicited general respiratory and systemic symptoms in the past year and symptoms likely unrelated to animals. Questions on host risk factors included a history of allergy or asthma, a family history of atopy, pets in the home or other outdoor livestock, symptoms from exposure to pets, and a history of tobacco use.

### PST/RAST

Eligible subjects ( $n = 253$ ) underwent PST on the volar aspect of the right forearm to the same antigen lot of positive (histamine) (Center

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Pharmaceuticals, Pompano Beach, FL) and negative (saline) (Greer, Lenoir, NC) controls as well as to environmental allergens (mixed grasses, mixed weeds, [both Hollister-Stier, Spokane, WA], mixed dust mite [Geer], cat [Greer], acetone-precipitated dog [Hollister-Stier], and horse [Greer]). Subjects were tested for allergy to LA on the left forearm (mouse [Center Pharmaceuticals and Greer], rat [Greer], rabbit [Center Pharmaceuticals and Greer], hamster [Greer], and guinea pig [Center Pharmaceuticals and Greer]). A positive reaction was a wheal response 3 mm greater than the negative control. Sixteen subjects taking medications that interfered with histamine-induced skin reactivity provided 10 ml of blood for IgE-RAST testing to the same panel of allergens (Pharmacia-CAP; Pharmacia and Upjohn Diagnostics Inc., Uppsala, Sweden). A positive response was defined as more than 0.35 kU/L on the basis of laboratory standards.

### Air Sampling

We obtained two to seven side-by-side samples for endotoxin and mouse allergen quantitation in two parallel closed face cassettes attached immediately adjacent to the worker to two separate pumps set at different flow rates. Samples were obtained for major work tasks defined in the questionnaire in (1) the animal facility, (2) research laboratories adjacent to animal-based experiments, (3) the same room remote from animal experiments, and (4) laboratories that do not conduct animal research. Endotoxin was sampled at 3 L/minute with an SKC AirCheck pump, eluted from 37-mm polyvinyl chloride filters using pyrogen-free water with 0.05% TWEEN-20 and assayed by the FDA-standardized Limulus Amoebocyte Lysate test QCL 1000 (Bio-Whittaker, Walkersville, MD). Results are reported in endotoxin units converted to pg/m<sup>3</sup> on the basis of 1 endotoxin unit = 100 pg and sampling times and rates. Mouse allergen was sampled at 15 L/minute with a GAST 1031 pump, eluted from 37-mm Teflon (polytetrafluoroethylene) filters and assayed with a polyclonal mouse urine protein antibody (Greer) ELISA inhibition assay and reported in nanograms per cubic meter on the basis of sampling times and rates. Particulates were measured in real time using a model 3320 aerodynamic particle sizer (TSI Corp, St. Paul, MN).

**Exposure Estimates.** Exposure estimates for endotoxin and mouse allergen were calculated individually for each subject. Mean task-specific airborne endotoxin (pg/m<sup>3</sup>) or mouse allergen (ng/m<sup>3</sup>) concentrations were multiplied by the number of hours/week the subject reported performing the task. The sum of all tasks performed in a typical work-week was divided by 5 (pg- or ng-hours/m<sup>3</sup> day), and multiplied by a standard volume of air breathed in an hour (0.5 L V<sub>T</sub>/breath × 15 breaths/minute × 60 minutes/hour = 0.45 m<sup>3</sup>/hour) to create an estimate of the daily endotoxin exposure in picograms (23) and of the daily mouse allergen exposure in nanograms. This value was multiplied by an estimate of 18 days worked per month and by the reported months in the current job to create individual cumulative endotoxin or mouse allergen exposure variables.

### Definition of Variables

**Mouse-sensitized** subjects were those who had a positive reaction to mouse allergen by PST or RAST. **Mouse-symptomatic** individuals were those who checked "yes" to questions asking if they "have ever had symptoms associated with mice: a) itchy, runny, stuffy, nose; b) sneezing; c) cough; d) chest tight or wheeze; e) shortness of breath; f) skin rash." Subjects who reported respiratory symptoms in the past year not related to mouse exposure were classified as "asymptomatic to mice."

**Exposures.** **Direct mouse exposure** was defined as any work with mice in the current job; **indirect mouse exposure** was defined as exposure to mice when coworkers used them in the lab; and **previous work with mice** was defined as any mouse work in previous jobs or educational settings.

### Analytic Plan

**Distribution of variables and associations between mouse symptoms and exposures.** We separated workers into two mutually exclusive categories of not sensitized or sensitized to mice and symptomatic or not symptomatic to mice. We characterized the groups by demographic, risk, and exposure factors and compared the differences between groups using  $\chi^2$  tests and analysis of variance for continuous variables or the Wilcoxon rank-sum test for nonnormally distributed variables. The significance was set at 0.05.

**TABLE 1. DEMOGRAPHIC FEATURES AND ANIMAL EXPOSURES OF ANIMAL HANDLERS, RESEARCH SCIENTISTS, AND TECHNICIANS**

| Variable                                | n (%)        |
|-----------------------------------------|--------------|
| No. of workers, (%)                     | 269/310 (87) |
| Mean age, (range)                       | 36.5 (18–63) |
| Male sex (%)                            | 132 (49)     |
| Ethnicity (%)                           |              |
| White                                   | 206 (76)     |
| Asian                                   | 50 (19)      |
| Hispanic                                | 10 (4)       |
| African American                        | 3 (1)        |
| Job title (%)                           |              |
| Animal handler                          | 18 (6)       |
| Lab technician                          | 85 (32)      |
| Research scientist                      | 155 (58)     |
| Research co-ordinator                   | 7 (3)        |
| Administrators                          | 4 (2)        |
| Animal exposures and sensitization      |              |
| Work with mice (%)                      | 135 (50)     |
| Sensitized to mice (%)                  | 57 (21)      |
| Symptoms to mice (%)                    | 60 (22)      |
| PST/RAST+ to any allergen (%)           | 196 (73)     |
| PST/RAST+ to two or more allergens (%)  | 168 (62)     |
| Symptoms and sensitized/symptomatic (%) | 26/60 (43)   |

Definition of abbreviation: PST = prick skin testing.

**Multivariate model for determinants of symptoms to mice.** Host risk and personal exposure variables significantly associated with symptoms to mice by univariate analysis (using a cutoff p value < 0.1) were included in the multivariate models stratified by mouse sensitization status. The primary exposure variables of interest were daily endotoxin and mouse allergen. Each model used nominal logistic regression to evaluate the contribution of explanatory risk factors and exposure variables to symptoms to mice. Odds ratios for continuous variables reflect the odds of a subject exposed to 1 SD or more above the mean value for that variable reporting symptoms to mice.

## RESULTS

### Distribution of Worker Characteristics and Host Risk Factors

Table 1 presents demographic data on the study cohort, which constituted 87% of the eligible workforce. Most workers were research scientists or laboratory technicians, and only 18 persons worked as animal handlers. Half of our cohort worked directly with mice. Almost three-quarters of the population was allergic to one or more environmental or animal allergens, and 62% were allergic to two or more environmental or animal allergens. Fifty-seven workers (21%) were positive for mouse sensitivity by PST, and sixty workers (22%) reported symptoms to mice. Of the 16 subjects evaluated by RAST, half reported symptoms to mice although none reacted to mouse allergen. Only 26 of the 60 (43%) mouse-symptomatic individuals were sensitized to mice.

We stratified workers as either sensitized or nonsensitized to mice and compared mouse-symptomatic with non-mouse-symptomatic subjects for type of symptoms and allergy risk factors (Table 2). Most workers were not sensitized to mice (n = 212, 79%). Significantly more mouse-sensitized workers reported symptoms to mice than did non-mouse-sensitized workers (46% [26/57] vs. 16% [34/212]). Age, sex, and ethnicity were similar in both groups of non-mouse-sensitized workers (data not shown). In the mouse-sensitized group, symptomatic workers were older (mean 41.9 vs. 35.6 years, p = 0.002) and were more likely to be female (59% of women had symptoms vs. 33% of males, p = 0.005).

The distribution of symptoms to mice was similar in both mouse-sensitized and non-mouse-sensitized workers. Both symp-

**TABLE 2. DISTRIBUTION OF REPORTED SYMPTOMS AND ALLERGY RISK FACTORS FOR WORKERS STRATIFIED BY SENSITIZATION AND SYMPTOMS TO MICE**

|                                          | Number of Workers (%)             |                  |         |                              |                  |         |
|------------------------------------------|-----------------------------------|------------------|---------|------------------------------|------------------|---------|
|                                          | Non-mouse-sensitized<br>(n = 212) |                  |         | Mouse-sensitized<br>(n = 57) |                  |         |
|                                          | No Symptoms to Mice               | Symptoms to Mice | p Value | No Symptoms to Mice          | Symptoms to Mice | p Value |
| Total number of workers (%)              | 178 (84)                          | 34 (16)          |         | 31 (54)                      | 26 (46)          | < 0.001 |
| Risk factors (%)                         |                                   |                  |         |                              |                  |         |
| Diagnosis of allergy made by a physician | 69 (39)                           | 26 (76)          | < 0.001 | 12 (39)                      | 18 (69)          | 0.020   |
| Diagnosis of asthma made by a physician  | 16 (9)                            | 14 (44)          | < 0.001 | 6 (19)                       | 10 (38)          | 0.109   |
| Family history of atopy                  | 101 (57)                          | 23 (68)          | 0.232   | 17 (55)                      | 20 (77)          | 0.079   |
| Aeroallergies by PST/RAST                | 113 (63)                          | 21 (62)          | 0.849   | 24 (77)                      | 24 (92)          | 0.114   |
| Pet allergy by PST/RAST                  | 76 (43)                           | 19 (56)          | 0.158   | 19 (61)                      | 23 (88)          | 0.017   |
| Current tobacco use                      | 9 (5)                             | 0                | 0.073   | 3 (10)                       | 3 (11)           | 0.820   |
| Previous tobacco use                     | 49 (27)                           | 7 (21)           | 0.390   | 11 (35)                      | 5 (19)           | 0.169   |
| Reported symptoms to mice                |                                   |                  |         |                              |                  |         |
| Nasal symptoms                           |                                   | 30 (88)          |         |                              | 23 (88)          | 0.978   |
| Chest symptoms                           |                                   | 9 (26)           |         |                              | 9 (35)           | 0.496   |
| Skin symptoms                            |                                   | 6 (18)           |         |                              | 9 (35)           | 0.134   |

Definition of abbreviation: PST = prick skin testing.

tomatic groups reported nasal and chest symptoms, although more mouse-sensitized workers reported skin symptoms (35 vs. 18%). Interestingly, significantly more symptomatic workers reported a diagnosis of allergy made by a physician irrespective of whether they were sensitized to mice. Non-mouse-sensitized workers were also more likely to report a diagnosis of asthma made by a physician. However, other markers of allergy such as a family history of atopy or a positive PST or RAST to pets were more frequent only in symptomatic mouse-sensitized workers. It is possible that in general atopic persons report more daily symptoms than do their nonatopic coworkers. However, atopic workers were not more likely than nonatopic workers to report symptoms unrelated to mouse exposure (data not shown), including upset stomach ( $p = 0.412$ ), joint aches ( $p = 0.429$ ), or flu-like symptoms ( $p = 0.160$ ). Current and previous tobacco use was similarly distributed among symptom groups, except that symptomatic non-mouse-sensitized workers were less likely to smoke currently.

#### Airborne Endotoxin and Mouse Allergen

Airborne endotoxin was detected throughout the animal care facility and in research laboratories, although levels varied even

among repeated samples in the same location (Table 3). Over 90% of particles were in the submicron particle range of less than 1  $\mu\text{m}$ . We also found mouse allergen throughout, with lesser variability between repeated samples. Samples reported as arithmetic or as geometric means were not normally distributed. Because we had limited sampling data and because we hypothesized that peak exposures were likely to influence symptom reporting, we reported arithmetic means for both endotoxin and mouse allergen concentrations. Endotoxin and mouse allergen concentrations sampled at the same time did not correlate in a pairwise analysis ( $n = 46$ , pairwise correlation 0.011,  $p = 0.946$ , Spearman  $\rho$  0.15,  $p = 0.328$ ). However, calculated individual endotoxin and mouse allergen exposures did correlate ( $n = 269$ , pairwise correlation 0.580,  $p < 0.0001$ , Spearman  $\rho$  0.750,  $p < 0.0001$ ).

#### Reported Job Tasks and Work with Mice

We compared length of employment, work in different settings, hours per week spent in animal-related and research activities, and work with mice between symptomatic and asymptomatic workers (Table 4). Mouse-symptomatic workers reported longer

**TABLE 3. AIR CONCENTRATIONS OF ENDOTOXIN AND MOUSE ALLERGEN DURING SPECIFIC WORK TASKS AND IN SPECIFIC WORK SITES**

|                               | Endotoxin |                              |                               | Mouse Allergen |                              |                               |
|-------------------------------|-----------|------------------------------|-------------------------------|----------------|------------------------------|-------------------------------|
|                               | n*        | Mean<br>(pg/m <sup>3</sup> ) | Range<br>(pg/m <sup>3</sup> ) | n*             | Mean<br>(ng/m <sup>3</sup> ) | Range<br>(ng/m <sup>3</sup> ) |
| Animal care facility          |           |                              |                               |                |                              |                               |
| Cage change                   | 4         | 315                          | 203–678                       | 2              | 65                           | 51–78                         |
| Cage wash dirty               | 4         | 305                          | 110–471                       | 3              | 75                           | 14–179                        |
| Cage wash clean               | 2         | 219                          | 24–414                        | 3              | 38                           | 0–92                          |
| Experimental rooms            | 7         | 566                          | 12–1,463                      | 5              | 40                           | 0–113                         |
| Administrative offices        | 3         | 157                          | 70–233                        | 3              | 10                           | 0–29                          |
| Research building #1          |           |                              |                               |                |                              |                               |
| Lab uses animals              |           |                              |                               |                |                              |                               |
| With animal experiment        | 6         | 228                          | 93–363                        | 2              | 75                           | 49–98                         |
| Away from animal experiment   | 5         | 71                           | 10–204                        | 5              | 44                           | 28–82                         |
| Nonanimal lab with experiment | 2         | 66                           | 25–108                        | 2              | 35                           | 33–37                         |
| Research building #2          |           |                              |                               |                |                              |                               |
| Lab uses animals              |           |                              |                               |                |                              |                               |
| With animal experiment        | 3         | 97                           | 46–187                        | 3              | 79                           | 55–121                        |
| Away from animal experiment   | 4         | 119                          | 14–432                        | 4              | 92                           | 25–271                        |
| Nonanimal lab with experiment | 3         | 29                           | 10–57                         | 3              | 24                           | 19–31                         |

\* Number = number of samples taken.

**TABLE 4. REPORTED ANIMAL EXPOSURES AND JOB TASKS, AND CALCULATED EXPOSURES TO ENDOTOXIN AND MOUSE ALLERGEN IN NONSENSITIZED AND SENSITIZED WORKERS**

|                                          | Non-mouse-sensitized<br>(n = 212) |                  |                 | Mouse-sensitized<br>(n = 57) |                  |                |
|------------------------------------------|-----------------------------------|------------------|-----------------|------------------------------|------------------|----------------|
|                                          | No Symptoms to Mice               | Symptoms to Mice | p Value         | No Symptoms to Mice          | Symptoms to Mice | p Value        |
| Number of subjects (%)                   | 178 (84)                          | 34 (16)          |                 | 31 (54)                      | 26 (46)          | < <b>0.001</b> |
| Reported mean months in job (range)      | 55 (1–359)                        | 69 (2–291)       | 0.1105          | 53 (4–288)                   | 92 (1.5–286)     | <b>0.017</b>   |
| Reported exposure location               |                                   |                  |                 |                              |                  |                |
| Any work in animal care facility (%)     | 63 (35)                           | 22 (65)          | < <b>0.001</b>  | 9 (29)                       | 15 (58)          | 0.058          |
| Work with mice in laboratory (%)         | 27 (15)                           | 7 (21)           |                 | 3 (10)                       | 3 (11)           |                |
| No work with mice (%)                    | 88 (49)                           | 5 (15)           |                 | 19 (61)                      | 8 (31)           |                |
| Reported job tasks, h/wk                 |                                   |                  |                 |                              |                  |                |
| Cage feed dump                           | 2.1                               | 2.5              | <b>0.003</b>    | 2.8                          | 0.8              | 0.350          |
| Animal experiments in animal facility    | 1.0                               | 3.2              | <b>0.0001</b>   | 0.9                          | 1.4              | <b>0.036</b>   |
| Experimental work in lab with animals    | 1.3                               | 4.2              | < <b>0.0001</b> | 1.0                          | 1.2              | 0.115          |
| Experimental work in lab without animals | 33.1                              | 32.2             | 0.645           | 36.8                         | 26.5             | 0.005          |
| Workers reporting mouse exposure, %      |                                   |                  |                 |                              |                  |                |
| Direct (%)                               | 81 (45)                           | 27 (79)          | < <b>0.001</b>  | 8 (26)                       | 17 (65)          | <b>0.002</b>   |
| Indirect (%)                             | 124 (70)                          | 26 (76)          | 0.416           | 19 (61)                      | 16 (61)          | 0.985          |
| Previous (%)                             | 91 (51)                           | 24 (71)          | <b>0.034</b>    | 13 (42)                      | 17 (65)          | 0.076          |
| Measured exposure levels                 |                                   |                  |                 |                              |                  |                |
| Daily endotoxin, pg                      | 398                               | 531              | <b>0.008</b>    | 414                          | 350              | 0.748          |
| Cumulative endotoxin, pg                 | 305,579                           | 627,140          | <b>0.008</b>    | 358,880                      | 544,753          | 0.396          |
| Daily mouse allergen, ng                 | 219                               | 244              | 0.207           | 247                          | 192              | 0.160          |
| Cumulative mouse, ng                     | 185,900                           | 285,685          | <b>0.022</b>    | 209,542                      | 287,262          | 0.701          |

tenure in their jobs than did asymptomatic workers. Significantly more mouse-symptomatic subjects performed experiments in the animal care facility, whereas significantly more asymptomatic subjects performed nonanimal based research. When workers reported the hours they spent each week in different research tasks, symptomatic non-mouse-sensitized workers spent more hours feeding and changing cages ( $p = 0.003$ ), performing animal experiments in the animal facility ( $p = 0.0001$ ), and in their own labs ( $p < 0.0001$ ) compared with asymptomatic workers. Symptomatic mouse-sensitized workers also spent longer hours in animal research in the animal facility ( $p = 0.036$ ) and fewer hours per week caring for animals or in laboratory work without mice ( $p = 0.005$ ). More symptomatic workers reported direct

and previous contact with mice in both the non-mouse-sensitized and mouse-sensitized groups. Those reporting indirect exposure to mice did not differ between symptom groups.

#### Measured Endotoxin and Mouse Allergen

We compared calculated measures of endotoxin and mouse allergen between symptomatic and asymptomatic subjects (Table 5). Endotoxin exposure differences were significant only in the non-mouse-sensitized group. The daily endotoxin dose was higher in those workers reporting any symptoms to mice ( $p = 0.008$ ), and cumulative endotoxin exposure in symptomatic workers was twice that of asymptomatic ones ( $p = 0.008$ ). Although daily mouse allergen did not differ significantly between those with

**TABLE 5. ASSOCIATION BETWEEN SYMPTOMS AND CALCULATED ENDOTOXIN AND MOUSE ALLERGEN EXPOSURES**

|                                             |              | Daily Endotoxin |              | Cumulative Endotoxin |              | Daily Mouse Allergen |              | Cumulative Mouse Allergen |              |
|---------------------------------------------|--------------|-----------------|--------------|----------------------|--------------|----------------------|--------------|---------------------------|--------------|
|                                             |              | (pg)            | p Value      | (pg)                 | p Value      | (ng)                 | p Value      | (ng)                      | p Value      |
| Subjects not sensitized to mice,<br>n = 212 |              |                 |              |                      |              |                      |              |                           |              |
| Any symptom to mice                         | No (n = 178) | 398             | <b>0.008</b> | 305,579              | <b>0.008</b> | 219                  | 0.207        | 185,900                   | <b>0.022</b> |
|                                             | Yes (n = 34) | 531             |              | 627,140              |              | 244                  |              | 285,685                   |              |
| Nasal symptoms to mice                      | No (n = 182) | 402             | <b>0.039</b> | 320,126              | <b>0.047</b> | 221                  | 0.391        | 192,860                   | 0.101        |
|                                             | Yes (n = 30) | 528             |              | 581,765              |              | 237                  |              | 256,767                   |              |
| Chest symptoms to mice                      | No (n = 203) | 406             | <b>0.002</b> | 338,892              | <b>0.011</b> | 218                  | <b>0.013</b> | 192,294                   | <b>0.034</b> |
|                                             | Yes (n = 9)  | 723             |              | 768,975              |              | 332                  |              | 418,647                   |              |
| Skin symptoms to mice                       | No (n = 206) | 417             | 0.062        | 338,223              | <b>0.007</b> | 222                  | 0.329        | 193,296                   | <b>0.010</b> |
|                                             | Yes (n = 6)  | 531             |              | 1,006,980            |              | 269                  |              | 497,402                   |              |
| Subjects are sensitized to mice,<br>n = 57  |              |                 |              |                      |              |                      |              |                           |              |
| Any symptom to mice                         | No (n = 31)  | 414             | 0.748        | 358,880              | 0.396        | 247                  | 0.160        | 209,542                   | 0.701        |
|                                             | Yes (n = 26) | 350             |              | 544,753              |              | 192                  |              | 287,262                   |              |
| Nasal symptoms to mice                      | No (n = 34)  | 412             | 0.444        | 424,241              | 0.909        | 240                  | 0.309        | 240,286                   | 0.961        |
|                                             | Yes (n = 23) | 345             |              | 472,377              |              | 196                  |              | 251,953                   |              |
| Chest symptoms to mice                      | No (n = 48)  | 395             | 0.346        | 442,902              | 0.818        | 228                  | 0.661        | 242,074                   | 0.751        |
|                                             | Yes (n = 9)  | 334             |              | 447,727              |              | 192                  |              | 260,564                   |              |
| Skin symptoms to mice                       | No (n = 48)  | 380             | 0.264        | 381,923              | <b>0.045</b> | 219                  | 0.630        | 212,401                   | <b>0.022</b> |
|                                             | Yes (n = 9)  | 441             |              | 772,953              |              | 236                  |              | 418,822                   |              |

**TABLE 6. UNIVARIATE ANALYSIS OF FACTORS PREDICTING SYMPTOMS TO MICE IN NON-MOUSE-SENSITIZED AND MOUSE-SENSITIZED WORKERS**

| Univariate Analysis                                                        | OR   | 95% CI      | p Value  |
|----------------------------------------------------------------------------|------|-------------|----------|
| Factors for workers nonsensitized to mice, n = 212                         |      |             |          |
| Months of employment, mean 57, SD 72                                       | 2.4  | 0.41, 11.9  | 0.300    |
| Work with mice, y/n                                                        | 4.6  | 2.0, 12.0   | 0.0007   |
| Previous work with mice, y/n                                               | 2.3  | 1.1, 5.3    | 0.040    |
| Diagnosis of allergy made by a physician, y/n                              | 5.1  | 2.3, 12.7   | 0.0002   |
| Diagnosis of asthma made by a physician, y/n                               | 8.0  | 3.4, 18.9   | < 0.0001 |
| Symptoms to pets (y/n)                                                     | 2.2  | 1.0, 4.7    | 0.058    |
| Duration of experiments in animal facility, h/wk, mean 1.4 h, SD 3.6 h     | 9.3  | 1.9, 49.3   | 0.006    |
| Duration of animal experiments in own lab, h/wk, mean 1.7 h, SD 4.2 h      | 10.3 | 2.6, 44.7   | 0.001    |
| Daily endotoxin, mean 420 pg, SD 291                                       | 8.1  | 1.4, 46.5   | 0.018    |
| Cumulative endotoxin, mean 357150 ng, SD 510943                            | 18.1 | 2.8, 131    | 0.003    |
| Factors for workers sensitized to mice, n = 57                             |      |             |          |
| Age, mean 38, SD 10                                                        | 15.2 | 1.5, 207.4  | 0.028    |
| Sex, male                                                                  | 2.9  | 1.01, 8.8   | 0.052    |
| Months of employment, mean 71, SD 75                                       | 9.0  | 1.1, 116.1  | 0.060    |
| Work with mice, y/n                                                        | 5.4  | 1.8, 17.9   | 0.004    |
| Previous work with mice, y/n                                               | 2.6  | 0.9, 7.9    | 0.080    |
| Diagnosis of allergy made by a physician, y/n                              | 3.6  | 1.2, 11.2   | 0.024    |
| Family history of atopy, y/n                                               | 2.7  | 0.9, 9.2    | 0.086    |
| PST positive to pets, y/n                                                  | 4.8  | 1.3, 23.6   | 0.028    |
| Symptoms to pets, y/n                                                      | 4.6  | 1.5, 14.8   | 0.007    |
| Duration of nonanimal experiments in own lab, h/wk, mean 32.1 h, SD 15.2 h | 0.05 | 0.003, 0.49 | 0.016    |
| Daily mouse allergen, mean 222 pg, SD 127                                  | 0.24 | 0.04, 1.3   | 0.104    |

Definition of abbreviations: CI = confidence interval; OR = odds ratio; PST = prick skin testing.

and without symptoms, cumulative mouse allergen exposure was higher in symptomatic non-mouse-sensitized workers ( $p = 0.0224$ ), although this may reflect the effect of longer months of employment. In contrast, mouse-sensitized symptomatic workers were exposed to lower daily endotoxin and mouse allergen than were asymptomatic workers, and cumulative endotoxin and mouse allergen exposures did not differ between symptom groups. This may reflect symptomatic workers moving out of exposure.

#### Association Between Specific Symptoms to Mice and Endotoxin and Mouse Allergen Exposure

We also evaluated which specific mouse-triggered symptoms were associated with higher endotoxin and mouse allergen exposures (Table 5). Of the 212 workers not sensitized to mice, workers reporting nasal symptoms to mice had significantly higher daily and cumulative endotoxin exposure than did those reporting no symptoms. Daily mouse allergen exposures did not differ significantly between the symptomatic and asymptomatic groups, although cumulative mouse allergen exposures did. In contrast, both endotoxin and mouse allergen exposures were associated with chest symptom reporting. Skin symptoms to mice were not associated with higher daily endotoxin or mouse allergen exposures, but they were associated with significantly higher cumulative endotoxin and mouse allergen exposures.

Interestingly, we could not demonstrate any significant associations in mouse-sensitized workers between mouse-triggered nasal or chest symptoms and daily or cumulative endotoxin or mouse allergen exposure. In fact, daily endotoxin and mouse allergen exposures were lower in symptomatic workers than in asymptomatic workers. Workers with skin symptoms to mice were exposed to higher daily and cumulative endotoxin and mouse allergen concentrations, but only differences in cumulative measures were significant.

We analyzed whether general symptoms reported in the past year, not related to mouse exposure, were associated with calculated endotoxin or mouse allergen exposure. Nose, chest, and skin symptoms were associated neither with endotoxin exposure

nor with mouse allergen exposure in non-mouse-sensitized or in mouse-sensitized workers (data not shown).

#### Multivariate Models for Prediction of Reactions to Mice

Demographic data, job description information including job title, length of employment and specific job tasks related to mouse work and to laboratory research, allergy risk factors, (PST or RAST results), and calculated endotoxin and mouse allergen exposures were first evaluated individually in univariate models (Table 6). We then incorporated those univariate factors significantly ( $p < 0.1$ ) associated with symptoms to mice into multivariate models for non-mouse-sensitized and mouse-sensitized workers and developed separate final multivariate models that included only significant predictor variables for each group of workers.

#### Multivariate Model for Non-mouse-sensitized Workers

Daily endotoxin exposure was the most significant predictor of symptoms to mice in non-mouse-sensitized workers. Several factors covaried with daily endotoxin exposure, including reporting any "work with mice," hours/week spent in experiments in the animal facility and hours/week spent in animal experiments in the worker's own laboratory. We retained daily endotoxin in the final model as it was the exposure of primary interest, and the odds ratio for mouse symptoms associated with endotoxin exposure was three to five times greater than those associated with the covariables in the multivariate model (data not shown). Cumulative endotoxin was also significantly associated with reports of symptoms to mice. Because this variable reflected both daily endotoxin and months of employment, we evaluated "months of employment" in a multivariate model and whether it modified the effect of endotoxin on predicting symptoms to mice. Longer duration of employment was a significant risk factor for symptoms to mice, suggesting a cumulative dose-response relationship. The effect of endotoxin on symptom reports was also significantly increased in those with longer months of employment, although the confidence intervals of this interaction term are wide and the estimate is unstable. A diagnosis of allergy

**TABLE 7. MULTIVARIATE ANALYSIS OF FACTORS PREDICTING SYMPTOMS TO MICE IN NON-MOUSE-SENSITIZED AND IN MOUSE-SENSITIZED WORKERS**

| Multivariate Analysis                              | OR    | 95% CI      | p Value  |
|----------------------------------------------------|-------|-------------|----------|
| Factors for workers nonsensitized to mice, n = 212 |       |             |          |
| Daily endotoxin, mean 420 pg, SD 291               | 30.8  | 3.4, 318.2  | 0.003    |
| Months employment, mean 57, SD 72                  | 9.7   | 1.1, 82.1   | 0.035    |
| Diagnosis of asthma made by a physician, y/n       | 8.1   | 3.0, 22.2   | < 0.0001 |
| Diagnosis of allergy made by a physician, y/n      | 3.9   | 1.5, 11.0   | 0.006    |
| Daily endotoxin × months employment                | 245.9 | 5.1, 25,596 | 0.010    |
| Factors for workers sensitized to mice, n = 57     |       |             |          |
| Work with mice, y/n                                | 52.9  | 7.9, 703.6  | 0.0004   |
| Diagnosis of allergy made by a physician, y/n      | 9.0   | 1.9, 65.6   | 0.011    |
| Daily mouse allergen, mean 222 pg, SD 127          | 0.02  | 0.0005, 0.2 | 0.008    |

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

made by a physician and a diagnosis of asthma made by a physician, each independently, predicted symptoms. Symptoms to pets lost significance when it was included with other markers of allergy in a multivariate model. Having a pet in the home did not contribute to symptoms to mice in the workplace (odds ratio = 1.1 [0.5, 2.4]). Daily mouse allergen exposure did not predict symptoms to mice in the non-mouse-sensitized workers (odds ratio = 2.31 [0.49, 11.12],  $p = 0.29$ ).

#### Multivariate Model for Mouse-sensitized Workers

Age, sex (male), and months of employment were not significant predictors of symptoms in the multivariate model. A diagnosis of allergy made by a physician strongly predicted reactions to mice in mouse-sensitized workers when covariables such as family history of atopy, PST or RAST reaction to pet allergens, and symptoms to pets were eliminated from the model. Reporting any current work with mice was the strongest predictor of symptoms, although those with symptoms to mice tended to avoid mice and had lower daily mouse allergen exposure than mouse-sensitized workers who did not report symptoms to mice. Workers who spent more time in nonanimal experiments were less likely to report symptoms to mice, although this was not significant in the final model. Daily endotoxin exposure did not predict symptoms in these workers (odds ratio = 0.29, [0.02, 3.13],  $p = 0.322$ ).

We found no significant confounders or effect modifiers in the multivariate models other than the interaction of endotoxin with length of employment in the non-mouse-sensitized workers only. Neither was cigarette smoking significantly associated with symptoms to mice nor were interaction terms that included current or former smoker and markers of atopy or interaction terms that included current or former smoker and endotoxin or mouse allergen exposure. In addition, smoking status did not modify the symptom response to endotoxin or to mouse allergen.

#### DISCUSSION

We report that daily endotoxin exposure strongly predicts symptoms to mice in non-mouse-sensitized animal handlers, research scientists, and laboratory technicians. In our population, 22% of workers reported symptoms to mice although only 43% of these were sensitized to mice. Nasal symptoms were the most frequently reported, followed by chest and skin symptoms. This distribution corresponds to other published reports (3, 6, 7) and suggests that our cohort of symptomatic and sensitized workers is typical of LA workers.

A strength of our study is that we obtained direct evidence of sensitization by PST or by RAST on all individuals. This enabled us to categorize workers on the basis of sensitive and specific tests for environmental and animal allergies rather than

relying on family history or symptom reporting alone. We used a screening mix of allergens that may have not included the relevant allergens for all subjects and might reduce the number of atopic workers we identified. However, our rates of atopy are similar to those reported in some recent studies (24–27), although they are somewhat higher than in others. This may reflect selection bias in that individuals attracted to work at an institution that specializes in allergic and pulmonary disorders may be more likely to be atopic themselves. We used two different extracts for mouse that increased our ability to capture as many sensitized individuals as possible. It is possible that some individuals sensitized to mice did not react to the epitopes contained in the commercially available allergen preparations, though this is likely to be a small proportion of our cohort. Our ability to accurately establish the mouse sensitization status of all individuals increased our power to explore dose–response relationships in different subgroups. The significant relationship between nasal and chest symptoms and endotoxin in our study may have been obscured in previous studies that did not establish the mouse sensitization status of all participants.

We measured mouse allergen and endotoxin concurrently during different mouse-related tasks and in different research settings. Endotoxin measurements varied greatly in the same setting and between different settings, whereas mouse allergen did not. Interestingly, endotoxin and mouse allergen did not correlate in our sampling. This suggests that these substances may bind to different particles having different aerodynamic properties or may persist differently in the environment. When particle counts were analyzed by size, less than 1  $\mu\text{m}$ , 1 to 5  $\mu\text{ms}$ , and more than 5  $\mu\text{ms}$ , over 90% were in the fine particle range of less than 1  $\mu\text{m}$ . These particles are more likely to deposit in the lower airways. We found only three published reports on endotoxin exposure in animal care settings (20–22), and exposure levels were similar to those we report. In our study, we chose to report the arithmetic mean of the endotoxin measurements and used it to create an exposure variable for each subject. In our opinion, the arithmetic mean better reflects excursions in exposure that may be important for intermittent symptom generation.

Previous studies report that nasal symptoms to mice or to rats are poorly associated with animal allergen exposure (6, 13). We found that nasal symptoms to mice reported by non-mouse-sensitized workers were strongly associated with both current and cumulative endotoxin exposure but not with mouse allergen. Interestingly, chest symptoms were associated with both endotoxin and mouse allergen exposures. These symptoms may have occurred in response to the irritant properties of endotoxin and not to allergen. It is possible that some PST-negative symptomatic workers may have developed airways IgE to animal allergens

that preceded a systemic IgE response detectable by PST. Symptoms to mice were also associated with locations of exposure to high endotoxin concentrations, including time spent in the research rooms of the animal facility and time spent in mouse research in individual laboratories.

Endotoxin has been shown to trigger respiratory symptoms and functional abnormalities in other occupational settings, including grain, cotton, poultry, and hog confinement workers (28–35). Endotoxin induces airflow obstruction, increased bronchial reactivity (30, 31) and symptoms of cough, dyspnea, chest tightness, sputum production and nasal stuffiness associated with an influx of neutrophils and upregulation of proinflammatory cytokines (36–38). Respiratory challenge with endotoxin increases bronchial hyper-reactivity and reproduces the upper and lower respiratory symptoms reported by these workers (28). Our study demonstrates an association between nasal and chest symptoms and higher endotoxin exposure in LA workers that has not been previously reported.

The levels of endotoxin associated with symptoms to mice in our study are lower than those reported to trigger symptoms in other settings (39). However, our results correspond to other published data on animal facilities (20–22), suggesting that these levels are reflective of actual exposures. In addition, the threshold dose for endotoxin effects has not yet been determined. The lowest dose of airborne endotoxin associated with airflow obstruction is between 64 pg/m<sup>3</sup> and 9 ng/m<sup>3</sup> (39). The mean dose of endotoxin associated with nasal symptoms to mice in our study was 528 pg/m<sup>3</sup> and ranged from 13 to 1,557 pg/m<sup>3</sup>. The dose associated with chest symptoms to mice was 723 pg/m<sup>3</sup> with a range of 428 to 1,462 pg/m<sup>3</sup>. These estimated endotoxin doses are within the ranges associated with symptom reports in other studies. Our results suggest that endotoxin triggers symptoms at lower concentrations and that such exposures deserve further study.

It is possible that our individual dose estimates of endotoxin, using hours reported each week for mouse-related and research tasks, may have been inaccurate. The average time spent per task per week is imprecise, as research may involve very intense exposure for weeks to months, followed by little to no animal exposure. Creating a cumulative exposure variable by extrapolating from current exposure also ignores fluctuations in exposure over time. However, the misclassification of exposure was likely equally distributed among all subjects. Using a standard volume of air breathed per workday may also introduce misclassification, as it varies by individual height, sex, and ethnicity. However, estimating differences for individuals would introduce more errors that we could not verify. Converting endotoxin and mouse allergen into an average daily exposure in picograms or nanograms per day had the advantage of estimating a dose that could be compared with other endotoxin-rich environments. Verification of these exposure estimates awaits further study.

The average daily exposure to mouse allergen predicted only chest symptoms to mice in non-mouse-sensitized workers but not nasal or skin symptoms. The concentrations of mouse allergen we measured are similar to those reported in other studies (40), suggesting that they accurately reflect work exposures. In mouse-sensitized workers, daily mouse allergen was *lower* in symptomatic workers. This finding may reflect a healthy worker effect in that sensitized workers may decrease their mouse exposure to avoid symptoms or that lower exposure to mouse allergen may trigger symptoms in the already sensitized host. Although direct work with mice predicted symptoms in mouse-sensitized workers, indirect exposure to mice did not.

Workers who reported symptoms to mice tended to be atopic irrespective of whether they were sensitized to mice. We considered that atopic persons in general might report more daily

symptoms than their nonatopic coworkers. However, atopic and nonatopic workers in this study reported symptoms unrelated to LA exposure at comparable rates. Workers who reported general nose, chest, or skin symptoms in the past year did not have higher endotoxin or mouse allergen exposures. Thus we interpret the relationship between higher daily endotoxin exposure and mouse-related symptoms to accurately reflect a specific association. Atopic subjects may be more reactive to endotoxin than nonatopic coworkers because of primed and upregulated antigen presenting cells, activation markers, or adhesion molecules expressed on respiratory airway cells or adjacent vascular endothelium. Some studies of endotoxin-challenged volunteers have demonstrated that atopic subjects respond with significantly more nasal eosinophils (41) and subjects with asthma express significantly higher constitutive sCD14 in bronchoalveolar lavage that correlates with polymorphonuclear leukocyte influx (42) as compared with subjects without asthma. Other studies, however, have not demonstrated increased endotoxin responsiveness in atopics (43, 44). Investigation of the role of atopy-associated differences in host response to endotoxin merits further study (45–47).

Our study was cross-sectional, which allows us only to describe associations between exposures and symptoms rather than demonstrate cause and effect. Future challenge studies using doses of endotoxin and mouse allergen similar to those we have measured may confirm these findings and elucidate mechanisms.

In summary, we report that endotoxin exposure is significantly associated with mouse-triggered symptoms in non-mouse-sensitized exposed workers. Research work with mice in the animal facility as well as in research laboratories elicit the most symptoms. Mouse allergen exposure does not predict symptoms in these workers. Atopic individuals are more likely to report symptoms related to mouse exposure irrespective of whether they are sensitized to mice. Further assessment of endotoxin concentrations, together with better control of endotoxin exposure, may diminish rates of work-related symptoms to mice.

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