

Reducing Exposure to Laboratory Animal Allergens

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Laboratory animal allergy is a serious health problem. We examined several possible allergen-reducing strategies that might be effective in the working mouse room. Ambient allergen concentrations were measured when mice were maintained under several conditions: conventional housing versus ventilated cage racks operated under negative or positive pressure. We found that housing mice in ventilated cages operated under negative pressure and using ventilated changing tables reduced ambient mouse allergen (Mus m 1) concentrations tenfold, compared with values when mice were housed in conventional caging and using a conventional (non-ventilated) changing table. Housing mice in positively pressurized cages versus conventional cages did not reduce ambient allergen values. Cleaning mouse rooms at an accelerated frequency also did not reduce ambient Mus m 1 concentration. We also quantified ambient allergen values in several areas of The Jackson Laboratory. A facility-wide survey of Mus m 1 concentrations indicated that allergen concentrations were undetectable in control areas, but ranged from a mean ($\pm$ SEM) 0.11 ± 0.02 ng/m³ to 5.40 ± 0.30 ng/m³ in mouse rooms with different cage types. The percentage of animal caretakers reporting allergy symptoms correlated significantly with ambient allergen concentrations: 12.9% reported symptoms in the rooms with the lowest allergen concentration (0.14 ± 0.02 ng/m³), but 45.9% reported symptoms in rooms with the highest concentration (2.3 ± 0.4 ng/m³). These data indicate that existing technology can significantly reduce exposure to laboratory animal allergens and improve the health of animal caretakers.

More than 90,000 workers in US scientific laboratories and rodent breeding facilities are exposed to rodent allergens each year (22). Cross-sectional surveys indicate that 10 to 46% of these exposed workers develop laboratory animal allergy (LAA) and, of those, 10% develop asthma (4, 6, 15, 22, 31). These statistics probably underestimate LAA prevalence, because many workers change jobs when they develop allergies and are not included in the cross-sectional surveys. The magnitude of this effect may be estimated by comparing two studies performed at The Jackson Laboratory (TJL). A cross-sectional survey indicated that 31% of workers have LAA (33); however, a prospective study of allergies in newly employed workers indicated that 55% develop LAA within the first two years (23).

Nationally, the costs of LAA are considerable. In 1998, asthmatic employees cost employers an estimated annual \$15,267 per person, compared with \$3,268 for matched-control non-asthmatic employees. These costs included direct costs of medical care and prescription drugs and indirect costs of absenteeism and disability claims (2); they did not include costs of hiring and training new employees when individuals with LAA leave jobs for health reasons. It is estimated that the total cost of replacing an animal caretaker is at least equivalent to the annual salary of the individual being replaced (19). Simply training animal caretakers to work in a large-scale production facility, such as JAX Research Systems (JRS), can cost as much as \$26,000 (5). The high costs associated with asthmatic employees, who are the most easily identifiable subset of LAA sufferers, increase the costs of animal care and production and would be reduced if

LAA could be reduced.

Mouse urine contains a number of major urinary proteins, lipocalins, that are synthesized in the liver (1). The family is polymorphic. Recognition of individuals is crucial to social behavior and impacts on the maintenance of social groups, parent-offspring or mate recognition, among other interactions (16). Mouse urinary proteins are the major vehicle for such recognition. Male mouse urine and serum contains approximately four times the concentrations found in samples from females (20). Unfortunately, these proteins also are major allergens for humans. Several practices have been found to reduce ambient concentrations of rodent allergens in animal rooms. By far, the most effective practices are housing mice in negatively pressurized ventilated cages and changing the bedding in those cages in pressurized ventilated changing tables (6, 8, 11, 12, 18, 27, 28, 34). Other practices that reduce allergen concentrations include increasing the relative humidity in animal rooms (18) and covering animal cages with fitted filter-bonnets (14, 17, 27). Some practices, such as increasing animal room ventilation rates from six to 20 air changes/h, do not significantly reduce ambient allergen concentrations (27).

Personal protective equipment (PPE) can reduce allergen exposure of caretakers as well as protect the mice from infectious agents that might be introduced by caretakers' skin, clothing, and shoes. Gloves, masks, bonnets, special shoes or shoe covers, and scrubs are worn in many facilities. However, the only PPE that can provide a high level of protection for severely allergic caretakers and cage wash support personnel are respirators. Many affected personnel find these to be quite uncomfortable and transfer to other areas of TJL to avoid potential exposure to ambient allergen. For this reason, our efforts toward ambient allergen reduction at TJL have concentrated on engineering

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controls (ventilated cages, laminar flow change tables, increased room humidity) and personnel practices.

The objective of the study reported here was to document that allergen-reducing strategies previously reported to be effective in a highly controlled experimental mouse room (28) are just as effective when applied in a working mouse room. We also wanted to document that the percentage of animal caretakers experiencing allergy symptoms increases as the exposure to allergens increases.

Materials and Methods

Animals, animal room, and caging. The 42.5-m² mouse room (B2A) used in this study housed approximately 25 inbred strains of mice, with mean ($\pm$ SEM) census of $4,016 \pm 296$ mice in 540 duplex polycarbonate cages ($31 \times 31 \times 14$ cm) with autoclaved white pine shavings (Crobb Box Co., Ellsworth, Maine). Mice had ad libitum access to bottled acidified water (pH 2.8 to 3.1) and pelleted feed (NIH 31, Purina Mills Inc., Richmond, Ind.). The mouse room was supplied with high-efficiency particulate air (HEPA)-filtered air at the rate of 19 air changes/h and was maintained at mean ($\pm$ SEM) relative humidity of $61 \pm 4\%$ and temperature of $22 \pm 2^\circ\text{C}$, and mice were exposed to a 14-h light:10-h dark cycle. The Institutional Animal Care and Use Committee of TJL approved all animal protocols.

Two types of cages were used: conventional cages covered with fitted filter-bonnet tops (28), and pressurized individually ventilated (PIV) cages (Maxi-Miser PIV; Thoren Caging Systems, Inc., Hazelton, Pa.) covered by snap-on filter tops (Reemay 2033, Thoren Caging Systems Inc.). By adjusting dampers in the rack supply and exhaust plenums, PIV cages could be positively or negatively pressurized with respect to the room (anemometer readings of 0.025 Pascals [0.0001 inches of water] for positive pressure; -0.2 Pascals [-0.0008 inches of water] for negative pressure). Where specified, the bedding in cages was removed and replaced on a ventilated table (Maxi-miser, Thoren Caging Systems, Inc.).

Collecting and quantifying Mus m 1 values. Because the bulk of Mus m 1 allergen adheres to mid-size (3.3 to $10 \mu\text{m}$ in diameter) particles (24), we measured aeroallergens on particles $< 10 \mu\text{m}$ in diameter. We collected these particles at a standardized room location, 1.5 m from the floor, using a 20 L/min air-sampling pump and an impactor (Air Diagnostics, Harrison, Maine). A stainless steel plate (Air Diagnostics) in the impactor captured air particles $> 10 \mu\text{m}$ in diameter, and a $2\text{-}\mu\text{m}$ Teflon filter (Chester LabNet, Tigard, Oreg.) captured particles $< 10 \mu\text{m}$ in diameter. The Mus m 1 was extracted by shaking the filters into a mixture of 2.5% Tween 20 and phosphate-buffered saline for 48 h (28). The eluate was stored frozen at -80°C and was subsequently lyophilized to concentrate the allergen. The lyophilized eluate was resuspended in one milliliter of distilled water, then was frozen at -20°C until it was assayed.

The Mus m 1 was quantified by use of a sandwich enzyme-linked immunosorbent assay (ELISA) (24). Briefly, wells were coated ($50 \mu\text{l}$ /well) with an affinity-purified monospecific anti-Mus m 1 sheep antibody (AP-SH407) in $0.05M$ borate buffer, pH 8.6 . After overnight incubation at 4°C , the wells were washed four times and triplicate test samples, as well as standard Mus m 1 samples, were incubated overnight at 4°C . After four additional washes, biotinylated AP-SH407 was added and the sample was incubated for one hour at room temperature, fol-

lowed by four more washes and the addition of horseradish peroxidase-conjugated streptavidin for 30 min. After further washing, 0.5 mg of orthophenylenediamine/ml and 0.006% hydrogen peroxide were added for 20 min, followed by optical density measurement at 495 nm. Optical densities for the wells containing the standards were converted to a standard curve with which test samples were compared.

Mouse room B2A experiment. During the first four weeks, we established baseline Mus m 1 concentrations in room B2A at standard operating conditions (condition 1): conventional cages covered with fitted bonnet tops; weekly cleaning of floors, work tables, ladders, stools, and chairs; and monthly cleaning of walls, lights, air vents, ceiling vents, and racks. During the second four weeks, the mouse room was cleaned at an accelerated frequency (condition 2): weekly cleaning tasks were done daily, and monthly cleaning tasks were done weekly. The rationale for accelerated room cleaning was that Mus m 1 is water-soluble; thus, lower concentrations might be expected if the room were cleaned frequently.

We then emptied the mouse room, cleaned it thoroughly, installed PIV caging systems and a ventilated changing table, returned the same mice, and allowed the room to equilibrate for three days. We then began a series of five additional combinations of cage pressure, changing table type, and cleaning frequency to examine which combination(s) would most effectively reduce ambient room concentrations of Mus m 1. The five conditions were tested in the following order: condition 3—negative PIV cages, ventilated changing table, accelerated cleaning schedule; condition 4—negative PIV cages, ventilated changing table, normal cleaning schedule; condition 5—negative PIV cages, conventional (not ventilated) changing table, normal cleaning schedule; condition 6—positive PIV cages, ventilated changing table, normal cleaning schedule; and condition 7—positive PIV cages, conventional changing table, normal cleaning schedule. The order in which conditions 3–7 were tested reflected increasingly higher expected allergen concentrations. At the end of each condition, the room was cleaned thoroughly, changed to a new condition, and allowed to equilibrate for two days.

A one-way analysis of variance (ANOVA) was originally used to determine whether there was a difference in mean allergen levels among the seven conditions used in mouse room B2A. However, because data did not meet parametric assumptions, a Kruskal-Wallis test (non-parametric form of ANOVA) was used. Alpha was set at 0.05 . The least significant difference in ranks test (26) was used to determine which conditions were significantly different from each other.

Concentrations of Mus m 1 throughout TJL. The Jackson Laboratory animal facilities are divided into two major work sites: the Research Animal Facility (RAF), where the research at TJL is conducted, and JRS, where mice are produced for worldwide distribution. We measured allergen in 26 RAF mouse rooms and the RAF cage washing room, 17 JRS mouse rooms and the JRS cage washing room, warehouse, cafeteria, offices, and the “clean supply area” (clean cages, bedding, and food are distributed to mouse rooms from this area). Filters were placed in each room from 8 a.m. to 4 p.m. Mondays through Thursdays (four filters collected over four days). Ambient allergen concentrations were measured as part of our ongoing health and safety program. That program, of necessity,

Table 1. Concentrations of Mus m 1 monitored in a room, using various caging systems and cleaning schedules

Cage type ¹	Number of filters ²	Pressure	Cleaning	Change table	Mus m 1 (ng/m ³)
(1) Conventional	13	NA	Normal	Conventional	1.24 ± 0.17 ^{5,a}
(2) Conventional	19	NA	Increased ³	Conventional	1.45 ± 0.33 ^a
(3) PIV	4	Negative ⁴	Increased	Ventilated	0.13 ± 0.02 ^b
(4) PIV	10	Negative	Normal	Ventilated	0.11 ± 0.03 ^b
(5) PIV	5	Negative	Normal	Conventional	0.31 ± 0.08 ^b
(6) PIV	5	Positive	Normal	Ventilated	1.35 ± 0.23 ^a
(7) PIV	5	Positive	Normal	Conventional	1.62 ± 0.56 ^a

¹Conventional cage studies were performed in August; ventilated cage studies were performed between late September and late November. Air changes per hour = 19.

²Each filter collected after eight hours except condition (3) for which filters were collected after 16 h. The first two conditions were measured for three to five days over four weeks to obtain a reliable baseline for comparison with other conditions. Conditions (5)–(7) were measured for four to five days over one week. For condition (4), we collected after 10 days during a two-week period because we expected low allergen concentrations and increased variability.

³For increased cleaning, all weekly cleaning tasks were done daily, all monthly cleaning tasks were done weekly.

⁴Negative and positive refer to the pressure difference; for positive, any leaking air flows from the cage into the room, for negative any leaking air flows from the room into the cage.

⁵Common superscript letters indicate conditions where mean Mus m 1 concentrations were not significantly different from each other. Alpha was set at 0.05.

Mus m 1 data are expressed as mean ± SEM.

NA = not applicable.

measures ambient concentrations because we have approximately 270 animal care technicians at TJL. On the basis of our experience of highly variable results measuring breathing zone allergen values, we could not test breathing zone values for a meaningful number of people. In the RAF, conventional cages are covered with fitted bonnet tops or flat sheets of lexon filters. In JRS, conventional cages are covered with Reemay snap-on filters.

A Mann-Whitney U test (non-parametric form of *t* test) was used to test for significant differences in allergen values between PIV and conventional cage mouse rooms in JRS. A Kruskal-Wallis test was used to test for differences in mean allergen values among four types of rooms (PIV, mixed, conventional with bonnet filter tops, and conventional with lexon filter tops) in the RAF. Alpha was set at 0.05. The least significant difference in ranks test (26) was then applied to determine which conditions were significantly different from each other.

Additional (non-animal) rooms in JRS and the RAF were sampled for allergen concentrations. Because these additional rooms were sampled in low numbers, these data were not included in the aforementioned tests, and are reported for qualitative comparisons only. All statistical tests were evaluated at $\alpha = 0.05$.

Laboratory animal allergy at TJL. As a first step to examine the prevalence of allergy symptoms among TJL employees, we distributed a one-page survey (available on request) to 277 employees (212 at JRS and 65 at the RAF). The survey, approved by The Jackson Laboratory Institutional Review Board, asked employees where they worked and which of the following symptoms they experienced each day for a week: sneezing, runny nose, sinus irritation, nose bleed, itchy eyes, skin rash, dry throat, cough, chest tightness, wheezing, shortness of breath, headache, dizziness, and fatigue.

Results

Mouse room B2A experiment. There was a significant difference in ambient Mus m 1 concentrations among the seven conditions used in the mouse room experiment (Kruskal-Wallis test, $P < 0.001$). Conditions 3–5 had negative PIV cages, and mean Mus m 1 concentration was < 0.35 ng/m³ (Table 1). The other four conditions (1, 2, 6, and 7) had either conventional or positive PIV cage types, and all had Mus m 1 concentration > 1.2 ng/m³ (Table 1). Conditions 3, 4, and 5 (mean ± SEM: 0.13 ± 0.02 , 0.11 ± 0.03 , and 0.31 ± 0.08 , respectively) were found to have allergen concentrations significantly lower than those associated with

conditions 1 (1.24 ± 0.17 ng/m³), 2 (1.45 ± 0.33 ng/m³), 6 (1.35 ± 0.23 ng/m³), and 7 (1.62 ± 0.56 ng/m³) (Table 1). Increased cleaning schedules did not appear to reduce Mus m 1 concentrations when cage type and changing table configuration were kept constant (condition 1 versus 2, and condition 3 versus 4; Table 1). Additionally, the changing table configuration did not appear to make a difference when cage type and cleaning schedule were kept constant (condition 4 versus 5, and condition 6 versus 7; Table 1), although the trends in the data supported this expectation.

Mus m 1 concentrations throughout TJL. We did not detect Mus m 1 in the control areas, including cafeteria, offices, the outlying computer center building, and the clean supply room (areas a, b, g, and h; Table 2). However, concentration in individual mouse rooms ranged from 0.11 to 5.40 ng/m³. Only two of the 17 sampled JRS mouse rooms had Mus m 1 concentration > 1 ng/m³. In the RAF, 10 of 26 rooms sampled had Mus m 1 concentration > 1 ng/m³.

At JRS, mean (± SEM) allergen concentration in rooms with positive PIV cages (area e, 0.52 ± 0.06 ng/m³) was significantly higher than that in rooms with conventional cages (area d, 0.30 ± 0.06 ng/m³, Table 2; Mann-Whitney U test, $P = 0.03$). In contrast, mean allergen concentration in RAF rooms with positive PIV cages (area i, 0.30 ± 0.06 ng/m³, Table 2) was lower than that in RAF rooms with either a mixture of PIV and conventional cages (area j, 0.81 ± 0.23 ng/m³), or with conventional cages only (areas k and l; 1.01 ± 0.18 ng/m³ and 3.40 ± 0.98 ng/m³, respectively; Table 2). A Kruskal-Wallis test indicated the mean allergen concentrations for the four types of mouse rooms sampled in the RAF were significantly different ($P = 0.002$). Post-hoc tests indicated that mean allergen concentration for the positive PIV cage rooms was not different from that for rooms with a mixture of PIV (positive) and conventional cages, but was significantly less than that for rooms with conventional cages ($P < 0.05$, Table 2). Mean allergen concentration for rooms with a mixture of cage types was no different from that for rooms with conventional cages (Table 2). Mean (± SEM) Mus m 1 concentration was highest in cage washing areas, with 7.00 ± 1.31 ng/m³ at JRS (area f), and a surprisingly high 346 ± 69 ng/m³ in the RAF (area m; Table 2).

Correlation of Mus m 1 concentration with prevalence of allergy symptoms. To determine whether the percentage of caretakers reporting allergy symptoms correlated with workplace concentration of ambient Mus m 1, we distributed a one-page survey to 277 animal caretakers working in the mouse

Table 2. Ambient allergen concentrations throughout the production (JRS) and research animal (RAF) facilities (November 1999–June 2000)

Area	Caging	No. of rooms	Mus m 1 (mean ng/m ³ ± SEM)
JRS			
(a) Cafeteria	NA ¹	1	Below detection
(b) Offices	NA	2	Below detection
(c) Warehouse	NA	1	0.04 ± 0.02
(d) Animal rooms	Conventional	8	0.30 ± 0.06 ^{2,a}
(e) Animal rooms	PIV (positive)	7	0.52 ± 0.06 ^b
(f) Cage washing room	NA	1	7.00 ± 1.31
RAF			
(g) Computer center building	NA	1	Below detection
(h) Room with clean supplies	NA	1	Below detection
(i) Animal rooms	PIV (positive)	5	0.30 ± 0.06 ^c
(j) Animal rooms	Mixture	5	0.81 ± 0.23 ^{c,d}
(k) Animal rooms	Conventional, bonnet filter tops	12	1.01 ± 0.18 ^d
(l) Animal rooms	Conventional, lexon filter tops	4	3.40 ± 0.98 ^d
(m) Cage washing room	NA	1	346 ± 69

¹ NA = not applicable.

²Common superscript letters indicate conditions in which mean Mus m 1 concentrations in animal rooms were not significantly different from each other. Comparisons are within JAX Research Systems (JRS) and within the research animal facility (RAF). Alpha is set at 0.05.

Each room was measured for four days, Monday–Thursday. The rooms with lexon filters, which are flat and fit loosely, have conventional caging. The rooms with a mixture of caging have PIV and conventional caging.

See Table 1 for key.

rooms we surveyed. For each day during the same week that Mus m 1 concentration was sampled, caretakers reported allergy symptoms they experienced. Of the 277 caretakers, 236 (85%) replied and 73 (31%) reported allergy symptoms. The symptoms most frequently reported, as a percentage of all symptoms, were rhinitis (60%), symptoms compatible with asthma (23%), and itchy or watery eyes (17%).

We designated all rooms with allergen concentration < 0.2 ng/m³ as low-exposure areas because this concentration is achievable with the existing technology of negatively pressurized cages and ventilated changing tables (Table 1). The percentage of workers with allergy symptoms was 12.9% in low-exposure rooms (seven rooms, mean allergen concentration of 0.14 ng/m² ± 0.02), the percentage of workers with symptoms in medium exposure rooms was 31.7% (25 rooms, mean allergen concentration of 0.52 ng/m² ± 0.03), and the percentage of workers with symptoms in high-exposure rooms was 45.9% (12 rooms, mean allergen concentration of 2.3 ng/m² ± 0.4); this rate of symptomatic workers differed significantly with increasing exposure (*P* = 0.014, χ^2 -analysis). These data indicate that the 10-fold reduction of allergen achievable by negatively pressurized PIV caging and ventilated changing tables produces a clinical benefit. If we considered only the percentage of workers with asthmatic symptoms (shortness of breath, wheezing, chest tightness), then none of the workers in the low-exposure rooms had asthmatic symptoms, compared with 15% in the medium exposure and 13% in the high-exposure rooms.

Discussion

When PIV rack and caging systems were invented, they were expected to decrease ambient allergen concentrations because most air exiting them would vent outside the room or, if vented into the room, exit through HEPA filters. By adjusting dampers in the rack supply and exhaust plenums, PIV cages can be positively or negatively pressurized with respect to the room. Essentially all of the air (and accompanying allergen) entering and leaving negative PIV cages passes through a HEPA filter. In contrast, a small fraction of the air (and its accompanying allergen) leaks from positively pressurized cages through the junctions of cage tops and cages. Ventilated change tables were also expected to reduce ambient room Mus m 1 concentration be-

cause the airflow pattern of filtered air in the enclosed table is designed to contain the particulates within the table.

Our results, for the most part, confirm results of previous studies (8, 12, 28) that ambient rodent allergens can be effectively reduced when rodents are housed in negatively pressurized ventilated cages that are changed in ventilated tables. In the mouse room B2A experiment, mean Mus m 1 concentration in the condition involving negatively pressurized PIV cages, a ventilated changing table, and a normal cleaning schedule was significantly less than mean concentration associated with conventional and positively pressurized PIV cages. It was somewhat surprising to find that Mus m 1 concentration for the treatment with negatively pressurized PIV cages, a ventilated changing table, and an increased cleaning schedule was not significantly different from that for treatments involving conventional and positively pressurized PIV cages. A quick post-hoc sample size analysis, with simplified assumptions, indicated that this is likely a sample size problem (a sample size of three was used for the latter negative PIV cage treatment). The trends in the mean concentrations for the various treatments also give credence to confirmation of results of previous studies (8, 12, 28).

Earlier studies (14, 27) indicated reductions in ambient rodent allergen concentrations when animal cages were covered with fitted filter-bonnets. Results of the survey of the RAF mouse rooms in this study indicated a trend that agrees with these findings, although the difference between rooms with fitted filter-bonnets and lexon filter tops was not statistically significant. Once again, small sample sizes may have reduced the power to detect significant differences.

The Mus m 1 concentration was significantly lower in rooms with conventional cages than in rooms with positive PIV cages in JRS. Although this result was not expected, it may be related to use of the same filter cover on both types of cages and the higher number of mice per unit of floor space (higher density housing) in rooms with ventilated caging. The opposite (and expected) results were found in the RAF: rooms with positive PIV cages had the lowest allergen concentrations. These results may be partly explained by different operating conditions and procedures in the two facilities. In JRS, cages are opened only weekly or biweekly for cleaning and husbandry. In the RAF, cages are

frequently opened for cleaning and research. The additional activity in the RAF may lead to increased opportunities for allergen dispersal from conventional cages. Another unexpected finding was the extremely high concentration of allergen in the RAF cage washing area relative to that in the JRS cage washing facility. A possible explanation is that the RAF cage washing area is in an old building with low ceilings and has old air-handling equipment. The JRS facility is in a new building with high ceilings and a modern air-handling system.

The allergen concentrations reported in this study and in that of Renstrom and co-workers (30) were orders of magnitude lower than those reported by Gordon and co-workers (9). The differences may be due to several factors: mouse allergens were measured in this study, whereas Gordon's group measured rat allergens; ELISA was used to quantify allergen concentrations in this study, whereas Gordon and colleagues used a radio-allergo-sorbent test (RAST); and polyclonal antibodies were used in assays in this study, whereas Gordon's group used pooled sera from allergic humans. Different assays can yield large differences in absolute allergen concentrations (13).

We found that caretakers exposed to the lowest allergen concentrations reported the fewest allergy symptoms. The percentage of caretakers working in low-, medium-, and high-exposure areas and reporting allergy symptoms were 12.9%, 31.7%, and 45.9%, respectively, which are significantly different from each other. This reduction in symptomatic employees from 45.9% in the high-exposure group to 12.9% in the low-exposure group confirms our hypothesis that the allergen reduction achievable by using negatively pressurized PIV caging and ventilated changing tables will have substantial clinical benefit (18).

Laboratory animal programs that monitor allergens report that sensitization can be prevented in most individuals (3, 7, 10, 32). Determining the threshold allergen concentrations below which new employees will not be sensitized will require a prospective study and, probably, further allergen reductions because sensitization occurs at lower concentrations than do symptoms (29). It may be an oversimplification to think that a single threshold would protect everyone because humans are so genetically variable and because environmental co-factors, such as smoking and air pollution, are important.

Any strategy to improve worker health must also be evaluated for its impact on animal health and welfare. The PIV caging systems are usually positively pressurized with respect to room air to prevent airborne pathogens from entering cages. Yet, any laboratory animal specialist would agree that mice are most vulnerable to airborne pathogens when their cages are opened and exposed to room air: the fewer pathogens in the air, the less mice are exposed to them. We suggest that mice housed in negatively pressurized cages and changed in ventilated changing tables are just as protected from airborne pathogens as are mice housed in positively pressurized cages. Using colony-forming units (CFUs) as surrogate measures for infectious organisms, we found similarly low CFUs for negatively and positively pressurized cages changed in ventilated changing tables (28). Also, in a nine-month study, we found that *Pneumocystis carinii*, which is known to be airborne, was not transmitted within or between negatively ventilated cage racks housing immunodeficient mice (21). Thus, we conclude that negative cage ventilation coupled with cage servicing within a containment device (such as a biological safety cabinet or venti-

lated changing station) is a viable strategy for suppressing the transmission of airborne infectious agents (depending on room operating procedures), and possibly protecting workers from LAA.

Use of personal protective equipment, specifically respirators, can contribute substantially to reducing allergen exposure in mouse housing and support areas. The cost of respirators is low, compared with an investment in expensive caging systems and changing tables; however, personnel find respirators to be hot and uncomfortable during prolonged use. For that reason, TJL has concentrated on engineering controls and refined practices to reduce exposure to mouse urine protein.

We have documented one combined practical and currently available method of reducing laboratory animal allergen concentrations in a relatively uncontrolled environment, the working mouse room: housing animals in negatively ventilated PIV cages. Changing the bedding in those cages in ventilated changing stations appears to be an additional step that could further reduce exposure to ambient allergen. The capital costs of converting from conventional to PIV housing systems are high, but they can be recovered because PIV cages can be changed every other week, a procedure that benefits animal health (29). The increased capacity afforded by PIV caging systems, the savings in labor for changing and washing cages, the reduced water and energy costs, and the longer life of cages resulted in a payback period of less than five years at this institution (25). Thus, these engineering controls coupled with employee education and medical surveillance should substantially reduce the potential prevalence of LAA and result in cost savings in the animal facility.

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