

Elimination of mouse allergens in the working environment: Assessment of individually ventilated cage systems and ventilated cabinets in the containment of mouse allergens

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Background: Laboratory animal allergy is an important occupational disease that is preventable by reduction of exposure to mammalian allergens.

Objective: The purpose of this investigation was to assess the efficacy of safety equipment in containing mouse urinary protein (MUP)—specifically, individually ventilated cage (IVC) systems and class I-type and class II ventilated cabinets.

Methods: Six IVC systems (which are used to house rodents) were operated at positive and negative pressure. Air samples (2 L/min) were collected at the cage front, cage back, air inlet, and air outlet, and the MUP was quantified by immunoassay. The background MUP of the IVC study room was compared with that of rooms where mice were housed conventionally or in isolators. Class I-type cabinets (n = 2) were tested during the disposal of soiled litter. Air samples were positioned on and behind the operator and inside the cabinet (n = 2). Personal samples were collected while scientific procedures were performed in a class II cabinet and in the open.

Results: All of the IVC systems contained MUP effectively (>250-fold) when operated at negative pressure. At positive pressure, the “unsealed” IVC racks leaked MUP (1- to 25-fold reduction) but the “sealed” IVC system did not. Class I-type cabinets reduced (10-fold) but did not eliminate exposure during “cleaning out.” No MUP was detected when procedures were performed in class II cabinets (protection factor, >10-fold).

Conclusion: Safety equipment can substantially reduce exposure to mouse allergen. Allergen exposure in holding rooms will be minimized if mice are housed in IVC systems operated at negative pressure or in “sealed” IVC systems at positive

pressure. Respiratory protection should be used whenever “unsealed” IVC systems are operated at positive pressure or during “cleaning out” in class I-type cabinets. (*J Allergy Clin Immunol* 2001;108:288-94.)

Key words: Laboratory animal allergy, mouse, aeroallergens, ventilated cage, exposure, occupational asthma

Epidemiologic studies show that up to one third of those exposed to laboratory animals will develop symptoms of laboratory animal allergy (LAA), such as rhinitis, conjunctivitis, and contact urticaria, and 1 in 10 of those exposed might develop asthma.¹⁻⁷

Awareness of the disease has increased; occupational asthma caused by allergy to laboratory animals became a prescribed industrial disease in 1982 in the United Kingdom.⁸ The National Institute for Occupational Safety and Health in the United States formally recognizes LAA as an occupational hazard and in 1998 issued recommendations for reducing exposure and the health surveillance of exposed staff.⁹ Several exposure-response studies show the intensity of exposure to animal allergens to be an important risk factor for the development of disease^{10,11} and the expression of symptoms.¹² However, these studies suggest that the level of allergen must be reduced by several orders of magnitude to minimize the risk of sensitization and symptoms.¹⁰ Encouragingly, reports from pharmaceutical companies in the United Kingdom and the United States show that improved work practices, including education programs and the mandatory use of respiratory protection, can greatly reduce the incidence of LAA.¹³⁻¹⁶ This means that if airborne allergen concentrations can be adequately controlled, the prevalence and incidence of this important occupational disease can be reduced.

There is a growing literature describing expensive equipment that can reduce exposure to laboratory animal allergens.¹⁷⁻²¹ The first aim of this study was to compare the efficacy of individually ventilated cage systems (IVCs) in containing allergen in animal housing rooms. The second aim was to assess the ability of class I-type and class II ventilated cabinets to contain mouse allergens during the high-exposure tasks of disposal of soiled litter and the handling of animals.

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Abbreviations used

HEPA: High-efficiency particulate air [filter]
IVC: Individually ventilated cage [system]
LAA: Laboratory animal allergy
MUP: Mouse urinary protein

METHODS

Study design

A summary of the study is shown in Fig 1. Additional air samples were collected from other areas of the animal unit to aid interpretation of the biological significance of the IVC and local exhaust ventilation results.

Assessment of IVCs

This equipment is designed primarily for control of the microbiological environment of rodents. Each cage is fitted with a lid, and air is supplied and exhausted from an integral air handling unit. The study took place late in 1998 and involved the use of the most current model of IVC racks available from each of 5 major manufacturers (A through E in Table I). Rack models A, B, and D were tested in situ by the manufacturers before the study commenced.

A plan of the study room is shown in Fig 2. The room was thoroughly cleaned before the study started, and contamination of the hard surfaces was monitored with the collection of "wall-wipe" samples (WW1 and WW2 in Fig 2), according to the method of Wood et al.²² The room was ventilated at a rate of 20 air changes per hour and maintained at positive pressure to the rest of the unit even when the connecting door was open. The temperature and humidity were measured twice daily and kept at averages of 21°C (range, 19.8°C to 22.9°C) and 33.5% (range, 22% to 45%), respectively. Three hundred adult male mice were used for the IVC tests and were housed according to the Home Office code of practice. The average age of the colony (3-4 months) was kept constant throughout the study. The animals were supplied with pelleted rodent food (Special Diet Services, Witham, Essex) and water ad libitum and were kept on sawdust bedding (grade BK8.15, Lignocel, RS Biotech). All husbandry procedures were performed outside the study room; the lids of the cages were not removed in the study room.

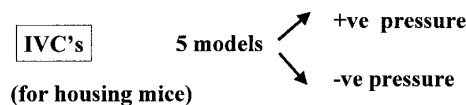
The fully stocked IVC was placed in the study room on the Friday afternoon before the air-sampling schedule began (Monday morning to Friday noon). Four areas of each rack were assessed for leakage of mouse aeroallergen: the exhausted air, the supply air, and the fronts and backs of the cages. Air samples (2 L/min) were collected for a total of approximately 30 hours through use of AFC 123 pumps (Casella London Ltd, London, United Kingdom) fitted with PTFE Millipore Fluoropore Membrane filters (pore size, 1.0 µm; Millipore [UK] Ltd, Watford, United Kingdom). A continuous background sample was also collected for approximately 98 hours through use of an electrically powered high-flow sampler (15 L/min; Casella Vortex Ultra Flow Sampling Pump, Casella Ltd, Bedford, United Kingdom; Fig 2). The experiments on the IVCs operating at negative pressure were done first.

Assessment of local exhaust ventilation

Both class I-type* and class II ventilated cabinets were assessed for their allergen containment properties. This type of equipment is classified according to the pattern of air flow within the cabinet. For

*We have used the suffix "type" because the units investigated in this study were mobile and not ducted to the main ventilation system (and hence did not strictly comply with British Standard BS5726).

Assessment of IVC's



Assessment of LEV



FIG 1. Study design.

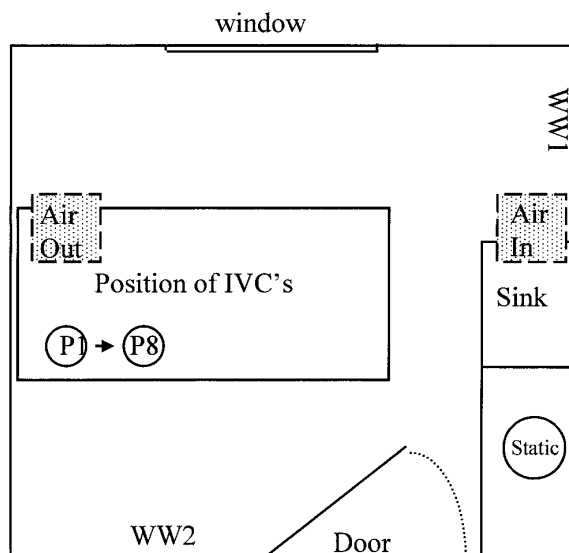


FIG 2. Plan of the study room (width, 2.9 m; length, 3.2 m, height 2.4 m) that was used to assess the IVC systems. The inlet and outlet vents of the ventilation system are at ceiling height (*Air In* and *Air Out*). The 2-L/min sampling pumps (*P1* through *P8*) are positioned at various places on the different IVC racks. The 15-L/min background air sampling pump is positioned at bench height (*Static*). Wall-wipe samples were collected at positions *WW1* and *WW2*.

class I cabinets, the air is drawn at high velocity directly from the room through the working aperture and then filtered through a high-efficiency particulate air (HEPA) filter before exhausting into the room. This protects the operator but not the work. For class II cabinets, the room air is drawn in and down at the front aperture and provides a clean air curtain after passing through a HEPA filter. Class II cabinets recycle approximately 70% of the air through a downflow HEPA filter; the rest is exhausted through a HEPA filter to the room. This affords protection to both the operator and the work. Further information on the use of local exhaust ventilation and other methods of controlling animal allergens has been presented by Harrison.²³

1. Class I-type ventilated cabinets. Cabinets from 2 manufacturers (cabinet 1 and cabinet 2) were assessed for their efficiency at reducing exposure to MUP during the cleaning-out of soiled litter. Cabinet 1 was a mobile workstation. When the hinged glazing at the front was in the "down" position, the face velocity of air flow was

TABLE I. Descriptions of the 5 IVC racks studied (manufacturers A through E)

Rack	No. of cages	Operational mode	Pressure (Pa): AM (SD)*	No. of air changes per hour	Ventilation design
A1	56	Negative	-3.77 (1.01)‡	100	Supply and exhaust at back of cage
A2	128	Negative	-20†	—	Sealed. HEPA-filtered air in and out back of cage
A2	128	Positive	+20†	—	As above
B	96	Negative	-12 (0)	120	"Minimum leakage" sealed lid. Supply and exhaust at back of cage
B	96	Positive	+12 (0)	120	As above
C	112	Negative	-5.6 (2.44)	100	Supply in cage lid back and exhaust in cage lid front
C	112	Positive	+2.3 (0.63)	100	As above
D	98	Negative	-29 (0.82)	98	Supply in cage lid front and exhaust in cage lid back
D	98	Positive	+9.5 (1.94)	100	As above
E	140	Positive	+0.36 (0.07)	30	Supply at back of cage, exhaust across lid and side to back of cage

Every cage was large enough to house 5 animals.

AM, Arithmetic mean; HEPA, high-efficiency particulate air [filter].

*The operational pressure within the IVC racks was monitored with a sample cage; the position of the cage in the rack was regularly changed to ensure that there was little or no variation in pressure across the rack.

†Operational pressure set by manufacturer.

‡Could not be operated at positive pressure.

more than 0.5 m/s. Cabinet 2 had the same specifications but operated at a face velocity of 0.7 m/s. Four samples (2 L/min) were taken for each assessment: 2 samples inside the hood (left-hand and right-hand sides), 1 personal sample, and 1 static sample positioned approximately 2 m behind the operator.

2. *Class II ventilated cabinets.* The efficiency of recirculating class II cabinets in containing MUP during the performance of scientific procedures was assessed at a separate facility. The MUP exposure of 50% of the scientists currently registered with the animal unit was measured through use of 2-L/min pumps. Because the exposure was expected to be low, whenever possible the filters were re-exposed and further pooled before analysis (3-fold concentration). Samples were also collected during the performance of procedures on an open bench.

Measurement of exposure at other locations in the facility

The background MUP levels of stockrooms housing 360 mice in 6 isolators (La-Calhène Ltd, Cambs, United Kingdom) and 1500 mice in 3 IVCs (models A, B, and D) were compared through use of pumps operated at 2 and 15 L/min for 24 hours. Air samples were also collected at 15 L/min from rooms where mice were housed in open cages (n = 5) and from cage wash and changing rooms (n = 3).

Elution and measurement of allergen

Allergen was eluted in 2 mL of 0.1 mol/L NH_4HCO_3 buffer (5 mL for the wall-wipe samples)—as described by Gordon et al²⁴—containing 0.1% v/v Tween 20 to maximize the elution of allergen. The filter eluates were lyophilized and reconstituted (250 μL). The allergen content was measured through use of an immunoassay specific for MUP²⁵ with the following modifications: Briefly, test samples (100 μL) were incubated overnight at 4°C with 100 μL of the antimouse antiserum (diluted 1:2000) in a microtiter plate (Immunolon II Removawell strips, Dynatech Laboratories, Billingshurst, United Kingdom) that had been previously coated with mouse urine antigen (200 μL of a 10- $\mu\text{g}/\text{mL}$ solution). Standards (mouse urine, 0.5 $\mu\text{g}/\text{mL}$ to 0.2 ng/mL) and controls (0.2 $\mu\text{g}/\text{mL}$, 0.05 $\mu\text{g}/\text{mL}$, and 0.01 $\mu\text{g}/\text{mL}$) were run in parallel. After washing, bound antibody was detected with ¹²⁵Iodine-labeled protein A (catalog number IM144; 100 μL of a 10-ng/mL solution incubated

overnight; supplied by Amersham International, Amersham, United Kingdom). After further washing, individual wells were counted in a gamma counter and the allergen content was determined according to the method described by Gordon et al.²⁵ The detection limit for the assay was 0.2 ng/mL—ie, 1 ng per filter. When samples were below the limit of detection for the assay, the maximum MUP exposure was assigned to each filter by dividing 1 ng by the volume of air sampled (in cubic meters).

RESULTS

Effectiveness of individually ventilated cage systems to contain mouse allergens

The test room wall-wipe samples contained MUP at 14.4 and >22 $\mu\text{g}/\text{m}^2$, which was reduced to 3.75 and 0.17 $\mu\text{g}/\text{m}^2$ after cleaning. The walls were therefore unlikely to be a significant source of MUP.

Table II shows the MUP concentrations obtained for each IVC rack. Of the IVCs operated at negative pressure, racks B and D were the most effective in containing MUP; no allergen could be measured, indicating that the background MUP levels were below 0.01 ng/m³ (ie, 1 ng in 89 m³). These racks operated at a high number of air changes per hour (120 and 100, respectively) and also at a substantial negative pressure differential—ie, they were the "most negative" of the racks tested (Table I). Racks A1 and C, while operating at a similar number of air changes per hour, were "less negative" and leaked low levels of MUP (respective background levels, 0.03 and 0.06 ng/m³). The source of the low levels of MUP leakage for rack A1 was the fronts and backs of the cages, and this might reflect poor fitting of the lid after autoclaving. The relatively high levels of MUP in the exhaust air of rack A1 was later (when checked by the manufacturer) attributed to a poorly fitting HEPA filter. The source of the allergen leakage from rack C could not be determined.

Under positive pressure, allergen leaked from all of the racks tested (ie, rack models B, C, D, and E).

TABLE II. MUP concentrations

		MUP concentration (ng/m ³)				
Rack	Pressure	Static	Supply	Exhaust	Cage front	Cage back
Operated at negative pressure						
A2	Negative	ND	ND	ND	ND	ND
B	Negative	ND	ND	ND*	Missing	ND
D	Negative	ND	ND	ND	ND	ND
A1 [†]	Negative	0.03	0.55	0.50*	0.30	0.10
C	Negative	0.06	ND	ND	ND	ND
Operated at positive pressure						
A2	Positive	ND	ND	ND	ND	ND
E [‡]	Positive	0.10	0.50	ND	3.00	0.30
C	Positive	0.30	ND	9.80*	ND	0.70
B	Positive	0.40	1.60	0.15	0.80	ND
D	Positive	1.92	6.90	4.65	11.60	6.70

Results are ranked in order of increasing static or background levels of MUP.
ND, No allergen detected.

*Represents a single measurement because of failure of sampling equipment.

[†]Could not be tested at positive pressure because of a technical problem that could not be resolved in time for the rack to be included in the study.

[‡]Is unable to operate at negative pressure.

Although model A1 is designed so that it can be operated at positive pressure, a maintenance problem with the fans prevented its inclusion in this part of the study. The lowest level of MUP leakage was measured with rack E (which also had the lowest number of air changes per hour, 30, and the lowest positive pressure differential). However, the background level of MUP was 10 times greater than that measured with the racks B and D when operated at negative pressure (0.10 ng/m³ compared to a maximum estimate of 0.01 ng/m³). For rack E, the most obvious source of the allergen leakage was the cage front. The magnitudes of the allergen leakage from racks C and B were similar (0.3 and 0.4 ng/m³, respectively) despite the semi-sealed design of the latter. The exhaust filter of rack C apparently leaked high levels of MUP. Large amounts of allergen leaked from all of the vulnerable points of rack D under positive pressure, which is in keeping with information supplied by the manufacturer; in this design, approximately 80% of the vented air can bypass the HEPA filter. Racks B, C, and D all operate at 3 to 4 times the number of air changes of rack E and at a much greater positive pressure differential.

When leakage of MUP, as measured by means of the static pump, was plotted against the operational pressure of the "standard" IVC racks (racks A1, C, D, and E), the allergen levels in the background of the room increased as the pressure increased (*solid diamonds* and *solid line* in Fig 3). The average MUP concentration measured from the "cage front" and "cage back" (*open diamonds* and *broken line* in Fig 3) can be taken as a measure of the efficacy of the fit or seal of the cage lid. For 2 of the racks tested, the "sealed" rack system (rack A2, shown as *squares* in Fig 3) and rack B (shown as *triangles* in Fig 3), MUP was barely detectable either in the background of the room (solid symbols) or close to the cage lid (open symbols). Both of these racks have very efficient seals within the cage lid, these being

designed to minimize the leakage of allergen; in addition, rack A2 incorporates HEPA filters within the cage lid.

Assessment of allergen containment equipment

1. *Class I-type ventilated cabinets.* Between 40 and 90 soiled cages were emptied during a sampling time of 70 to 125 minutes; the average rate of cage-emptying was 0.57 cages/min for cabinet 1 and 0.67 cages/min for cabinet 2. Both of the models tested were effective at containing mouse allergen (Table III). When the MUP exposure of the operator (*Outside—personal* in Table III) was compared with that measured inside the cabinet (*Inside—RHS* and *Inside—LHS* in Table III), exposure to MUP during the removal of soiled litter was reduced approximately 2.4- to 10-fold. The cabinet that operated with the highest face velocity had the highest protection factor. Static samples situated 2 m behind the operator detected low levels of allergen (Table III), showing that allergen was not significantly leaking into the room.

2. *Class II ventilated cabinets.* Eighteen samples were collected from scientists working with mice within class II ventilated cabinets. The procedures undertaken included the postmortem collection of tissue (n = 12) and the shaving of conscious mice (n = 4). The duration of sampling was between 1 and 3 hours, and the numbers of mice handled were between 10 and 30. Despite efforts to collect and concentrate (3-fold) material for analysis, no MUP could be detected. Inasmuch as the volume of air sampled was 0.15 to 0.3 m³, the scientists were exposed to less than 2.2 ng/m³ (1 ng/0.15 m³/3). Only 2 of the 6 open bench samples contained detectable MUP: the removal of the thymus in 10 mice (12.5 ng/m³) and post-mortem examination and tissue removal in 4 mice (15.2 ng/m³). It is possible that the use of alcohol to wet the fur before the surgical procedures contributed to the low lev-

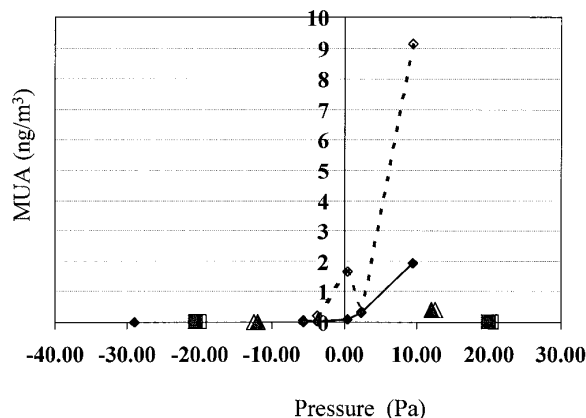


FIG 3. Relationship of the operational pressure of the IVC to leakage of MUP. The background (or static) MUP level of the room (for "standard" racks) is shown by the *solid line and solid diamonds*. The MUP leaking out of the cage lid is shown by the *broken line and open diamonds*. The measurements from rack B (which featured an improved design of the seal of the lid) are plotted as *triangles*; those from rack A2 (which featured a "sealed" design) are plotted as *squares*.

TABLE III. Comparison of class I-type cabinets for the containment of allergen during the removal of soiled litter

	MUP: AM (range)	
	Cabinet 1 (N = 3)	Cabinet 2 (N = 4)
Inside—RHS	47.9 (30.6-60.7)	111.8 (3.9-212.8)
Inside—LHS	44.8 (3.2-123.6)	27.4 (1.7-80.3)
Outside—personal	19.1 (10.2-33.6)	6.6 (2.9-11.0)
Outside—static*	4.3 (3.1-5.5)	5.5 (3.8-6.2)

AM, Arithmetic mean (in ng/m³); N, number of observations; RHS, right-hand side; LHS, left-hand side.

*Sample positioned 2 m behind the operator.

els for some of the samples. These limited data suggest that when small numbers of unconscious or dead mice are handled on the open bench, the exposure to MUP is minimal. However, when the maximum exposure levels recorded are compared with the minimum detectable limit, it is seen that the use of class II ventilated cabinets can reduce exposure to MUP by approximately 10-fold.

Measurement of exposure to MUP at other locations in the facility

No MUP was detected in the isolator suite (sampled on 3 occasions for 24 hours) or in the IVC suite (sampled on 4 occasions), even when the higher-volume pump was used. The level was therefore below 0.05 ng/m³ (ie, 1 ng in 21.6 m³). In contrast, a high level of MUP was measured in areas where mice were housed in open cages (arithmetic mean, 4.6 ng/m³; n = 5; range, 2.4-7.1 ng/m³). Areas of indirect animal contact, such as the cage wash and changing areas, had airborne MUP concentrations of 1 ng/m³ or less. These values are consistent with the value previously reported.²⁵

In this animal unit, the typical background level of MUP measured in stockrooms where mice were housed in traditional open cages was 4.6 ng/m³ (n = 5). When the number of male mice present was corrected for, it was estimated that each male mouse contributed 0.008 ng/m³ of MUP to the environment. Three hundred male mice would therefore produce an estimated background MUP level of 2.5 ng/m³. Comparison with this figure suggests that the protection factor for the "unsealed" racks varied between 1.3 and 250 when they were operated at positive pressure and between 42 and 250 when they were operated at negative pressure.

DISCUSSION

Studies conducted within the pharmaceutical industry suggest that LAA can be prevented in most individuals.¹³⁻¹⁶ The key requirements for a successful prevention strategy include training staff and establishing (and following) a code of practice to minimize exposure to allergens. The "tasks" associated with highest exposure to laboratory animal allergens are those that involve direct contact with the animals or their waste products (eg, soiled litter).^{19,23,25} Although exposure can be reduced by respiratory protective equipment, it is ethically more acceptable to control the allergens at source. Strategies to prevent LAA increasingly rely on expensive engineering solutions. However, there is currently little information available about the relative efficacy of these "control measures" or about the biological significance of the reduction in exposure that can be achieved. This information is required for the accurate assessment of risk and the education of staff.

A previous study of workers newly exposed to rat allergen found that to reduce the risk of sensitization to rats by 6-fold, exposure to rat allergen must be reduced 600-fold.¹⁰ To minimize LAA caused by rats, exposure should be reduced to 0.1 µg/m³ (approximately equivalent to the exposure when one is working with rat tissue). Because of the apparent differences in magnitude of exposure between the 2 species (which is probably attributable to differences in the immunologic assays used to quantify aeroallergen exposure), a comparable level for mice would be approximately 3 ng/m³.²⁵ Preliminary results of longitudinal exposure-response data collected from newly exposed mice workers show that the risk of sensitization to mice and the development of symptoms is increased at MUP concentrations above approximately 5 ng/m³ (unpublished data). For the purposes of discussion of the current study, it will be presumed that it is desirable to reduce MUP concentrations to 5 ng/m³ or less.

Previous studies have described how individual models of IVC racks contain allergen.^{18,19,21} As far as we are aware, the present investigation is the first to compare several different models of IVCs under controlled conditions and to interpret the biological significance of the observations. This study was performed in a busy animal facility. To minimize disruption, measurements to compare the IVCs were performed only in duplicate. Increasing the number of measurements would not have affected our

main observation that all of the IVC models were highly effective in containing MUP when operated at negative pressure to the environment. In this mode, the ambient concentration of MUP in the study room was low (0.06 ng/m³ or less), suggesting a reduction of 250-fold in comparison with housing of the same number of mice in conventional open-top cages. However, the IVC racks were less effective at containing MUP when operated at positive pressure to the environment, ambient concentrations of MUP being reduced only 25-fold. The main factors affecting the allergen containment were the differential pressure and effectiveness of the seals, especially those of the cage lids. Personnel would therefore be at risk of exposure to high levels (>5 ng/m³) of MUP when working in close proximity to an IVC system operating at positive pressure, particularly one with poorly fitting cage lids.

Data collected from other rooms in the animal unit demonstrate that our observations in the controlled test room are also valid in the "real" working environment. We were unable to detect MUP in a stockroom containing approximately 1000 male animals housed in 3 IVCs operated at negative pressure. We were also unable to detect any MUP in an isolator suite (housing approximately 900 female mice), suggesting that the efficiency of allergen containment of IVCs at negative pressure is comparable to (or possibly greater than) that of flexible film isolators.

For an effective allergen control program, exposure to allergen must also be controlled during known high-exposure procedures. We have shown that class I-type cabinets reduced exposure to MUP during the dumping of soiled litter by approximately 10-fold. It is of note that exposure to MUP, though reduced, was not eliminated and that the operator was still exposed to levels of MUP greater than 5 ng/m³, suggesting that respiratory protection should also be used.

Of the 2 models of class I-type ventilated cabinet tested, the cabinet with the highest face velocity was also the most efficient at containing the MUP. Further studies are required to determine more exactly the relationship between allergen containment and the air handling properties of this type of equipment. Similarly, a minimum protection factor of 10 was found for scientists performing experimental procedures in class II ventilated cabinets. The scientists and husbandry staff who took part in the study were not specially selected, but all had been proven to comply with the correct use of the containment equipment and hence had negligible exposure to mouse allergens.

In conclusion, we would recommend any of the IVC models tested as an efficient method of reducing the airborne allergen concentration in an animal stockroom, particularly when used at negative pressure. These IVCs thus have an important contribution to make to any LAA prevention program, provided that they are appropriately commissioned before use and that the manufacturers' advice on effective maintenance programs to ensure optimum performance of the seals and filters is followed at all times. The selection of one particular model rather than another can be based on economic, ergonomic, and animal welfare considerations. However, if the priority is to maintain microbio-

logical protection of the animals, the only model currently available that combines this facility with effective allergen containment is a "fully sealed" system. Such a system has important implications for animal welfare. Although the additional use of respiratory protection might be required for tasks such as "cleaning out," we believe that it is possible to obtain a virtually allergen-free environment when animals are housed in efficient IVCs and when class I-type and class II ventilated cabinets are used for all procedures.

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