

Reduction of exposure to laboratory animal allergens in the workplace

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Summary

Objectives Evidence is now accumulating that the prevalence of allergy to laboratory animals is related to the intensity of exposure to animal allergens. Whilst airborne animal allergen concentrations may be influenced by the litter type, cage design and stock density, the effectiveness of methods to reduce personal exposure has not been objectively assessed.

Methods Air samples were collected at 2 L/min and 180 L/min onto polytetrafluoroethylene (PTFE) filters and the rat urinary aeroallergen (RUA) and mouse urinary aeroallergen (MUA) concentrations were measured by radioallergosorbent test (RAST) inhibition.

Results When 545 mice (11.1 mice/m³) were housed in ventilated cages (Thoren Maximiser cage system) operated at positive pressure to the environment, the static MUA concentration ($n = 24$, median = 0.10 $\mu\text{g}/\text{m}^3$) was reduced sevenfold when compared with conventional cage systems ($n = 12$, median = 0.67 $\mu\text{g}/\text{m}^3$, $P < 0.001$). MUA could be further reduced if the ventilated cage system was operated at lower pressure; static samples ($n = 1$) collected at 180 L/min at negative, ambient and positive pressure registered < 0.003, 0.02 and 0.28 $\mu\text{g}/\text{m}^3$, respectively. During cleaning out, the intensity of personal exposure to RUA was apparently reduced twofold when soiled litter was removed by vacuum ($n = 17$, median = 22.87 $\mu\text{g}/\text{m}^3$) when compared with tipping ($n = 18$, median = 38.15 $\mu\text{g}/\text{m}^3$), $P = 0.002$ although the task took twice as long to perform. The RUA exposure associated with handling rats was reduced 25-fold when performed in a ventilated cabinet ($n = 21$, median = 2.67 $\mu\text{g}/\text{m}^3$) compared with handling of rats on an open bench ($n = 17$, median = 54.39 $\mu\text{g}/\text{m}^3$, $P = 0.0001$).

Conclusions Effective reduction of exposure to animal allergens can now be achieved by the use of ventilated systems both for housing and handling rats and mice providing safety equipment is used correctly. The vacuum removal of soiled litter during the task of cleaning out was less efficient and additional respiratory protection is therefore recommended for this procedure.

Keywords: aeroallergen, laboratory animal allergy, rat urine

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Introduction

Allergy to laboratory animals is an important occupational health problem affecting those working with rats, mice and

other laboratory animals. Approximately one in three people with direct contact with these animals, or their dander or urine, will develop allergic symptoms [1,2]. Asthma, the most serious symptom, may be experienced by one in 10 people with laboratory animal exposure and laboratory animals are consistently cited as being one of the three most common causes of occupational asthma reported to SWORD (surveillance of work-related and occupational respiratory disease) in the UK [3].

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Information about the occupational exposure of laboratory animal workers to rat and mouse urine proteins has only recently become available [4–7]. These reports highlight the high levels of animal allergen exposure experienced by those with direct animal contact; the tasks of cleaning out and handling the animals are particularly associated with very intense aeroallergen exposure [8,9]. The effect that this exposure may have on the prevalence and incidence of allergy to these animals has been examined and a relationship between the intensity of exposure to RUA and MUP with sensitization and symptoms, especially skin symptoms, has been demonstrated [10]. The results from the epidemiological exposure-response study of Cullinan *et al.* strongly suggest that a reduction of exposure to laboratory animal allergens may be associated with a reduction in the risk of developing the disease. This hypothesis is supported by the observations of Botham *et al.* who noted a decrease in the incidence of laboratory animal allergy (LAA) symptoms when the mandatory use of respiratory protection was adopted [11].

Although effective methods to reduce the background concentrations of rat urinary aeroallergen (RUA) in an animal house have previously been described [12], there is little information about the efficacy of safety equipment to reduce personal exposure to animal allergens during high risk procedures such as the handling of animals and the removal of soiled litter. This paper aims to assess objectively the effectiveness of safety equipment used to reduce exposure to rat and mouse allergens.

Materials and methods

Collection of air samples

Static and personal air samples were collected at 2 L/min using AFC 123 pumps (Casella London Ltd, London, UK). The dust was impacted onto PTFE filters (1.2 µm pore size, Sartorius Instruments Ltd, Epsom, UK) and the allergen eluted as described previously [13]. Additional air samples were collected using a high volume air sampler (180 L/min, Air Sentinel, Quan-Tec-Air Inc., Rochester, MN, USA) and the PTFE filters (0.2 µm pore size, Quan-Tec-Air Inc.) eluted in 15 mL of buffer.

Measurement of allergen

The content of the air sample eluates was quantified using radioallergosorbent test (RAST) inhibition. The rat and mouse urinary antigens were prepared and coupled to cyanogen bromide activated discs as described in detail before [4]. The mouse urine RAST inhibition assay employed pooled serum from five mouse urine allergic subjects (used at a dilution of 1:16) and had an assay

range of 0.5 µg/mL to 1.0 mg/mL. The detection limit for the mouse urine RAST inhibition assay was 0.5 µg/mL. The rat urine RAST inhibition assay was performed exactly as described previously and the detection limit for the rat urine RAST inhibition assay was 0.05 µg/mL [4]. The final aeroallergen concentration was obtained after correction for any dilution factors and the volume of air sampled. If no detectable allergen was measured, the filter was assigned an allergen level of half of the detection limit of the assay [4,14] and was corrected for the volume of air sampled. This enables an estimate of the exposure to be included for the purpose of statistical analysis.

Animal husbandry

Adult rats (male, CBH/cbi) and mice (female, DBA2 and Balb/C supplied by Medical Research Council, National Institute of Medical Research, London) were housed in separate rooms and according to Home Office code of practice. They were supplied with expanded rodent diet (Special Diet Services, Witham, Essex) and water *ad libitum*.

Assessment of 'safety' equipment

A summary of the experimental comparisons is shown in Table 1.

Effectiveness of positive individually ventilated (PIV) cage system to contain animal allergens

The PIV cage system (Thoren Maximiser PIV cage rack) was obtained from Thoren Cage Systems, Hazelton, PA, USA by their suppliers Lab Care Precision, Aldington, Kent. The cages were suspended on runners from a hollow metal shelf through which the air supply was delivered and removed. Each cage was capped with a removable spun polyester filter (7–10 µm pore size). Forced ventilation was provided by two fans, one of which maintained the flow of supply air and one the exhaust air. Each fan unit was provided with a pre-filter and high efficiency HEPA filter and adjustable dampers permitted the

Table 1. Summary of the experimental comparisons

Husbandry equipment	Species
PIV cage system vs conventional cages	Mouse
PIV cage system – 'Box change'	Rat
Handling. Bench vs ventilated cabinet	Rat
Litter removal. Vacuum vs 'tipping'	Rat

air flow to be regulated so that the cage could be operated at positive, equal (ambient) or negative pressure to the room.

Mice Five hundred and forty-five female mice (stock density 11.1 mice/m³) were housed in the PIV cage system, five mice per cage, on sawdust litter (grade 6, Special Diet Services, Witham, Essex). Where comparisons were to be made, the animals were also housed subsequently 15 per cage in open top, solid base cages (type RC1, North Kent Plastic Cages Ltd, Erith, Kent) on sawdust bedding. Air samples were collected under the following conditions.

i) Static samples were collected at 2 L/min ($n=7$, 4 h) with no animals present in order to establish baseline levels of MUA.

ii) The mice were housed in the PIV cage system which was then operated for 1 week each at negative, positive and then ambient pressure to the room. To allow the MUA concentrations to equilibrate after each husbandry change, 3 days were allowed to elapse before the commencement of the air sampling. Cleaning out took place on a non-sampling day. The effect on static (or background) MUA concentration was monitored by collecting air samples at 2 L/min for ≈ 8 h at six sites around the room (Fig. 1 left). One addi-

tional static sample was collected at 180 L/min for 16 h for each pressure change (Fig. 1 left, high vol). These were collected on the first day of sampling only (day 3).

iii) For comparison, all the mice were housed in open top, solid based cages. Six static samples were collected as before at 2 L/min for ≈ 8 h (2 days, $n=12$).

Rats Each PIV cage system (Fig. 1 right) was stocked with, on average, 45 rats (stock density 3.6 rats/m³), 5 rats per cage on sawdust litter and was operated at positive pressure only. Air samples were collected under the following conditions.

(i) Before the room was stocked with animals, baseline samples were collected at 2 L/min ($n=10$). Five static samplers were placed around the room (Fig. 1 right, positions P1-P3, plus on the bench at the rear of the room and in the centre of the room). Sampling times of 24 h per filter were achieved by re-exposure of the filter, in the same position, on 3 consecutive days and storing the filter at 4°C overnight in the sealed sampling head.

ii) On non-cleaning out days (days 1–3), static samples were collected at the same positions. Re-exposure of the filters on 3 consecutive days gave a total sampling time of 24 h. Two sets of filters were collected ($n=10$).

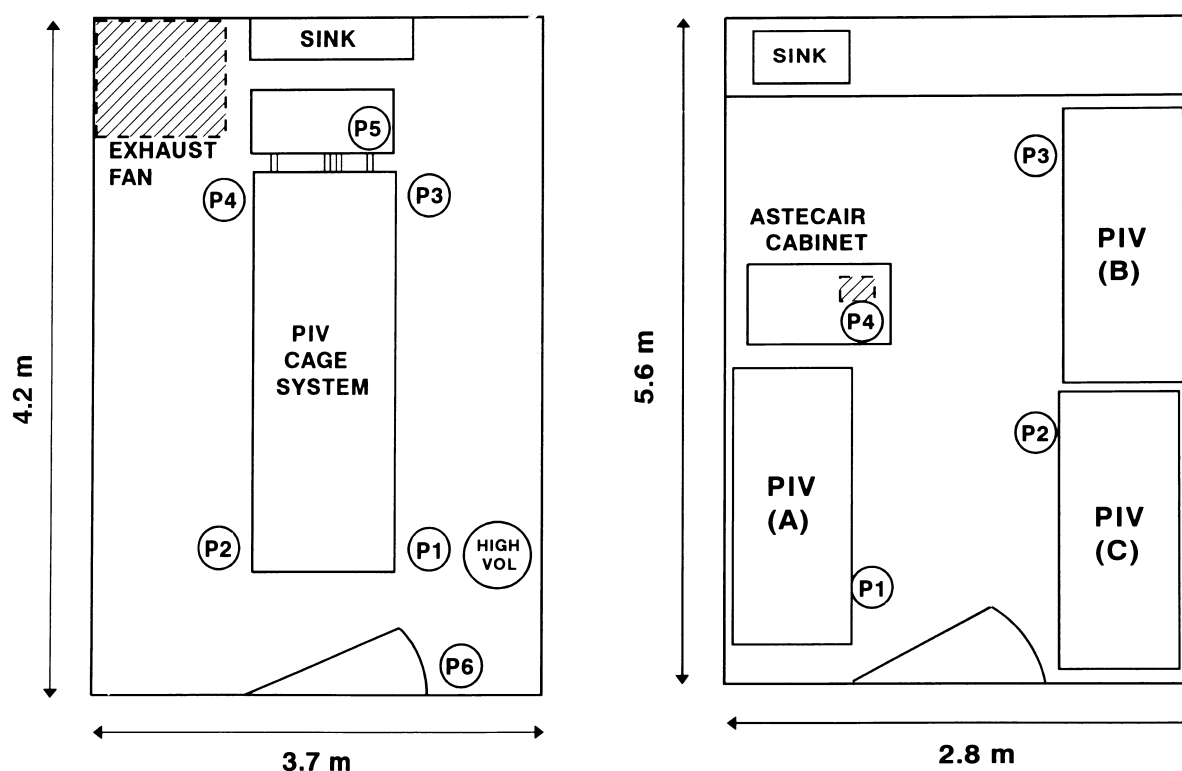


Fig. 1. Plan of the study rooms showing the relative positions of the air sampling pumps (P1, etc.) and the PIV cage systems. Left. Plan of study room for the mouse experiments. Right. Plan of the study room for the rat experiments performed on cleaning out days. An additional pump (P5) was worn by the operator of the Astecair cabinet.

iii) On cleaning out days, once per week (day 4), the animals from PIV cage system A (Fig. 1 right) were transferred into clean cages (a 'box change') within an Astecair 4000E filtration fume cupboard, supplied by Astec Environmental Systems, Weston Super Mare, UK. For this procedure, the Astecair cabinet was operated with the front open which is against manufacturers' recommendations. The soiled cages were then removed from the study room and cleaned elsewhere. Static samples were collected at positions P1-P3 only (Fig. 1 right). An air sampler was also placed on top of the Astecair cabinet to sample the HEPA filtered exhaust air. The operator performing the cleaning out tasks wore a personal air sampler.

In total, seven sets of the air samples (from cleaning out and non-cleaning out days) were collected and the sampling time was for the duration of the task only (1–2 h, $n = 35$).

Effectiveness of safety equipment to reduce personal exposure to rat allergen

Removal of soiled litter The RUA exposure associated with two different methods of removal of soiled litter was compared. Eighteen personal air samples were collected when soiled litter was emptied by tipping into another container and compared with samples collected when the litter was removed by vacuum ($n = 16$; Nilfisk model GS-82, HEPA filtered and with separate waste hopper, supplied by Nilfisk UK, Suffolk). The number of boxes handled and the time taken to perform the task were noted.

Handling of rats The RUA exposure associated with the handling of rats on an open bench ($n = 17$) was compared with that when the animals were handled in an Astecair 4000E filtration fume cupboard, operated with the front down ($n = 21$). The procedure involved transferring the rats from one cage to another for periods of 1 h.

Statistical analysis

All statistical analyses were performed in Minitab (Minitab Inc., 3081 Enterprise drive, State College, PA, USA) on untransformed data. As the data were not normally distributed, non-parametric tests were used; the statistical significance was determined by the Mann–Whitney *U*-test (adjusted for ties) unless otherwise stated.

Results

Effectiveness of PIV cage system to contain animal allergens

Mice All of the filters collected at 2 L/min when the

rooms were empty (background, $n = 7$), or stocked with animals housed in the PIV cage systems operated at negative ($n = 24$), ambient ($n = 24$) or positive pressure ($n = 24$), had insufficient MUA for quantification in the immunoassay. When a value of half of the detection was assigned to these filters the median MUA concentrations obtained were; background = $0.12 \mu\text{g}/\text{m}^3$, negative = $0.085 \mu\text{g}/\text{m}^3$, ambient = $0.085 \mu\text{g}/\text{m}^3$ and positive = $0.100 \mu\text{g}/\text{m}^3$. However, when the high volume air sampler (180 L/min) was used some MUA was detected; at negative, ambient and positive pressure the MUA concentrations were < 0.003 , 0.02 and $0.28 \mu\text{g}/\text{m}^3$, respectively. This indicates that slight leakage of mouse allergen occurred when the rack was operated at positive pressure.

When the same animals were housed in conventional open cages, MUA could be measured with the air samplers operating at 2 L/min (Fig. 2). The median MUA concentration measured was $0.67 \mu\text{g}/\text{m}^3$ ($n = 12$, range 0.290 – $0.940 \mu\text{g}/\text{m}^3$). When operated at positive pressure, the least effective operating condition for allergen containment, the use of the PIV cage system therefore reduced the

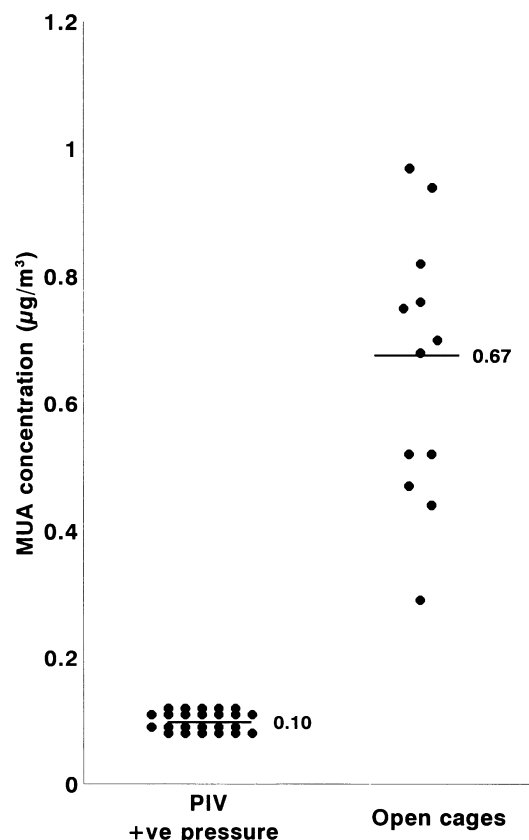


Fig. 2. Comparison of cage type. Mouse urinary aeroallergen concentrations measured when 545 mice were housed in a PIV cage system and in open cages. The median is indicated.

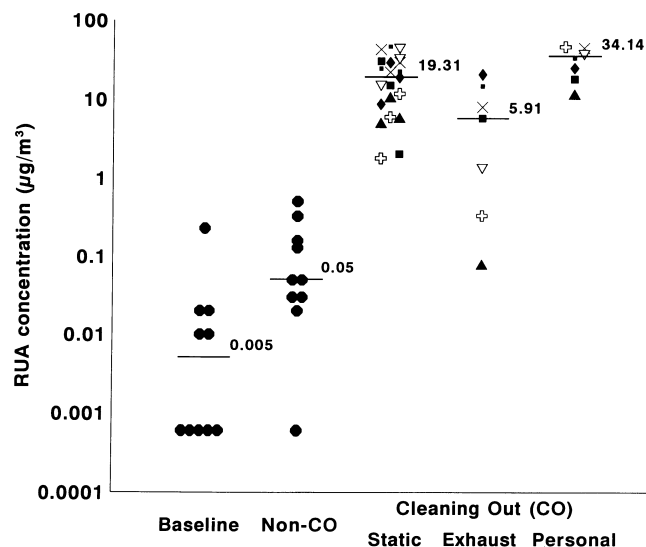


Fig. 3. Levels of RUA measured in the rat study room during the cleaning out of the PIV cage system. The median is indicated. The different symbols used for the cleaning out days represent the measurements made on different days.

MUA concentrations by $\approx$ sevenfold when compared with conventional cage systems (Fig. 2, $P < 0.001$).

Rats The PIV cage system was also effective at containing RUA (Fig. 3). Static samples measured on non-cleaning out days (non-CO) indicated that the median background concentration of RUA ($0.050 \mu\text{g}/\text{m}^3$, $n = 10$) was increased 10-fold over the baseline concentrations measured when the room was empty ($0.005 \mu\text{g}/\text{m}^3$, $n = 10$, $P = 0.009$). The background levels of RUA within the room increased further when cleaning out was in progress and rose to approximately half that of the personal exposure experienced by the operator during the 1–2 h that the rats were being cleaned out (median CO = $19.31 \mu\text{g}/\text{m}^3$, $n = 21$, median personal = $34.14 \mu\text{g}/\text{m}^3$, $n = 7$). The use of a ventilated cabinet for the ‘box change’ did not prevent this rise and significant amounts of allergen appeared to

have escaped through the open front (the front of the cabinet was left open against manufacturers’ instructions) and the exhaust of the cabinet (median RUA concentration = $5.91 \mu\text{g}/\text{m}^3$, range 0.08 – $19.54 \mu\text{g}/\text{m}^3$, $n = 7$). How long the RUA levels remained elevated within the study room is unknown.

Effectiveness of safety equipment to reduce personal exposure to rat allergen

The RUA levels measured during the removal of soiled litter and the handling of rats with and without the use of safety equipment are shown in Table 2. Whilst there is an apparently twofold reduction in the personal RUA exposure associated with the removal of soiled litter when the vacuum device was used ($P = 0.002$), both the number of boxes handled and the time taken to perform the task were significantly greater in the group where the vacuum device was used ($P < 0.0001$, Table 1). To assess the contribution of these factors to this apparent decrease in exposure, the amount of RUA captured on each filter was determined and corrected for the number of boxes handled. There was no significant difference in the RUA released per box emptied between the two groups ($P = 0.60$, Table 1) indicating that the ‘total dose’ of allergen experienced by the operator when doing this task was the same for both systems of litter removal.

A highly significant, 20-fold reduction in RUA exposure was observed when animals were handled in the ventilated cabinet when compared to handling on the open bench (ventilated cabinet median = $2.67 \mu\text{g}/\text{m}^3$, $n = 21$, range 0.05 – $11.20 \mu\text{g}/\text{m}^3$. Open bench median = $54.39 \mu\text{g}/\text{m}^3$, $n = 17$, range 3.99 – $126.41 \mu\text{g}/\text{m}^3$, $P < 0.0001$). As all filters were exposed for 1 h, the significance of this test was not affected.

Discussion

In 1992 we reported on the effectiveness of simple modifications to husbandry practise in reducing the background

Table 2. Comparison of the effectiveness of safety equipment to reduce personal exposure to RUA

Exposure group		<i>n</i>	RUA $\mu\text{g}/\text{m}^3$		μg on filter/ <i>n</i> $^\circ$ of boxes	
			median	range	median	range
Cleaning out	+ vacuum removal	17	22.87	1.25–37.42	0.0191	0.0017–0.0360
	– vacuum removal	18	38.15	4.31–90.65	0.0195	0.0027–0.0459
Handling	+ vent. cabinet	21	2.670	0.05–11.20	–	–
	– vent. cabinet	17	54.39	3.99–126.41	–	–

levels of RUA in a rat stock room [12]. Whilst keeping the stock density to a minimum and replacing in-contact wood-based litters with non-contact absorbent litter significantly reduced the concentrations of RUA in the room, the levels were further reduced by housing the animals in filter-top cages for a short period of time. Since, for the foreseeable future, there is still a need for animals in medical research, close contact between research staff and laboratory animals cannot be avoided. Whilst much can be done to contain allergen when the animals are housed within cages, the greatest risk of high exposure to animal allergens is when the animals are undergoing procedures such as being handled or cleaned out. We have now extended our studies to assess objectively methods to contain animal allergens both when the animals are being used and when in their cages.

Our original study with filter-top cages demonstrated their efficacy at containing allergen but could not be applied to the long-term housing of animals due to the low air-exchange rate within each cage and the detrimental effect that this would have on the animals' health. This has been overcome by using a cage system where each cage is individually ventilated. The conditions for the animals within these cages has been previously tested and shown to be satisfactory [15]. The primary purpose of the PIV cage system is to provide a cage barrier system to protect the animals within from airborne contamination by the provision of individual ventilation with the supply and exhaust of filtered air to each filter capped cage. For the maintenance of optimum barrier conditions, the cage system should be operated at positive pressure to the room. Under these conditions, the leakage of a small amount of airborne MUA could be detected in the background air of the room with the high volume air samplers (180 L/min); the amount of allergen escaping from the cage system was too low to be detected with the low flow rate air samplers (2 L/min). Even when operated at positive pressure to the environment, the PIV system was an effective way of containing both rat and mouse aeroallergen and is recommended for housing animals where allergen containment is important.

The efficacy of the other safety equipment tested appeared to be more variable. The Astecair ventilated cabinet when used according to the manufacturers' instructions was very effective at containing RUA resulting in a 20-fold reduction in exposure when compared with handling rats on the open bench. When used with the front up for the 'box changing' of rats however, the effectiveness of the ventilated cabinet was considerably reduced. The efficacy of the vacuum system for removing soiled litter was more difficult to assess as it took twice as long to empty the cages of the soiled litter with the vacuum device as compared to simply tipping the soiled litter from the cages into an open receptacle. A twofold reduction was observed in the

intensity of RUA exposure when the vacuum system was used although the total allergen or 'dose' captured on the filter during these procedures was the same. We do not know whether this is a consequence of the operator being exposed at a lower intensity for a longer time, or whether the peaks of exposure are the same with both methods (e.g. still significant disturbance of litter with vacuum removal or leakage from equipment). It now needs to be determined whether this reduction in intensity of exposure when using the vacuum system is of any safety value even though the apparent dose of allergen remained the same.

The aim of controlling exposure to allergens is to reduce the levels of allergen below that eliciting asthma and other symptoms in sensitized workers, and ultimately to prevent subjects becoming sensitized in the first place. It is generally believed that the level of allergen required to sensitize an individual is lower than that to elicit symptoms in sensitized workers. Approximate threshold values have been established for domestic allergens such as Der p 1 (the major house dust mite allergen) below which the risk of becoming sensitized (2 µg/g of household dust) and eliciting symptoms in sensitized asthmatics (10 µg/g household dust) is reduced [16]. A similar threshold level (> 8 µg/g household dust) for the elicitation of asthmatic symptoms in cat sensitized individuals has also been proposed for the major cat allergen, Fel d 1 [17]. It is now thought that this approach may be simplistic for several reasons the main ones of which are non-atopic individuals may become sensitized at higher exposure levels than atopic (and possibly other immunogenetically susceptible individuals); the relationship between current exposure and symptoms may be complex and subject to individual variation; and the nature of the relationship between allergen exposure and sensitization and/or symptoms is not known (i.e. whether it is linear, sigmoidal or some other dose-response curves) [reviewed 18].

Although the relevant threshold values have yet to be fully determined for occupational exposure to rats, the interesting findings of Botham *et al.* would suggest that a threshold phenomena exists. They have shown that where vigorous hygiene standards have been enforced (including the use of respiratory protection), whilst the reporting of LAA type symptoms by animal exposed staff has been reduced, the number of subjects becoming immunologically sensitised has not [19]. This observation would therefore seem to parallel those seen for domestic allergens and is the first evidence that a lower threshold for sensitization than elicitation of asthmatic symptoms would also exist for laboratory animal allergens. Whilst this study is encouraging, it also demonstrates that highly efficient and practicable methods are needed to reduce RUA sufficiently to prevent sensitization.

Although LAA caused by rat urine has been recognised for some 20 years [20] the prevalence of the disease in this

period has not significantly decreased [21–24] despite engineering attempts to improve the environment in animal houses both for employees and the animals. During this period however, the procedures used to handle the animals have remained much the same partly due to the nature of the work, but also due to a general reluctance to use ventilated cabinets and other safety equipment. Consequently exposure to animal allergens during handling and experimental procedures has probably not changed significantly. Whilst at this stage the effect of the use of the equipment studied here on the incidence rate of LAA can not be assessed, anecdotally, it may be possible for pre-sensitized staff to continue their work with laboratory animals under these conditions; to date three sensitized workers in the unit where this work was carried out, and who have been working exclusively with the PIV cage system and performing procedures in correctly operated ventilated cabinets, have remained symptom free for 12 months. Unfortunately the subjects predominantly described nasal symptoms and there are no supporting objective data. From the results presented here the level of airborne RUA below which these subjects have remained symptom free is $\approx 10 \mu\text{g}/\text{m}^3$ (as measured by RAST inhibition assay and when strictly adhering to the correct use of safety equipment). Further evidence now needs to be sought to confirm the existence of a threshold of RUA exposure to elicit LAA symptoms and to establish the value by additional methods of RUA quantitation.

In summary we have shown that ventilated cages are an effective way of containing animal allergens whilst the animals are not being handled. When animals have to be handled for procedures such as 'box changing', examinations and experimental procedures, the use of a ventilated cabinet, when operated correctly, can significantly reduce exposure to animal allergens. There is currently insufficient evidence to suggest that the vacuum removal of soiled litter is effective in reducing the total RUA exposure received, and additional safety precautions such as the use of respiratory protection should be considered. With the introduction of these safer handling procedures, the prevalence and incidence of LAA to rats and mice should be minimized.

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