

Asthma and Rhinitis Related to Laboratory Rats: Use of a Purified Rat Urinary Allergen to Study Exposure in Laboratories and the Human Immune Response

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ABSTRACT

Using the major rat allergen as a model has made it possible to study both natural exposure and the human immune response to an important laboratory animal. The close correlation between positive skin tests to whole rat urine and IgE antibody to the major urinary allergen in this and previous studies supports the use of this protein as a model rat allergen. Measurements of airborne rat allergen confirm that the maximum levels are higher than those reported with pollen or mite allergens. However, it is possible that exposure to rat allergens is comparable to levels of exposure to cat salivary allergens in houses with cats. The clear implication is that the high levels of exposure are responsible for the fact that a large proportion of exposed individuals develop IgG antibodies. Our results suggest that the prevalence of IgG antibodies (not indi-

vidual levels) in a group of workers would be a good guide to exposure. This leaves unresolved why some of the individuals who develop IgG ab also develop IgE ab and become at risk for developing asthmatic responses. Only part of this risk is related to atopy. A striking feature of all the studies on animal allergy is the close association between IgE ab and asthma. It appears clear that it is those immune responses that include IgE ab that are a risk factor for asthma. It is not sensible for anyone to remain consistently sick with asthma and continue working with laboratory animals because there are well documented examples of occupational asthma that has not resolved after ceasing exposure. Finally the risk of acute attacks of asthma on exposure to animals must be regarded as a serious health problem both on an individual basis and also in the design and management of research facilities.

INTRODUCTION

Allergic reactions to laboratory animals are a major problem both in academic and commercial research. After working with animals for as little as a few months up to 15% of exposed individuals may develop rhinitis, conjunctivitis, urticaria, dermatitis and/or asthma.¹⁻⁴ The rhinitis and skin rashes though not dangerous may be sufficiently severe to interfere with the patients' employment. Asthma related to exposure to laboratory animals occasionally presents as a gradual worsening of pre-existing chronic asthma, however attacks usually develop rapidly on exposure to animals and may be acute or even life threatening. In many cases these attacks of asthma make it impossible for the individual to continue working with animals. Although

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individuals vary in the animals to which they react, there is a tendency to develop either generalized sensitivity to rodents or to become allergic to a new species if they change their exposure. Many different animals can cause symptoms but in several series rats have been reported as the most important cause.^{1,2,5,6} In our studies we have used the major urinary allergen of the rat as a model both of rat allergens and of laboratory animals in general.^{5,7,8,9} This protein can be radiolabelled easily and can be used either for measuring isotype specific antibodies or for measuring rat allergen. In the present short review we will focus on the conclusions of these studies measuring exposure to rat allergen in laboratories, the immune response to rat allergen and the relevance of these results to control measures.

EXPOSURE TO RAT URINARY ALLERGEN

The important or major allergens from many different sources are freely soluble proteins with a molecular weight of 10,–40,000 Daltons. In many cases these major allergens also turn out to be the protein present in the largest quantity.^{10,11} Laboratory rats lose surprisingly large quantities of protein in their urine, greater than 2 mg/ml for healthy male rats and up to 0.5 mg/ml in urine from females.^{7,8} Thus, it is not surprising that for rats, and many other rodents, urine is increasingly being recognized as the major source of allergen. It is also obvious that the major accumulation of rat allergen will be in the cage litter. Indeed we have measured up to 3 grams of rat allergen in a single cage. The high concentration of rat allergen in urine makes it relatively easy to purify this allergen.⁷ There are two forms of the allergen in rat urine, a prealbumin and a euglobulin. Although those proteins have distinctly different molecular weight and only partially cross react using rabbit antisera on crossed immunoelectrophoresis, they appear to fully cross react as seen by human IgE antibodies.⁹ The protein we have used is the alpha-2-euglobulin (moll. weight 18,000). Alpha 2 is its electrophoretic mobility and the suffix eu- indicates that it precipitates when dialysed against distilled water. This same protein has previously been referred to as alpha-2_u-globulin where the "u" indicates its urinary origin. When radiolabelled with ¹²⁵Iodine, this protein can be bound either by rabbit antibodies or by human IgG or IgE antibodies. To measure allergen we used an inhibition radioimmunoassay (RIA) where the first antibody was the IgG ab present in the serum of a rat allergic patient, and the second antibody was goat anti-human IgG.⁸ This RIA is capable of detecting ≤1 ng of rat allergen and is standardized relative to measurements of protein made on purified rat allergen.

Since most rat allergic workers are clear that their allergic reactions are caused by entering sites where rats

Table I		
Rat and Dust Mite Allergen in House Dust and Rat Litter*		
	Rat Allergen ng/g	Antigen P ₁ ng/g
Selected house dust samples		
i	<8	233,000
ii	<8	25,000
iii	<8	1,218
iv	<8	340
Samples from rat room		
Dirty litter	855,000	<60
Clean sawdust	38	112
Shelf dust	340,000	294
	ng/ml	ng/ml
Skin test reagents		
Rat hair	842	<1.0
<i>D. pteronyssinus</i>	<0.4	61,000
House dust	<1.0	820
Male rat urine	a) 2,166,000 b) 4,660,000	<2 <2
Female rat urine	a) 30,000 b) 540,000	<2 <2

* Allergens measured by double antibody inhibition radioimmunoassay. Values for dust or litter obtained from 100 mg fine dust extracted with 2 ml buffered saline values calculated as ng/g.

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are kept, it is not surprising that we found high levels of rat allergen in animal facilities and no allergen either in laboratories where rats were not present or in house dust (Table I).⁸ Sampling the air in laboratories by pulling air through a millipore filter we found significant allergen airborne in all rat rooms (1–50 ng/m³) with a modest increase during changing of the cages. In rooms where rats were kept on litter the airborne allergen levels were slightly higher than in rooms where the rats were kept on wire mesh suspended above the litter. Initial experiments to look at the quantities of allergen that became airborne during cage cleaning gave very confusing results, i.e. very high and very low levels on different occasions. These differences appeared to be partly

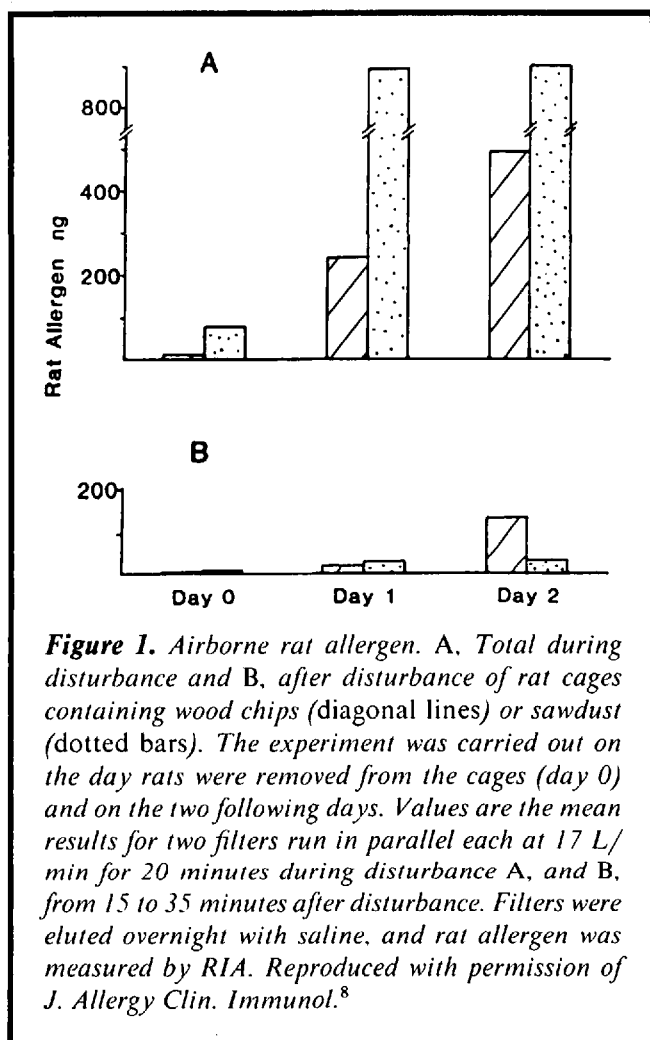


Figure 1. Airborne rat allergen. A, Total during disturbance and B, after disturbance of rat cages containing wood chips (diagonal lines) or sawdust (dotted bars). The experiment was carried out on the day rats were removed from the cages (day 0) and on the two following days. Values are the mean results for two filters run in parallel each at 17 L/min for 20 minutes during disturbance A, and B, from 15 to 35 minutes after disturbance. Filters were eluted overnight with saline, and rat allergen was measured by RIA. Reproduced with permission of J. Allergy Clin. Immunol.⁸

due to differences between sawdust and wood chips, however, the major variable was the saturation of the litter with urine. Shaking wet wood chips or wet sawdust produced very little airborne allergen but after one or two days of drying the quantities becoming airborne increased dramatically, up to 800 ng collected on a filter run at 17 liters/min for 20 mins (Fig. 1). Thus there were already two important differences between airborne rat allergen and airborne dust mite allergen: first, rat allergen was always detectable in the air of rat rooms whereas mite allergen is generally undetectable in the air of an undisturbed room, even when the floor dust contains very high levels; second, the maximum levels of rat allergen that become airborne during cage disturbance were much higher than the levels of airborne mite allergen we have measured in houses.¹² The high levels of rat allergen are in keeping with values obtained by Dr. Swanson and his colleagues at the Mayo Clinic in experiments on airborne mouse and guinea pig allergens.^{13,14}

The next experiments were designed to examine the size of particles carrying airborne rat allergen. Sampling the air with a cascade impactor gave a mean particle size carrying rat allergen of 7 μ m diameter which was significantly smaller than the size of particles carrying airborne mite allergen. In keeping with that size airborne rat allergen was shown to fall significantly more slowly than mite allergen, i.e. in about 15–20 minutes rather than in $\sim$ 5 minutes. The mean results for experiments on particle sizes carrying mite or rat allergens are shown in Table II. Airborne rat allergen was found on smaller particles than mite allergen, however, in both cases the sizes of particles carrying allergen were significantly greater than the size of droplets produced by a nebulizer (Table II).

THE HUMAN IMMUNE RESPONSE TO RAT URINARY ALLERGEN

It is well established that most patients who develop rhinitis or asthma on exposure to laboratory animals have positive immediate skin tests to dander, serum or urine of the relevant animal.^{1,2,3,5,6} We were interested in the immune response to rat allergen not only in the symptomatic patients but also in the majority of laboratory workers who do not develop symptoms. In 1981 John Edwards and his colleagues studied 213 laboratory workers and animal handlers and reported their results in the Lancet.¹ That study confirmed the presence of positive skin tests to animal urine in most of the symptomatic individuals and found that reactions to rat urine were the single most important problem. In addition that study suggested that the correlation between animal allergy and atopy was not very marked. They concluded that screening applicants to exclude atopic individuals would be an ineffective approach to controlling animal allergy.¹ That survey was also designed to look for cases of chronic fibrotic lung disease or of Allergic Alveolitis, among laboratory workers. The results including lung function studies and tests for serum precipitins did not reveal any evidence that animal exposure could cause lung disease apart from asthma. Following that study we assayed sera from all 213 individuals where symptoms, past history, skin tests and lung function results were already available.

The sera were assayed for IgG and IgE antibodies using ¹²⁵I labelled rat urinary allergen. The technique used was radioimmuno precipitation in which, after binding radiolabelled allergen, the immunoglobulin of each class was precipitated separately by monospecific goat anti-IgG or goat anti-IgE.^{9,10,15} The results were analyzed relative to symptoms, exposure history and atopic status.⁹ The correlation between IgE antibodies to rat allergen and positive skin tests to rat urine was excellent; IgE ab was detected in 14 out of 22 sera from

Table II

Summary of Results on the Distribution of Rat Urinary Allergen and Mite Allergens (Antigen P₁) on a Cascade Impactor

Impactor Stage	Approximate Diameter of Particles Collected μm	Rat Urinary Allergen			Dust Mite Antigen P ₁			Nebulized** Aqueous Extract	
		Rat Rooms* (n = 5) %	Artificial Disturbance†		Houses‡ (n = 5) %	Artificial Disturbance $\diamond$		Rat (n = 8) %	Mite (n = 2) %
			During (n = 8) %	Post (n = 8) %		During (n = 6) %	Post (n = 9) %		
1	>20-6	26.9	44.3	8.7	88	76.1	26.2	0.6	<2
2	15-2	45.4	45.9	42.3	6.7	13.2	36.2	9.0	10.4
3	5-1	11.8	4.5	17.4	1.8	5.5	15.4	12.3	11.5
4	2.5-0.3	5.7	2.6	21.2	1.4	3.9	13.5	55.9	73.5
Final filter	<0.5	10.3	2.7	11.1	1.3	1.2	8.6	22.1	<6

* Values from Table 2.

† Values from Table 3 and 6 further experiments.

‡ Values from Tovey et al. 1981, obtained during housecleaning.

$\diamond$ Values from Table 4 plus 7 further experiments.

** The mean percent of nebulized extract collected on the first two stages of the impactor (9.6%) was significantly lower than comparable results for Antigen P₁ post-disturbance (62.5%, $p < 0.001$) or rat allergen post-disturbance (51.2%, $p < 0.005$).

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individuals with positive skin tests and only three out of 191 sera from individuals with negative skin tests. Equally striking was the correlation between a history of asthma on exposure to rats and serum IgE ab to rat allergen; 12 of 18 individuals reporting asthmatic reactions had IgE ab, while only two out of 182 asymptomatic individuals had detectable IgE ab to rat allergen. By contrast IgG ab to rat allergen was present in sera from many individuals who had negative skin tests and reported no symptoms on exposure to rats (Fig. 2). Thus while 83% of patients reporting asthma on exposure to rats had IgG antibodies, 30% of asymptomatic individuals also had IgG antibodies. Using comparable techniques we have found that less than 10% of asymptomatic normal individuals have IgG antibody to common allergens such as grass pollen or dust mite.

When we analyzed the relationship between IgG antibodies to rat allergen and the degree of exposure to rats we found a clear positive correlation (Fig. 3). However this correlation was with the hours/day spent in the laboratory/animal house area rather than with the number of years spent working with rats. The impli-

cation is that a particular level of allergen over a limited period of time, i.e. days or weeks is critical in initiating an immune response to rat protein. The results also allowed us to re-examine the relationship between "atopy" and immune responses to rat urine. Using a positive skin test to dust mite, cat and/or grass pollen as the definition of atopy we found an increased incidence of both symptoms and IgE antibodies among atopic individuals. Thus, 12 of 18 (66%) of patients with asthma related to rats were atopic, while 71 of 179 (40%) of asymptomatic individuals were atopic. Equivalent figures were found showing a modest correlation between atopy and IgE antibody to rat allergen but no effect of atopy on the prevalence of IgG antibody to rat allergen.

RELEVANCE OF THESE RESULTS TO DESIGN OF MEASURES TO PREVENT RAT ALLERGY

Control of laboratory animal allergy can involve several different approaches: (a) selection of employees to avoid those at high risk, (b) reduction of exposure at work, and (c) medical management.

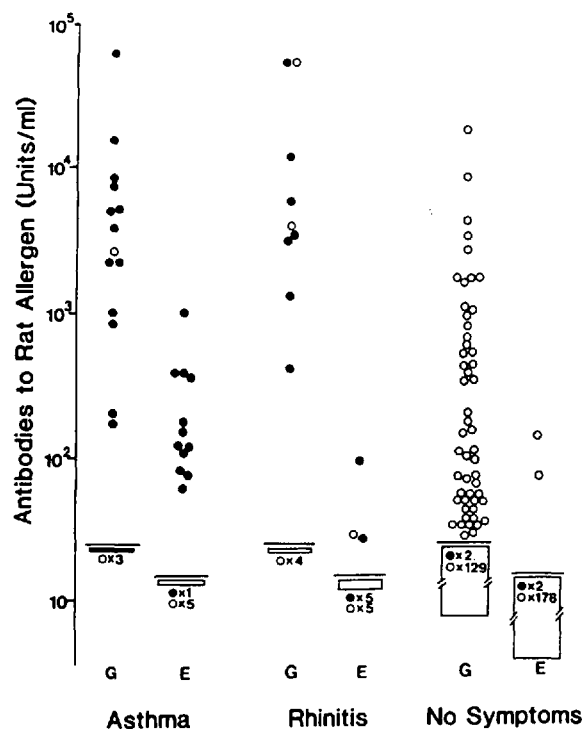


Figure 2. Isotype-specific antibodies to rat allergen in sera from individuals reporting asthma, rhinitis or no symptoms. IgG antibodies (G) and IgE antibodies (E) were measured by radioimmunoprecipitation and values are given in arbitrary units. The asymptomatic group includes the 54 sera from unexposed individuals. Individuals who had positive skin tests to rat urine are indicated by solid circles (●). The number of sera showing no detectable antibodies is shown below the line for the lower limit of the assay. Reproduced with permission of *J. Allergy Clin. Immunol.*⁹

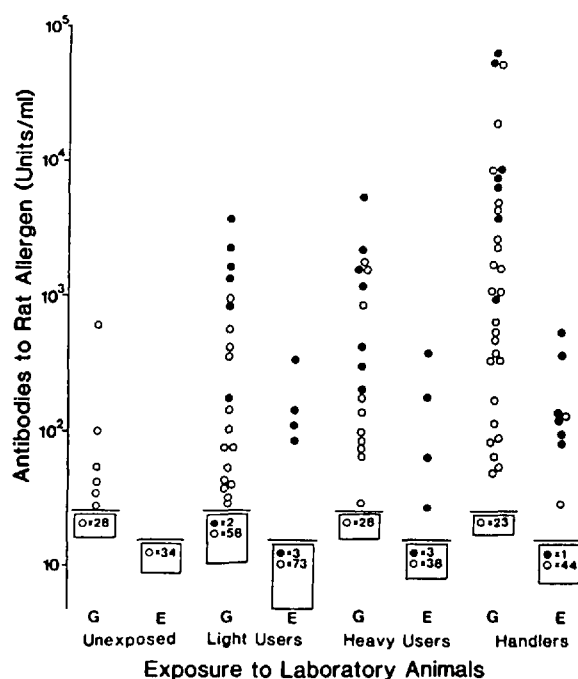


Figure 3. IgG (G) and IgE (E) antibodies to rat allergen in sera from groups with different degrees of exposure to laboratory animals. Individuals with a positive skin test to rat urine are indicated by solid symbols (●). Exposure is divided into animal handlers (working with animals all day); heavy users (>2 hours/day), and light ones (<2 hours/day). Reproduced with permission of *J. Allergy Clin. Immunol.*⁹

(a) Selection of Employees

Several studies in the past have recommended that employers should screen prospective laboratory workers to avoid those with a high risk of developing allergy. This could be done by taking histories of allergy, by skin testing, or by *in vitro* assays of IgE antibody. In 1981 Edwards, et al. concluded that excluding patients with an allergic history would *not* be an effective way of reducing allergic disease.¹ Our data on rat allergy suggests that excluding 71 out of 179 (atopic by skin tests) applicants would only have prevented 66% of the cases of laboratory animal asthma. Excluding the 41 individuals who had both a prior history of allergic disease and positive skin tests would only have avoided

33% of the cases of asthma. The clear conclusion was that although there is a statistically increased risk of rat allergy among atopic individuals, a large proportion of rat allergic individuals were not previously atopic, and the majority of atopic individuals did not become allergic when they worked with rats. By contrast there is a general consensus that individuals who are currently under treatment for asthma should be advised not to work with laboratory animals.

(b) Reduction of Exposure

The second approach is to try to control the quantities of allergen that are inhaled by animal handlers, technicians and scientists. The first approach used was

to improve filtration of air in animal facilities, both by fitting better filters and by increasing the frequency of circulation. However, this was not uniformly successful because increased airflow has a tendency to increase the amount of allergen coming off the rats and the litter. Indeed in some institutions increasing airflow appeared to increase the severity of allergic reactions. A more useful approach is to design animal facilities so that the air flowing over the cages does not flow towards the handlers. However it is difficult to change airflow patterns after a facility has been built. Our results also show that minor changes in cage design could alter the quantities of allergens that become airborne. Thus, wood chips produce less airborne allergen than sawdust, rats housed on wire mesh produce less allergen, and most important dry litter produces far more allergen than wet litter. There are also techniques for housing animals on forms of paper which might further reduce the quantities that become airborne. Although none of the results on cages are significantly advanced to recommend changes in cage design they certainly suggest that real advantages could be obtained and that further studies should be encouraged.

In general it is not possible for animal handlers to avoid exposure to rats as there are very few establishments which are sufficiently large for individuals to work away from the rodents. For scientists and technicians it is possible to reduce exposure in a variety of ways.

i) Have animals transferred to clean cages and moved to a separate experimental room away from the main animal area. However, it is a mistake to have laboratory animals around the normal laboratory because this will increase exposure of others.

ii) Use female animals where possible, or in some cases use other non rodent species, e.g. musk shrews. Changing one rodent species to another generally gives only temporary relief.

iii) Wear masks, if possible; surgical masks help, a helmet with filtered air may be fully effective if it can be tolerated.

(c) Medical Management

Medical management of allergic reactions to laboratory animals is along familiar grounds. It is reasonable to try to control both rhinitis and asthmatic responses using antihistamines, local steroids and Cromolyn. In our experience controlling asthmatic reactions is similar to controlling exercise induced asthma, i.e. inhaled Cromolyn, and B₂ agonists (plus or minus antihistamines) prior to exposure. In some cases it may be justifiable to use oral xanthines either on a regular or occasional basis. However any patient who requires either maximal doses of Theophylline or oral steroids should not be allowed to continue exposure to labora-

tory animals. Similarly it is difficult to become enthusiastic about using immunotherapy for a problem that can be solved in other ways. Interestingly, the major problem with asthma due to laboratory animals appears to be acute attacks at the time of exposure. Thus, gradual development of chronic asthma without acute attacks at work appears to be rare. In keeping with this, Levy, et al.,¹⁶ reported that some patients who had acute attacks of asthma associated with rats did *not* have increased non specific bronchial reactivity. This may reflect the fact that natural exposure to rat allergen often represents a high dose carried on "small" particles which approximates more closely to bronchial provocation.¹⁷ If this is so then adequate control of the acute attacks with Cromolyn and B₂ agonists may be sufficient and the risk of developing chronic changes is small. However, all workers who develop acute attacks of asthma must be *at risk* for developing chronic bronchial reactivity which may or may not be reversible, and they should be warned.

CONCLUSIONS

Using the major rat allergen as a model has made it possible to study both natural exposure and the human immune response to an important laboratory animal. The close correlation between positive skin tests to whole rat urine and IgE ab to the major urinary allergen in this and previous studies supports the use of this protein as a model rat allergen. Indeed comparison with studies on other laboratory animal allergens suggests that these results are reasonably typical of laboratory animal allergens in general. Measurements of airborne rat allergen confirm that the maximum levels are higher than those reported with pollen or mite allergens. However, it is possible that exposure to rat allergens is comparable to levels of exposure to cat salivary allergens in houses with cats. The clear implication is that the high levels of exposure are responsible for the fact that a large proportion of exposed individuals develop IgG antibodies. Our results suggest that the prevalence of IgG antibodies (not individual levels) in a group of workers would be a good guide to exposure. This leaves unresolved why some of the individuals who develop IgG ab also develop IgE ab and become at risk for developing asthmatic responses. Only part of this risk is related to atopy. Another issue that is not yet resolved is whether annual skin tests and/or annual measurements of IgG antibody would successfully identify those individuals who were going to develop symptoms. A striking feature of all the studies on animal allergy is the close association between IgE ab and asthma. It appears clear that it is those immune responses that include IgE ab that are a risk factor for asthma.

Screening applicants is neither a reasonable nor an effective approach to reducing allergic disease caused by laboratory animals. However, improving the design of cages and animal facilities could be a useful approach. Changes in bedding or airflow might dramatically decrease exposure. However, any measures would have to be carefully worked out taking into account the health needs of the animals. Keeping rats on wet paper with very low airflow would not be wise (rats develop pneumonia if the airflow is reduced). What is needed is progressive modifications to a series of rooms combined with monitoring airborne allergen levels. A true study to establish that a given design would decrease the prevalence of disease would be a much bigger undertaking. In the meanwhile, the simple practical measures that can be taken to reduce exposure should not be ignored, as they can make major differences in individual cases. On the other hand asthmatic reactions in laboratory animals are a serious clinical problem. It is not sensible for anyone to remain consistently sick with asthma and continue working with laboratory animals. There are well documented examples of occupational asthma that has not resolved after ceasing exposure. Finally the risk of acute attacks of asthma on exposure to animals must be regarded as a serious health problem both on an individual basis and also in the design and management of research facilities.

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