

Task-related variation in airborne concentrations of laboratory animal allergens: Studies with *Rat n I*

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To define airborne allergen exposure during various tasks with rats in a laboratory, concentrations of allergen Rat n I were measured by radioimmunoassay in extracts from filters in personal air sampling devices that were worn by laboratory workers while they were performing these tasks. The tasks included feeding, cage cleaning, handling, injection, surgery, and sacrifice. Median concentrations encountered during feeding or cleaning (21 ng/m³) and injection or handling (13 ng/m³) were higher than those associated with surgery or sacrifice (3.1 ng/m³; p < 0.01). Area samples in animal-holding rooms contained 3.4 ng/m³ during animal handling and 2.3 ng/m³ at other times. Very low concentrations were found in air outside the handling room, in unused laboratories, or outside air. We concluded that certain tasks incur a higher risk of allergen exposure but that exposure may occur anywhere within an animal laboratory environment. (J ALLERGY CLIN IMMUNOL 1989;84:347-52.)

Allergy to laboratory animals develops when genetically susceptible workers are exposed to airborne allergens in the workplace. Only recently have immunoassays been developed that have the sensitivity to quantitate the airborne concentrations of these allergens to document exposure. With these techniques, measurable quantities of rat, guinea pig, mouse, and cat allergen¹⁻⁶ can be demonstrated in the air in laboratories, and certain activities, such as cage cleaning, have been demonstrated to increase these concentrations significantly.^{2, 3}

As part of an ongoing prospective study of the risk factors for developing laboratory animal allergy, it was important to define the allergen exposure during various laboratory tasks. To determine the exposure, it was first necessary to develop a method to measure allergen concentrations as various tasks were performed. The method chosen was to measure concentrations within workers' personal breathing zones while they performed specified tasks. The personal breathing zone, defined as a volume of air within a 1 foot radius of the head, has been demonstrated in other industrial settings to provide a more accurate measure of the quantity of respirable particles to which a worker is exposed and provides the basis for workplace standards of Occupational Safety and Health Administration.^{6, 7} Portable sampling devices are available for industrial monitoring. Although their use has not been demonstrated in monitoring airborne allergen exposure, intuitively they should be useful for this purpose as well. The present article describes task-related personal breathing zone concentrations of rat allergen in animal workers.

METHODS

Air sampling

Personal breathing zone samples were obtained with a battery-powered sampling pump (model P-2500, E.I.

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TABLE I. *Rat n I* antigen collected in personal breathing zone samples during laboratory tasks

	Cleaning/feeding	Handling/injection	Surgery/sacrifice
<i>Rat n I</i> concentrations (ng/m ³)			
Median	21	13	3.1
Range	(8.1-69)	(ND-45)	(ND-12)
N	7	16	33
Normalized concentrations (ng/m ³ /rat)			
Median	0.36	0.61	0.38
Range	(0.03-1.5)	(ND-3.3)	(ND-1.6)
N	6	14	32

ND, Not detectable.

DuPont, Wilmington, Del.) calibrated at a flow rate of approximately 2.04 ± 0.01 L/min. Attached to the pump by a length of Tygon (Norton Performance Plastics, Wayne, N.J.) tubing was a plastic cassette containing a 37 mm glass-fiber filter (Millipore Corp., Bedford, Mass.) with a thick paper support pad. The filter was 99.9% efficient in collecting spherical particles $>0.3 \mu\text{m}$ in diameter. The pump was worn at waist level with the filter cassette attached to the person's collar. The pump was activated for the duration of the worker's rat exposure. The cassettes were stored at -20°C until extraction.

Area samples were collected on identical glass-fiber filters mounted in plastic cassettes. Air was drawn through the cassette with rotating vane vacuum pumps (Gast Mfg. Corp., Benton Harbor, Maine) regulated at a flow rate of either 16.3 ± 0.05 L/min or 4.21 ± 1.05 L/min. Samplers were placed at the center of each area at a height of 4 feet.

Personal breathing zone sampling was conducted in 29 locations in five buildings. In each location, ventilation for rooms containing animals was separated from the ventilation system of the main building and provided 10 to 15 vol exchanges per hour. All animals present during sampling were Sprague-Dawley strain, and 60% to 100% of animals present were male adults.

Antigen extraction and assay

Antigen was extracted from the filters with methods described previously.³ The cassettes were dismantled, and the supportive paper pad was removed. The cassettes and filters were reassembled, and 3 ml of PIPES buffer (25 mmol/L of piperazine-*N,N*-bis-(2-ethanesulfonic acid), 110 mmol/L of NaCl, 5 mmol/L of KCl, pH 7.4, containing 0.3% human serum albumin) was injected into the cassette. After overnight shaking at 4°C , the fluid was collected, centrifuged at 3000 *g* to remove debris, and stored at -20°C . Regardless of the volume of the fluid recovered, the total volume of the allergen solution obtained from each filter was considered to be 3 ml, because the recovered and unrecovered solutions were presumed to have the same allergen concentration after overnight shaking.

Rat n I concentrations in the extracts were determined by an inhibition radioimmunoassay with methods very similar to methods used previously to assay for cat allergen *Feld*

I.³ *Rat* α_{2u} -globulin (*Rat n I B*) was radioiodinated with ¹²⁵I by the chloramine-T method. The filter extract (0.3 ml) was mixed with 0.05 ml of pooled immune human serum (diluted 1:20 in borate-buffered saline) and 0.05 ml of the radio-labeled *Rat n I B* (containing 1 ng of antigen) in a total volume of 0.4 ml of borate-buffer containing 0.6% bovine serum albumin. The human serum pool was selected from a panel of 10 different human sera based on its relatively high content of antibody against *Rat n I*. After a 4-hour incubation at 23°C , 0.1 ml of goat antihuman IgG was added in slight antibody excess, and precipitates were allowed to form overnight at 4°C . The precipitates were then washed four times with borate-buffered saline, and the bound ¹²⁵I was quantitated. Allergen concentration in the extracts was computed by comparing these values with a standard inhibition curve obtained in parallel experiments with a preparation of rat urinary protein that contained known amounts of *Rat n I B*. The assay was capable of detecting 0.04 ng of the allergen per assay tube. Since the assay used an aliquot (0.3 ml) that represented one tenth of the total allergen extracted, the sensitivity limit of the assay was 0.4 ng per filter.

To determine the airborne allergen concentration, the antigen mass collected was expressed as the airborne allergen concentration (mass divided by [flow rate $\times$ sampling time] $\times 1000$ L/m³) with units of nanograms per cubic meter of air. In preliminary experiments, samples collected for a short time, as well as samples collected during certain tasks, were found to contain antigen masses below the level of sensitivity of the assay. In later experiments, in which low allergen concentrations were suspected, the extracts from more than one filter were pooled, lyophilized, and reconstituted with water to 3 ml.

Laboratory tasks

From a comprehensive list of tasks performed in the animal laboratory, three tasks were chosen for study because of the reliability of their definition from work diaries and direct observation, as well as the frequency of occurrence and the duration of each task. The three categories studied were defined as follows:

Cleaning/feeding. This task involved transferring rats to a cage containing clean bedding and then pouring soiled

bedding material (fine wood chips) into an open trash receptacle. A food hopper and water bottle were usually filled at the same time. The task generally took 10 to 155 minutes and from two to 725 rats were handled.

Handling/injection. A conscious rat was held for purposes such as weighing, shaving, or palpating, or was pierced with a needle for purposes such as injecting medication or collecting blood or urine specimens. This task took from 15 to 570 minutes and from six to 75 rats were handled.

Surgery/sacrifice. During this task, an unconscious rat underwent surgery or was sacrificed by cervical dislocation. This task took from 20 to 835 minutes and from one to 59 rats were handled.

Statistics

Because the data were not normally distributed, statistical analysis used nonparametric techniques, usually the Wilcoxon signed-rank test

RESULTS

Eighty-eight filters from personal breathing zone samples were analyzed. Preliminary analysis demonstrated that when samples were collected for less than 1 hour, the quantity of *Rat n I* collected on the filter frequently was not adequate to produce detectable concentrations in the 3 ml extraction volume. Of 13 samples collected for less than 1 hour, nine (69%) did not contain detectable antigen. Accordingly, the protocol was changed, and any remaining filters exposed for less than 1 hour were combined with one or two others obtained during similar tasks to provide a cumulative collection time of more than 1 hour. Collectively, 73 filters were combined to form 56 samples, and the 13 samples collected for less than 1 hour were excluded from the data set. Of samples excluded, four were obtained during cleaning/feeding, two during handling/injection, and seven samples during surgery/sacrifice; therefore, the excluded samples were distributed evenly between the various tasks.

With data from 56 samples collected for more than 1 hour, the antigen mass (i.e., the total weight of *Rat n I* collected on a filter) was found to vary widely during each task, ranging from undetectable to 22.1 ng per filter, but was not statistically different among the three tasks ($p > 0.1$).

The sampling times were quite different during the various tasks, however, shortest during cleaning/feeding (10 to 155 minutes; median, 82) and longest during surgery/sacrifice (20 to 835 minutes; median, 240). Since flow rates during each collection were the same, different volumes of air were thus being sampled. Therefore, since the antigen mass collected was similar, the airborne *Rat n I* allergen concentration (mass per volume of air) was considerably different during the various tasks. As presented in Table I and Fig. 1,

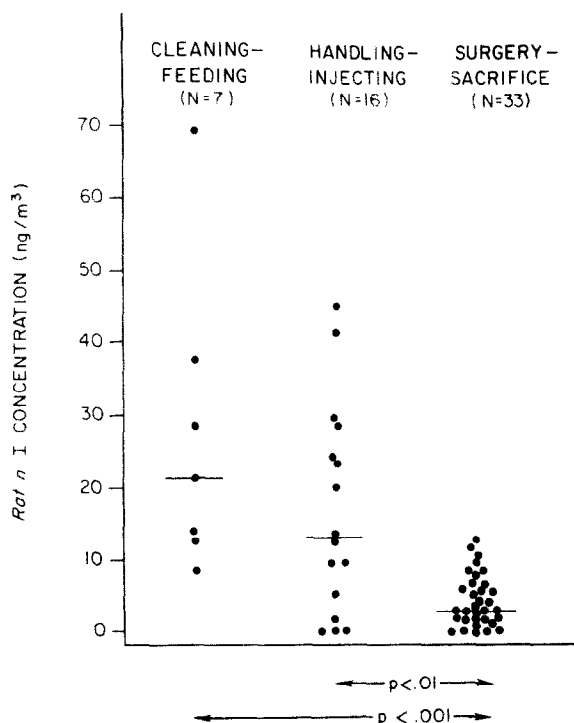


FIG. 1. *Rat n I* concentrations in personal breathing zone samples. Each point represents the airborne concentration from a sample collected for at least 1 hour. Bars are drawn at median values for each task. Concentrations found during surgery/sacrifice are lower than during handling/injection ($p < 0.01$) or during cleaning/feeding ($p < 0.001$).

the *Rat n I* concentration was significantly lower during surgery/sacrifice than during cleaning/feeding (median, 3.1 versus 21 ng/m³; $p < 0.001$) or during handling/injection (median, 3.1 versus 13 ng/m³; $p < 0.01$). Both the median and the range of concentrations observed during cleaning/feeding were the highest observed among all three tasks, but the difference did not reach statistical significance.

One possible determinant of these differences in airborne *Rat n I* concentrations was the number of rats present during procedures. This was analyzed first by normalizing antigen concentrations for the number of rats present. In four instances there was no information about the numbers of rats present during the sampling period. For the rest, the median *Rat n I* values in nanograms per cubic meter per rat are presented in Table II. It is apparent that the median values for cleaning/feeding (0.36 ng/m³ per rat) were almost identical to values during surgery/sacrifice (0.38 ng/m³ per rat). However, handling/injection was associated with concentrations (0.61 ng/m³ per rat) that were significantly higher than either of the other two tasks ($p < 0.05$). In addition, when a relationship was sought between the number of rats and *Rat n I* allergen

TABLE II. *Rat n I* concentrations in air samples collected with area sampling technique*

Site	Concentration (ng/m ³)		
	Range	Median	N
Animal storage room with cleaning activity	0.62-7.0	3.4	8
Animal storage room	ND-3.1	2.3	6
Hallway			
Night	ND-0.41	ND	4
Day	ND-0.18	ND	4
Hallway			
At laboratory door	ND-0.18	ND	4
75 cm from door	ND-0.18	ND	4
Rat laboratory	ND	ND	3
Nonrat laboratory	ND	ND	12
Outside	ND	ND	6

ND, Not detectable.

*Sampled at 4 L/min or 16.3 L/min for 8 to 16 hours.

concentration with regression analysis (Spearman rank-correlation test), a weak but significant relationship was found during surgery/sacrifice ($r_s = 0.41$; $n = 32$; $p < 0.02$) but not during handling/injection ($R_s = 0.31$; $n = 14$; $p > 0.1$) or cleaning-feeding ($r_s = 0.09$; $n = 6$; $p > 0.1$). Thus, it would appear that the wide ranges of allergen concentrations found cannot be explained only by the number of rats present, and other important variables are contributing, such as the proximity of the worker to the animal during surgery or the extent of contamination of the bedding during cleaning/feeding.

Since *Rat n I* antigen is believed to be excreted largely,⁸ but not exclusively,⁹ in male rat urine, the proportion of female rats handled by the workers was another potential source of variation in airborne concentrations. Of the 56 samples, the number of female animals was specified in 54. In 41 samples, all the animals present were male, and in only 11 were less than half of the rats present male. Most interestingly, in six samples all animals were female, yet in only one case was *Rat n I* antigen undetectable, and the highest concentration was 23.2 ng/m³.

The results of area sampling are presented in Table II. Samples obtained in an animal storage room had somewhat higher *Rat n I* content with cleaning activity (median, 3.4 ng/m³) than at other times with no activity (2.3 ng/m³), but the difference was not significant ($p > 0.1$). In the hallway near the storage room, very low *Rat n I* concentrations were found either in the day or night, and levels were not related to the proximity to the storage room door. Samples collected in the rat laboratory, when no animals were present, in another laboratory in which rats were not used, or

outside the building did not contain detectable levels of *Rat n I* antigen.

DISCUSSION

To measure reliably the allergen concentrations that an individual laboratory worker encountered during various tasks, it was important to be able to sample air from the personal breathing zone. Since exposure involved a small allergen source, measurements at any distance would be diluted enormously in area air samples and would be a poor estimate of task-related exposure. Furthermore, in an environment with a large amount of random air mixing from ventilation equipment and from the worker's movements, airborne antigen mixing was likely to be uneven and to distort further the task-related exposure. Available personal sampling devices were designed to be worn comfortably by workers, and the airflows generated (2 L/min) were substantially lower than airflows used in previous studies of airborne allergen sampling.¹⁻⁶ Thus, the demonstration that most samples contained detectable antigen levels with these relatively low airflows was an important first step in measuring task-related differences in airborne allergen concentrations.

Airborne urinary allergen was found in association with every task performed in the laboratory. Those tasks which involved handling conscious rats or contaminated bedding exposed a worker to four to five times higher concentrations than did tasks with unconscious rats, probably because antigen on the fur or bedding became airborne during handling. Another, equally important variation, was in the allergen concentration found with repeated samples of the same task, which ranged tenfold to 50-fold. This blurred

the distinction between tasks somewhat and suggested that any task in the laboratory can at some time expose a worker to significant allergen concentrations.

Two obvious causes for these variations were examined, that is, the numbers and sex of animals, and individual differences between technicians handling them. Neither factor provided a consistent explanation, although the numbers of rats present appeared to influence concentrations to some extent, especially during surgery/sacrifice. The relationship was not conclusive, and future studies will examine other variables, such as regional ventilation during a given task and the individual animal's allergen production.

The major allergens in rat urine, *Rat n I B* (α_{2u} -globulin) and *Rat n I A* (prealbumin), are partially cross-reactive with each other.^{10, 11} The α_{2u} -globulin (relative molecular mass, 16,000 to 17,000; pI, 5.4, 5.6, and 5.8) is the major component, whereas the prealbumin (relative molecular mass, 20,000 to 21,000; pI, 4.5 and 4.6) is present in comparatively lower amounts. The two proteins appear to have similar amino acid composition, but their carbohydrate content differs. Each protein has several "isoantigenic forms" that appear to be immunologically indistinguishable.¹² The competitive inhibition radioimmunoassay used in the present studies used a labeled preparation of *Rat n I B*, but because of partial antigenic cross-reactivity, prealbumin was probably detected as well.

The concentration of rat allergen detected in these studies was comparable to that found by other investigations in laboratory environments. With a RAST-inhibition assay and a sampler with a flow rate of 1190 L/min, Twiggs et al.⁴ found 59 ng/m³ of mouse urine protein and 8.25 ng/m³ of mouse pelt protein in a mouse care unit housing 2000 animals. Platts-Mills et al.² found 0.9 to 24 ng/m³ of rat allergen in undisturbed rat-holding room and substantially higher amounts when cages were cleaned and disturbed. Van Metre et al.³ found up to 11 ng/m³ of cat *Fel d I* antigen in an enclosed animal-holding room; concentrations could be increased tenfold when settled dust was vigorously disturbed. Our results are much lower than results reported by Swanson et al.¹ They found 17,010 ng/m³ of guinea pig urinary allergen in area samples from an animal-holding room and 20 ng/m³ in an animal laboratory; however, their assay used unpurified urine and may not be comparable to other results.

The concentration of rat allergens measured are well within the range of airborne allergen that should produce symptoms in sensitized individuals. Van Metre et al.³ demonstrated that 10 cat-sensitive subjects with asthma demonstrated a 20% fall in FEV₁ when they were exposed to a room with airborne concentrations

of *Fel d I* in the range of approximately 1.1 to 128 ng/m³. Three of their 10 patients developed asthma at concentrations of <11 ng/m³; all patients developed rhinitis at both concentrations. Evans et al.¹³ demonstrated asthmatic responses in five rat-allergic individuals exposed to cleaning/feeding tasks. Thus, the airborne concentrations of rat allergen observed in all personal breathing zone samples collected during laboratory tasks would be likely to produce symptoms in sensitized individuals. At the same time, the data of Van Metre et al.³ suggest that symptom induction has a steep dose dependence; therefore, the concentrations of *Rat n I* measured during surgery/sacrifice would be less likely to induce symptoms than concentrations during cleaning/feeding. Thus, in the sense that symptoms are more or less likely to be induced, it is possible to designate certain tasks as high risk, such as handling/injecting or cleaning/feeding.

This study was conducted as part of a larger prospective project that seeks to define risk factors in the development of laboratory-animal allergy. This study is less concerned with the induction of symptoms in sensitized workers than with risks of sensitization itself; until it is completed, we cannot really know the sensitizing potential of the various tasks described here. At the same time, the data are consistent with reported differences in sensitization rates among laboratory animal workers.^{11, 14-17} For example, Platts-Mills et al.⁹ found the highest prevalence and titer of IgE antibody in animal handlers who would have performed cage cleaning, the task we found associated with highest allergen levels. They also found that six of 34 clerical workers in adjacent offices had IgG antibody in the absence of IgE, suggesting that an immune response can follow exposure to low allergen levels or occasional exposure to higher levels. It is hoped that the prospective study now under way will allow better understanding of the significance of the different task-related concentrations in inducing sensitivity.

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