

Difference in exposure to airborne major rat allergen (Rat n 1) and to endotoxin in rat quarters according to tasks

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Summary

Background Endotoxins found in occupational settings constitute a risk factor in the severity of respiratory allergic symptoms.

Objectives To assess the airborne concentrations of major rat allergen (Rat n 1) and endotoxin under various circumstances.

Methods We took 483 airborne samples from 12 sites: 114 individual samples for endotoxin measurements and 113 for Rat n 1, from 38 workers (nine animal technicians, nine laboratory technicians, nine scientists and 11 students); and 256 static samples in rat rooms and experimental rooms, with or without disturbance, for simultaneous endotoxin and Rat n 1 measurements. Rat n 1 was measured with a two-site monoclonal ELISA and endotoxins with the *Limulus* method.

Results Airborne Rat n 1 and endotoxin were significantly higher in rat rooms than in experimental rooms. Animal technicians had the greatest exposure to both Rat n 1 and endotoxin. Cage cleaning and rat feeding induced the highest exposure to Rat n 1 and endotoxin. Furthermore, we observed no significant difference in endotoxin exposure between researchers with or without rat contact during the sample period. There was no correlation between the number of rats present and airborne endotoxin concentrations.

Conclusions Exposure to airborne Rat n 1 and endotoxin is higher during cleaning and feeding tasks than during any other task, we feel that a major source of both is contaminated bedding that becomes airborne during disturbance.

Keywords: endotoxin, rat allergen, Rat n 1, airborne allergens, airborne Rat n 1, airborne endotoxin, rat quarters, occupational asthma

Clinical and Experimental Allergy, Vol. 31, pp. 1449–1456. Submitted 30 December 1999; revised 7 March 2001; accepted 26 March 2001.

Introduction

According to the report of the Surveillance of work-related and occupational respiratory disease (SWORD) project, allergies to laboratory animals are the fifth leading cause of occupational asthma in the United Kingdom [1]. Different studies report a prevalence of symptoms among occupationally exposed individuals that varies from 19% to 31% [2,3] and a

prevalence of asthma estimated at between 6% and 10%. Various studies reveal a relatively close concordance between skin tests and symptoms (81%) [4]. However, it has also been reported that only 63% of persons with symptoms are sensitized to rat allergens [5]. This suggests that other agents present in animal facilities may play some role.

Endotoxins are one such class of agents that may influence the pathogenesis of bronchial symptoms [6,7]. The lipopolysaccharide (LPS) that is part of endotoxins has been found to be an important immunoadjuvant for the development of allergic reactions to latex protein [8]. Moreover, Michel *et al.*

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have suggested that exposure to endotoxins can increase asthma severity in subjects sensitized to house dust mites and exposed to high levels of mite allergens [9]. Airborne endotoxin measurements have been performed in many different occupational environments [10]. Laboratories using animals, however, have not been tested often: two studies reported endotoxins, at low levels, in five animal facilities (rats/mice), measured on static samples [11,12].

The aims of our study were to determine:

- airborne endotoxin and Rat n 1 exposures in rat quarters;
- variations of those airborne exposures according to tasks; and
- the relation between airborne exposure to major rat allergen (Rat n 1) and endotoxin.

Methods

Air sampling techniques

Individual air samples for Rat n 1 and endotoxin measurements were collected with a portable pump (Gilair, Panametrics, La Garenne-Colombes, France and Casella AFC 123, Casella London, Bedford, UK). A 37-mm glass fibre filter (Millipore, AP 4003705, St Quentin, France) contained in a plastic cassette (Millipore, PAT No. 2879207) was connected to the pump. The airflow was adjusted to 2 L/min, according to the manufacturer's recommendations. The pump was worn at waist level with the filter cassette attached to the subject's collar. Each subject carried a portable sampler on six occasions (three times for 6 h for Rat n 1 and three times for 4 h for endotoxin).

Static samples were collected on the same glass fibre filters and plastic cassettes as the individual samples. Cassettes were connected to a vacuum pump (IP 20, Bioblock SA, Strasbourg, France) at a flow rate of 20 L/min. Each sample was taken three times, each for 60 min. The sampler was located 1.5 m above the floor in the middle of the room. Rat allergen and endotoxin were measured simultaneously from static samples. All air samples with stationary devices were collected by the same researcher.

For individual and static sampling, the airflow was adjusted with a bubble airflow meter (Gillibrator, Panametrics) in compliance with French regulations for sampling airborne allergens [13].

Extraction and assay

Rat major allergen (Rat n 1) extraction and assay Allergen was extracted from the filter in 1 mL of phosphate buffer saline, with 1% of bovine serum albumin (PBS BSA 1%). It was kept overnight at 4 °C; the eluate was then collected by compressing the filter in a plastic syringe and stored at -20 °C until assayed with a two-site MAb-based

ELISA, which has previously been described [14]. Monoclonal antibody-6 in PBS at 10 µg/mL was coated to 96-well microtitre plates (Immulon* 2 flat-bottomed ELISA plates, Dynatech, Alexandria, VA, USA) overnight at room temperature (20°C). Next, 100 µL aliquots of either affinity-purified rat urinary allergen standard or eluates from the filter, either pure or diluted (1/5 or 1/10) in PBS-T, were added to the wells in duplicate and incubated for 60 min at room temperature, then 100 µL biotinylated mAb-1 was added and incubated for a further hour. After another wash, 100 µL neutravidin-horseradish peroxidase was added and incubated for 60 min. Then, substrate TMB (Sigma) was added. The reaction was terminated after 20 min with 1 mol/L H₂SO₄ and absorbance was read at 450 nm and analysed. A control curve for each assay was established over the range of 0.31–20 ng/mL of Rat n 1. The detection limit was 0.31 ng/mL, equivalent to a detection limit of 0.25 ng/m³ for air sampling for 1 h at 20 L/min. The mean intra-assay coefficient of variation (CV) was 11% for duplicate wells ($n = 12$) and the mean interassay CV was 9.5% ($n = 20$).

Endotoxin extraction and assay Endotoxins were extracted from the filter in 5 mL of a pyrogen-free buffer (PBS, pH 7.2, Biomérieux, Craponne, France) in a plastic syringe that was shaken on a rotator for 60 min at room temperature. The fluid was then collected by compressing the filter in the syringe and centrifuged at 1000 *g* for 10 min. The supernatant was then stored at -80 °C before use in the analysis. The endotoxin assay used pyrogen-free glassware and laboratory instruments. The following standard endotoxin concentrations were assayed on each microtitre plate: 5, 0.5, 0.05 and 0.025 IU/mL, as recommended by the manufacturer (Chromogenix AB, Möndal, Sweden) [9]. *Escherichia coli* 055/B5 was used; it is calibrated for its *Limulus* procoagulant activity according to USA Food and Drug Administration (FDA) and World Health Organization (WHO) standards (1 ng is equivalent to approximately 12 endotoxin units). Unknown samples were run both neat and diluted (1/2 and 1/10).

We then added 100 µL LAL (Coamatic®Chromo-LAL distributed by Chromogenix AB) to each well [9]. A kinetic LAL method with an automated microtitre plate reader was used to assay the endotoxin at 37 °C (LabSystems®iEMS MF, Cergy Pontoise, France). Standards and unknown samples in duplicate were run with optical density (OD) every 30 s for 50 min to establish a rate constant. The reader determined the endotoxin concentration by measuring the time it takes for the OD at 405 nm to increase 0.200 absorbance units. This method has been published previously [15]. The airborne concentration of endotoxin was expressed in ng/m³. The detection limit of the test was 0.02 ng/m³. The intra-assay CV was calculated for three

values of the standard curve (5, 0.5 and 0.05 IU/mL) on 12 occasions and for the fourth point (0.025 IU/mL) on 11 occasions in duplicate ($n = 24$ and 22 , respectively) on the same microplate. For all four points, the intra-assay CV varied from 1.5% to 6.8%. The interassay CV was calculated for two values chosen in the standard curve (5 and 0.5 IU/mL) on 20 different plates run on 20 different days by the same technician. Inter-assay CVs were 4.9% for 5 IU/mL and 8.6% for 0.5 IU/mL.

Study design

From December 1997 to April 1998, we enrolled 107 workers at 12 rat facilities affiliated with universities or research institutes in Strasbourg. They were divided into four groups based on their occupation: scientists, laboratory technicians, animal technicians and students. Members of each group gave information about the duration and frequency of their activities:

- For animal technicians: cleaning rat rooms, cleaning cages, changing bedding and feeding rats, tasks performed at the same time.
- For laboratory technicians, students and scientists: handling rats, collecting blood and urine specimens, surgery and sacrifice.

Each animal facility was visited by the same researcher, who recorded information about how it was cleaned, the number of rats and the ventilation system. Only one rat facility had windows. Windows in the laboratory were not opened during air sampling, which took place in the winter. Air samples were taken from three locations: the rat rooms, experimental rooms (which were close to the rat housing) and offices (where students and scientists had their desks and rats were forbidden). Different samples were taken to evaluate:

- Job-dependent exposure: 227 individual samples during a work shift, for 38 subjects (nine scientists, 11 students, nine laboratory technicians and nine animal technicians).
- Task-dependent exposure in rat quarters and in experimental rooms: 256 static samples during different tasks (cleaning/feeding in rat rooms and handling rats in experimental rooms) and to determine base level (without any disturbance in rat rooms and with no rats in experimental rooms).
- Task-dependent exposure in offices: 37 individual air samples from researchers (scientists and students) during shifts without any rat contact.

In all, 483 airborne measurements were taken in 12 research units. Individual samples were taken successively for Rat n 1 ($n = 113$) and for endotoxin ($n = 114$). Static samples were taken concomitantly for Rat n 1 ($n = 128$) and for endotoxin ($n = 128$). All the data across sites were grouped to analyse room-dependent, job-dependent and task-dependent exposures.

Statistics

All the values were log-transformed on the model ' $\log_{10}(1 + x)$ '; those transformations permitted us to analyse results using parametric tests (analysis of variance: one way and two way ANOVA). After transformation and with univariate analysis of variance, airborne Rat n 1 and endotoxin individual and static levels were separately compared between sites and between the four professional categories. The comparison of airborne Rat n 1 and endotoxin concentrations according to tasks and rooms used multivariate analyses of variance and Neuman-Keuls tests. Airborne levels for researchers with or without contact with rats were compared with unpaired Student *t*-tests. Correlation analysis were performed between results of individual and static samples, between Rat n 1 and endotoxin concentrations and between the number of animals and exposure to rat allergen or endotoxin. The significance level was set at 5%.

Results

Characteristics of rat quarters

The principal characteristics of the rat rooms are summarized in Table 1. The mean number of rats per facility was 640, with a range of 30–2700. The cage designs and litter (wooden chips) were the same at all sites, except site H, which used the same litter but steam-sterilized it. The rooms and cages were washed twice a week in all facilities. Most of the facilities used quaternary ammoniums as disinfectants for cleaning rat rooms, but sites F and L used a blend of amino acids, and site K used bleach. All but one facility ventilated the rat rooms mechanically (site D). The

Table 1. Site characteristics

Site	Number of rats in rat rooms	Disinfectant used for rat rooms	No. persons exposed to rats
A	2700	IV amm*	10
B	30	IV amm	3
C	300	IV amm	7
D	230	IV amm	6
E	350	Other	16
F	80	Amino acid	7
G	115	IV amm	16
H	80	IV amm	6
I	500	IV amm	3
J	1600	IV amm	8
K	230	Bleach	6
L	1500	Amino acid	19

*IV amm = quaternary ammonium.

air exchange rate was planned to be 15 ACH (air changes per hour). The mean number of persons who carried an individual sampler was three at each site (range: two–six).

Global airborne measurements

Rat n 1 airborne concentrations The geometric mean airborne Rat n 1 levels found in each site, with portable and static samples, are summarized in Table 2. The results varied widely from site to site, ranging from a minimum value of less than 0.25 ng/m³ (detection limit of the assay) to a maximum of 537 ng/m³. The mean exposure for individual and static air samples taken together was 34 ng/m³ ($n = 241$).

Endotoxin airborne concentrations The geometric mean airborne endotoxin concentrations found in each site, with portable and static samplers, are summarized in Table 2. These results also varied widely, ranging from a minimum value of less than 0.02 ng/m³ (detection limit) to a maximum of 15.46 ng/m³. The mean exposure, individual and static samples combined, was 0.224 ng/m³ ($n = 242$).

Site-dependent exposure

Three categories of sites were identified: in sites I and K, airborne Rat n 1 levels were higher, they were intermediate in other sites, and lower in sites F and B ($P = 0.0001$). Concerning endotoxin, levels were the highest in site K, and the lowest in sites H and J ($P = 0.002$).

Room-dependent exposure

Mean levels of airborne Rat n 1 and endotoxin from static samples were significantly higher in rat rooms (AM = 53.1 ng/m³, $n = 65$, and 0.284 ng/m³, $n = 65$, respectively) than in experimental rooms (AM = 9.7 ng/m³, $n = 56$, and 0.095 ng/m³, $n = 56$, respectively) ($P = 0.02$ and $P = 0.05$, respectively).

Job-dependent exposure

In all, 113 personal full-shift airborne samples were collected to evaluate airborne Rat n 1 concentrations, and 114 for airborne endotoxin concentrations. Each person took samples three times on 3 different days, and there were no significant differences between the 3 days. The highest personal exposure to Rat n 1 and endotoxin levels during a shift was measured for animal technicians (arithmetic mean (AM) of 72.3 ng Rat n 1/m³ and 1.57 ng endotoxin/m³), compared with laboratory technicians, students and scientists (AM = 21.2, 33.7, and 24.0 ng Rat n 1/m³, respectively; and AM = 0.238, 0.381, and 0.187 ng/m³ of endotoxin, respectively) (Fig. 1).

Task-dependent exposure

Cage cleaning and rat feeding in rat rooms induced the highest exposure to Rat n 1 and endotoxin (AM = 91.1 ng Rat n 1/m³, $n = 34$, and 0.479 ng endotoxin/m³, $n = 34$) ($P = 0.03$ and $P = 0.001$, respectively). Rat n 1 airborne levels were significantly higher during base level in rat

Table 2. Geometric means and geometric standard deviations of airborne Rat n 1 and endotoxin levels (ng/m³) with static and individual samples in the 12 sites

Site	Rat n 1						Endotoxin					
	Static sample			Individual sample			Static sample			Individual sample		
	$n^{(1)}$	GM ⁽²⁾	GSD ⁽³⁾	n	GM	GSD	n	GM	GSD	n	GM	GSD
A	9	21.51	1.12	12	1.80	1.07	9	0.32	0.67	12	0.21	0.85
B	6	0.43	1.00	nd	nd*	nd*	6	0.06	0.70	nd	nd*	nd*
C	nd*	nd*	nd*	9	8.16	0.82	nd	nd*	nd*	9	0.15	0.66
D	12	7.44	0.93	4	27.02	1.12	12	0.10	0.84	3	0.54	0.86
E	15	2.18	0.85	14	1.77	0.86	15	0.11	0.70	15	0.15	0.77
F	10	1.71	1.12	12	0.49	0.83	10	0.12	0.96	12	0.14	0.64
G	12	3.20	1.05	20	4.02	0.51	12	0.07	0.84	21	0.24	0.83
H	12	1.19	0.77	6	4.86	1.37	12	0.04	0.70	6	0.08	1.34
I	12	20.95	0.60	6	42.35	0.87	12	0.10	0.62	6	0.11	1.05
J	12	9.91	0.86	12	7.93	0.86	12	0.03	0.79	12	0.22	0.82
K	13	27.36	0.73	6	48.96	0.92	13	0.57	0.73	6	1.42	1.15
L	15	3.35	1.10	12	1.68	1.08	15	0.04	1.03	12	0.16	0.67

⁽¹⁾ n = number of samplings, ⁽²⁾GM = geometric mean, ⁽³⁾GSD = geometric standard deviation, *nd = not done.

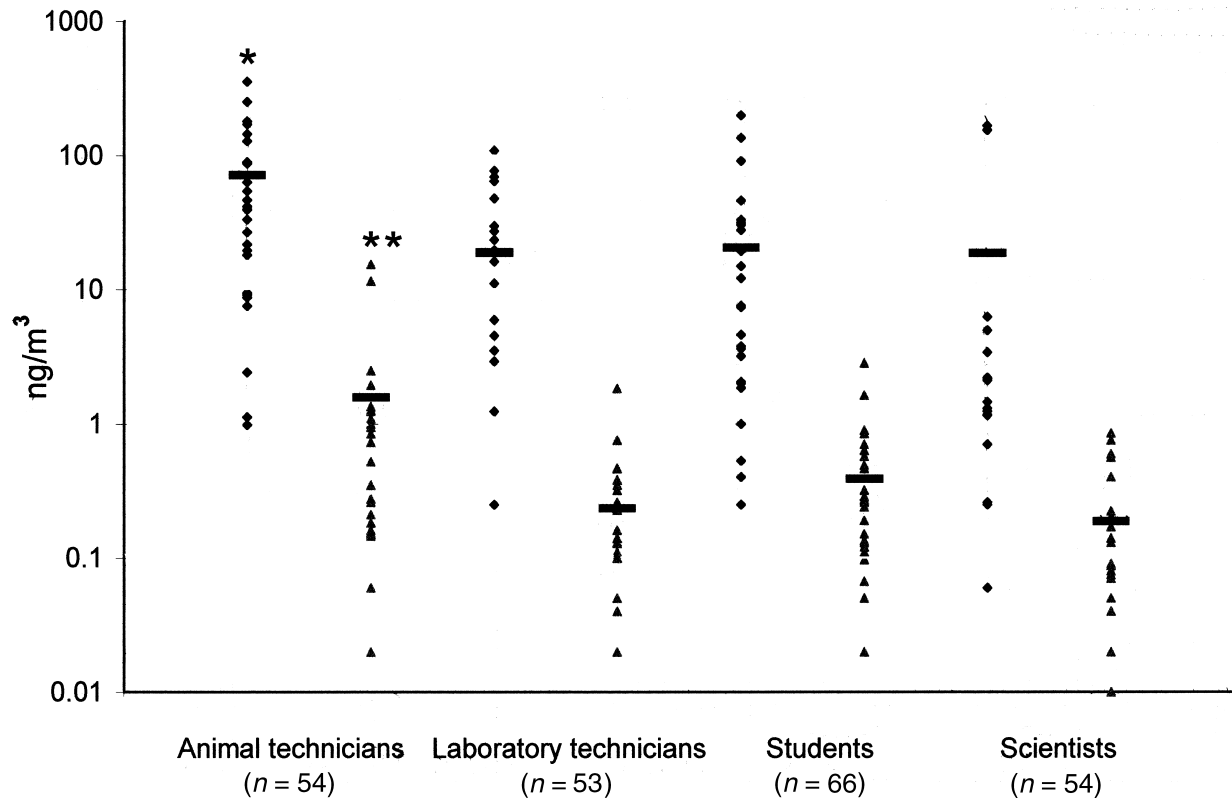


Fig. 1. Means of airborne Rat n 1 (◆) and endotoxin (▲) (ng/m^3) concentrations according to professional categories: animal technicians (AT), laboratory technicians (LT), students (St) and scientists (Sc). *Rat n 1 concentrations were higher for AT than for LT, St and Sc ($P = 0.004$). **Endotoxin concentrations were higher for AT than for LT, St and Sc ($P = 0.01$).

rooms ($\text{AM} = 35.7 \text{ ng}/\text{m}^3$, $n = 31$) than both measurements in experimental rooms ($\text{AM} = 5.4 \text{ ng}/\text{m}^3$ during rat handling ($n = 31$) and $1.9 \text{ ng}/\text{m}^3$ during base level ($n = 32$)). Airborne endotoxin concentrations did not differ under the other three conditions: the base level in rat rooms and both measurements in experimental rooms ($\text{AM} = 0.135$, $n = 31$, 0.062 , $n = 31$, and $0.083 \text{ ng}/\text{m}^3$, $n = 32$, respectively) (Fig. 2).

In offices, levels of airborne Rat n 1 were significantly lower for researchers without any contact with rats ($\text{AM} = 0.4 \text{ ng}/\text{m}^3$, $n = 16$) than for those who had contact during that shift ($\text{AM} = 26.2 \text{ ng}/\text{m}^3$, $n = 45$) ($P < 0.05$). In contrast, no significant difference was found in endotoxin exposure between researchers with or without contact with rats during their shift ($\text{AM} = 0.306 \text{ ng}/\text{m}^3$, $n = 43$; and $0.244 \text{ ng}/\text{m}^3$, $n = 21$, respectively) (Fig. 3).

Correlation analysis

Significant correlations were found between the airborne Rat n 1 levels measured with static and portable samplers ($r = 0.69$; $P < 0.001$; $n = 241$), and between the airborne endotoxin levels measured with these two types of samplers

($r = 0.88$; $P < 0.0001$; $n = 242$). A weak correlation between airborne Rat n 1 and endotoxin was found only in rat rooms ($r = 0.62$; $P < 0.002$) but no significant correlation was observed in experimental rooms and in offices. A correlation was found between Rat n 1 airborne concentrations and the number of rats ($r = 0.83$; $P = 0.005$). In contrast, airborne endotoxin levels were not significantly correlated with the number of rats.

Discussion

Our results demonstrate that airborne endotoxin is present in laboratory rat quarters. They also assessed the variation in rat major allergen and in endotoxin according to occupational tasks, as well as the relations between these two airborne exposures.

The present results confirmed that animal technicians experience a significantly greater exposure to Rat n 1 than do other workers [16–18]. Like other studies [19–21], we found that the number of rats and such tasks as feeding and cleaning cages were determinant factors in elevating airborne Rat n 1 concentrations.

To measure endotoxin, we used a *Limulus* amoebocyte

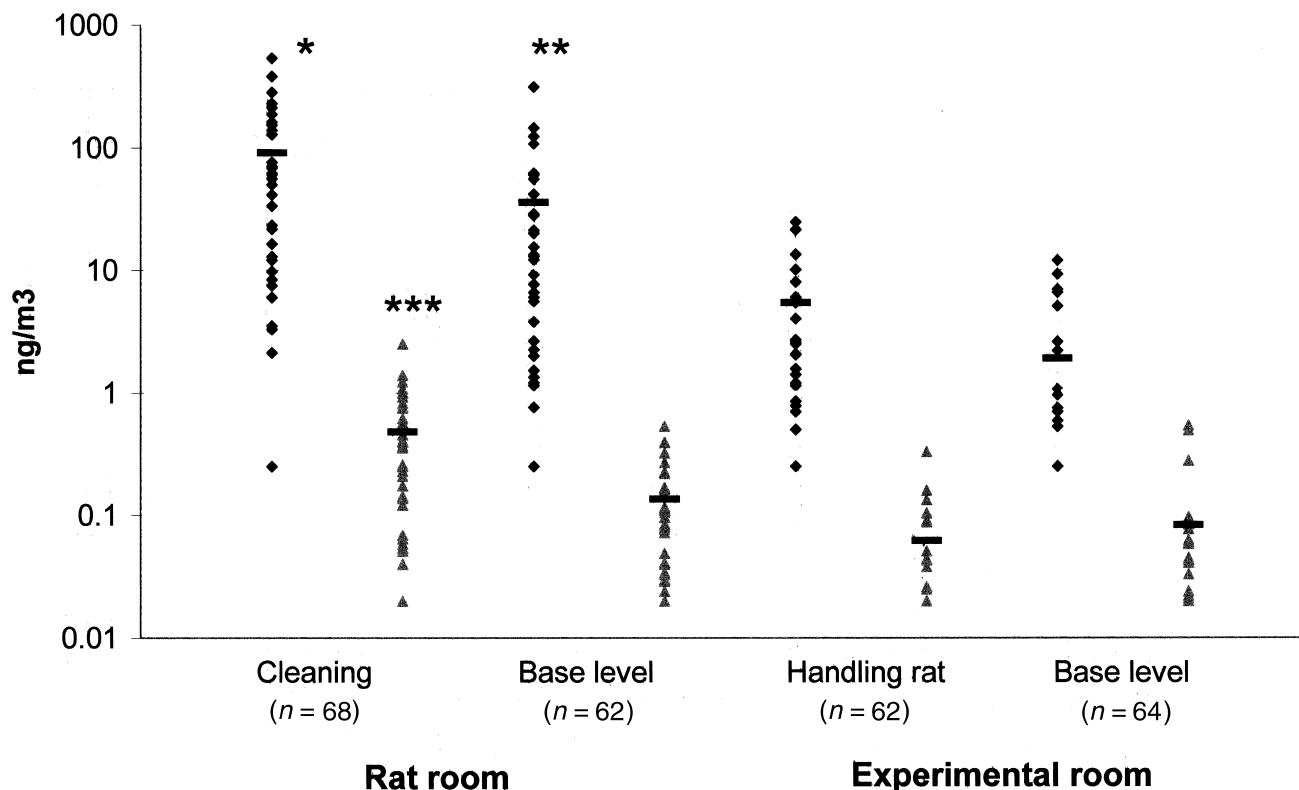


Fig. 2. Means of airborne Rat n 1 (♦) and endotoxin (▲) (ng/m^3) concentrations according to tasks in rat room (cleaning/feeding and base level) and in experimental room (handling rat and base level). *Rat n 1 levels were highest during cage cleaning and animal feeding in rat room ($P = 0.03$). **Rat n 1 concentrations were higher during base level in rat rooms than both measurements in experimental rooms ($P = 0.03$). ***Endotoxin concentrations were higher during cage cleaning/animal feeding in rat rooms ($P = 0.001$).

lysate chromogenic kinetic assay that has been used in other previously published studies [12,15]. Our results were relevant, since our method could measure amounts from 0.02 to 4 ng/m^3 with a relative standard deviation of 5.3%, which is close to the best standard deviation reported for this method [15,22]. We observed that the 12 sites varied significantly in airborne endotoxin concentrations, with a mean value of 0.224 ng/m^3 . These levels are close to those reported in an earlier pilot study [11] and in a recently published study [12] of animal facilities. Our results indicate that rat rooms are the main reservoir of endotoxin: the highest endotoxin concentrations were measured there. The role of disturbance is important in endotoxin exposure: the highest concentrations of airborne endotoxin were observed in rat rooms while cages were cleaned, bedding changed and animals fed ($P = 0.01$). Furthermore, animal technicians, who performed those tasks in rat rooms, were four–eight times more exposed to endotoxin than other workers. Rats by themselves did not appear to be a source of endotoxin, since the experimental rooms did not differ in their airborne endotoxin levels according to whether rats were present. This finding was confirmed by the absence of

correlation between the number of rats and airborne endotoxin concentrations. Moreover, exposure to airborne endotoxin was similar in scientists handling rats and those who had no contact with rats during their sampling. Thus, the source of endotoxin would appear to be the rat food or contaminated bedding, as demonstrated by Smid *et al.* who found endotoxin in the animal feed industry (average 8-h personal inspirable exposure = 25 ng/m^3 endotoxin) [23]. Indeed, airborne endotoxin levels were significantly lower in site H, in which rat litter and food were steam-sterilized. The presence of endotoxins in offices far from the rat rooms suggests that endotoxins may be carried on clothes, on shoes or on airborne particles that can circulate from one room to another, like rat and mouse allergens [16,24].

Rhinitis and asthma related to workplace exposure to rats have been reported at prevalences of 19–31% [2–4,25]. The peak expiratory flow of workers who reported asthmatic symptoms due to working with rats decreased significantly on days when they worked with laboratory rats [26]. One study of rat-exposed workers found that 37% of symptomatic workers were not sensitized to rats [5]. In a recent study [12], only one subject among 20 animal

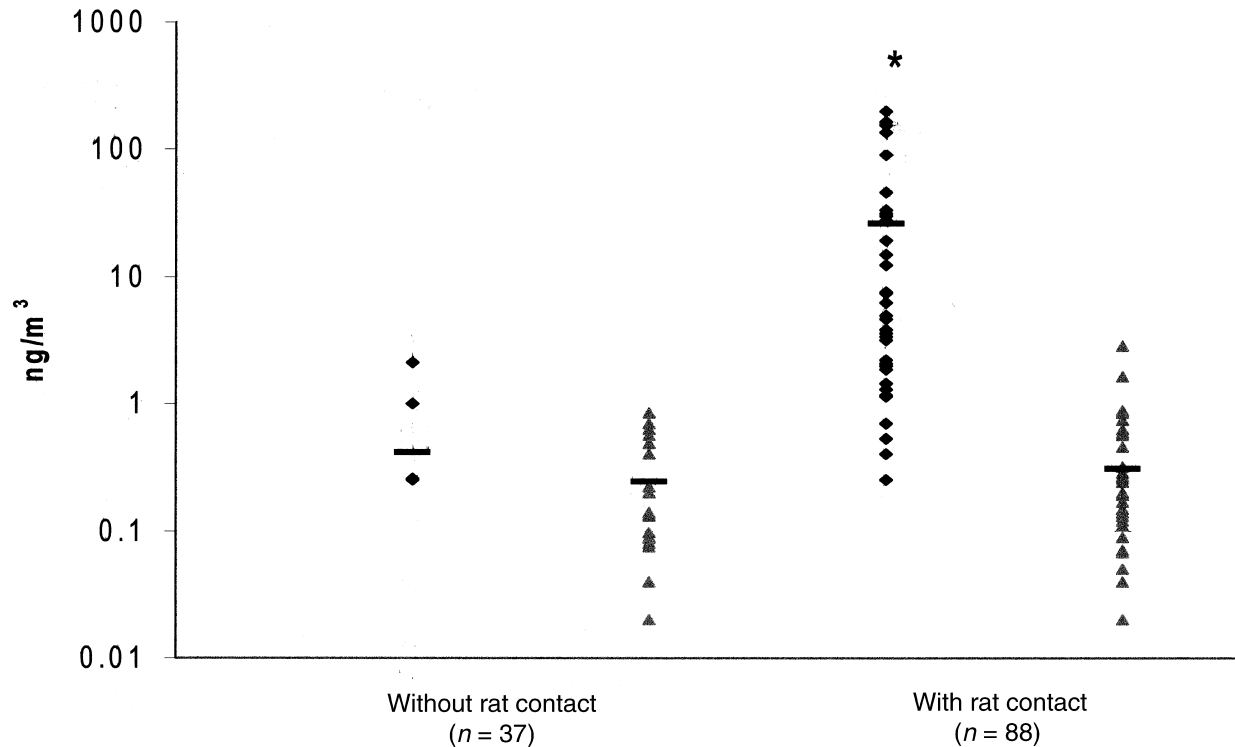


Fig. 3. Means of airborne Rat n 1 (◆) and endotoxin (▲) (ng/m³) concentrations for researchers with or without rat contact. *Rat n 1 concentrations for researchers were higher during shift with rat contact compared with shifts without rat contact ($P = 0.05$). No significant difference was found in endotoxin exposure.

workers was sensitized to common laboratory animal allergens, while 16 reported more than one symptom. Other factors could be responsible for the rhinitis and/or asthma related to rat exposure in workers who are not sensitized to rat. Airborne endotoxin concentrations measured in the 12 sites were from 100 to 1000 times lower than in other occupational settings in which endotoxins were considered responsible for respiratory symptoms [10]. Endotoxin exposure in a potato processing industry was related to across-shift peak flow changes and the occurrence of work-related respiratory symptoms, but endotoxin airborne exposure was more than 100-fold higher [27].

Castellan *et al.* have demonstrated that a decrease in FEV₁ was obtained in healthy subjects exposed to levels above 10 ng of endotoxin/m³ for 6 h [28]. On two occasions, we measured levels higher than 10 ng/m³ in a rat room during disturbance. We do not know the levels of endotoxins needed to induce symptoms in atopic subjects. Moreover, we can suppose that levels inducing symptoms during a bronchial challenge test are dramatically higher than those inducing symptoms in realistic conditions, as is the case for cat allergens [29,30]. It seems probable, however, that the dose of endotoxin responsible for

symptoms varies widely from one person to another, so that threshold values for symptoms are not always relevant [31]. Moreover, at low airborne endotoxin levels, equivalent to those we found, the peripheral blood of animal facility workers shows a cellular response to LPS [12], with significant increases of BPI (bactericidal permeability-increasing protein) and of sTNF-R75 (75-kDa soluble tumor necrosis factor receptor), two LPS-response mediating proteins. We cannot reject the hypothesis that endotoxin in patients not sensitized to rats may be one of the factors responsible for respiratory symptoms among rat-exposed workers.

In conclusion, our findings show that measurable airborne endotoxin concentrations exist in rat quarters and that Rat n 1 and endotoxin behave differently in the air. Airborne Rat n 1 levels were related to rats; in contrast, airborne endotoxin was probably related to contaminated bedding or rat feed. Animal technicians can be exposed to peak airborne Rat n 1 and endotoxin levels during cleaning and feeding. In contrast, other professional categories are exposed to peak Rat n 1 levels only in rat rooms, but are constantly exposed to low levels of airborne endotoxin. Determination of particle sizes carrying endotoxin should help explain these findings.

Acknowledgements

The authors thank Dr O. Michel (Hôpital Universitaire Saint Pierre, Bruxelles, Belgique) and Prof. J. Duchateau (Hôpital Brugman, Bruxelles, Belgique) for their advice on endotoxin measurements.

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