

Detection of mouse and rat urinary aeroallergens with an improved ELISA

Anne Korpi, PhD,^a Rauno Mäntyjärvi, MD,^b Jaakko Rautiainen, PhD,^b
Eila Kaliste, PhD,^c Pentti Kalliokoski, PhD, LicEng,^a Anne Renström, PhD,^d and
Anna-Liisa Pasanen, PhD^a Kuopio, Finland, and Stockholm, Sweden

Background: Risk analysis of laboratory animal work presupposes allergen monitoring with sensitive methods. Commercial ELISA kits have recently become available for the detection of mouse (Mus m 1) and rat (Rat n 1) urinary allergen from settled dust samples and air samples with high allergen levels.

Objective: Our aims were to enhance the sensitivities of the commercial ELISA kits for low aeroallergen levels (less than 1 ng/m³) and to test these methods with air samples collected from an animal facility.

Methods: Personal and stationary air samples were collected from an animal facility during various tasks of laboratory animal work and from various premises of the animal facility. **Results:** The sensitivities of the ELISA assays were improved with a careful choice of analysis parameters and reagents. The detection limits of 0.1 ng/m³ for Mus m 1 and 0.8 ng/m³ for Rat n 1 were established. The sensitized assays enabled detection of mouse and rat aeroallergens also from premises in which animals or dirty cages were not present during sampling.

Conclusion: These sensitive assays will help to perform risk assessment in laboratory animal work. However, there remains a lack of standardized analytic procedures and occupational exposure limits for laboratory animal allergens. (*J Allergy Clin Immunol* 2004;113:677-82.)

Key words: Air sampling, ELISA, laboratory animal allergy, Rat n 1, Mus m 1, rat, mouse, allergen

Exposure to laboratory animal allergens, such as rat and mouse allergens, is known to pose a significant risk for the development of IgE-mediated hypersensitivity reactions. Approximately one third of laboratory animal workers are sensitized to animal allergens, and a third of them develop symptomatic asthma.^{1,2} Allergenic components become airborne when animals are treated and maintained. In addition to inhalation, skin contact is an exposure route. The nature of the relationship between the level of allergen

Abbreviations used

CV: Coefficient of variation
HRP: Horseradish peroxidase
TMB: Tetramethylbenzidine

required to sensitize an individual and that to elicit symptoms in sensitized workers is presently not known.³

Exposure assessment of animal allergens is an important stage in the risk analysis of laboratory animal work.⁴ Similarly, characterization of the work environment as well as quantification and evaluation of the effectiveness of measures to control occupational allergen exposure (risk management) presuppose allergen monitoring with sensitive methods.⁵ Airborne dust levels in animal facilities are generally low with varying allergen contents; therefore, it is not possible to quantify exposure to animal allergens by gravimetric means.⁶

The predominant allergens of rat and mouse, Rat n 1 and Mus m 1, are members of the lipocalin superfamily of small extracellular proteins. Mus m 1 (of the major urinary protein complex, MUP) has a molecular weight of 17.5 to 19 kd. Sources of this allergen comprise urine, hair follicles, and dander.^{1,4,7} The rat allergen Rat n 1 (16 to 21 kd) also originates from urine as well as from hair, dander, and saliva.⁷ The homology between Rat n 1 and Mus m 1 results in cross-reactivity between the 2 proteins.⁴

Several immunoassays have been described for quantifying airborne animal allergens, including RAST, RIA inhibition assay, and ELISA. Unlike ELISA assays, the RAST and RIA inhibition methods require large pools of human allergic sera containing relevant antibodies and radiolabeling of either the purified allergenic component or anti-human IgE. The advantages of ELISA include easy performance and improved work safety, combined with specificity and a high sensitivity.⁸ For measuring mouse allergen from air samples with ELISA, polyclonal antibodies produced in rabbit or sheep^{9,10} and rendered monospecific by purification of the immunizing protein¹¹⁻¹³ or by affinity purification of the antibodies¹⁴⁻¹⁷ have been used. The ELISA assays for rat allergen measurements have been based on polyclonal^{9,10,18} or monoclonal antibodies.^{11,14,19-22} However, a standardized assay for quantifying the exposure to laboratory animal allergens is not yet available. In addition, no occupational exposure limits have been set for the allergens.

From the ^aDepartment of Environmental Sciences, University of Kuopio,

^bDepartment of Clinical Microbiology, University of Kuopio, ^cNational Laboratory Animal Centre, University of Kuopio, and ^dInstitute of Environmental Medicine, Karolinska Institute.

Supported by The Finnish Work Environment Fund (101297).

Received for publication October 2, 2004; revised November 13, 2004; accepted for publication November 25, 2004.

Reprint requests: Anne Korpi, PhD, University of Kuopio, Department of Environmental Sciences, Research and Development Unit of Environmental Health, Biotecknia 2, PO Box 1627, 70211 Kuopio, Finland.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2003.11.039

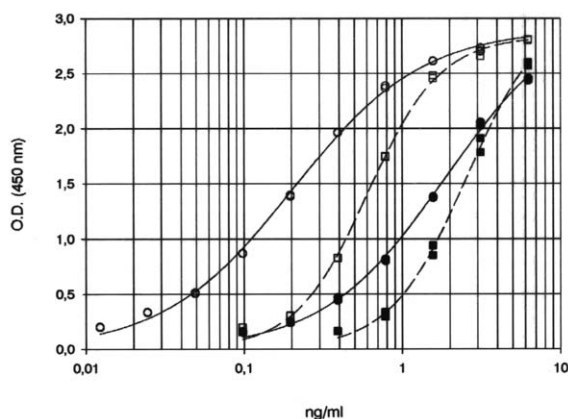


FIG 1. Comparison between Mus m 1 (s, d) and Rat n 1 (h, n) standard curves obtained with AMDEX conjugate (s, h) and ABC conjugate (d, n). The detection limits for 2-fold diluted samples are the lowest standard points, equivalent to background means + 2 SD.

The aim of this study was to introduce a new ELISA application with commercial reagents that have recently become available. The commercial ELISA kits for the detection of mouse (Mus m 1) or rat (Rat n 1) urinary allergen are instructed for settled dust samples, thus being suitable for air samples with high allergen levels. In the present study the assays were modified for measuring lower allergen levels (less than 1 ng/m³) in the air to constitute a useful tool for risk assessment purposes. With these methods, Mus m 1 and Rat n 1 levels were analyzed from air samples taken during various tasks of laboratory animal work.

MATERIALS AND METHODS

Assay optimization

The commercial ELISA kits for the detection of Mus m 1 and Rat n 1 were launched recently (Indoor Biotechnologies, Inc, Cardiff, United Kingdom, and Mabtech AB, Nacka, Sweden), and the antibodies in these kits were used as a basis for the following ELISA methods. The Rat n 1 assay antibodies have been described in the literature.²¹ According to the manufacturer, the sensitivity of the assay was 200 pg/mL for Mus m 1 and 500 pg/mL for Rat n 1, especially suitable for the analysis of settled dust samples and for airborne allergen exposure in high exposure areas. The kit protocols were modified and optimized to enhance the sensitivity of the assays for air samples not taken from the immediate proximity of rodents. This was done by testing the effect of different working volumes, antibody concentrations, coating, blocking, dilution, and washing buffers, and conjugate, substrate, and stopping solutions. Two commercial avidin/streptavidin—horseradish peroxidase (HRP) conjugates, ie, Vectastain Elite ABC reagents at 1:500 (Vector Laboratories, Inc, Burlingame, Calif) and AMDEX (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) (a signal amplification conjugate with streptavidin conjugated to multiple HRP molecules on a dextran backbone) at 1:1000 to 1:8000, were compared for sensitivity, and the latter was chosen for the assay.

In the final assays, flat-bottomed 96-well microtiter plates (Nunc-Immuno MaxiSorp Plates; Nunc A/S, Roskilde, Denmark) were coated overnight at 4°C with polyclonal rabbit anti-Mus m 1 antibodies (Indoor Biotechnologies Inc) at 1 µg/mL or monoclonal

antibody RUP-6 (Mabtech AB) at 5 µg/mL diluted in PBS, pH 7.4. After washing (PBS with 0.05% Tween 20) and blocking (PBS with 1% BSA), the standards (Mus m 1 allergen standard [Indoor Biotechnologies Inc] at 6 to 3125 pg/mL; Rat n 1 standard [Indoor Biotechnologies Inc] at 12 to 6250 pg/mL) and samples diluted (from 1:2 to 1:256) in the blocking buffer were incubated for 90 minutes at room temperature. After washing, a biotinylated secondary antibody (Rabbit anti-Mus m 1 polyclonal antibodies at 1:1000, Indoor Biotechnologies Inc; monoclonal antibody RUP-1 at 1:1000, Mabtech AB) was added and incubated for 1 hour at room temperature. After washing the wells, AMDEX streptavidin-HRP conjugate (1:8000) was added and incubated for 30 minutes. Thereafter, the tetramethylbenzidine (TMB) substrate (0.1 mol/L Na-acetate buffer [pH 6] with 0.15% urea-H₂O₂ [Fluka Chemie AG, Buchs, Switzerland] and 1% TMB solution [10 mg/mL TMB; Merck, Darmstadt, Germany; in dimethyl sulfoxide]) were added. The color development was stopped with 2 mol/L H₂SO₄ after 10 minutes and read at 450 nm with HTS 7000 Plus Bio Assay Reader (Perkin Elmer, Norwalk, Conn). For duplicate standard or eluate wells, a coefficient of variation (CV) of 10% was accepted.

Assay validation, precision, and specificity

The intra-assay precision was assessed by using 10 replicate analyses of 2 kit standard concentrations (50 and 200 pg/mL). Interassay precision was assessed by analyzing 2 concentrations (Mus m 1, 195 and 390 pg/mL; Rat n 1, 390 and 780 pg/mL) in duplicate on 10 different days. For both concentrations, mean, SD, and CV were calculated, and the mean of the 2 CVs was calculated for both precision types.

Description of the animal facility used for testing the assays

The animal facility with 2-corridor system was built at the beginning of the 1980s and was about to be renovated. The volume of the animal housing rooms was either 46 or 68 m³, and the animal loading ranged from 3.3 to 8.7 animals/m³. In the animal housing rooms, the ventilation was arranged with 1 supply air and a maximum of 4 exhaust air vents per room. The animal rooms were without any safety hoods or local exhaust ventilation. The air exchange rates were approximately 8 to 12 times per hour, and the relative humidity was adjusted to 50% ± 10% and temperature to 22°C ± 1°C. The bedding (aspen chip) was changed once a week for mice and twice a week for rats by transferring animals from soiled to clean cages. The mice were brought into the adjacent laboratories only for operation on tables. The ventilation rate in the laboratories was approximately 3.5 times per hour. In the cage washing room, the soiled litter was dumped into a container (without exhaust ventilation) before placing the emptied cage into a tunnel washer. The washer's dirty end was connected to local exhaust ventilation. To clean the premises, floors and other surfaces were swept and wiped with moist cloth, and no vacuum cleaning was performed.

Air sampling

Air samples were collected on 25-mm Teflon filters (FALP 02500, 1.0 µm pore size; Millipore, Billerica, Mass) with IOM Inhalable Dust Samplers (SKC Inc, Eighty Four, Pa) by using portable pumps with a flow rate of 2 L/min.^{15,16} This sampler has been found to allow aerosol measurements according to the international criteria for the inhalable particle fraction.²³

Personal task samples with 36- to 172-minute sampling times were collected during (1) cage changing, (2) animal handling, (3) cleaning other premises than animal rooms, and (4) manual emptying of cages.

TABLE I. Mus m 1 allergen concentrations during different tasks of laboratory animal care taking

Task/area	Stationary (S) or personal (P) sampling	No. of samples	Sampling time, min.	Mean Mus m 1 concentration, ng/m ³		Range, ng/m ³	No. of handled cages or animals
				AM	GM		
Changing room	S	1	965	0.3	0.3		0
Cleaning the animal premises other than animal housing rooms	P	2	110-118	0.9	0.9	0.7-1.1	0
Laboratory when mice were handled on an open bench	S	1	123	9	9		22 mice
Handling mice in laboratory on an unventilated table; animals 20 cm from breathing zone	P	2	88-124	9	4	1-16	21-22 mice
Background level in a mouse room	S	1	370	17	17		227 mice
Mouse room during cage change	S	5	52-368	41	32	10-96	225-446 mice*
Cage changing	P	4	53-59	81	69	42-172	78-400 mice
Manual emptying of cages and automated cage washing	P	3	36-38	251	180	104-545	95-105 mouse cages
Cage emptying and washing	S	3	36-38	233	156	35-375	95-105 mouse cages
Rat room during cage change	S	5	50-95	<0.1	<0.1		231-485 rats*
Change of rat cages	P	4	50-68	0.1 [†]	0.08 [†]	nd-0.4	101-213 rats
Manual emptying of rat cages and automated cage washing	P	2	42-67	1.5	1.4	1-2	86-200 rat cages

The detection limit for Mus m 1 is 0.1 ng/m³ for 1-hour sampling.

AM, Arithmetic mean; GM, geometric mean; nd, not detected.

*Total number of animals in the room; not all were changed from dirty to clean cages during sampling.

[†]Detection limit divided by 2 was used as the concentration in each sample with a Mus m 1 concentration less than detection limit.

TABLE II. Rat n 1 allergen concentrations during different tasks of laboratory animal care taking

Task/area	Stationary (S) or personal (P) Sampling	No. of samples	Sampling time, min	Mean Rat n 1 concentration, ng/m ³		Range, ng/m ³	No. of handles cages or animals
				AM	GM		
Changing room	S	1	965	0.2	0.2		0
Cleaning the animal premises other than animal housing rooms	P	2	110-118	0.6*	0.5*	nd-0.7	0
Handling rats in a rat room	P	2	42-146	15	14	12-17	42-63 rats
Background level in a rat room	S	3	42-369	15	13	9-25	285-392 rats
Rat room during cage change	S	5	50-95	57	53	34-98	231-485 rats [†]
Cage changing	P	4	50-68	60	59	38-69	101-213 rats
Manual emptying of cages and automated cage washing	P	2	42-67	109	93	53-164	86-200 rat cages
Cage emptying and washing	S	2	41-67	256	226	136-376	86-200 rat cages
Mouse room during cage change	S	4	52-58	<0.8	<0.8		264-400 mice [†]
Change of mouse cages	P	4	53-59	<0.8	<0.8		78-400 mice
Manual emptying of mouse cages and automated cage washing	P	3	36-38	<0.8	<0.8		95-105 mouse cages

The detection limit for Rat n 1 is 0.8 ng/m³ for 1-hour sampling.

AM, Arithmetic mean; GM, geometric mean; nd, not detected.

*Detection limit divided by 2 was used as the concentration in each sample with a Rat n 1 concentration less than detection limit.

[†]Total number of animals in the room; not all were changed from dirty to clean cages during sampling.

During these tasks the personnel carried the pumps on a belt with the sampler head fastened in the breathing zone.

Stationary samples with 36- to 965-minute sampling times were collected from (1) a changing room, (2) undisturbed animal rooms, (3) animal rooms during cage change, (4) a cage washing room, and (5)

a laboratory when 22 mice were handled. The samplers were fixed at the height of 1 to 1.6 m, depending on whether the worker was sitting or standing during the task. The distance of the sampling stand from walls was minimum of 60 cm, and the samplers faced the room center.

The filters were stored at -20°C until extraction.

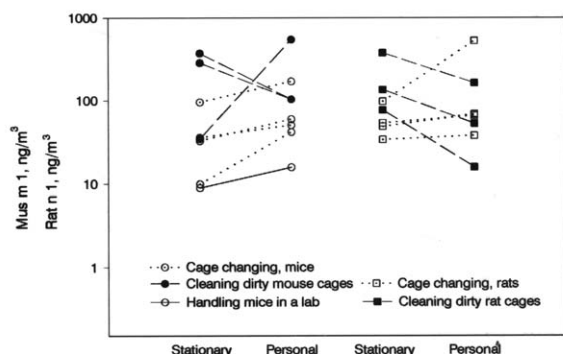


FIG 2. Comparison of allergen levels obtained by stationary and personal sampling during the same task.

Extraction of filters

The filters were allowed to thaw slowly before elution in 3-mL Minisorp tubes (Nunc A/S) in 1 mL PBS with 0.5% Tween 20 under gentle rotation for 2 hours.¹⁵ The filter was then discarded, and BSA (Fraction V; Sigma Chemical Co, St Louis, Mo) was added to 1% (w/v) for protein stabilization.¹⁵ The eluates were stored at -20°C until analysis for the level of Mus m 1 and Rat n 1 by using the ELISA assays described above. This elution method has been tested to be efficient compared with 2 other elution methods.²⁴

RESULTS

Validation of the ELISA procedure

The selection of the conjugate proved critical for achieving the required sensitivity. The Mus m 1 and Rat n 1 standard curves obtained with 2 conjugates are illustrated in Fig 1. Consequently, the AMDEX was chosen for the final assay because of the greater than 4-fold better sensitivity compared with ABC conjugate.

The validation of the final ELISA procedures was performed with samples collected from an animal facility. The sensitivities of the assays were determined to be 12 pg/mL for Mus m 1 and 98 pg/mL for Rat n 1 for 2-fold diluted samples, defined as the concentration higher than the mean absorbance of blanks + 2 * SD. This was equivalent to the detection limits of 0.1 ng/m³ and 0.8 ng/m³ for Mus m 1 and Rat n 1, respectively, for air sampling for 1 hour at 2 L/min. For statistical calculations (mean concentrations) all samples below the detection limit were assigned a value of half of the detection limit for 1-hour samples, ie, 0.05 ng/m³ for Mus m 1 and 0.4 ng/m³ for Rat n 1.

The mean intra-assay CV of the two Mus m 1 concentrations prepared from the kit standard was 9.1%, and the mean interassay CV was 5.6%. For Rat n 1, the intra-assay and interassay CV values were 5.4% and 4.1%, respectively.

Mouse and rat allergen levels in air samples

The Mus m 1 concentration was above the detection limit (0.1 ng/m³) in 77% of the air samples. The samples with Mus m 1 concentrations below the detection limit were taken when rats or dirty rat cages were handled (Table I). The lowest Mus m 1 levels were measured in the

changing room and when cleaning other than animal rooms. In these premises without animals, Mus m 1 concentrations were below 1 ng/m³. The highest Mus m 1 concentrations, from 10s to 100s of nanograms per cubic meter, were measured during the cage change and cage cleaning tasks. During the cage change, the Mus m 1 levels exceeded the background level (ie, allergen levels without disturbance) by more than 2-fold in stationary sampling and more than 4-fold in personal sampling.

Rat urinary allergen was detected in 65% of the air samples; the Rat n 1 concentrations were below the detection limit when mice or dirty mouse cages were handled (Table II). Because of the longer sampling periods in the changing room and on cleaning other than animal rooms, the Rat n 1 concentrations in these samples could be determined even below 0.8 ng/m³. The cage cleaning involved highest Rat n 1 levels from 10s to 100s of nanograms per cubic meter. The cage changing approximately quadrupled the background Rat n 1 levels in the rat rooms.

The mouse and rat urinary allergen concentrations measured by both stationary and personal samplings during the same task are compared in Fig 2. The personal sampling during cage changing and animal handling resulted in up to 6 times higher aeroallergen concentration than the stationary sampling. The allergen levels measured by the 2 sampling types differed up to 16-fold during cleaning dirty cages, but neither of the sampling types yielded a higher concentration consistently.

DISCUSSION

It is generally agreed that international standardization should be sought at the earliest opportunity for the measurement of laboratory animal allergens in the air including collection of the air sample, elution of the filter, and especially the immunoassay of the eluate.^{4,22,24} All these steps were taken into account in this study. Besides this study, the IOM-sampler has also been used for collection of inhalable dust for the measurement of airborne animal allergens.^{9,10,14-16,18,21,25-27} Likewise, the extraction method used in this study has been shown to be efficient and to produce stable extracts.²⁴ Different assay types produce large differences in the absolute allergen concentration as was shown recently.²⁴ For example, an inhibition RIA was found to give approximately 7 times higher rat urinary allergen concentrations than the ELISA, and differences in the slope of the standard curves of these methods were observed.²⁸ However, even within the same assay type (ELISA), nearly 2-fold differences were found in the allergen levels between laboratories because of procedural variation.²⁴ Obtaining sufficient volumes of positive human IgE serum for long-term usage of RAST assays might be difficult and might result in reduced repeatability and thus make this assay an unsuitable candidate for method standardization.⁸ Even though ELISA methods might underestimate the clinical allergenic potency of an air sample,²⁹ they still represent

the best chance of obtaining a standard method for environmental allergen measurement.⁸

High enough sensitivity and intralaboratory and interlaboratory reproducibility are the main requirements for the analysis of airborne allergens. As a result of this study, sensitive methods for airborne Mus m 1 and Rat n 1 were introduced. These methods are easily adaptable in research laboratories with basic ELISA equipment and knowledge. Two different conjugates were tested and found to result in greater than 4-fold difference in the sensitivity. When compared with the sensitivities informed in the ELISA kits, the improvement was 5-fold (from 500 to 98 pg/mL) for Rat n 1 and 16-fold (from 200 to 12 pg/mL) for Mus m 1. So far the laboratory animal allergen assays with comparable sensitivities have used expensive signal amplification requiring preceding screening of low allergen level samples with an unamplified method.^{15,16,30} As for the reproducibility between different laboratories, the assessment would need further testing. Equally important would be a discussion of the acceptable variation, considering the differences of several orders of magnitude in the allergen concentrations during the same tasks and differences in the individual sensitivity to get allergy.

The sensitive methods described here enable detection of mouse and rat aeroallergens also from premises in which (respective) animals or dirty cages are not present during sampling. Defects in ventilation and allergen transport with clothing from mouse rooms to rat rooms and vice versa might explain the low Mus m 1 levels detected when rat cages were washed. Also, rat urinary allergens could be detected in samples from a mouse room (0.3 ng/m³) when sampling time was increased up to 6 hours (data not shown), indicating allergen leakage or transport to secondary exposure sites. However, reciprocal cross-reaction as a result of the homology between Rat n 1 and Mus m 1 might not be a significant problem in these assays because mouse urinary allergens were detected only in 1 of the 9 samples collected during change of rat cages when the Rat n 1 levels were high and ranged from 34 to 98 ng/m³. The presence of small amounts of mouse or rat urinary allergen in air samples as a result of allergen leakage or inefficient ventilation is probably insignificant on the practical level of the exposure assessment.

It is known that allergen levels correlate with the number of animals present in the room (animal loading) and the degree of activity during sampling.⁹⁻¹¹ This was also the case in the present study. It was also observed that the number of cages worked with, different working habits, and technical interventions (such as local exhaust ventilation systems) explained the variation. Generally, the highest exposure to Mus m 1 or Rat n 1 has been found to occur during cage cleaning, changing animals from dirty to clean cages, feeding, and animal handling.^{9,10,15,16,19,31-33} However, within the same task or area category in this study, the allergen concentrations within a task or area varied. This has been observed also in other studies, eg, the measured Mus m 1 concentrations differed by greater than 4000-fold during emptying dirty

cages.^{9,10} Because the number of handled animals (cages) during each task sampling differs and thus affects the allergen levels, it remains difficult to establish reference levels for good hygiene in various tasks. Thus, the mere description of a work task might not give reliable indication of the exposure level and thus should not be used as a surrogate for exposure assessment, and the need to measure allergen concentrations prevails. It has been suggested that measuring room concentrations of allergens might significantly underestimate the levels generated locally and hence underestimate potential exposure.^{4,17,31,34,35} In this study, however, the stationary sampling was observed to occasionally also overestimate the personal allergen exposure (Fig 2). If exposure levels can be related to sensitization and symptoms, the significance of the differences in aeroallergen levels as a result of sampling types can be estimated, and suggestions can be given for the exposure assessment and management.

In conclusion, the sensitive methods introduced here can be used for the estimation of airborne mouse and rat allergen exposure in animal units and research laboratories. Moreover, the methods allow the evaluation of the effectiveness of technical interventions to decrease exposure levels. The reagents used in these assays are commercially available for research purposes.

Camilla Kallin at the Karolinska Institute is heartily acknowledged for cooperation and discussion. The personnel in the animal facility are thanked for very helpful and pleasant collaboration.

REFERENCES

- Bush RK, Wood RA, Eggleston PA. Laboratory animal allergy. *J Allergy Clin Immunol* 1998;102:99-112.
- Bush RK. Assessment and treatment of laboratory animal allergy. *ILAR J* 2001;42:55-64.
- Gordon S, Wallace J, Cook A, Tee RD, Newman Taylor AJ. Reduction of exposure to laboratory animal allergens in the workplace. *Clin Exp Allergy* 1997;27:744-51.
- Gordon S, Tee RD. Allergenic hazards. In: Wood M, Smith MW, editors. *Laboratory animal handbooks no 13: health and safety in laboratory animal facilities* London: Laboratory Animals Ltd; 1999. p. 17-47.
- Harrison DJ. Controlling exposure to laboratory animal allergens. *ILAR J* 2001;42:17-36.
- Nieuwenhuijsen MJ, Gordon S, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. Exposure to dust and rat urinary aeroallergens in research establishments. *Occup Environ Med* 1994;51:593-6.
- Wood RA. Laboratory animal allergens. *ILAR J* 2001;42:12-6.
- Gordon S. Laboratory animal allergy: a British perspective on a global problem. *ILAR J* 2001;42:37-46.
- Hollander A, van Run P, Spithoven J, Heederik D, Doekes G. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. *Clin Exp Allergy* 1997;27:617-26.
- Hollander A, Heederik D, Doekes G, Kromhout H. Determinants of airborne rat and mouse urinary allergen exposure. *Scand J Work Environ Health* 1998;24:228-35.
- Price JA, Longbottom JL. ELISA method for measurement of airborne levels of major laboratory animal allergens. *Clin Allergy* 1988;18:95-107.
- Jones RB, Kacergis JB, Paigen B, MacDonald MR, Ohman JL, McKnight FT, Turner WA. The effect of relative humidity on mouse allergen levels in an environmentally controlled mouse room. *AIHA J* 1995;56:398-401.
- Kacergis JB, Jones RB, Reeb CK, Turner WA, Ohman JL, Ardman MR, Paigen B. Air quality in an animal facility: particulates, ammonia, and volatile organic compounds. *AIHA J* 1996;57:634-40.

14. Renström A, Karlsson A-S, Malmberg P, Larsson PH, van Hage-Hamsten M. Working with male rodents may increase risk of allergy to laboratory animals. *Allergy* 2001;56:964-70.
15. Renström A, Björing G, Höglund AU. Evaluation of individually ventilated cage systems for laboratory rodents: occupational health aspects. *Lab Anim* 2001;35:42-50.
16. Thulin H, Björkdahl M, Karlsson A-S, Renström A. Reduction of exposure to laboratory animal allergens in a research laboratory. *Ann Occup Hyg* 2002;46:61-8.
17. Ohman JL Jr, Hagberg K, MacDonald MR, Jones RR Jr, Paigen BJ, Kacergis JB. Distribution of airborne mouse allergen in a major mouse breeding facility. *J Allergy Clin Immunol* 1994;94:810-7.
18. Hollander A, Heederik D, Doekes G. Respiratory allergy to rats: exposure-response relationships in laboratory animal workers. *Am J Respir Crit Care Med* 1997;155:562-7.
19. Lieutier-Colas F, Meyer P, Larsson P, Malmberg P, Frossard N, Pauli G, de Blay F. Difference in exposure to airborne major rat allergen (Rat n 1) and to endotoxin in rat quarters according to tasks. *Clin Exp Allergy* 2001;31:1449-56.
20. Lieutier-Colas F, Meyer P, Pons F, Hedelin G, Larsson P, Malmberg P, Pauli G, De Blay F. Prevalence of symptoms, sensitization to rats, and airborne exposure to major rat allergen (Rat n 1) and to endotoxin in rat-exposed workers: a cross-sectional study. *Clin Exp Allergy* 2002;32:1424-9.
21. Renström A, Larsson PH, Malmberg P, Bayard C. A new amplified monoclonal rat allergen assay used for evaluation of ventilation improvements in animal rooms. *J Allergy Clin Immunol* 1997;100:649-55.
22. Renström A, Gordon S, Larsson PH, Tee RD, Newman Taylor AJ, Malmberg P. Comparison of a radioallergosorbent (RAST) inhibition method and a monoclonal enzyme linked immunosorbent assay (ELISA) for aeroallergen measurement. *Clin Exp Allergy* 1997;27:1314-21.
23. Bartley DL. Inhalable aerosol samplers. *Appl Occup Environ Hyg* 1998;13:274-8.
24. Hollander A, Gordon S, Renström A, Thissen J, Doekes G, Larsson PH, Malmberg P, Venables KM, Heederik D. Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis of air samples. *Allergy* 1999;54:142-9.
25. Almqvist C, Larsson PH, Egmar A-C, Hedrén M, Malmberg P, Wickman M. School as a risk environment for children allergic to cats and a site for transfer of cat allergen to homes. *J Allergy Clin Immunol* 1999;103:1012-7.
26. Emenius G, Larsson PH, Wickman M, Härfast B. Dispersion of horse allergen in the ambient air, detected with sandwich ELISA. *Allergy* 2001;56:771-4.
27. Wickman M, Egmar A-C, Emenius G, Almqvist C, Berglind N, Larsson P, van Hage-Hamsten M. Fel d 1 and Can f 1 in settled dust and airborne Fel d 1 in allergen avoidance day-care centers for atopic children in relation to number of pet-owners, ventilation and general cleaning. *Clin Exp Allergy* 1999;29:626-32.
28. Renström A, Gordon S, Hollander A, Spithoven J, Larsson PH, Venables KM, Heederik D, Malmberg P. Comparison of methods to assess airborne rat and mouse allergen levels. II. Factors influencing antigen detection. *Allergy* 1999;54:150-7.
29. Renström A. Allergy to laboratory animals: risk factors for development of allergy and methods for measuring airborne rodent allergens [thesis]. Arbete och hälsa 26. Stockholm: Karolinska Institute; 1997.
30. Renström A, Karlsson A-S, Tovey E. Nasal air sampling used for the assessment of occupational allergen exposure and the efficacy of respiratory protection. *Clin Exp Allergy* 2002;32:1769-75.
31. Eggleston PA, Newill CA, Ansari AA, Pustelnik A, Lou S-R, Evans R III, Marsh DG, Longbottom JL, Corn M. Task-related variation in airborne concentrations of laboratory animal allergens: studies with *Rat n* I. *J Allergy Clin Immunol* 1989;84:347-52.
32. Gordon S, Tee RD, Nieuwenhuijsen MJ, Lowson D, Harris J, Newman Taylor AJ. Measurement of airborne rat urinary allergen in an epidemiological study. *Clin Exp Allergy* 1994;24:1070-7.
33. Nieuwenhuijsen MJ, Gordon S, Harris JM, Tee RD, Venables KM, Newman Taylor AJ. Variation in rat urinary aeroallergen levels explained by differences in site, task and exposure group. *Ann occup Hyg* 1995;39:819-25.
34. Botham PA, Lamb CT, Teasdale EL, Bonner SM, Tomenson JA. Allergy to laboratory animals: a follow up study of its incidence and of the influence of atopy and pre-existing sensitization on its development. *Occup Environ Med* 1995;52:129-33.
35. Renström A. Exposure to airborne allergens: a review of sampling methods. *J Environ Monit* 2002;4:619-22.