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Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis of air samples

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Airborne laboratory-animal allergens can be measured by several methods, but little is known about the effects of important differences in methodology. Therefore, methods used in research projects in The Netherlands, the UK, and Sweden were compared. Seventy-four sets of three parallel inhalable dust samples were taken by a single operator in animal facilities in the three countries, and analyzed in parallel by the three institutes for rat and mouse urinary allergen. Rat-allergen levels measured by RAST inhibition (UK) were 3000 and 1700 times higher than levels measured by enzyme immunoassay (EIA)-sandwich methods with polyclonal rabbit (The Netherlands) or monoclonal mouse (Sweden) antibodies, while the difference between the two EIA-sandwich methods was much smaller: a factor of 2.2. For mouse allergen, an inhibition radioimmunoassay (RIA) with rabbit antimouse antibodies (UK) gave 4.6 and 5.9 times higher concentrations than sandwich EIAs with rabbit polyclonal antibodies (Sweden and The Netherlands), while the difference between the two sandwich EIAs was, on average, 1.6-fold. Thus, although levels of rat and mouse aeroallergens are significantly correlated, the assay type gives large differences in absolute concentrations, and interlaboratory technical differences affect even the same assay type. Conversion factors can aid comparison between studies, and, in the long term, assay standardization is desirable.

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Abbreviations. NHLI: National Heart and Lung Institute (UK); WAU: Wageningen Agricultural University (The Netherlands); NIWL: National Institute for Working Life (Sweden); EIA: enzyme immunoassay; RAST: radioallergosorbent test; RIA: radioimmunoassay; MUA: mouse urinary allergen; RUA: rat urinary allergen; BSA: bovine serum albumin; HSA: human serum albumin; PBS: phosphate-buffered saline; PTFE: polytetrafluoroethylene (Teflon); CI: confidence interval.

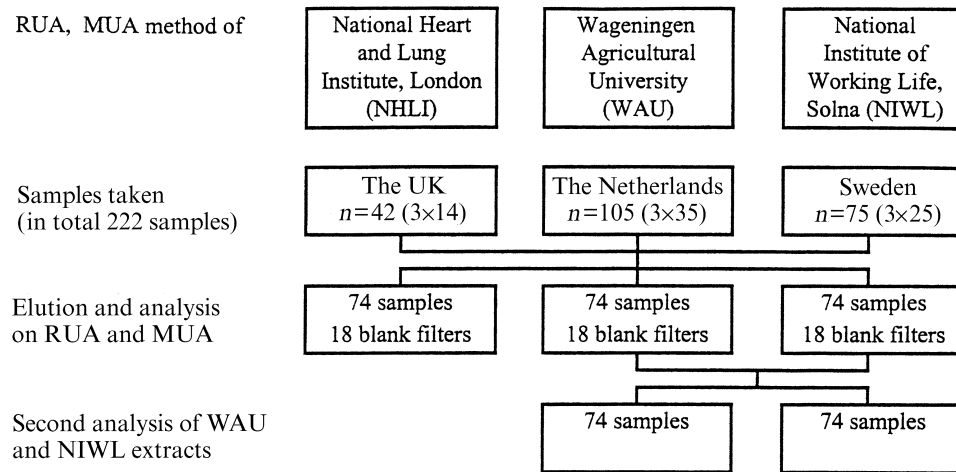


Figure 1. Study design.

Laboratory animal workers are at high risk of developing laboratory-animal allergy (LAA) (1, 2). The risk increases with the level of allergen exposure as measured by immunoassays (3–5), which can also be used to identify determinants of laboratory-animal allergen exposure (6–15). Yet, allergen concentrations should be compared with care (16). The reported allergen levels may differ for methodological reasons: sampling equipment, extraction methods, reference allergens, antibodies, and the design of the immunoassay used.

Therefore, we compared methods to measure rat urinary aeroallergens (RUA) (13, 17, 18) and mouse urinary aeroallergens (MUA) (18–20) in inhalable dust samples as part of the concerted action program, “Epidemiology of occupational allergic asthma and exposure to bioaerosols”, supported by the European Union. These methods have been applied in epidemiologic and other studies by three European research groups at the National Heart and Lung Institute, London, UK (NHLI) (4, 9–12, 16, 21); Wageningen Agricultural University (WAU), The Netherlands (5, 18); and the National Institute for Working Life, Solna, Sweden (NIWL) (13, 16, 20).

Material and methods

Parallel ambient air inhalable dust samples were taken in The Netherlands ($3 \times 35 = 105$), the UK ($3 \times 14 = 42$), and Sweden ($3 \times 25 = 75$) (Fig. 1). Samples were taken in triplicate in rat rooms, mouse rooms, and cage-cleaning rooms. Additionally, 3×18 (54) blank filters were collected by mounting them in sampling heads, packing them in the same way as the test filters, and taking them to the sites of

sampling, but not unpacking them. Sampling was performed by one operator (J.T.) using portable pumps at 2 l/min airflow, following in each country the current method of the participating institution (Table 1). For each set, the three sampling heads were randomly placed approximately 15 cm apart on the sampling stand. Sampling time varied between 40 min and 20 h (mean 460 min) to provide a sufficiently wide range of RUA and MUA concentrations. Each institution received one filter from each set (74 test samples plus 18 blanks=92), without knowing its type and origin, and eluted and assayed them within 3–6 months after sampling for both rat and mouse allergens, following its published procedures (13, 17–20) (Table 1).

The separate effects of elution method and immunoassay were studied by exchanging filter extracts produced at WAU ($n=74$) and NIWL ($n=74$), which were reanalyzed approximately 9 months after the first analysis. Both institutions simultaneously reanalyzed the retained extracts to control for insufficient reproducibility or effects of more prolonged storage.

The detection limit of each method has been described elsewhere (13, 17–20) (Table 1). These limits and the mean sampled volume (0.92 m^3) were used to calculate detection limits per m^3 . Statistical analyses were performed with SAS software (version 6.09). Median RUA and MUA levels were calculated, because distributions were not clearly normal or log-normal. Agreement between methods was determined by calculating the geometric mean of the ratio among allergen levels detected by different methods in parallel samples (22), in which both methods gave detectable levels, and a 95% confidence interval (CI) for the mean ratio was calculated for each comparison.

Table 1. Essential features of and differences between methods developed by three institutions to measure airborne rat and mouse urinary allergen levels

Institution	NHLI	WAU	NIWL
Sampling method			
Inhalable dust sampler	Seven-hole	IOM	IOM
PTFE filter, pore size	1.2 µm	1.0 µm	1.0 µm
Elution method			
Buffer	2 ml 0.1 M NH ₄ HCO ₃ plus 0.5% Tween 20	2 ml 0.15 M PBS	1 ml 0.15 M PBS plus 0.5% Tween 20
Method (extracts were all stored at -20 °C)	Vortexed, and after 2 h, centrifuged and lyophilized. Reconstituted in PBS plus 0.3% w/v HSA before assay to get 10-fold concentrated extract	Vortexed 2 min, sonicated 2 min, vortexed 5 min, sonicated 2 min, centrifuged	Rotation 1 h, filter discarded, and 1% w/v BSA added
RUA immunoassay			
Immunoassay	RAST inhibition	EIA sandwich	EIA sandwich
Rat urine standard	From male, postpubertal Wistar rats	From young/old and male/female Wistar rats	Rat n 1.02 from 3–4-month-old male Sprague Dawley rats
Antibodies	IgE pool of eight rat-allergic workers	Polyclonal antibodies against RUA	Monoclonal antibodies against Rat n 1.02
Detection limit assay	50 ng dry weight (16.5 ng protein)/ml	0.075 ng protein/ml	0.10 ng protein/ml (unamplified protocol)
Detection limit method*	10 ng per filter (10.9 ng/m ³) (3.3 ng per filter [3.6 ng/m ³] as protein)	0.15 ng per filter (0.16 ng/m ³)	0.10 ng per filter (0.11 ng/m ³)
Specificity	Rat urine allergens	Rat urine proteins	Rat n 1.02
Reproducibility	Interassay CV** 7.0%	Interassay CV** 12.9%	–
MUA immunoassay			
Immunoassay	Competitive inhibition RIA	EIA sandwich	EIA sandwich
Mouse urine standard	From male, postpubertal mice	From young/old and male/female Balb/c mice	Mus m 1 from postpubertal male NMRI mice
Antibodies	Polyclonal antibodies against MUA	Polyclonal antibodies against MUA	Polyclonal antibodies against Mus m 1
Detection limit assay	0.5 ng dry weight (0.09 ng protein)/ml	0.075 ng protein/ml	0.10 ng protein/ml
Detection limit method**†	4.0 ng per filter (4.3 ng/m ³) (0.72 ng per filter [0.8 ng/m ³] as protein)	0.15 ng per filter (0.16 ng/m ³)	0.10 ng per filter (0.11 ng/m ³)
Specificity	Mouse urine proteins	Mouse urine proteins	Mus m 1
Reproducibility	Interassay CV** 20%	Interassay CV** 12.9%	–

*Expressed in relation to 0.92 m³, average volume of air sampled.

**CV: coefficient of variation.

†Samples less than detection limit in NHLI MUA assay were assigned value of 1.8 ng per filter (19).

Table 2. Median and range of RUA and MUA levels (ng/m³) found in ambient air dust samples taken in facilities provided for study by three participating institutions. Levels are stratified by institution analyzing filters

Filter source	Samples taken in NHLI			Samples taken in WAU			Samples taken in NIWL		
Method	n	Median (ng/m ³)	Range	n	Median (ng/m ³)	Range	n	median (ng/m ³)	range
RUA									
NHLI*	13	11 000	172–52 900	35	3730	<10.9–47 200	25	775	<10.9–21 700
WAU	13	0.37	<0.16–15.0	35	0.86	<0.16–31.9	25	<0.16	<0.16–3.6
NIWL	14	1.95	<0.11–11.8	35	2.0	<0.11–43.4	25	0.71	<0.11–11.6
MUA									
NHLI*	10	9.92	0.89–162	21	11.0	0.8–4610	20	9.37	0.74–82.5
WAU	13	<0.16	<0.16–32.6	34	1.1	<0.16–1560	25	<0.16	<0.16–3.0
NIWL	14	0.24	<0.11–71.5	35	2.8	0.13–446	25	0.36	<0.11–6.1

*To compare values adjusted for protein content, multiply NHLI RUA by 0.35 and NHLI MUA by 0.17 (see Table 1).

Results

Descriptive statistics

Table 2 shows the descriptive statistics of the RUA and MUA levels found by the three methods. Some of the 74 samples were missing due to problems with elution or because of insufficient extract. The allergen concentrations measured at NHLI were considerably higher than the concentrations found in parallel samples by the other institutions (Table 2). These differences were most pronounced, i.e., several orders of magnitude, for RUA, and were observed for all samples, irrespective of the country where they had been taken.

The frequency of detection and nominal RUA and MUA levels in test samples and blank filters are shown in

Table 3. The methods of NHLI and NIWL were more sensitive and detected aeroallergens in both rat and mouse rooms more frequently than the methods of WAU. The levels detected with all RUA methods in rat rooms were considerably higher than those in mouse rooms and vice versa. All methods detected allergen on some blank filters, particularly the NHLI RUA method, but blank filter values were orders of magnitude lower than values from test samples.

RUA and MUA methods

Samples with RUA or MUA concentrations above the detection limit were used to compare the three methods. The number in each comparison varied, because ratios were calculated only when the methods of both institutions gave

Table 3. RUA and MUA levels (ng/m³) found on filters taken in rat rooms, mouse rooms, and on blank filters by methods of three institutions

Filter source	Rat rooms				Mouse rooms				Blank filters		
Method	n	n _d *	Median (ng/m ³)	Positive test result (%)**	n	n _d *	Median (ng/m ³)	Positive test result (%)**	n	n _d *	Range (ng/m ³)
RUA											
NHLI**	38	1	6960	97	21	2	330	90	18	10	<10.9–1400
WAU	38	9	0.62	76	22	20	<0.16	9	17	16	<0.16–0.45
NIWL	38	1	2.1	97	22	17	<0.11	23	18	15	<0.11–0.78
MUA											
NHLI**	29	11	6.64	62	13	1	15.9	92	10	7	<4.3–6.1
WAU	38	27	<0.16	29	21	7	3.0	67	16	15	<0.16–0.47
NIWL	38	12	0.29	68	22	3	17.3	86	18	17	<0.11–0.20

*n_d, number of samples below detection limit; ** $(n-n_d)/n\%$.

**To compare values adjusted for protein content, multiply NHLI RUA by 0.35 and NHLI MUA by 0.17 (see Table 1).

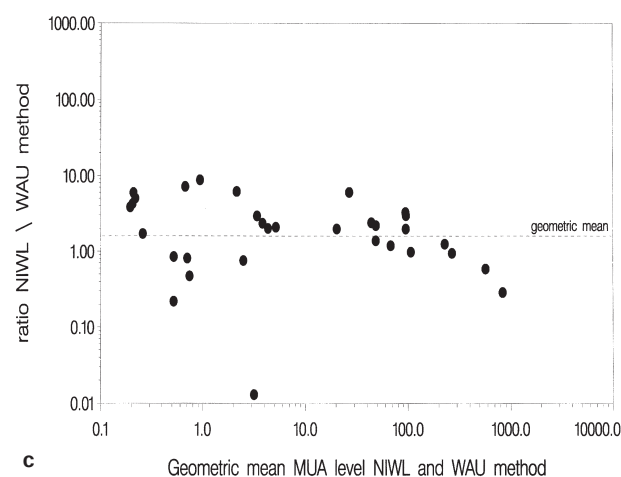
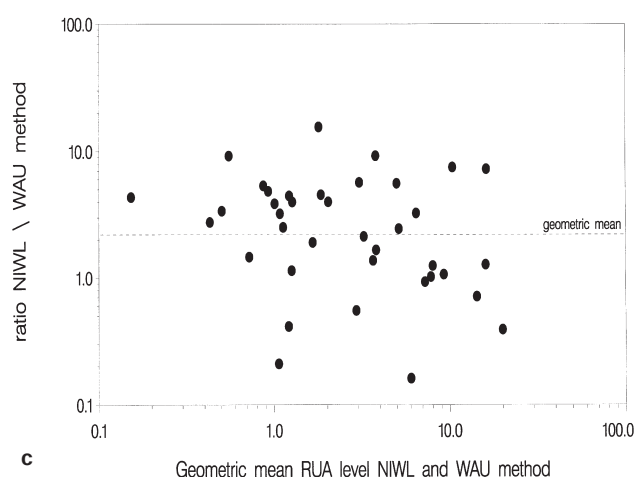
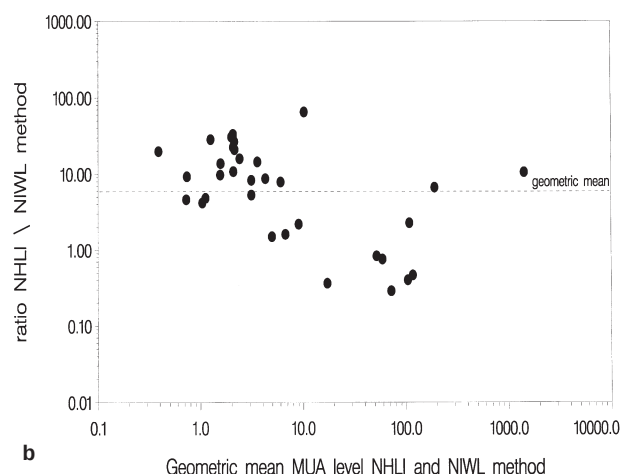
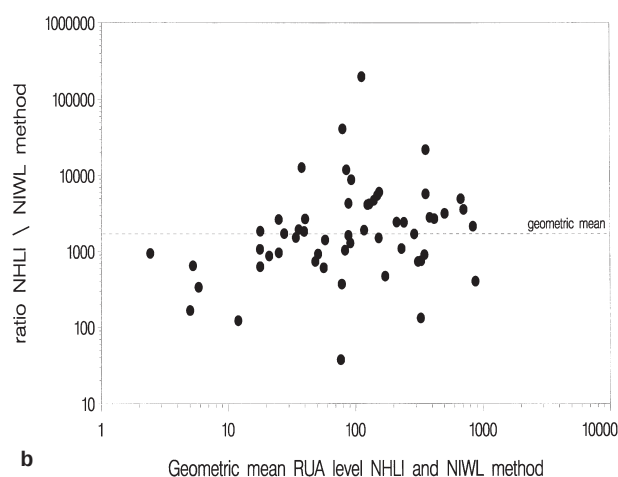
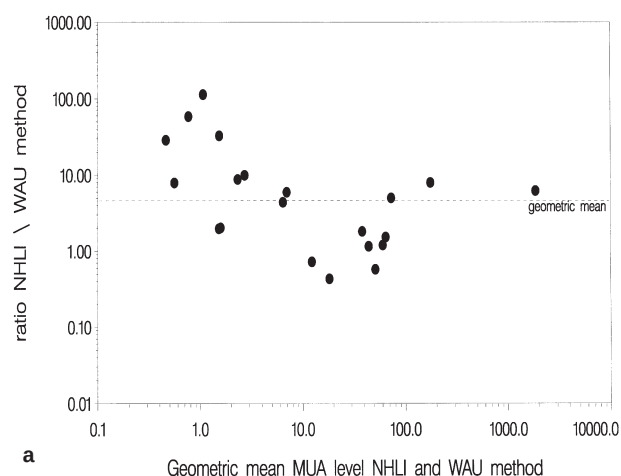
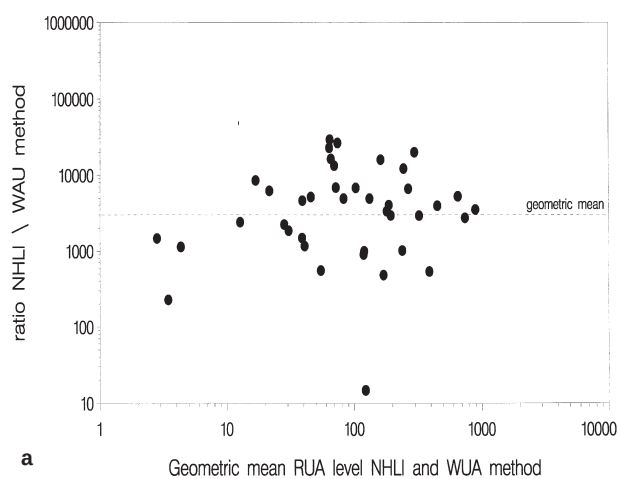


Figure 2. Relative differences (ratios) between rat urinary allergen (RUA) concentrations measured by different institutions in parallel airborne dust samples. Ratios for RUA levels found by two institutions are plotted against geometric mean of two values. Dotted lines give geometric mean of ratios for comparison between RUA levels measured at National Heart and Lung Institute (NHLI) and Wageningen Agricultural University (WAU) (2a), NHLI and National Institute for Working Life (NIWL) (2b), and NIWL and WAU (2c). To compare values adjusted for protein content, multiply NHLI RUA by 0.35 and NHLI MUA by 0.17 (see Table 1).

Figure 3. Relative difference (ratio) plotted against geometric mean of mouse urinary allergen (MUA) levels measured by methods of three institutions: NHLI, WAU, and NIWL. Format as in Fig. 2. Multiply NHLI RUA by 0.35 and NHLI MUA by 0.17 to convert for calculations using dry weight on filter.

detectable values. Close agreement between methods would have resulted in a distribution of ratios of around 1. RUA concentrations found at NHLI were 3000 (95% CI 1900–4900; $n=40$) and 1700 (95% CI 1200–2500; $n=56$) times higher than those measured at WAU and NIWL, respectively (Fig. 2a,b). The geometric mean of the ratios between RUA levels measured by NIWL and WAU was much smaller: 2.2 (95% CI 1.6–3.1; $n=38$) (Fig. 2c). MUA concentrations measured at NHLI were also higher than those measured at WAU (4.6 times, 95% CI 2.3–9.1; $n=21$) and NIWL (5.9 times, 95% CI 3.5–9.8; $n=34$) (Fig. 3a,b), but the differences were much smaller than for RUA. The mean difference between MUA results obtained by NIWL and WAU was smaller: 1.6 (95% CI 1.0–2.5; $n=32$) (Fig. 3c). The MUA methods agreed best at levels of approximately 3–100 ng/m³.

Stability of allergen extracts

The second analysis by WAU of its own extracts showed RUA allergen levels that were, on average, 63% (95% CI 53–71%; $n=35$) and MUA allergen levels 38% (95% CI 30–50%; $n=28$) of the previously obtained values. The NIWL second analysis results were, on average, 77% (95% CI 71–91%; $n=50$) and 109% (95% CI 100–120%; $n=57$) of the initial RUA and MUA levels, respectively. Thus, extracts prepared at WAU appeared to be less stable over time than those prepared at NIWL.

Differences due to elution method

Second analysis of extracts prepared at WAU and NIWL after exchange of extracts revealed that in both the monoclonal sandwich assay of NIWL and the polyclonal sandwich assay of WAU the extracts made at NIWL gave higher results for both RUA and MUA than extracts from parallel filters that had been eluted at WAU. For pairs of filters with detectable levels of allergen in both laboratories, the ratios (NIWL/WAU) were, on average, 10 (95% CI 6.8–14.6; $n=46$) for RUA, and 5.1 (95% CI 3.7–7.1; $n=67$) for MUA. Thus, filter extraction at NIWL appeared to be more efficient.

Differences due to immunoassay

Within the same series of secondary measurements, a comparison was made between values provided by the NIWL and WAU assays for duplicate samples from the same filter extracts. Of the assays for RUA, the WAU assay gave, on average, 4.1 times (95% CI 3.4–4.9; $n=59$) higher results for each center's extracts than the assay of NIWL. This

suggests that the WAU immunoassay for RUA detected more allergen than the NIWL assay. In contrast, the difference between the values provided by the two MUA immunoassays was small and not consistent, with an average NIWL/WAU ratio of 1.1 (95% CI 0.91–1.3; $n=68$).

Discussion

At present, there is no standard method for measuring laboratory animal allergen in airborne dust samples. The present study aimed to compare methods currently available for the measurement of airborne rat and mouse allergens. The methods had been independently developed for specific studies by three research groups and used to investigate the exposure–response relationship between exposure to allergen and the development of LAA. This study showed that results obtained by different methods give similar relative values but that absolute concentrations showed large differences. Some of the main determinants causing these differences could be identified, while a more detailed analysis is presented in an accompanying paper (23).

In theory, differences could be due to dust-sampling method, filter elution, storage in the laboratory, or immunoassay. This study did not attempt to evaluate the effect of dust sampling because this has been studied by others (24, 25). All three institutions sampled the inhalable dust fraction. Although they did use different makes of sampling head, this is not estimated to make more than a 1.2-fold difference in total dust collected. However, sampling heads designed to collect different dust fractions would be expected to give large differences.

It has been shown previously that the addition of detergent increases the recovery of rat urinary allergen from PTFE and glass-fiber filters (26), especially at low levels of allergen, when most of the allergen load is likely to be deeply embedded in the filter. There have been similar findings for grass pollen (27) and for other biologically relevant components such as endotoxins, the size of the effect probably depending on solubility (28–30). In the present study, the elution factors which varied were use of a detergent, amount of agitation, and duration of elution. The filter-exchange study by NIWL and WAU confirmed that the use of a detergent increases allergen yield by one order of magnitude. NHLI did not join in this study but used detergent and had the longest duration of elution (Table 1).

Extensive agitation of PTFE filters is not needed for the efficient elution of rat urinary allergen (26). Furthermore, rat urinary allergens may not be stable if sonicated (S. Gordon,

unpublished observation); therefore, prolonged sonication for 4 min, as used by WAU, especially in the absence of added protein, may destroy allergen and reduce the yield. Another factor explaining the low yields at WAU is the halving in allergen yield after storage of frozen extracts for approximately 9 months. WAU, unlike NHLI and NIWL, did not store its extracts with added detergent or protein, which may prevent adherence of proteins to vial walls and pipette tips.

An important finding of this study is that air samples need to be processed in the same way before immunoassay in order to make meaningful comparisons between air samples taken at different times or in different working areas. Otherwise, erroneous conclusions could be drawn about, for example, the effectiveness of control measures. The variations due to elution and storage methods discussed above were of a magnitude sufficient to explain most of the differences observed between the MUA levels measured by the three centers. The MUA immunoassays were similar, each employing rabbit polyclonal antibody, but there may be a small assay effect, which is discussed further in the companion paper (23).

Elution and storage effects do not explain all the differences in RUA levels, and the remaining differences must be attributable to differences in immunoassay. This is explored further in the companion paper (23). When elution and storage effects were controlled, the monoclonal immunoassay (NIWL) detected less RUA than the rabbit polyclonal immunoassay (WAU). This is not surprising, since a monoclonal assay is designed to detect only one component, in this case, the major allergen Rat n 1.02 (13), whereas rat-room air is complex, containing many major and minor allergens. Assays using experimentally induced animal polyclonal antibodies (as at WAU) or human polyclonal antibodies from occupational sensitization (as at NHLI) would be expected to capture a greater range of antibodies. It is of interest that the greater yield of allergen from filters by NIWL (and NHLI) methods appeared to outweigh the narrow specificity of the monoclonal technology, because the RUA levels measured by NIWL in parallel filters were consistently higher than those reported by WAU (Fig. 2c).

There are also arithmetic factors which differ between the immunoassays. Both the WAU and NIWL results were expressed in relation to the protein content of the standard employed. However, the NHLI results were not corrected for protein and were simply expressed as dry weight. This is primarily because carbohydrate or glycosylated allergens in the dust or standard urine extract may contribute to the

inhibition seen in the UK assays, but also because the protein content of dust samples is usually much lower than that of urine (31). As the protein content of the NHLI RUA and MUA extracts was 35.4% and 17.0%, respectively, the NHLI values should be reduced by multiplying by 0.35 and 0.17 if comparison of the protein content of the filters is required.

Allergen was detected on blank filters by each method, most frequently in the NHLI assays. This phenomenon has been noted before, particularly for mouse (19). However, the positive values for blank filters were consistently some orders of magnitude lower than for test filters. Since these filters were taken into the animal-house environment but not exposed, this effect may reflect minor contamination of sampling heads or filters. Alternatively, these may be false positives due to inherent problems in the detection of low levels of allergen caused by a shallow standard curve at low concentrations.

The assays were, as expected, immunologically specific, detecting RUA in rat rooms and MUA in mouse rooms. All three systems also detected RUA in mouse rooms and vice versa, although at much lower levels than in the homologous rooms (Table 3). The NHLI assay had a greater number of nonhomologous positives. The most likely explanations are residual allergen from prior occupation of the room by the other species, and contamination through doors, on staff clothing, or via ventilation. Contamination of sampling heads or in the analyzing laboratories is less likely, since that should have equally affected the results for the blank filters. Cross-reaction between species in all assays was minimal (23).

In the absence of a "gold standard", centers will continue to use the assay which appears best for their purposes. There are reasons not addressed in this study for choosing one assay rather than another. Monoclonal technology, as used by NIWL for the RUA assay, offers good reproducibility, so that comparisons can be made at very different time periods. Techniques using human IgE pools, as used by NHLI in the project contributing to this study, can be expected to measure allergens of clinical relevance, while assays using other antibody sources always require further validation. These issues aside, this comparative study has shown that identical air samples would give different results depending on the assay technology employed.

These differences are by orders of magnitude and would clearly be important if quantitative comparisons between workplaces or over time were to be made without taking account of sample processing, storage, assay, and arithmetic assumptions. The three methods, however, gave rankings

similar to the air samples (Figs. 2 and 3), so that internal comparisons within studies are valid whatever assay is used and semiquantitative approaches – for example, using percentiles of the exposure distribution – may also be valid. In principle, the use of conversion factors, obtainable, for example, from the results of this study, could make data

from allergen measurements comparable between studies. For future work, however, standardization of assays is desirable.

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References

- Hunnskaar S, Fosse RT. Allergy to laboratory mice and rats: a review of the pathophysiology, epidemiology and clinical aspects. *Lab Anim* 1990;**24**:358–374.
- Gordon S, Newman Taylor AJ. Animal, insect and shellfish allergy. In: Bernstein IL, Chan-Yeung M, Malo J-L, Bernstein DI, editors. *Asthma in the workplace*. 2nd ed. New York: Marcel Dekker, 1999;(in press).
- Kibby T, Powell G, Cromer J. Allergy to laboratory animals: a prospective and cross-sectional study. *J Occup Med* 1989;**31**:842–846.
- Cullinan P, Lowson D, Nieuwenhuijsen MJ, et al. Work related symptoms, sensitisation, and estimated exposure in workers not previously exposed to laboratory rats. *Occup Environ Med* 1994;**51**:589–592.
- 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**:562–567.
- Davies GE, Thompson AV, Rackham M. Estimation of airborne rat-derived antigens by ELISA. *J Immunoassay* 1983;**4**:113–126.
- Corn M, Koegel A, Hall T, Scott A, Newill C, Evans R. Characteristics of airborne particles associated with animal allergy in laboratory workers. *Ann Occup Hyg* 1988;**32 Suppl 1**:435–446.
- Eggleston PA, Newill CA, Ansari AA, et al. Task-related variation in airborne concentrations of laboratory animal allergens: studies with Rat n 1. *J Allergy Clin Immunol* 1989;**84**:347–352.
- Gordon S, Tee RD, Lowson D, Wallace J, Newman Taylor AJ. Reduction of airborne allergenic urinary proteins from laboratory rats. *Br J Ind Med* 1992;**49**:416–422.
- Nieuwenhuijsen MJ, Gordon S, Harris J, Tee RD, Venables KM, Newman Taylor AJ. Determinants of airborne allergen exposure in an animal house. *Occup Hyg* 1995;**1**:317–324.
- 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**:819–825.
- 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**:744–751.
- 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**:649–655.
- Platts-Mills TAE, Heyman PW, Longbottom JL, Wilkins SR. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J Allergy Clin Immunol* 1986;**77**:850–857.
- Ohman JL, Hagberg K, MacDonald MR, Jones RR, Paigen BJ, Kacergis JB. Distribution of airborne mouse allergen in a major mouse breeding facility. *J Allergy Clin Immunol* 1994;**94**:810–817.
- 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**:1314–1321.
- 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**:1070–1077.
- Hollander A, van Run P, Spithoven J, Doekes G, Heederik D. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. *Clin Exp Allergy* 1997;**27**:616–626.
- Gordon S, Kiernan LA, Nieuwenhuijsen MJ, Cook AD, Tee RD, Newman Taylor AJ. Measurement of exposure to mouse urinary proteins in an epidemiological study. *Occup Environ Med* 1997;**54**:135–140.
- Renström A, Karlsson AS, Larsson PH, Malmberg P. High prevalence of symptoms and specific IgE to laboratory animal allergens in spite of low exposure among research personnel. *Eur Respir J* 1996;**9 Suppl 23**:120s.
- 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**:593–597.
- Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;**1**:307–310.
- Renström A, Gordon S, Hollander A, et al. Comparison of methods to assess airborne rat and mouse allergen levels. II. Factors influencing antigen detection. *Allergy* 1999;**54**:150–157.
- Vaughan NP, Chalmers CP, Botham RA. Field comparison of personal samplers for inhalable dust. *Ann Occup Hyg* 1990;**34**:553–573.
- Kenny LC, Aitken R, Chalmers C, et al. A collaborative European study of personal inhalable aerosol samples performance. *Ann Occup Hyg* 1999;**41**:135–153.
- Gordon S, Tee RD, Lowson D, Newman Taylor AJ. Comparison and optimization of filter elution methods for the measurement of airborne allergen. *Ann Occup Hyg* 1992;**36**:575–587.
- Jensen J, Poulsen LK, Mygind K, Weeke ER, Weeke B. Immunochemical estimations of allergenic activities from outdoor aeroallergens collected by a high volume air sampler. *Allergy* 1989;**44**:52–59.
- Douwes JE, Versloot P, Hollander A, Heederik DJ, Doekes G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995;**61**:1763–1769.
- Zock J, Hollander A, Doekes G, Heederik D. The influence of different filter elution methods on the measurement of airborne potato antigens. *Am Ind Hyg Assoc J* 1996;**57**:567–570.
- Vittinghus E. Preanalytical handling of stored urine samples, and measurement of beta 2-microglobulin, orosomucoid, albumin, transferrin and immunoglobulin G in urine by enzyme-linked immunosorbent assay (ELISA). *Scand J Clin Lab Invest* 1990;**50**:843–849.
- Gordon S, Tee RD, Newman Taylor AJ. Analysis of the allergenic composition of rat dust. *Clin Exp Allergy* 1996;**26**:533–541.