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VARIATION IN RAT URINARY AEROALLERGEN LEVELS EXPLAINED BY DIFFERENCES IN SITE, TASK AND EXPOSURE GROUP

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Abstract—We describe how much of the variation in rat urinary aeroallergen (RUA) levels may be explained by differences in site and exposure group in research establishments and we identify task categories associated with high RUA levels. In this population 73% of the variability in RUA levels could be explained by exposure group, site and their interaction term. Exposure group alone explained the majority of the variation (69%). The task category associated with the highest RUA level was cleaning out, followed by indirect contact with rats and handling rats. The task categories associated with the lowest levels were slide production, post mortem and experiments. These results may help choose appropriate exposure measures for epidemiological studies in research establishments in the future and may help reduce the incidence of laboratory animal allergy.

INTRODUCTION

Studies examining the influence of site differences, job categories and exposure groups on exposure levels in the workplace are rare although they may reveal useful information regarding determinants of exposure patterns. This could, in turn, provide a rational basis for the choice of exposure measures in epidemiological studies, and for the reduction of exposure. In this paper we describe how much of the variation in rat urinary aeroallergen (RUA) levels can be explained by differences in site, task category and exposure group in research establishments, and we identify task categories that were associated with high RUA levels.

Laboratory animal allergy is frequently reported in workers exposed to rats and cross-sectional epidemiological studies have shown that up to one-third of those exposed report allergic symptoms (Cockcroft *et al.*, 1981; Slovak and Hill, 1981; Venables *et al.*, 1988). The animals' urine has been identified as major source of allergen (Newman Taylor *et al.*, 1977).

Previously we have reported two RUA studies: the first described RUA exposure levels in two research establishments (Nieuwenhuijsen, 1993; Nieuwenhuijsen *et al.*, 1994a), and the second the influence of rooms, day, job category, ventilation and stock density on RUA levels in one animal house (Nieuwenhuijsen, 1993; Nieuwenhuijsen *et al.*, 1995). In the first study, animal technicians were the exposure group with the highest average RUA levels. The mean RUA levels were low for tasks involving a small number and/or unconscious rats and high where there was a large number of conscious rats involved. In the second study, in an analysis of variance task category was the

second most important determinant of RUA exposure. Blanchet (1988) and Swanson *et al.* (1990) also reported that the highest levels of RUA were found amongst animal technicians. Other studies (Blanchet, 1988; Eggleston *et al.*, 1989; Gordon *et al.*, 1992) reported that the tasks associated with the highest RUA exposure were cleaning, feeding, handling and injection of animals.

Analysis of variance or regression techniques have rarely been used to study the influence of site, exposure group or task on exposure in animal houses. They have been used in Vermont granite sheds (Eisen *et al.*, 1984), in the animal food industry (Heederik *et al.*, 1991), in bakeries and flour mills (Nieuwenhuijsen, 1993; Nieuwenhuijsen *et al.*, 1994b) and the rubber industry (Kromhout *et al.*, 1994) to study the influence of site and exposure group on exposure levels, and in the rubber industry (Kromhout *et al.*, 1994) and printing industry (Hansen and Whitehead, 1988) to investigate the influence of tasks.

MATERIALS AND METHODS

Sites, exposure groups and task categories

Site, exposure group, descriptions of task category and mean RUA levels of the exposure groups have been reported previously (Nieuwenhuijsen, 1993; Nieuwenhuijsen *et al.*, 1994a). Briefly, at two research sites, departments where there was work with rats were selected. An occupational hygienist (M. J. Nieuwenhuijsen) visited each site and divided the employees into nine exposure groups as follows: scientists and laboratory technicians; cage cleaners; supervisors; animal technicians; workers in the office; in slide production; in maintenance; in post mortem; and a miscellaneous group. Employees in each exposure group were asked to wear a personal sampler for a whole work shift and to keep a work record detailing times spent performing tasks which were grouped into nine categories. These categories were: corridor work; non/minimal contact; slide production; experiments on rats in laboratories; post mortem of rats in laboratories; cage wash; indirect contact with rats in an animal room (entering an animal room without handling the animals); cleaning out cages; and handling of rats in an animal room.

Air sampling, elution, RUA assay

Personal samples were collected during a whole work shift using samplers (Casella AFC123, Casella London Ltd, Bedford, U.K.) connected to seven-hole sampling heads (Casella London Ltd, Bedford, U.K.) each containing pre-weighed polytetrafluoroethylene (PTFE) filters (1.2 μm pore size, 25 mm dia.; Sartorius Instruments Ltd, GB-Belmont, Surrey, U.K.). The flow rate was 2 l. min⁻¹. After sampling the filters were eluted, the eluates freeze dried and reconstituted before assaying. The RUA content was measured by RAST inhibition, using pooled sera from eight patients allergic to rat urine (Gordon, 1994). Half the value of the detection limit (50 ng ml⁻¹) was assigned to samples where RUA was not detectable. (For further details, see Gordon *et al.*, 1992; Nieuwenhuijsen, 1993; Gordon, 1994.)

Statistical analysis

The statistical software package SAS (SAS Institute Inc., Cary, North Carolina, U.S.A.) was used for the following statistical analyses: descriptive statistics; normality

testing; and analysis of variance. A generalized linear model using the gamma distribution (GLIM version 3.77, Royal Statistical Society, London, 1985) was used to identify tasks affecting exposure levels and estimate exposure levels. The gamma distribution is the sum of a number of exponential distributions and it allows for increasing variances. For the analyses shown, it was necessary to split the data into three separate groups to fit the models. The amount of allergen on the filter was used as a dependent variable and the amount of air pulled through the filter as an independent variable. In this analysis, the amount of allergen on the filter is assumed to be proportional to the length of time spent in the task. The regression coefficients estimate the RUA concentration for each task.

RESULTS

For all the following analyses only measurements where contact with rats, rat tissue and rat faeces or urine was reported were used. This resulted in a total of 205 measurements available for analyses; 106 measurements for animal technicians, 31 for scientists and laboratory technicians, 16 for cage cleaners, two for supervisors, 20 for post mortem staff, 29 for slide production workers and one for maintenance workers. The measurements followed an approximately log-normal distribution and log transformed values were therefore used for some of the analyses.

In univariate analyses of variance with the log transformed RUA concentrations as a dependent variable, significant contributions to the model were observed for both site ($F=6.5$, $P=0.01$, $R^2=0.03$) and exposure group ($F=72.2$, $P=0.0001$, $R^2=0.69$). A multivariate analysis of variance model with log transformed RUA concentrations as a dependent variable and exposure group, site and their interaction as independent variables is shown in Table 1. These three independent variables together explained 73% ($R^2=0.73$) of the variation in RUA. Estimates of the RUA concentrations, obtained from the model, for site and exposure group are shown in Table 2. Only three exposure groups are present at both sites. In particular for cage cleaners there are large differences in RUA levels between the sites.

Table 3 shows the frequency and duration for the different job categories. A multivariate regression model with log transformed RUA concentrations as a dependent variable and dummy variables for each job category and site as an independent variable explained 63% of the variation in RUA concentration. However,

Table 1. Analysis of variance model of log transformed RUA concentrations ($N=205$) ($R^2=0.73$)

Source	df	MS	F	P
Site	1	66.1	26.4	0.0001
Exposure group	6	138.6	55.5	0.0001
Site $\times$ exposure group	2	31.4	12.6	0.0001
Error	195	2.5		

R^2 = explained variance.

df = degrees of freedom.

MS = mean square.

F = F-test.

P = significance level for F-test.

Table 2. Estimates of RUA concentrations, expressed as geometric means ($\mu\text{g m}^{-3}$), by exposure group and site

Exposure group	Site A	Site B
Animal technicians	47.9	38.5
Scientists	0.3	6.7
Cage cleaners	1.2	26.1
Supervisors	0.6	—
Post mortem	1.8	—
Slide production	0.0	—
Maintenance	—	0.3

— = not present.

Table 3. Characteristics of job categories ($N = 205$ measurements)

Job category	Frequency of occurrence	Duration (min)			
		AM	SD	Min	Max
Slide production	29	323	39	250	390
Post mortem	28	199	106	10	375
Experiments	7	230	123	85	380
Handling of rats	104	84	64	5	357
Indirect contact	94	63	53	4	275
Cleaning out	74	42	33	2	184
Cage wash	16	188	120	10	345

 N = total number of whole shift measurements.

AM = arithmetic mean.

SD = standard deviation.

Min = minimum amount of time recorded.

Max = maximum amount of time recorded.

several dummy variables were associated with each other and the residuals showed an increase with an increase in RUA concentration.

A new model had to be considered that would take into account the amount of time spent in the various tasks. This model, a generalized linear model using the gamma distribution, required the data set to be split into three groups. RUA estimates for the various tasks in each group are given in Table 4. Estimates from the model shows that job categories where there is contact with frozen tissue (slide production) or a small number of and/or unconscious animals (post mortem and experiments) were associated with low RUA levels. Task categories with contact with a large number of conscious rats (handling rats and indirect contact) were associated with much higher RUA levels. Cage cleaning was associated with the highest level of RUA.

Models with site, exposure group and task grouped together were attempted but the statistical package would not fit the models. A few predicted values became negative, which does not correspond with the assumptions of a gamma model.

DISCUSSION

This paper describes how much of the variation in rat urinary aeroallergen (RUA) levels can be explained by differences in sites and exposure groups in research

Table 4. Estimates of the RUA concentrations ($\mu\text{g m}^{-3}$) by job using three separate generalized linear models

Variable	Parameter estimate	SE
Model 1 (N = 29)		
Intercept	0.0	0.0
Slide production	0.1	0.0
Model 2 (N = 29)		
Intercept	0.3	0.3
Post mortem	3.1	1.3
Experiments	0.1	0.1
Model 3 (N = 141)		
Intercept	-0.3	0.7
Handling rats	105.1	19.7
Indirect contact	133.4	42.3
Cleaning out	496.4	120.3
Cage wash	26.4	9.0

SE = Standard error.

N = number of samples.

establishments. It also identified task categories associated with high RUA levels and estimated these levels.

Differences between exposure groups explained a much larger percentage of the variability in RUA levels than differences between sites. This is similar to findings in bakeries and flour mills (Nieuwenhuijsen, 1993; Nieuwenhuijsen *et al.*, 1994b) and in the animal food industry (Heederik *et al.*, 1991) where the influence of site was also small compared with exposure group or job category. Therefore site differences could be ignored and attention focused on exposure group differences. This is different from results of a study by Kromhout *et al.* (1994) in the rubber industry where the interaction between site and production function appeared to be the most important variable in a model ($R^2 = 0.39$) with site, function and their interaction. In the present study, differences in exposure group explained 69% of the variability in RUA. This is high compared with previous reports of bakeries and flour mills where exposure groups explained approximately 45% of the variability in dust levels and a much lower percentage of flour aeroallergen levels (Nieuwenhuijsen *et al.*, 1994b), of postural load on the back where exposure groups explained between 47 and 72% (Burdorf, 1992), and of Vermont granite sheds where jobs explained 12% of the variability in respirable granite dust (Eisen *et al.*, 1984). In the latter study site differences explained 23% of the variability in dust concentrations.

A secondary aim of the study was to identify task categories with high RUA levels and estimate these levels. We found, in an analyses of variance model, that tasks explained 63% of the variation in RUA, but several (dummy) variables were associated with each other and the residuals showed an increase with an increase in RUA concentration. Tasks can explain a large percentage of exposure variability as was shown in a printing plant where the number of times the worker performed a certain 'hazardous task' explained 57% of the variability in solvent exposure in a regression analysis (Hansen and Whitehead, 1988). In a previous report (Nieuwenhuijsen, 1993; Nieuwenhuijsen *et al.*, 1995) in one animal house, task categories explained only 12% of the variability in RUA levels, but only one exposure group and three task categories

were examined. Jobs and ventilation explained approximately 40% of the variability in dust particulates in a study by Kromhout *et al.* (1994) in the rubber industry.

The present study has shown that task categories where there was contact with frozen tissue (slide production) and/or a small number of unconscious animals (post mortem and experiments) RUA levels were low. Task categories with contact with a large number of rats (handling rats and indirect contact) had much higher RUA levels. Cage cleaning was associated with the highest level of RUA. This is similar to other studies (Blanchet, 1988; Eggleston *et al.*, 1989; Gordon *et al.*, 1992; Nieuwenhuijsen, 1993; Nieuwenhuijsen *et al.*, 1994a, 1995) which reported that tasks associated with the highest RUA exposure were cleaning, feeding, handling and injection of animals, while tasks with the lowest RUA exposure were sacrifice, surgery or slide production.

Unfortunately it was not possible to examine exposure group, site and task category in the same model since the statistical package used was not able to fit the model. A model with all the variables would have given a more informative answer to the question, what is the most important determinant of exposure? Modelling is difficult because of issues such as increase in variance with an increase in a continuous dependent variable, the expected or most likely relationship between dependent and independent variables, and association between independent variables.

The study has shown that a large proportion of variability in RUA levels can be explained by simple grouping of workers. Task categories with high RUA levels could be identified by whole shift sampling and filling in a work record sheet. Either groups of workers or task categories with high RUA levels can subsequently be targeted for reduction of exposure and related disease.

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