Exposure-response relationships for work-related sensitization in workers exposed to rat urinary allergens: Results from a pooled study

Dick Heederik, PhD,[a] Katherine M. Venables, MD, FRCP,[b] Per Malmberg, MD, PhD,[c] Albert Hollander, PhD,[a] Anne-Sophie Karlsson, MSc,[c] Anne Renström, PhD,[c] Gert Doekes, PhD,[a] Mark Nieuwenhijsen, PhD,[b] and Susan Gordon, PhD,[b] *Wageningen, The Netherlands, London, United Kingdom, and Solna, Sweden*

Background: Recent studies in a few industries have shown that the likelihood of IgE-mediated sensitization increases with increasing exposure. The shape of the exposure-response relationships and modification by age, sex, and smoking habit has hardly been studied.
Objective: The purpose of this study was to determine exposure sensitization relationships for rat sensitization and to evaluate the influence of atopy, smoking habits, and sex.
Methods: Data from 3 cross-sectional studies in The Netherlands, the United Kingdom, and Sweden were used and involved 1062 animal laboratory workers. Selection criteria were harmonized, and this resulted in a study population of 650 animal laboratory workers (60.6% female) with less than 4 years of exposure. Air allergen levels were assessed previously and converted on the basis of an interlaboratory allergen analysis comparison. Available sera were analyzed for the presence of specific antibodies against common allergens (house dust mite, cat, dog, and grass and birch pollen) and work-related allergens (rat and mouse urinary proteins). Questionnaire items on work-related respiratory symptoms, hours worked with rats per week, job performed, smoking habits, and sex were used in this analysis
Results: The prevalence of work-related sensitization to rat urinary allergens (IgE >0.7 KU/L) was 9.7 % (n = 63). Thirty-six of the sensitized workers had work-related symptoms (asthma or rhinitis). Two hundred forty-eight workers (38.2%) were atopic (defined as specific IgE to 1 of the common allergens). The sensitization rate increased with increasing air allergen exposure. Atopic workers exposed to low levels of allergen had a more than 3-fold increased sensitization risk compared with nonexposed atopic workers. For atopic subjects, the risk increased little with increasing exposure, whereas for nonatopic subjects, a steadily increasing risk was observed. Smoking and sex did not modify the sensitization risk.
Conclusion: Rat urinary allergen–sensitization risk increased with increasing exposure intensity. Workers who were atopic had a clearly elevated sensitization risk at low allergen exposure levels. (J Allergy Clin Immunol 1999;103:678-84.)

Key words: *Laboratory animal allergy, sensitization, allergen exposure, rat urinary allergens*

Workers in industry can be exposed to allergens that are not present in the general environment. Exposure to occupational allergens can therefore serve as a convenient model to study the effects of environmental allergens. Laboratory animal workers can become sensitized as a result of exposure to rat urinary proteins (RUPs).[1] Work-related sensitization is often followed by the development of symptoms and bronchial hyperresponsiveness.[2] Recent studies in a few industries have shown that the likelihood of IgE-mediated sensitization increases with increasing exposure intensity.[3,4] In a retrospective cohort study it was shown that the risk of the development of work-related symptoms increased with the number of hours per week that a worker was exposed to rats.[5] Application of immunoassays in epidemiologic studies that allow measurement of allergen levels as low as the nanogram per cubic meter range, by use of the specificity of the immunoglobulin response, allows exploration of exposure-sensitization relationships.[6] The risk for the development of sensitization is higher for workers who are atopic compared with workers who are not atopic.[3,4] The risk of sensitization might be modified by smoking[7] and sex,[8] but this has not been evaluated in a satisfactory way. Previous studies have not had sufficient power to detect elevated risks for specific subcategories, such as smokers and male versus female subjects. The shape of the exposure-response relationship has therefore hardly been studied, and studies published so far were too small to be able to describe potential effect modification by sex and smoking quantitatively.

For the present study, we used data from 3 independent studies undertaken in Sweden, the Netherlands, and the United Kingdom. Some of the methods applied in these studies (questionnaires) were comparable. The

From [a]the Department of Environmental Sciences, Environmental and Occupational Health Group, Wageningen University and Research Center; [b]the Department of Occupational and Environmental Medicine, National Heart and Lung Institute, Imperial College School of Medicine, London; and [c]the Department of Occupational Medicine, National Institute for Working Life, Solna.

Funded in part by the European Community (contract #BMH1-CT94-1446; data pooling and serology) and by the Netherlands Organization for Research (statistical analyses).
Received for publication June 16, 1998; revised Oct 19, 1998; accepted for publication Dec 8, 1998.

Reprint requests: Dick Heederik, PhD, Department of Environmental Sciences, Environmental and Occupational Health Group, Wageningen University and Research Center, PO Box 238, 6700 AE Wageningen, The Netherlands.

0091-6749/99 $8.00 + 0 **1/1/96452**

Abbreviations used
MUA: Mouse urinary allergen
MUP: Mouse urinary protein
RUA: Rat urinary allergen
RUP: Rat urinary protein

design of the cross-sectional studies could be further harmonized by applying the same enrollment criteria a posteriori. By a reanalysis of sera, comparable information on IgE against common and occupational allergens was obtained for all 3 studies. The assays used to assess the exposure to allergens in the 3 studies have been compared earlier in an interlaboratory comparison.[8,9] This comparison facilitates a conversion of exposure levels measured by one of the assays into exposure levels measured by the other assays. By the use of this information, a job exposure matrix could be developed that estimated the exposure for all workers from the 3 countries. This allowed pooling of the data into 1 large data set and a combined statistical analysis.

METHODS

Population

The study population came from laboratory animal facilities in Sweden, the Netherlands, and the United Kingdom. Swedish laboratory animal workers (n = 38) came from an earlier study among students from laboratory technician training schools with exposure for more than 5 months at follow-up,[2] and another 90 Swedish subjects came from a cross-sectional study in university facilities.[10] Dutch laboratory animal workers came from a cross-sectional study among laboratory animal workers in 4 universities, 3 commercial or industrial laboratories, and students from a laboratory school.[4] Laboratory animal workers in the United Kingdom came from 3 institutions (2 commercial and 1 academic) specializing in small animal research.[3]

The participation rates in all 3 countries ranged from 77% (the Netherlands) to 88% (United Kingdom). Because criteria for enrolment in the study differed between countries, the most stringent criteria from each country were applied to all 3 populations. Only laboratory animal workers with a working history of less than 4 years were included because this criterion was used in the UK study. This resulted in a total of 74 Swedish, 219 Dutch, and 357 UK laboratory animal workers. The number of observations can differ from earlier published analyses because serum and not the information on skin prick test reactivity was used in this study.

Questionnaire

Questionnaires were completed in all 3 countries. All subjects answered questions on personal and family history of allergic symptoms and smoking habits. Questions were also asked about upper and lower respiratory symptoms suggestive of allergy (chest tightness [asthma], runny or sneezing nose, and animal species causing the symptoms) and about temporal relationships with work. Other questions covered exposure to laboratory animals, tasks, duration of exposure (hours/week), type of animal causing symptoms, and frequency of exposure.

Serologic information

IgE against 5 common allergens (house dust mite [*Dermatophagoides pteronyssinus*], grass pollen [1:1 mixture of *Lolium perenne* and *Phleum pratense*], birch pollen [*Betula verrucosa*], cat fur, and dog fur) were measured with an assay developed at the Department of Environmental Sciences at the University of Wageningen, The Netherlands.[10] Microwells were coated overnight at 4°C with commercially available lyophilized extracts (ALK Benelux, Houten, The Netherlands). Coating concentrations were 25 μg/mL of protein. Diluted sera (1:10) in PBS-Tween containing 0.2% gelatin were incubated for 2 hours at 37°C, and bound IgE was measured by subsequent incubations with monoclonal mouse anti-human IgE, biotinylated affinity-purified rabbit anti-mouse Ig, avidin-peroxidase, and *o*-phenylenediamine. An OD_{492} exceeding the OD + 0.05 of the reagent blank (no serum control) was interpreted as a positive reaction. Total IgE was measured by a sandwich enzyme immunoassay.[11] Briefly, mouse monoclonal anti-IgE was coated in microwells. Sera were added in 4 dilutions and incubated for 2 hours at 37°C. Bound IgE was measured after incubation with peroxidase-labeled mouse monoclonal anti-IgE for 1 hour at 37°C, followed by a 30-minute incubation with *o*-phenylenediamine at 20°C in the dark. The reaction was terminated by the addition of HCl, and the OD was read at 492 nm. Each microtiter plate included a serially diluted reference sample (10-9123-01; Kabi-Pharmacia, Woerden, The Netherlands).

IgE against animal urinary allergens was measured with an assay developed at the National Institute for Working Life (Stockholm, Sweden). Briefly, RUP and mouse urinary protein (MUP; 10 μg/mL in PBS) were coated to Maxisorp Microtitre plates (Nunc, Roskilde, Denmark). Standards and sera in PBS dilution buffer (0.1% Tween, 1% BSA, and 0.15% Kathon), supplemented with 10% normal human serum, were added in duplicates and incubated overnight. Biotinylated rabbit anti-human IgE was diluted 250-fold and 125-fold for RUP-IgE and MUP-IgE, respectively, in PBS dilution buffer and incubated for 1 hour, followed by steptavidin coupled to alkaline phosphatase diluted 250-fold in PBS dilution buffer for 1 hour. Binding was visualized with *p*-nitrophenyl phosphate in substrate buffer (1 mol/L diethanolamine, pH 9.8) after 30 and 90 minutes for RUP-IgE and MUP-IgE, respectively. For RUP-IgE, standards of 3 sera were used with known IgE concentrations (9, 40, and 255 KU/L) when analyzed with a CAP-RAST (Pharmacia), giving a final concentration of 21.4 KU/L. For MUP-IgE a mouse positive serum was used with a concentration of 4 KU/L) when analyzed with a CAP-RAST. The standard curves had a range from 0.01 to 1 KU/L. The detection limit was 0.1 KU/L, with a minimum serum dilution of 1:10.

Elevated total serum IgE was defined as a level above 100 KU/L. Atopy was regarded as a positive IgE (OD_{492} exceeding the OD + 0.05 of the reagent blank [no serum control]) to at least 1 of the common allergens. Sera were considered positive against rat urinary allergen (RUA) or mouse urinary allergen (MUA) if the specific IgE level was above 0.7 KU/L. Rat or mouse allergy was defined as presence of work-related symptoms caused by rats or mice and a positive specific IgE to rats or mice, respectively.

Allergen exposure

In all 3 countries elaborate exposure assessment studies had been undertaken.[6,12,13] The exposure studies comprised more than 500 full shift RUA in air measurements (Sweden, 18 personal samples, 46 static samples; the Netherlands, 251 personal samples; the UK, 271 personal samples). Average RUA levels were calculated by job title and work area (or facility).

Three different assays had been used in the 3 original studies to measure RUAs in the air.[8,9,14] The Swedish and Dutch groups had each used an EIA sandwich assay with mAbs against Rat n I and rabbit polyclonal antibodies against RUA, respectively. The British group had developed a RAST inhibition assay using IgE antibodies from 8 workers who were allergic to rat allergen. Details on the 3

TABLE I. Basic characteristics of 650 laboratory animal workers by country

	Sweden (n = 74)	The Netherlands (n = 219)	United Kingdom (n = 357)
Age (y)	32.0 (11.6)	28.5 (6.8)	27.5 (9.7)
Smokers (%)	16 (21.6)	55 (25.1)	91 (25.5)
Exsmokers (%)	13 (17.6)	36 (16.4)	44 (12.3)
General respiratory symptoms			
Asthma (%)	7 (9.5)	21 (9.6)	32 (9.0)
Rhinitis (%)	15 (20.3)	58 (26.5)	81 (22.7)
Cough (%)	3 (4.1)	13 (5.9)	25 (7.0)
Phlegm (%)	3 (4.1)	13 (5.9)	20 (5.6)
Female workers (%)	51 (68.9)	122 (55.7)	221 (61.9)
Atopic workers (%)	18 (24.3)	82 (37.4)	148 (41.5)
Pos IgE birch (%)	7 (9.5)	28 (12.7)	28 (7.8)
Pos IgE cat (%)	9 (12.2)	32 (14.7)	45 (22.6)
Pos IgE dog (%)	2 (2.7)	9 (4.1)	13 (3.6)
Pos IgE grass (%)	7 (9.5)	42 (19.4)	99 (27.7)
Pos IgE house dust mite (%)	5 (6.8)	50 (23)	91 (25.5)
Total IgE >100 kU/L (%)	16 (21.6)	49 (22.4)	124 (34.7)
Sensitized to RUA (%)	5 (6.8)	28 (12.8)	30 (8.4)
Work-related asthma symptoms	2 (2.7)	11 (5.0)	9 (2.5)
Work-related rhinitis symptoms	9 (12.2)	33 (15.1)	35 (9.8)
Rat allergy* (%)	4 (5.3)	18 (8.2)	14 (3.9)
RUA level† (ng EQ/m^3)	0.09 (0.13)	0.45 (0.6)	1.9 (3.8)
Hours/week worked with rats†	5.6 (9.8)	5.3 (8.8)	9.1 (14.2)
Time · average exposure† (ng RUA·hr/m^3·week)	0.85 (1.8)	4.3 (11.0)	60.9 (137)

Standard deviations are given in parentheses.
*Defined as a positive IgE against RUA and work-related asthma or rhinitis symptoms
†Arithmetic mean.

assays can be found in the literature.[8] The 3 assays have been compared previously by taking parallel air samples in 3 laboratories in each country.[8] Results from this study showed that the correlation between results obtained with each assay were sufficiently high to derive conversion factors. Results in this study have all been converted to concentrations obtained with the Dutch polyclonal sandwich assay. This arbitrary choice was driven by the fact that this assay has been used for the exposure assessment in a study that described an exposure-response relationship for rat sensitization and rat allergy (defined as sensitization plus symptoms).[3,6] The conversion factors were based on the ratio of the median exposure in the 74 parallel samples measured with each assay. For conversion from Swedish to Dutch allergen levels, Swedish results were multiplied by a conversion factor 0.45. For conversion of the British results, a multiplier of $3.3.10^{-4}$ was used. The air allergen concentration was expressed in nanogram equivalent per cubic meter.

Statistical analyses

All statistical analyses were performed with SAS software (version 6.12; SAS Institute, Inc, Cary, NC). Simple analysis of variance or χ^2 statistics were used to test differences between subpopulations in continuously respectively dichotomous outcomes measured. Recent insights have shown that odds ratios, as a measure of association between the exposure and response, can be heavily biased when the prevalence of the response is relatively high. We therefore analyzed the relationship between the prevalence rate of RUA sensitization and level of exposure, atopy, and sex of the subject by using a proportional hazard model (Cox's regression model) with the SAS procedure PHREG to obtain prevalence ratios as an estimate of the relative risk.[15] The model assumes Poisson variability in the response variable and by defining the model for a closed cohort and a constant risk period direct estimates of the prevalence rate can be obtained. The prevalence rate can be calculated from the regression coefficient as e^{β}. It is known that the standard error of the regression coefficient is an overestimation of the true standard error.[15] As a result, confidence intervals are wider, and statistical testing is known to be more conservative. Statistical significance was reached at the 5% level (2-sided).

RESULTS

Basic characteristics of the 3 populations of laboratory animal workers are given in Table I. Although numbers of smokers and exsmokers and the male/female ratio are roughly similar in the 3 countries, distinct differences exist in the number of workers who were atopic, individuals with high total IgE, and the exposure to RUA. A lower atopy rate was found in Swedish individuals compared with the other nationalities (χ^2, $P < .05$). This difference could mainly be attributed to differences in prevalence of positive reactions to house dust mite, although the number of cat-, dog-, and grass-positive results was lower in at least 1 of the other countries as well (χ^2, $P < .05$). The UK laboratory animal workers worked longer hours with laboratory animals, which partly explains the higher cumulative exposure than the Dutch and Swedish laboratory animal workers (*t* test, $P < .05$). The differences in exposure also reflect differences in work practices in the 3 national samples. Almost all Dutch and Swedish laboratory animal workers came from university laboratories, and these workers tended to have intermittent exposure patterns in contrast to the UK workers, most of whom came from 1 large commercial laboratory. All 3 samples had similar distributions of

TABLE II. Basic characteristics of 650 laboratory animal workers by atopy and sex

	Nonatopic workers		Atopic workers	
	Male (n = 145)	**Female (n = 256)**	**Male (n = 110)**	**Female (n = 138)**
Age (y)	29.8 (9.9)	28.4 (9.6)	27.3 (8.1)	27.4 (8.2)
Smokers (%)	37 (23.2)	69 (27.0)	27 (24.5)	29 (21.0)
Exsmokers (%)	15 (10.3)	40 (15.6)	20 (18.2)	18 (13.0)
Asthma (%)	4 (2.7)	7 (2.7)	26 (23.6)	23 (16.9)
Rhinitis (%)	9 (6.2)	20 (7.8)	54 (49.1)	71 (51.4)
Cough (%)	7 (4.8)	20 (7.8)	3 (2.7)	12 (8.7)
Phlegm (%)	9 (6.2)	12 (4.7)	7 (6.4)	9 (6.5)
Pos IgE birch (%)	—	—	34 (30.9)	29 (21.0)
Pos IgE cat (%)	—	—	40 (36.4)	46 (33.3)
Pos IgE dog (%)	—	—	12 (10.9)	12 (8.7)
Pos IgE grass (%)	—	—	73 (66.4)	75 (54.3)
Pos IgE house dust mite (%)	—	—	72 (65.5)	74 (53.6)
Total IgE >100 kU/L (%)	20 (13.7)	35 (13.7)	67 (60.9)	67 (48.6)
Sensitized to RUA (%)	9 (6.2)	9 (3.5)	20 (18.2)	25 (18.1)
Work-related asthma symptoms (%)	1 (0.7)	4 (1.6)	8 (7.3)	9 (5.6)
Work-related rhinitis symptoms (%)	9 (6.2)	23 (9.1)	21 (19.1)	24 (17.5)
Rat allergy* (%)	4 (2.7)	2 (0.8)	17 (15.5)	13 (9.4)
RUA level† (ng EQ/m^3)	1.5 (3.3)	1.2 (3.0)	1.3 (3.1)	0.9 (2.5)
Hours/week worked with rats†	8.5 (13.5)	7.5 (12.6)	8.2 (11.7)	5.4 (10.2)
Time · average exposure† (ng EQ.hr/m^3.week)	45.2 (118)	35.8 (108)	36 (108)	22 (84.0)

Standard deviations are given in parentheses.
*Defined as a positive IgE against RUA and work-related asthma or rhinitis symptoms.
†Arithmetic mean.

duration of exposure to laboratory animals as a result of the selection procedure (inclusion of workers with 4 years of exposure only). The overall prevalence of RUA sensitization was 9.7%. For MUA this figure was lower (3.5%). However, the prevalence for MUA sensitization is an underestimation because few workers had regular contact with conscious mice (<58% of the population). Twenty of the 23 MUA-sensitized workers were RUA sensitized as well. Because the prevalence rate of MUA sensitization was lower and fewer workers had regular contact with mice, elaborate exposure-response modeling was not applied to the MUA data, which would have resulted in unstable predicted exposure-response models.

Table II gives some basic characteristics broken down by atopy and sex. Male workers who were atopic had a higher prevalence of specific antibodies against birch and grass pollen (χ^2, $P < .05$). Fewer female atopic RUA-sensitized individuals were symptomatic so that the prevalence of rat allergy was similar in male and female workers who were atopic. Interestingly, female workers who were atopic had been exposed to slightly lower RUA exposure levels than male workers who were atopic and workers who were not atopic, and they had worked fewer hours with rats than the others. As a result, their time multiplied average exposure was considerably lower than others (*t* test, $P < .05$).

There was a clear difference in the age of the laboratory animal workers between the 3 national samples. Atopic laboratory animal workers tended to be somewhat younger than nonatopic workers (*t* test, $P < .05$).

Table III gives a breakdown of the number of RUA-sensitized workers by exposure for the 3 exposure proxies available (level, time, and level · time). The prevalence of RUA sensitization increases with increasing exposure for all 3 exposure proxies as is most clearly illustrated by the crude (uncorrected) prevalence ratios. The RUA-sensitized workers in the lowest exposure category (10 individuals) had been exposed while working with unconscious rats, rat tissue, or rat urine or possibly had indirect exposures, probably through ventilation systems. The specific IgE titers were considerably lower for these 10 individuals (1.4 KU/L [SD 0.8]) compared for instance to those with exposures 0 to 0.5, 0.5 to 8, and more than 8 ng/m^3 · hrs/wk. The latter groups had average specific IgE titers against RUA of, respectively, 11.5 (SD 22), 7.6 (SD 16.1), and 8.2 (SD 10.0) KU/L. Tabular analysis and multiple proportional hazard regression analysis did not reveal a relationship between rat sensitization and duration of employment (years of exposure; not presented). Analyses using the job with the highest exposure ever experienced instead of the present job did not yield different results.

Results of the multiple proportional hazard regression models are given in Table IV. Because clear differences were observed for atopic and nonatopic workers, separate analyses were performed for these 2 categories. All models generally showed a clearly increasing sensitization risk with increasing exposure for nonatopic workers. For atopic workers, the exposure-response relationships were less steep. A more than 3-fold increased risk could be observed for low-exposure atopic subjects, and this risk increased little with increasing exposure. From a statistical perspective, the model with the time-multiplied exposure was superior over the other models because

TABLE III. Prevalence of RUA sensitization by exposure in 650 laboratory animal workers

	Cases (n/N)	Prevalence (%)	PR (CI)
Not working with conscious rats	10/251	4.0	1 (—)
0-2 h/wk	11/85	13.0	3.2 (1.4-7.6)
2-8 h/wk	19/159	12.0	3.0 (1.4-6.5)
>8 h/wk	23/155	14.8	3.7 (1.8-7.8)
No exposure	10/252	4.0	1 (—)
0-0.25 ng EQ/m^3	13/117	11.0	2.8 (1.2-6.4)
0.25-1.25 ng EQ/m^3	28/205	13.7	3.4 (1.7-7.1)
>1.25 ng EQ/m^3	12/76	15.8	4.0 (1.7-9.2)
No exposure	10/242	4.0	1 (—)
0-0.5 ng EQ/m^3 · h/wk	15/125	12.0	3.0 (1.4-6.7)
0.5-8 ng EQ/m^3 · h/wk	19/155	12.3	3.1 (1.4-6.6)
>8 ng EQ/m^3 · h/wk	19/118	16.1	4.1 (1.9-8.7)

PR, Prevalence ratio; *CI*, 95% confidence interval.

TABLE IV. Relationship between RUA sensitization and exposure, sex, and smoking status for atopic and nonatopic workers from a pool of 650 laboratory animal workers

	Nonatopic workers		Atopic workers	
	PR	CI	PR	CI
Not working with conscious rats	1.0	—	1.0	—
0-2 h/wk	2.6	0.5-13.1	3.4	1.3-9.6
2-8 h/wk	2.8	0.7-12.0	2.8	1.1-7.0
>8 h/wk	3.4	0.9-13.3	4.1	1.6-10.1
Sex (F vs M)	0.6	0.2-1.6	1.1	0.6-2.0
Smoker (smoker vs nonsmoker)	1.4	0.5-3.8	0.9	0.5-1.9
Exsmoker (exsmoker vs nonsmoker)	1.0	0.2-5.0	0.8	0.3-2.0
No exposure	1.0	—	1.0	—
0-0.25 ng EQ/m^3	0.8	0.1-8.0	3.1	1.2-7.9
0.25-1.25 ng EQ/m^3	3.4	0.9-12.7	3.5	1.5-8.3
>1.25 ng EQ/m^3	5.0	1.2-21.1	3.7	1.2-10.9
Sex (F vs M)	0.7	0.3-1.8	1.1	0.6-2.0
Smoker (smoker vs nonsmoker)	1.3	0.5-3.5	0.9	0.4-1.9
Exsmoker (exsmoker vs nonsmoker)	1.0	0.2-4.8	0.8	0.3-2.0
No exposure	1.0	—	1.0	—
0-0.5 ng EQ/m^3 · h/wk	1.5	0.2-8.8	3.1	1.2-8.0
0.5-8 ng EQ/m^3 · h/wk	3.1	0.8-12.4	3.1	1.2-7.8
>8 ng EQ/m^3 · h/wk	4.4	1.1-17.1	4.2	1.6-11.2
Sex (F vs M)	0.6	0.3-1.6	1.1	0.6-2.0
Smoker (smoker vs nonsmoker)	1.3	0.5-3.6	0.9	0.4-1.8
Exsmoker (exsmoker vs nonsmoker)	1.0	0.2-4.8	0.8	0.3-2.0

PR, Prevalence ratio; *CI*, 95% confidence interval.

the likelihood ratios were highest for atopic workers and for nonatopic workers. No differences were observed among smokers, exsmokers, and nonsmokers. The models did not change when information on the number of pack-years smoked was included in the analyses. For instance, a comparison of heavy smokers with nonsmokers (to obtain the most contrast possible) did not change the outcomes of the analyses. Sensitization risk also did not differ among atopic smokers, exsmokers, and nonsmokers and males and females. Results presented were not corrected for age because such a correction did not change any of the regression coefficients of variables of interest and age was not related to sensitization risk ($P > .80$).

The magnitude of the conversion factors to convert concentrations measured with the 3 assays to one arbitrarily chosen reference level was estimated from an interlaboratory study.[8] Only samples above the detection limit were considered in the interlaboratory study. If samples below the detection limit were used and were set at 2:3 of the detection limit, a common procedure in occupational hygiene, considerably larger (150% to 250%) conversion factors would have been obtained. Analyses using different conversion factor scenarios showed that the shape of the exposure-response relationship depended to some extent on the magnitude of the exposure conversion factors chosen. The shape varied between a monotonically increasing exposure-response relationship and one with a risk plateau for workers with intermediate exposures. The risk for the high-exposure category changed little.

DISCUSSION

In this study we observed clear exposure-response relationships for RUA exposure and specific IgE antibodies against laboratory rats. The risk for development of sensitization was clearly increased in atopic workers compared with nonatopic workers. The hours worked with conscious rats and the product of hours worked with conscious rats and exposure level gave the best discrimination between workers with and without anti-RUA sensitization.

Similar results were obtained when the job with the highest exposure was used in the analysis instead of the present exposure. Only a few workers were categorized differently with regard to their exposure status. It could be that the time frame is too short for selective migration from high- to low-exposure jobs, although it is also possible that those workers who are likely to migrate from high- to low-exposure jobs (sensitized workers with severe work-related symptoms) have already left the industry. If the latter is true, this study most likely underestimates the relationship between allergen exposure and work-related sensitization. The absence of a relationship for duration of exposure might have similar explanations. Only longitudinal studies can address these hypotheses adequately.

The exposure-response relationship appeared to be robust and not sensitive to changes in the magnitude of the conversion factors for the immunoassays for assessment of air allergen levels. This makes sense, because earlier exposure studies revealed that the difference in exposure level between high-exposure workers (animal technicians and cage cleaners) and low-exposure workers (office workers and workers in slide production) was considerably larger than the uncertainty in conversion factors. A 175- to 800-fold difference between high- and low-exposure workers was found in the UK study,[12] and almost a 30-fold difference was found in the Dutch study (average difference over all facilities).[6] The difference between high- and low-exposure job titles in the Swedish workers was less than a factor of 10. Therefore even considerable changes in the choice of conversion factors had only a limited impact on the ranking of exposed individuals on the exposure axis. On the other hand, changes in conversion factors will only partially account for differences in assays with regards to the allergens detected by each assay.[8,9] The effect of changes in the conversion factor had the most effect on the relative placement of the Swedish laboratory animal workers on the exposure axis. This is because Swedish workers had low exposure compared with British and Dutch laboratory animal workers, and the contrast between high- and low-exposure Swedish laboratory animal workers was low.

Interestingly, this study suggests an increased sensitization risk at very low exposure levels. Atopic workers exposed for only a few hours per week with low-exposure levels between 0 and 0.5 ng EQ/m^3 · hrs/wk (exposure category arithmetic mean exposure 0.18 ng EQ/m^3 · hrs/wk) had a more than 3-fold likelihood of being sensitized than nonexposed workers. If conversion factors were changed (from 30% to 300%), the prevalence ratios changed for this exposure category, but the sensitization risk remained higher than 2.5. The risk curve increased little at higher exposure levels. Atopic workers in the highest exposure category with exposure levels above 8 ng EQ/m^3 · hrs/wk had an almost 4 times increased sensitization risk, but their average exposure is more than 1000-fold higher than observed for the lowest exposed category (exposure category arithmetic mean exposure 188 ng EQ/m^3 · hrs/wk). The increase in risk seemed steeper in nonatopic subjects. The lowest exposure levels observed in this study seem sufficient to sensitize a considerable portion of the atopic subjects, whereas the risk for nonatopic subjects to become sensitized at these levels is almost negligible and noticeable only at higher exposure levels.

The criterion of a specific IgE level above 0.7 KU/L for sensitization to RUPs is rigid for an epidemiologic study. If a cut-off level of 0.35 KU/L was chosen, almost twice as many workers would have been characterized as sensitized (97 of 650 workers [14.9%]). However, multiple proportional hazard regression analyses with this cut-point for work-related sensitization lead to a less steep exposure-response relationship because the prevalence of rat sensitization in the reference group increased most likely because of a reduced specificity.

The hours worked with conscious rats and the product of hours worked with conscious rats and exposure level were better predictors of work-related sensitization than the current job-average concentration. This is probably caused by some specific characteristics of this population. The time spent with conscious rats varied strongly between exposed individuals from a few hours per week (scientists and students) to a full working week (animal caretakers).

The observation with regard to the modifying effect of atopy is in agreement with findings from numerous other studies on high molecular weight sensitizers. No earlier observations with regard to work-related sensitization to high molecular weight sensitizers are available from the literature for male and female workers separately. Our results suggest that atopy and an elevated total IgE level is more common in male workers than in female workers. This has also been observed in some large-scale open-population studies.[16,17] However, rat-specific sensitization rates were similar in female and male atopic workers. Interestingly, the sensitization pattern to common allergens also differed between countries and sex. This has also been observed in some other studies for mites and cats.[18-20]

Sensitization to cats and dogs has been mentioned in the literature as a risk factor for the development of laboratory animal allergy.[21,22] Also, in this study cat and dog sensitization was somewhat more strongly associated with rat sensitization than was sensitization to house dust mite, grasses, and pollen (prevalence ratio for house dust mite, grasses, and pollen, 3.0 [confidence interval, 1.8-5.0] vs prevalence ratio for cat and dog, 4.1 [confidence

interval, 2.5-6.8]), even after stratification for country. This raises the hypothesis that the increased risk observed for atopic women might be explained by an increased sensitization rate to cats and dogs. The role of cat or dog sensitization therefore remains to be confirmed in longitudinal studies. The question why different relationships between cat and dog sensitization for each country exist needs clarification as well.

In conclusion, this study, in which data were pooled from 3 earlier published studies, confirmed the presence of recently published exposure-response relationships.

We thank Prof A. Newman Taylor for helpful discussions during preparation of the manuscript.

REFERENCES

1. Newman Taylor AJ, Longbottom JL, Pepys J. Respiratory allergy to urine proteins of rats and mice. Lancet 1977;2:847-9.
2. Renström A, Malmberg P, Larsson K, Sundblad B-M. Allergic sensitization is associated with increased bronchial responsiveness: a prospective study of allergy to laboratory animals. Eur Respir J 1995;8:1514-9.
3. Cullinan P, Lowson D, Nieuwenhuijsen MJ, S Gordon, RD Tee, KM Venables, JC McDonald, et al. Work related symptoms, sensitisation, and estimated exposure in workers not previously exposed to laboratory rats. Occup Environ Med 1994;51:589-92.
4. 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.
5. Kruize H, Post W, Heederik D, Martens B, Hollander A, van der Beek A. Respiratory allergy in laboratory animal workers: a retrospective cohort study using pre-employment screening data. Occup Environ Med 1997;11:830-5.
6. Hollander A, van Run P, Spithoven J, Heederik D, Doekes G. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. J Allergy Clin Immunol 1997;99:286-92.
7. Venables KM, Upton JL, Hawkins ER, Tee RD, Longbottom JL, Newman Taylor AJ. Smoking, atopy and laboratory animal allergy. Br J Ind Med 1988;45;667-71.
8. Hollander A, Gordon S, Renström A, Thissen J, Doekes G, Larsson P, et al. Comparison of methods to assess airborne rat and mouse urinary allergen levels I: analysis of air samples. Allergy 1998. In press.
9. Renström A, Gordon S, Hollander A, Heederik D, Doekes G. Comparson of methods to assess airborne rat or mouse urinary allergen levels: II factors influencing antigen detection. Allergy 1998. In press.
10. Renström A, Karlsson A-S, Larsson PH, Malmberg P. High prevalence of symptoms and specific IgE to laboratory animal allergens in spite of low exposure among research personnel [abstract]. Eur Respir J 1998;9:1205.
11. Doekes G, Douwes J, Wouters I, de Wind S, Houba R, Hollander A. Enzyme immuno-assays for total and specific IgE in population studies. Occup Environ Med 1996;57:567-70.
12. Nieuwenhuijsen M, Gordon S, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. Exposure to dust and rat urinary allergens in research establishments. Occup Environ Med 1994;51:593-6.
13. Renström A, Malmberg P, Larsson K, Sundblad BM, Larsson PH. Prospective study of laboratory-animal allergy: factors predisposing to sensitization and development of allergic symptoms. Allergy 1994;49:548-52.
14. Gordon S, Tee RD, Newman Taylor AJ. Analysis of rat urine proteins and allergens by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. J Allergy Clin Immunol 1993;92:298-305.
15. Thompson ML, Meyers JE, Kriebel D. Prevalence odds ratio or prevalence ratio in the analysis of cross sectional data: What is to be done? Occup Environ Med 1998;55:272-7.
16. Barbee RA, Halonen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlations with age, sex and allergen skin reactivity. J Allergy Clin Immunology 1981;68:106-11.
17. Kerkhof M, Droste JHJ, de Monchy JGR, Schouten JP, Rijcken B. Distribution of total serum IgE and specific IgE to common aeroallergens by sex and age, and their relationship to each other in a random sample of the Dutch general population aged 20-70 years. Allergy 1996;51:770-6.
18. Tee RD, Gordon DJ, van Hage Hamsten M, Gordon S, Nunn AJ, Johansson SG, et al. Comparison of allergic responses to dust mites in UK bakery workers and Swedish farmers. Clin Exp Allergy 1992;22:233-9.
19. De Groot H, Stapel SO, Aalberse RC. Statistical analysis of IgE antibodies to the common inhalant allergens in a defined population. Ann Allergy 1990;65:97-104.
20. Ommenaas E, Bakke P, Elsayed S, Hanaoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. Clin Exp Allergy 1994;24:530-9.
21. Hollander A, Doekes G, Heederik D. Cat and dog allergy and total IgE as risk factors of laboratory animal allergy. Clin Exp Allergy 1996;98:545-54.
22. Bryant DH, Boscato LM, Mboloi PN, Stuart MC. Allergy to laboratory animals among animal handlers. Med J Aust 1995;163:415-8.