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Predictors for Increased and Reduced Rat and Mouse Allergen Exposure in Laboratory Animal Facilities

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Abstract

Introduction: Exposure to rat and mouse allergens during work in laboratory animal facilities represents a risk for being sensitized and developing allergic diseases, and it is important to keep the exposure level as low as possible. The objective of this study was to characterize the personal Mus m 1 and Rat n 1 exposure during work in laboratory animal facilities, and to investigate the effect of identified predictors of increased and reduced exposure.

Methods: Mus m 1 and Rat n 1 were analysed in whole day or task-based personal air samples by enhanced sensitivity sandwich enzyme-linked immunosorbent assay. Information about cage-and-rack systems, tasks, and other conditions known to influence the allergen exposure was registered. Predictors for allergen exposure were identified by multiple linear regression analyses.

Results: The median allergen exposure was 3.0 ng m^{-3} Mus m 1 and 0.5 ng m^{-3} Rat n 1, with large task-dependent variations among the samples. The highest exposed job group were animal technicians. Cage emptying and cage washing in the cage washroom represented the highest exposure, whereas animal experiments in the lab/operation room represented the lowest exposure, with laminar airflow bench being an exposure-reducing determinant. Cage changing was the highest exposed task in the animal room, where individually ventilated cages (IVCs) were predictors of reduced exposure for both Mus m 1 and Rat n 1, whereas cage-rack systems with open shelves and sliding doors were predictors of increased Rat n 1 exposure. Cages of IVC type with positive air pressure (IVC+) as well as open shelves and sliding doors were strong predictors of increased exposure during cage emptying and cage washing.

Conclusions: Significant different exposure levels depending on type of work and task imply different risks of sensitization and allergy development. The fact that IVC+ cages have opposite impact on Mus m 1 and Rat n 1 exposure during different tasks may have positive clinical implications when taken into account.

Keywords: allergen; exposure assessment; exposure predictors; IVC; laboratory animal facilities; Mus m 1; Rat n 1; task-based

Introduction

Working with laboratory animals is a well-known risk for developing allergic diseases. Typical airway symptoms include allergic inflammation in the nasal mucosa, conjunctivitis, and asthma, whereas urticaria is the most common skin reaction. Frequent contact with animals increases the probability of health effects. As many as 10–30% of the workers in laboratory animal facilities develop allergy against rat or mice (Hunskaar and Fosse, 1990; Renstrom *et al*., 1995; Palmberg *et al*., 2015; Simoneti *et al*., 2016), which is considerable compared with other occupations such as bakers or dental technicians (Gautrin *et al*., 2000). Between 5 and 40% develop allergy during the first 1–2 years of exposure (Renstrom *et al*., 1994; Palmberg *et al*., 2015). According to a pooled analysis of 13 studies, 80% individuals with allergic symptoms have inflammation in eyes or nasal mucosa (53–100%), 40% (13–70%) have skin reactions and 35% (13–71%) have asthma (Hunskaar and Fosse, 1990). Over 60% of individuals with laboratory animal allergy (LAA) have immunoglobulin (Ig) E antibodies specific for the animal allergens (Gordon and Preece, 2003; Palmberg *et al*., 2015). Atopic individuals have 11 times higher risk of developing allergy (Renstrom *et al*., 2001b), and develop LAA four times faster than non-atopics (Botham *et al*., 1995).

Allergens from mice and rats induce more allergies in humans than other animal allergens, primarily because mice and rats are used more often than other animals and not necessarily because the other animals are less allergenic (Aoyama *et al*., 1992). The main allergens from rat and mice, Rat n 1 and Mus m 1, respectively, are lipocalin proteins produced by the liver and excreted in the urine. The excretion is hormonally controlled, and the level of Mus m 1 in urine is, due to androgen stimulation, about four times higher in male mice compared to female mice (Renstrom *et al*., 2001b). The allergens may be retrieved in hair, dandruff, and urine of the animals, as well as low levels in serum. Mice- and rat-allergens are mainly carried by particles of 3–10 µm in aerodynamic diameter (Ohman *et al*., 1994; Pacheco *et al*., 2006).

The number of animals killed for experiments have been reduced in some areas of science by utilizing *in vitro* systems as an alternative, but more recently genome-based research has led to increased use of experimental animal models, using particularly genetically modifiable mice (Feary and Cullinan, 2016). This may have resulted in both increased potential for exposure and increased number of employees in the laboratory animal facilities. On the other hand, modern equipment and facilities may have reduced the exposure risk. Although animal technicians have been the highest exposed job group, and scientists among the lowest exposed (Lieutier-Colas *et al*., 2001; Curtin-Brosnan *et al*., 2010), the work performed by the exposed job groups may change depending on the type of the facility. Several have expressed their concern about lacking task-based exposure assessments (Jones, 2015; Feary and Cullinan, 2016). Thus, there is need for a more detailed task-based exposure assessment of today's laboratory animal facilities.

Different methods for allergen measurements hamper direct comparisons of concentrations reported between studies. However, with reasonable consistency, the allergen exposure has been shown to vary with the facility, the cage systems, the number of animals in the room, and the type and duration of performed tasks (Eggleston *et al*., 1989; Ohman *et al*., 1994; Nieuwenhuijsen *et al*., 1995; Hollander *et al*., 1998; Thulin *et al*., 2002; Glueck *et al*., 2012). Use of equipment such as individually ventilated cages (IVCs) has been shown to reduce the allergen exposure in animal rooms (Gordon *et al*., 2001; Renstrom *et al*., 2001a), whereas handling dirty cages is a high-exposed task (Lieutier-Colas *et al*., 2001; Thulin *et al*., 2002; Korpi *et al*., 2004).

It is difficult to establish occupational exposure limits for allergens, partly due to the lack of standardized measurement methods and partly because of complex exposure-response relationships (Jones, 2015) and the influence of genetic susceptibility, such as atopy (Palmberg *et al*., 2015). To reduce the incidence of allergen sensitization and LAA, it is therefore important to keep the allergen exposure in laboratory animal facilities as low as possible. It is thus necessary to identify the conditions and tasks that can give the lowest possible exposure as well as predictors of high exposure that may provide a basis for priority setting of preventive measures. The aim

of this study was to characterize the personal exposure to rat and mice allergens, and to investigate the effect of identified predictors of increased and decreased exposure.

Methods

Study population

The study population consisted of employees in laboratory animal facilities of Norway's four largest universities with contact with mice and rats, and included animal technicians, laboratory technicians, scientists, and PhD students. Laboratory technicians, scientists, and PhD students were working at the lab/operation room, whereas animal technicians were working in animal room and cage-washing room.

Work environment

The laboratory animal facilities consisted of clean and dirty zones with a sluice between the zones. Clean zone consisted of corridors, offices, lunchroom, wardrobes, and clean side of the washing room, where the preparation of new cages took place. Work clothes, consisting of laboratory coat or coveralls, hairnet, gloves, shoes, and respiratory protective equipment, were put on in the sluice. The dirty zone consisted of animal rooms for unpacking of animals, laboratories, storage rooms, and cage washrooms including station for emptying of used bedding from cages. Strains, and number of mice and rats that were present during the study, varied between the facilities. All facilities had separate stables for mice and rats. There were separate breeding rooms and various contamination regimes. The animal cages were either open or IVCs with positive (+) or negative (–) air pressure. IVCs were connected to separate ventilation units that controlled air exchange, air humidity, and temperature in the cages, and filtered the exhaust air through high efficiency particulate air (HEPA) filters. In some facilities, the air was recirculated because the exhaust air was not always connected to the general ventilation in the room. The animal rooms had balanced ventilation and negative pressure relative to the surroundings, except rooms with stricter isolation regimes, which had positive pressure to protect the animals. Exhaust channels from the stables had HEPA filters to prevent emission of allergens outside the animal facility. When open cages were used in open shelves, sliding doors of plexiglass were mounted in front of the shelves to prevent the release of allergens out in the room, and the rooms had special ventilation that extracted the air from the room, past and out behind the shelves. All facilities used aspen bedding. A description of the work in each room, as well as the type of workbench, cage, and racks registered in this study, is presented in Table 1.

Sampling strategy

Ten series of allergen sampling at six different laboratory animal facilities were performed during 2009–2015 by occupational hygienists employed at the universities, according to a standardized protocol for this study. The allergens were collected on Teflon filters (diameter 25 mm, pore size 1.0 µm, Millipore FALP 02500), placed in IOM cassettes (SKC Inc., Valley View, PA, USA) connected to a battery-powered pump with an airflow of 2 l min^{-1}. Personal sampling (n = 184) of airborne allergens was performed either during the whole workday or during specific tasks (task-based strategy). The filter was placed in the breathing zone of the employees, outside of any respiratory protective equipment. The time of sampling was 15–380 min (median 133 min). Information about type of room, cage and cage rack, number of animals, sex of the animals, ventilation, work tasks and routines, job title, and use of personal protective equipment was registered in the sampling protocol.

Determination of Mus m 1 and Rat n 1 allergen

After sampling, filters were transferred to 3 ml Nunc Minisorp tubes with the exposed surface rolled in towards the centre of the tube. Allergens were extracted by incubating the exposed filters in 2-ml phosphate buffered saline (PBS) containing 0.5% Tween-20 and 0.15% ProClin (Sigma Aldrich) for 2 h with rotation. After 10 min centrifugation at 1000 g, the eluate was transferred into a fresh Minisorp tube containing 20-mg heat-fractionated Bovine serum albumin [BSA (Sigma-Aldrich)]. The BSA was dissolved by rotation for 5 min, and the eluate was aliquoted into 5 tubes and stored at –20°C until analysis. The samples were slowly thawed before analysis. Of the 184 collected samples, Mus m 1 and Rat n 1 allergen were analysed in 136 and 139 extracts, respectively. Both allergens were analysed in 91 of the samples. The allergens were detected by enzyme-linked immunosorbent assay (ELISA) using commercial Mus m 1 and Rat n 1 ELISA kits (Indoor Biotechnologies Inc., Wiltshire, UK) as basis for sandwich ELISAs with enhanced sensitivity, as previously described (Korpi *et al*., 2004). In brief, flat-bottomed 96-well microtiter plates were coated overnight at 4°C with polyclonal rabbit anti-Mus m 1 at 1 µg ml^{-1} or monoclonal mouse IgG1 anti-Rat n 1 antibody (RUP-6) at 5 µg ml^{-1} diluted in 50 mM carbonate-bicarbonate buffer, pH 9.6. After washing with PBS containing 0.05% Tween-20 and blocking with Casein buffer (Fitzgerald Industries Int., Concord, MA, USA) at 1:20, a 9-step dilution row from 5 to 1280 pg ml^{-1} of Mus m 1 or Rat n 1 standards (Indoor Biotechnologies Inc., Wiltshire, UK) and samples diluted 1:2 or 1:20 in casein buffer were added and

Table 1. Work descriptions.

Room type	Work	Work description	Bench type, cages, and racks
Lab/operation room	Experiments	Minor surgery on animals under anaesthesia, behaviour experiments with registration of brain activity, blood sampling, and euthanization	*Bench type:* ventilated, LAF, other
Animal room	Changing cages	Transfer of animals from used cages with soiled beddings to clean cages with new bedding was primarily done in changing stations with LAF, but some places on ordinary tables/benches	*Bench type:* ventilated, ordinary, LAF, other/not provided *Cage type:* open, filter top, IVC+, IVC–, other/not provided *Cage-racking system:* open shelves, open shelves + sliding doors, IVC, other/not provided
	Animal caring and feeding	Checking general condition of animals, refill of food and water, medication, separation of offspring. Almost all tasks implied opening of cages	
Washing room	Emptying cages	Dirty cages with used bedding were emptied in particular emptying stations, mainly with bedding transport band and LAF, in the unclean zone of the washing room. Cage tops were removed, feeders were emptied in the cages, and the cages were stacked on the floor or trolleys at the side of the emptying station. One cage at a time was emptied by turning the cage upside down on the transport band, and subsequently knocking and scraping the rest of the bedding out	*Bench type:* ventilated, other *Cage type:* open, IVC+, IVC–, other/not provided *Cage-racking system:* open shelves, open shelves + sliding doors, IVC, other/not provided
	Washing cages	Cage washing was mainly an automatic process where the cages were washed either by stacking on a washing stand that was rolled into the washing machine, or by placing cages on a transport band that transported the cages through a tunnel washer	
	Preparation of new cages	Adding new bedding, food, and water in clean cages were done on the clean side of the washing room	
All rooms	Cleaning	Cleaning of surfaces was done successively after work operations throughout the day. IVC units were cleaned regularly with HEPA vacuum cleaning of the filters, and dismantling and cleaning of the ventilation channels. Cleaning of the LAF stations was done after each cage changing session in addition to a weekly main cleaning with HEPA vacuum cleaning. The emptying station and the floor around it were vacuum cleaned after each session of emptying. Main cleaning of all rooms in the facilities was done weekly in some facilities. Existing routines for control and service of IVC ventilation units, emptying station, and the washing machines were followed	

incubated for 90 min at room temperature. After washing, biotinylated rabbit anti-Mus m 1 polyclonal antibody at 1:10 000 dilution or monoclonal IgG_1 anti-Rat n 1 antibody (RUP-1) diluted 1:2000 was added and incubated for 60 min at room temperature. The plates were washed, and streptavidin-polyHRP 80 conjugate (Fitzgerald Industries International, North Acton, MA, USA) was added at 1:20 000 dilution for Mus m 1 and 1:10 000 for Rat n 1, and incubated for 60 min at room temperature, before washing again. Thereafter, 1-step ultra tetramethylbenzide substrate (Thermo Scientific) was added and the reaction stopped after 15 min by

adding 1N H_2SO_4. The optical density (OD) of the resulting colour was read at 450 nm in a Spectramax i3 spectrophotometer (Molecular Devices LLC., Sunnyvale, CA, USA) and adjusted for background signal read at 650 nm. A standard curve was obtained by 4- or 5-parameter best-fit evaluation, and upper and lower detection limit (DL) as well as the sample values were determined based on the standard curve. Determinations with coefficient of variation (CV) <10% of the response value for the duplicates, with CV <20% for different dilution of the same samples, and with background signal with OD <0.2, were accepted. Samples with OD <0.2 were treated as below the DL. The DL was between 18 and 80 pg ml^{-1} for both Mus m 1 and Rat n 1. Sample values were adjusted for dilution and air volume and expressed as allergen exposure in ng m^{-3}.

Data analyses

The exposure values were skewed to the right and were ln-transformed to obtain near normal distribution before statistical analyses. Measurements below the DL of the ELISA were substituted by the DL/√2 for inclusion in the statistical analyses. The general exposure concentrations are presented by arithmetic mean (AM), median, minimum (min), and maximum (max). In addition, the 90 and 95 percentiles are presented for different room categories. Group differences in the ln-transformed mean of the personal measurements were tested by analysis of variance and subsequent *post hoc* pairwise tests with Bonferroni correction for multiple comparison, and by independent sample *t*-test for comparison of two groups. A *P*-value of 0.05 was regarded as statistically significant. Samples were grouped into exclusive room categories suitable for regression modelling of room-dependent exposure determinants (Fig. 1). Measurements not exclusive for any of the room categories were not included in regression analyses, but the exposure level was for the record collectively presented as a separate category. The most important determinants for allergen exposure in each room were identified by backwards selection in multiple linear regression analyses with the concentration of Mus m 1 or Rat n 1 as dependent variables and different determinants of exposure as independent variables. General linear regression models were built based on this, and the strength of the determinants is given as a factor related to the constant in the model. Exposure levels of different combinations of determinants can be computed from the regression models as follows:

$$E = e^{c+b1+b2..} = e^{c} \times \text{effect}_{\text{determinant1}} \times \text{effect}_{\text{determinant2}}$$

where E = exposure, c = intercept of the regression model, and $b1$ and $b2$ = regression coefficients of the determinants 1 and 2.

The IBM software package SPSS version 24 was used for the statistical analyses (IBM Corp, Armonk, NY, USA).

Results

Allergen levels in laboratory animal facilities

The median allergen exposure was 3.0 ng m^{-3} Mus m 1 and 0.5 ng m^{-3} Rat n 1. The variation among the samples was large (Table 2). The mean Mus m 1 exposure among animal technicians was much higher than among scientists/students ($P = 0.003$) and laboratory technicians, although the difference to the latter was not statistically significant. The number of samples from scientists and laboratory technicians was too few for meaningful statistical analyses of the Rat n 1 exposure differences. However, when scientists and laboratory technicians were combined, their exposure levels were statistically significantly different from animal technicians, regarding both rat and mouse allergens ($P = 0.03$ and $P < 0.001$, respectively).

Allergen exposure by room category and task

The allergen exposure variability was very high both among different laboratory animal facilities and within each series representing a facility (Fig. 2), as well as among tasks (Fig. 3). The exposure measurements stratified by tasks covered several tasks and work in several room types. Because the room type mainly determined the tasks, the measurements were grouped into exclusive room categories (lab/operation room, animal room, and cage washroom) to refine the exposure assessment. The personal exposure levels for mouse and rat allergens in the different room categories are presented in Table 3. The exposure levels of both mouse and rat allergens were highest in the cage washroom and lowest in the lab/operation room. The mean exposure differences between the exclusive room groups were statistically significant ($P \leq 0.001$ for Mus m 1 and $P = 0.01$ for Rat n 1). The pairwise comparison showed that the Mus m 1 exposure was significantly different between all room categories ($P = 0.03$ to $P < 0.001$), whereas the difference in Rat n 1 exposure was statistically significant only between lab/operation rooms and cage washrooms ($P = 0.02$). The exposure level measured during work in several room categories was also high (Table 3).

Predictors for allergen exposure

Cage changing increased the Rat n 1 exposure by a factor of 4.2 compared with any other task in the animal room category (Table 4). The cage-racking system with open shelves and sliding doors was identified as a strong

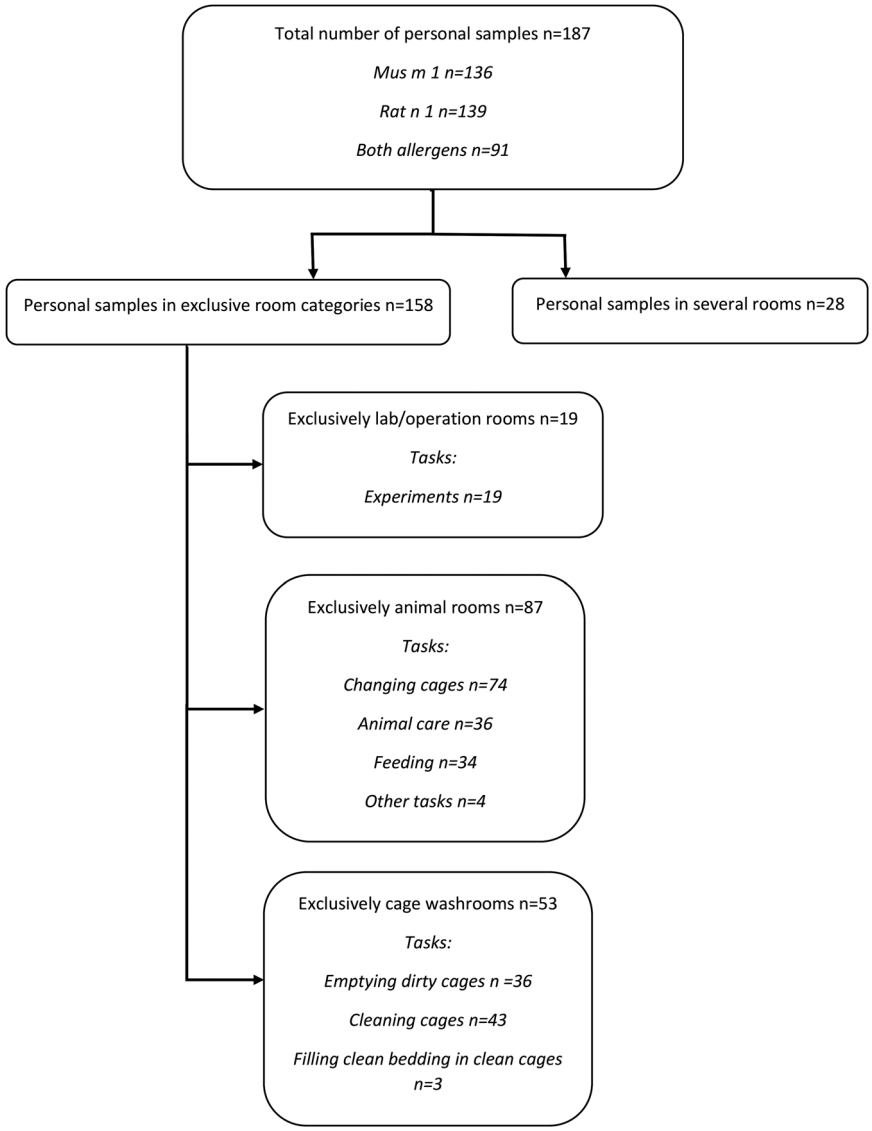

Figure 1. Flow chart of the grouping of personal samples into exclusive room categories suitable for regression modelling of determinants of Mus m 1 and Rat n 1 exposure. Work in lab/operation room was tantamount with experiments in the exclusive room category. 'Other tasks' consisted of one measurement of vacuum cleaning of rack aggregate and filter, one measurement of floor cleaning and wash/disinfection of cage changing station, one measurement of floor cleaning and filter change, and one measurement of floor cleaning and garbage handling.

predictor for Rat n 1 exposure increase, associated with a 14-times increase compared with any other cage-racking system in the animal room. IVC– or IVC+ cages were predictors of significant Rat n 1 exposure reduction (Table 4).

Mus m 1 exposure in animal rooms was high regardless of task, but IVC+ cages were associated with 10 times lower Mus m 1 exposure, and IVC– cages were associated with 50 times lower Mus m 1 exposure than any other cage type. The use of an ordinary workbench was also associated with Mus m 1 exposure reduction compared with a laminar air flow (LAF) bench (Table 4).

In cage washrooms, IVC+ cages and cage-rack system with open shelves and sliding doors were predictors of very high exposures (Table 4). IVC+ cages were associated with 160 times higher Mus m 1 exposure and 26 times higher Rat n 1 exposure than any other cage type. Furthermore, cage-racking systems consisting of open

Table 2. Exposure to rat and mouse allergens in all personal samples and by job title.

	Mus m 1 (ng m^{-3})						Rat n 1 (ng m^{-3})					
	n	*n* < *DL*	AM	Median	Min	Max	*n*	*n* < DL	AM	Median	Min	Max
All personal samples	136	8	74	3.0	0.02	2261	139	48	8.4	0.5	0.01	413
Animal technician	120	4	83.7	5.0	0.02	2261	126	37	9.3	0.6	0.1	413
Scientist and students	11	4	1.2	0.3	0.05	6.7	7	7	0.3	0.1	0.1	1.0
Laboratory technicians	5	0	3.6	0.3	0.06	11	6	4	0.2	0.2	0.08	0.5

The median of the samples when including non-exchanged values for samples <DL was 3.0 ng m^{-3} Mus m 1 and 0.23 ng m^{-3} Rat n 1. *n* = number of samples; *n* < DL = number of samples below the detection limit.

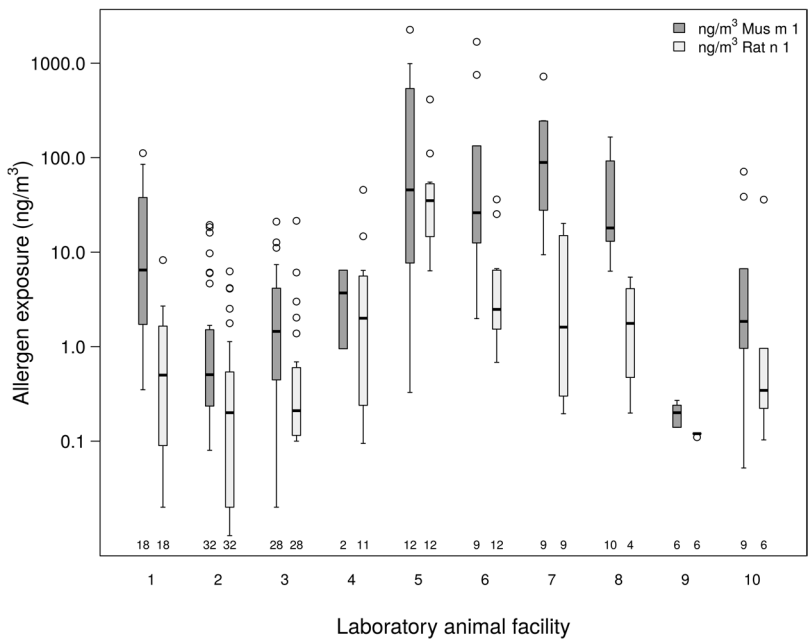

Figure 2. Personal exposure for rat and mouse allergens in Norwegian laboratory animal facilities. The boxes represent all samples analysed for Rat n 1 and Mus m 1 in each of 10 series of samples from different laboratory animal facilities. The black horizontal line inside the boxes indicates the median; the bottom and top line of the box indicates the 25 percentile and the 75 percentile, respectively; the whiskers indicate the 95 percentile and the points indicate outliers. The number of samples is presented below each box.

shelves with sliding doors were associated with 71 times higher Mus m 1 and 455 times higher Rat n 1 exposure, whereas IVC cage-racking systems were predictors of Mus m 1 exposure reduction (Table 4).

In the lab/operation rooms, the use of an LAF bench during mouse experiments was associated with significantly lower Mus m 1 exposure, whereas the use of a ventilated bench was associated with a significantly higher Rat n 1 exposure during rat experiments (Table 4).

The models explained a large proportion of the variance in exposure (r^2_{adj} = 21–75%).

Discussion

This study has characterized the personal exposure for rat and mice allergens in today's Norwegian university laboratory animal facilities. Collected information of cage and rack systems, tasks, and other conditions that are known to influence the allergen exposure has enabled the identification of several predictors for high and low exposure that may be used to obtain the lowest possible exposure in similar facilities. The influence of job groups, room types, tasks, and cage systems on the exposure was investigated.

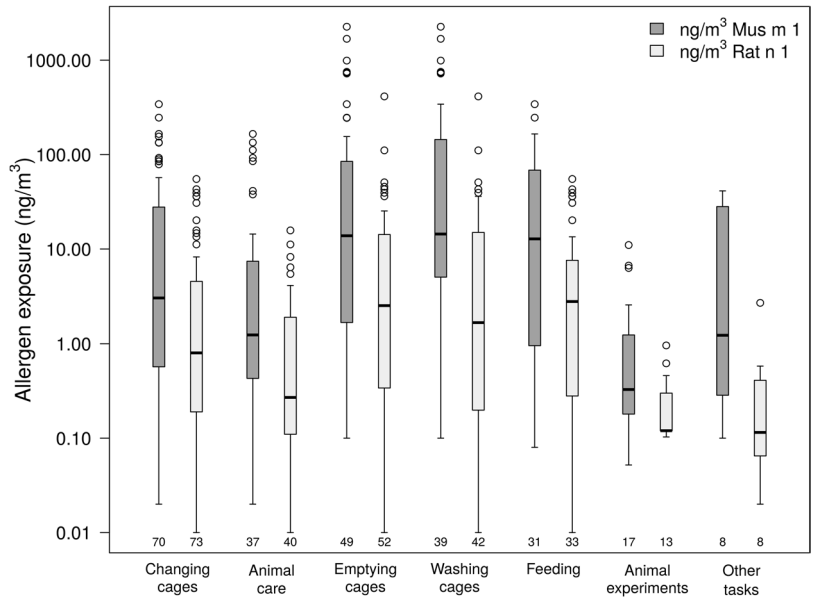

Figure 3. Exposure to Mus m 1 and Rat n 1 allergens by task. Boxes represent non-exclusive categories of tasks; i.e. the exposure level for one task also includes the exposure level for another task if both tasks were performed during the same sampling period. The black horizontal line inside the boxes indicates the median; the bottom and top line of the box indicates the 25 percentile and the 75 percentile, respectively; the whiskers indicate the 95 percentile and the points indicate outliers. The number of samples is presented below each box.

The general exposure level of median 3.0 ng m^{-3} Mus m 1, range 0.02–2261, was higher than the previously reported general median exposure of 0.7 ng m^{-3}, 25–75%ile 0.09–9.88 (Peng *et al.*, 2011), and the range from 1.2 to 563 ng m^{-3} (Ohman *et al.*, 1994) in Swedish and US laboratory animal facilities. Although the particular exposure levels among the relevant job groups may vary from study to study, the pattern of animal technicians being the highest exposed job group and the laboratory technicians, scientists, and students markedly lower is in agreement with previous observations of exposure by job title (Lieutier-Colas *et al.*, 2001; Curtin-Brosnan *et al.*, 2010). However, some facilities may have a different exposure pattern by job title, such as phenotyping facilities, where scientific staff may acquire exposure levels similar to animal technicians (Feistenauer *et al.*, 2014). To identify determinants of low and high exposure for assessment of possible exposure reduction, more information than job title is needed.

The grouping of exposure measurements according to room categories connected the exposure levels closer to the exposure source, and revealed that work in the cage-washing room gave the highest median exposures, the second highest were in animal rooms, and the lowest in lab/operation rooms. This is in agreement with several other studies using both personal and stationary sampling. Renstrom *et al.* (2001b) showed that rat and mouse allergen exposure levels in animal rooms were higher than in research departments (median 1.5 ng m^{-3} and <0.26 ng m^{-3} Rat n 1 and median <DL Mus m 1 in both rooms, respectively). This was lower than the median exposure of 2.0 ng m^{-3} and 0.3 ng m^{-3} Mus m 1 in this study, but higher than the 0.3 ng m^{-3} and 0.1 ng m^{-3} Rat n 1 in animal rooms and lab/operation rooms. A room- and task-dependent assessment with stationary sampling showed that the Rat n 1 allergen concentration in air in the Swedish facilities was significantly higher in animal rooms (AM 53.1 ng m^{-3}) compared with experimental rooms (AM 9.7 ng m^{-3}) (Lieutier-Colas *et al.*, 2001). Both of these levels are higher than the exposure levels of AM 3.3 ng m^{-3} and 0.2 ng m^{-3} Rat n 1 in the respective rooms in this study.

Because employees in laboratory animal facilities usually perform several tasks during a shift, task-based exposure assessment is necessary to get detailed information of the exposure during particular tasks. The median 0.3 ng m^{-3} Mus n1 exposure level during animal experiments in the lab/operation rooms represented the lowest exposure level in the dataset, and was similar to the levels previously reported during laboratory experiments (Curtin-Brosnan *et al.*, 2010). There was even a significant potential for reducing Mus m 1 exposure when performing animal experiments by an LAF workbench instead of any other type of workbench. The LAF

Table 3. Allergen exposure levels by room category.

	Mus m 1 (ng m^{-3})						Rat n 1 (ng m^{-3})					
	n	*n* < DL	AM	Median	(min–max)	90%ile	95%ile	*n*	*n* < DL	AM	Median	(min–max)	90%ile	95%ile
Total	136	8	74	3.0	(0.02–2261)	118	399	139	48	8.4	0.5	(0.01–414)	18	39
Lab/operation room[a]	16	4	1.9	0.3	(0.05–11)	8	—	12	10	0.2	0.1	(0.08–0.5)	0.4	—
Animal room[a]	71	2	17	2.0	(0.02–166)	74	100	72	20	3.2	0.3	(0.01–55)	7	21
Cage washroom[a]	34	1	226	12	(0.1–2261)	872	1829	39	16	19	0.5	(0.01–414)	46	111
Several rooms[b]	15	1	78	38	(0.1–342)	285	—	16	2	12	5.5	(0.2–43)	40	—

Measurements represent work in more than one room category; *n* = number of samples; *n* < DL = number of samples below the detection limit.
[a]Exclusive room categories.
[b]Non-exclusive categories.

workbench did not turn out as a significant exposure-reducing predictor for Rat n 1 in the regression models. As LAF benches were the main workbench in this group, the reducing effect is reflected in the low model intercept, whereas use of a ventilated workbench during animal experiments increased the exposure by a factor of 2.8. Animal work on a ventilated bench has previously been shown to give a Mus m 1 exposure of geometric mean (GM) 2.1 ng m^{-3} compared with GM 87 ng m^{-3} outside the ventilated bench (Thulin *et al.*, 2002). A much lower Mus m 1 exposure level of GM 4 ng m^{-3} was measured during handling of 21 and 22 mice on an unventilated table (Korpi *et al.*, 2004).

Cage changing is a well-known high-risk task. In agreement with this, high Mus m 1 exposure levels were observed in animal rooms [median (min–max) 2 ng m^{-3} (0.002–166)], and cage changing was identified as one of the predictors of high allergen exposure in the Rat n 1 regression model in animal rooms. Mus m 1 exposure levels during cage changing have previously been reported as high as GM (CI) 63 (4–907) ng m^{-3} (Pacheco *et al.*, 2006), GM 69 ng m^{-3} (78–400 mice), and GM 87 ng m^{-3} on unventilated table (Thulin *et al.*, 2002; Korpi *et al.*, 2004), and 17 ng m^{-3} on a ventilated wagon (Thulin *et al.*, 2002). The mean exposure level of 17 ng m^{-3} Mus m 1 in animal rooms in this study was similar to the exposure level on the ventilated wagon. The mean Mus m 1 exposure level in animal room was 38 ng m^{-3} when estimated from the regression model without adjustment of any other determinants. However, the exposure level was 10 times lower if the cages were IVC– (estimated mean 3.8 ng m^{-3}). Whereas the use of cage-changing stations has been shown to reduce the exposure during cage changing (Feistenauer *et al.*, 2014), this did not turn out as a significant predictor in this study. In fact, working on an ordinary bench was associated with significantly lower exposure than working in an LAF bench in the Mus m 1 model. Disturbance of the protective laminar flow by wrong use of the LAF changing stations was observed by the occupational hygienist, and could partly explain this. The cage types were more important for exposure, as demonstrated by the IVC being strong exposure-reducing determinants during cage changing. IVC has become state-of-the-art caging system for many facilities, and both IVC– and IVC+ cages were, as expected, strong determinants of reduced Mus m 1 exposure. IVC+ cages reduced Mus m 1 exposure five times more than IVC– cages, a difference also others have observed (Renstrom *et al.*, 2001a). It is, however, not suitable for all facilities to use IVC systems. Open shelves with sliding doors, the cage-racking system for open cages, were also a strong determinant for

Table 4. Determinants of Mus m 1 and Rat n 1 exposure in lab/operation rooms, animal rooms, and cage washrooms of Norwegian laboratory animal facilities.

Ln Mus m 1	B	SE	*P*	95% CI	Effect	Ln Rat n 1	B	SE	*P*	95% CI	Effect
Lab/operation room (r^2_{adj} = 0.21)						*Lab/operation room (r^2_{adj} = 0.74)*					
Intercept	−0.07	0.52	0.90	−1.19 to 1.05		Intercept	−2.12	0.09	<0.001	−2.33 to −1.92	
Bench type: LAF	−1.77	0.79	0.04	−3.46 to −0.08	**0.2**	Bench type: ventilated	1.03	0.18	<0.001	0.62 to 1.43	**2.8**
Animal room (r^2_{adj} = 0.41)						*Animal room (r^2_{adj} = 0.50)*					
Intercept	3.65	0.46	<0.001	2.72 to 4.57		Intercept	−1.07	0.52	0.04	−2.11 to −0.03	
Bench type: ordinary	−1.82	0.60	0.004	−3.01 to −0.62	**0.2**	Task: changing cages	1.43	0.51	0.006	0.42 to 2.45	**4.2**
Cage: IVC+	−2.11	0.62	0.001	−3.34 to −0.88	**0.1**	Cage: IVC−	−2.22	0.38	<0.001	−2.98 to −1.47	**0.1**
Cage: IVC−	−3.78	0.54	<0.001	−4.85 to −2.70	**0.02**	Cage-racking system: open shelves with sliding doors	2.65	0.82	0.002	1.16 to 4.28	**14**
Cage washroom (r^2_{adj} = 0.71)						*Cage washroom (r^2_{adj} = 0.75)*					
Intercept	2.82	0.54	<0.001	1.70 to 3.94		Intercept	−1.73	0.36	<0.001	−2.47 to −0.99	
Cage: IVC+	5.08	0.85	<0.001	3.31 to 6.84	**160**	Cage: IVC+	3.24	0.62	<0.001	1.95 to 4.53	**26**
Cage-racking system: IVC	−2.73	0.79	0.002	−4.37 to −1.09	**0.07**						
Cage-racking system: open shelves with sliding doors	4.26	1.03	0.001	2.11 to 6.40	**71**	Cage-racking system: open shelves with sliding doors	6.12	0.76	<0.001	4.54 to 7.69	**455**

The effect of the determinants, e^B, where B is the coefficient of the determinant, is a factor that multiplied with e^B for the intercept of the exclusive room will give the exposure effect of the determinant. That is, work in animal room with changing IVC− rat cages will give the following Rat n 1 exposure: $e^{B(\text{intercept for animal room})} \times e^{B\text{Task: changing cages}} \times e^{B\text{Cage: IVC negative}} = 0.3 \times 4.2 \times 0.1 = 0.13$ ng m^{-3}

increased Rat n 1 exposure. Such a one-way airflow system has previously been shown to effectively reduce the allergen levels in the centre of the room (Hollander *et al*., 1998), although this was based on stationary sampling, not comparable with personal exposure measurements.

The highest exposure level was measured during cage emptying and cage washing, in agreement with several other studies (Lieutier-Colas *et al*., 2001; Thulin *et al*., 2002; Korpi *et al*., 2004; Pacheco *et al*., 2006). However, the general exposure levels of median 12 ng m^{-3} Mus m 1 and 0.5 ng m^{-3} Rat n 1 in cage-washing rooms were lower than the GM 180 ng m^{-3} Mus m 1 (104–545, n = 3, 95–105 mouse cages) and GM 93 ng m^{-3} Rat n 1 (53–194, n = 2, 86–200 rat cages) during manual cage emptying and automatic cage washing in a Finnish study (Korpi *et al*., 2004). Both cage emptying and washing were mainly done in the same work session in this study. Thus, most measurements included both tasks, as opposed to the study of Thulin *et al*. (2002) that investigated emptying only, and reported GM 367 ng m^{-3} during manual emptying, 0.5 ng m^{-3} during automated emptying on one side of the machine, and 6.8 ng m^{-3} on the other side of the machine (where the cages were stacked). Automatic emptying and the use of a vacuum disposal system for bedding have been shown to reduce the exposure levels during this task (Thulin *et al*., 2002; Feistenauer *et al*., 2014).

Although cage emptying and cage washing represented the highest exposed tasks in this study, and resulted in a generally high exposure in the cage-washing rooms, cage type and cage rack types were identified as additional exposure determinants. Interestingly, our results showed that whereas IVC+ cages significantly reduced the Mus m 1 and Rat n 1 exposure during work in the animal rooms, IVC+ cages highly increased the exposure during cage emptying and cage washing. The exposure during cage emptying and cage washing estimated from the cage washroom models increased from 17 ng m^{-3} to 2684 ng m^{-3} Mus m 1 and from 0.18 ng m^{-3} to 4.6 ng m^{-3} Rat n 1 when working with IVC+ cages compared with any other cage types. Previously, median 466 ng m^{-3} Mus m 1 has been reported during cage cleaning with IVC– cages occupied by males (Renstrom *et al*., 2001a). The task-dependent exposure duality associated with IVC+ cages may be related to how the positive pressure first contain the allergens inside the cage, then releases the allergens upon cage dismantling, and particularly when emptying and washing the cages. The concentration of allergens in the IVC+ cages may be higher than any other cage types due to the direction of the air pressure, and when the cages are opened, more allergens are released compared with other cage types, where allergens are removed actively by negative pressure or are passively released into the surroundings (open cages).

To assess the exposure related to tasks for employees that perform a variety of tasks on a work shift, task-based exposure measurements are preferable, but not always practically feasible, and not necessarily sufficient. The results in this study show that information of caging systems should accompany a task-based exposure assessment to give sufficient information for correct risk assessment. Statistical modelling of the exposure during work including several tasks and information of determinants such as cage types is an alternative possible for a reasonable sized dataset and the only possible alternative if tasks and determinants partly overlap.

Identifying determinants for low and high exposure can be useful for the evaluation of possible exposure-reducing measures. Furthermore, it can be helpful for estimating the risk of sensitization or induction of symptoms in occupational or environmental settings. However, both duration and intensity of the exposure are important (Pacheco *et al*., 2006). A stable moderate exposure may be strongly associated with the development of allergic sensitization, whereas variable high-level exposure may be more strongly associated with IgG_4 response (Peng *et al*., 2011), although this does not necessarily protect against the development of sensitization and allergic symptoms (Krop *et al*., 2011).

There are several uncertainties related to the results in the present article. Short sampling time for several of the task-based measurements may have resulted in samples <DL when the allergen concentration was low. Task-based sampling is, however, the best way of gaining information of important exposure determinants, and is thus also a strength in the study. There is still a need for more standardized task-based measurements in future studies.

The method applied for determining allergen exposure used antibodies that are specific to the main allergens from rat and mice, Rat n 1 and Mus m 1. Other allergens from the animals were therefore not detected in these analyses. The grouping of measurements and statistical analyses was limited by the number of samples and the partially incomplete information collected during sampling. Other factors of importance for the exposure that have not been registered during the sampling or that did not have the required quality for data analyses, were animal density, ventilation (air exchange) related to room size, other activity in the rooms during sampling, frequency of main cleaning of the facilities, and lab classification. The number of animals, percentage of males, and strains may also affect the exposure level, but data on these parameters were too

incomplete for generalization. Between-worker variations related to duration, physics, and working methods may also influence the exposure. The results are based on data from laboratory animal facilities in the four largest universities in Norway and are thus representative for similar facilities. Facilities with significantly different organization may have other exposure levels and other important determinants for exposure. Results comparisons between studies are in general often also limited by different designs and analytical methods. Regression modelling was a strength of the study that enable statistical assessment of the complex information that was collected simultaneously with the exposure measurements, and that showed how the exposure is dependent on a set of complex variables that were not always the same for all tasks. Modelling adjusts for this, and identifies the variables with statistically strongest impact on exposure. Several of the regression models were also successful in explaining most of the exposure variance.

Conclusions

Collectively, this study has showed that animal experiments in lab/operation rooms gave the lowest exposure for mouse and rat allergens, whereas cage emptying and cage washing gave the highest exposure. Mus m 1 exposure was generally high during work in animal rooms, but IVCs were strong exposure-reducing predictors. Rat n 1 exposure was particularly high during cage changing. IVC– cages reduced the exposure, whereas open shelves and sliding doors largely increased the Rat n 1 exposure in the animal rooms. On the other hand, use of IVC+ cages largely increased the allergen exposure in cage washrooms. Cage-racking systems consisting of open shelves and sliding doors were also a strong determinant for increased allergen exposure in the washrooms. Significant different exposure levels during work with different tasks imply different risks of sensitization and allergy development. Special consideration to the fact that IVC+ cage types have opposite impact on Mus m 1 exposure during different task may have positive clinical implications when taken into account.

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