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Concentration and Emission of Airborne Contaminants in a Laboratory Animal Facility Housing Rabbits

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Characterization of animal housing conditions can determine the frequency of bedding and cage changes, which are not standardized from facility to facility. Rabbits produce noticeable odors, and their excreta can scald and stain cages. Our facility wanted to document measurable airborne contaminants in a laboratory rabbit room in which excreta pans were changed weekly and cages changed biweekly. Contaminants included particulate, endotoxin, ammonia, carbon dioxide, and a rabbit salivary protein as a marker for rabbit allergen. Concentrations were measured daily over a 2-wk period in a laboratory animal facility to determine whether they increased over time and on days considered to be the dirtiest. Except for ammonia, concentrations of all airborne contaminants did not differ between clean and dirty days. Concentrations were lower than occupational health exposure guidelines for all contaminants studied, including ammonia. After measurement of concentration, a model was applied to calculate mean emission factors in this rabbit room. Examples of emission factor utilization to determine airborne contaminant concentration in rabbit rooms under various environmental conditions and housing densities are provided.

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; CMS, completely mixed space; EMB, experimental mass balance; EU, endotoxin units; OSHA, Occupational Safety and Health Administration; RSP, rabbit salivary protein

Allergic reaction to animals is common and an important occupational health concern. Estimates of workers exposed to animals or animal products range from 40,000 to 2 million.[36,55] Studies of animal allergy prevalence show rates that range from 4% to 22% of exposed workers.[6,9] In 1 study,[14] the annual incidence rates of animal allergy and asthma were 15% and 2%, respectively.

Rabbits are a common laboratory species, especially for polyclonal antibody production, and represent a considerable allergic risk.[55] When housed at high density in research facilities, their waste can be particularly unpleasant for workers. Rabbits produce urine that is thick, concentrated, and difficult to clean; they produce large volumes of feces; and they shed fur. Airborne contaminants that can be generated by rabbits include particulate, endotoxin, ammonia, carbon dioxide, and rabbit salivary secretions, which contain high levels of rabbit allergen.[10,64] These compounds also have been detected in the air of rooms housing other animal species in laboratory and farm environments.[23-24,35,43] Several studies have recognized an association between these airborne contaminants and acute and chronic respiratory or mucous membrane irritation.[15,48] However, few investigations have focused on airborne contaminants in rabbit rooms in a laboratory-animal environment,[27] and to our knowledge, no reports have thoroughly documented factors associated with room ventilation and airborne contaminant emission.

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The frequency of excreta tray and cage changes in laboratory animal facilities is often left to the discretion of the animal facility manager and attending veterinarian, who consider animal welfare, facility cleanliness, current applicable regulations, and minimizing labor and ultimately overall cost of maintenance. Selection of appropriate bedding can decrease odor and contaminants, and rationing of food can help minimize animal excreta. The Animal Welfare Act[4] dictates that rabbit excreta pans must be changed at least once weekly and that cage changes for rabbits must be done at least once a month. The current *Guide for the Care and Use of Laboratory Animals*[38] indicates that primary enclosures should be sanitized at least every 2 wk but allows changes according to professional judgment of the situation. The *Guide* indicates that odor should not be the sole indicator of when cages should be sanitized, but it can be used in conjunction with the ammonia concentration, appearance of the animals and their cage, cage density, and bedding.[38] In the current literature, no articles discuss the frequency of excreta pan and cage changes for rabbit facilities, and only a few articles[27,28] describe airborne contaminants in rabbit rooms; these studies indicated the frequencies of excreta pan and cage changes as twice weekly and once weekly, respectively. However, sampling for airborne contaminants occurred in the center of the room,[27,28] and room ventilation was not fully characterized in either article, preventing extrapolation of these data to other animal facilities. Our goal was to determine the concentration of contaminants in our facility during weekly excreta pan changes and biweekly (that is, every 14 d) cage changes.

Airborne contaminants in rabbit rooms include fur, epidermal cells, feces, urine, saliva, feed, and bedding.[50] Common airborne contaminants that may be present in rabbit housing rooms are particulate, endotoxin, ammonia, and carbon dioxide. Human

exposure to airborne particulate has been linked to irritation, allergic sensitization, rhinitis, bronchial inflammation, allergic alveolitis, and occupational asthma.[31] Endotoxin is a lipopolysaccharide produced from decomposition of gram-negative bacterial cell walls in animal waste.[27] Several studies have implicated atmospheric endotoxin as a promoter of asthma; therefore, if present in high concentrations in an animal facility, endotoxin may be an occupational health concern.[21,22,42,57] Ammonia is produced from urease-positive bacteria in feces and is a powerful irritant of the upper respiratory tract.[27,40] Carbon dioxide is produced as a waste gas of respiration and can cause headache, dizziness, and dyspnea if present in high concentration.[29] In the present study, we measured the concentrations of airborne contaminants to determine whether they increased over time and whether they were highest on the days considered to be the dirtiest.

Rabbit allergens are of interest because they are present on smaller particles of dust, occur in higher concentrations, and persist in the air longer than are allergens of other common laboratory animals, such as mice and rats.[51] There are at least 2 major rabbit allergens—Ory c 1 and Ory c 2—and several minor allergens.[51] Ory c 1 is found in rabbit saliva, urine, and dander, whereas Ory c 2 is found in hair, urine, and dander.[10,64] Similar to mouse, rat, dog, and cat allergens, rabbit allergens are members of a family of low-molecular–weight carrier proteins called lipocalins, which are necessary in eliciting an IgE antibody response.[10] In rabbits, the most potent and allergenic extract identified by radioallergosoerbent assays, a test to determine a person's level of IgE (and therefore allergy) to a specific substance, is saliva. Protein electrophoresis and immunoblotting have identified 12 protein bands in rabbit saliva,[5] which contains 2 lipocalin allergens of 18 (Ory c 1) and 21 kDa.[5] Although various rabbit allergens have been characterized,[50,51,63] a rabbit allergen assay is not yet commercially available. The present study uses a detectable rabbit salivary protein (RSP) as a marker for rabbit allergen because of the ease of obtaining saliva from rabbits and measuring the same-sized protein deposited on the filters used to sample air in rabbit rooms.

The primary objectives of this study were to quantify the concentration of particulate, endotoxin, ammonia, carbon dioxide, and RSP detectable in a laboratory animal facility room that houses rabbits and in which excreta pans are changed weekly and cages are changed biweekly. A secondary objective was to determine contaminant emission factors for estimating airborne contaminant levels associated with projected room conditions.[11-13,58-62]

Materials and Methods

Animals. Airborne contaminant sampling was performed in a room housing New Zealand White rabbits (*Oryctolagus cuniculi)* obtained from a commercial specific pathogen-free vendor (Harlan, Indianapolis, IN) for use on a long-term cardiology research study. Prior to arrival for the study, the animals were tested quarterly and had negative serology for *Encephalitozoon cuniculi*, *Treponema cuniculi*, *Clostridium piliforme*, myxoma virus, calicivirus, and *Toxoplasma* spp.; had negative culture results for pathogenic enteric and respiratory bacteria; and were negative for all endo- and ectoparasites. Rabbits were weighed on day –6 and at the conclusion of the sampling period. Two additional adult specific pathogen-free New Zealand White rabbits were obtained from a commercial vendor (Covance, Madison, WI) for saliva collection. Quarterly health status surveys indicated that these animals were negative for *Pasteurella multocida*, *P. pneumotropica*, *T. cuniculi*, *C. piliforme*, cilia-associated respiratory bacillus, *Salmonella* spp., *Yersinia pseudotuberculosis*, *Listeria monocytogenes*, *Francisella tularensis*, *E. cuniculi*, *Eimeria steidae*, *Toxoplasma gondii*, *Passalurus ambiguus*, *Taenia pisiformis*, *Psoroptes cuniculi*, *Cheyletiella parasitovorax*, *Listrophorus gibbus*, Shope fibroma virus, Shope papilloma virus, myxoma virus, and dermatomycosis. Rabbits were housed individually in stainless steel cages (63.5 × 76.2 × 40.6 cm) with slatted floors and excreta pans with nonantibiotic-impregnated crepe-paper pads lined with polyvinyl (Omni-Pad, Harlan, Madison, WI) in an AAALAC-accredited animal facility. Rabbits were fed 130 g of a commercial pelleted diet daily (High-fiber Rabbit Diet 2031, Harlan, Madison) and were allowed ad libitum access to water via an automatic watering system. Cardboard tubes (Armbrust Paper Tubes, Chicago, IL) were provided at each cage change for nonnutritive environmental enrichment.

Two female Hartley guinea pigs (Charles River Laboratories, Kingston, NY) were certified by the vendor to be free of: Sendai virus, pneumonia virus of mice, reovirus, lymphocytic choriomeningitis virus, *E. cuniculi*, guinea pig adenovirus, *Bordetella bronchiseptica*, *Helicobacter* spp., *Streptococcus zooepidemicus*, *S. moniliformis*, *S. pneumoniae*, *C. piliforme*, *Mycoplasma pulmonis*, and all endo- and ectoparasites. Guinea pigs were housed in a different room but in the same vivarium as the rabbits. Guinea pigs were pair-housed and maintained on contact hardwood bedding (Beta-chip, Northeastern Products, Warrensburg, NY) changed twice weekly, fed commercial guinea pig diet (Guinea Pig Diet 7006, Harlan Teklad), and provided with polyvinyl chloride pipes to hide in. Animals were maintained according to the recommendations in the *Guide for the Care and Use of Laboratory Animals*;[38] the University of Illinois–Chicago's Animal Care Committee approved all experimental animal procedures.

Room and husbandry conditions. Rabbit cages were changed biweekly, and excreta pans changed weekly. Pans were removed from the cages inside the room, placed on a tray rack, and moved to the cage wash area (located in a separate area of the building), where they were emptied. When cages were changed, a clean rack was brought into the room, rabbits were moved to their new cages, and the dirty rack and pans were moved to the same cage wash area. The room was stocked with 5 stainless steel rabbit racks, each containing 6 individual rabbit cages, for a total of 30 rabbits in the room. Rabbits were maintained in a suite consisting of an anteroom and the rabbit room (Figure 1). The anteroom is used as a staging area for donning personal protective equipment. Air pressure was positive from the rabbit room to the anteroom, and air pressure in the anteroom was negative to the corridor. The total volume of the rabbit room was 36.37 m^3. In the anteroom, a circular supply plenum (diameter, 61.0 cm) was located in the ceiling, and rectangular plenums (90.1 × 54 cm) were located in the 2 open, empty cubicles. For exhaust, a rectangular exhaust grille (13.3 × 18.4 cm) was located approximately 16 cm from the ground on each of 3 walls. In the rabbit room, a circular supply plenum (diameter, 106.7 cm) was located in the ceiling, and a single exhaust grille (13.3 × 18.4 cm) was located 16 cm from the ground. During the sampling period, air supply and exhaust flow rates were measured (balometer model 6465, Alnor Instrument Company, Skokie, IL) in the rabbit room daily at 1600. The velocity of air exiting from the animal housing room to the anteroom was measured daily at the center and bottom gaps between the doors by using a portable air-velocity meter (Velocicalc 8350, TSI, Shoreview, MN) and was added to the exhaust airflow rate. The rabbits, racks, cages, and sampling equipment were the only items in the animal housing room during sampling.

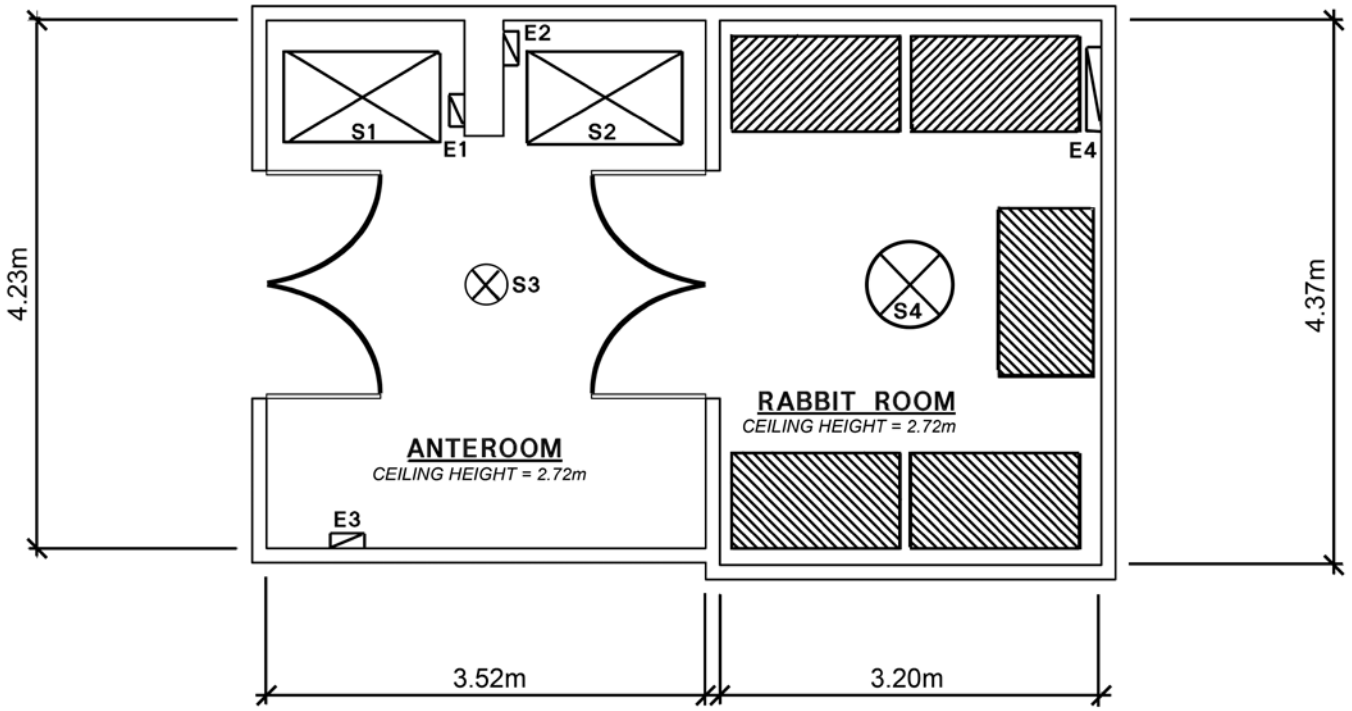

Figure 1. Room layout. The anteroom is where staff donned personal protective equipment. The rabbit room is where rabbits were housed. S1 through 4 represent air supply plenums located in the ceiling; E1 through 4 represent air exhaust grilles located on the wall near the ground. The air supply is conditioned outdoor air with no recirculation. S1 and E1 and S2 and E2 are located in open Illinois cubicles. Shaded rectangles in rabbit room represent rabbit racks, each housing 6 rabbits. Air sampling for supply plenums occurred at S4 and for exhaust grilles occurred at E4.

Contaminant screening. Room characterization and airborne contaminant sampling were conducted over a 2-wk period. In light of the cage- and rack-changing schedules, days 1 and 8 were designated as the cleanest days. Excreta pans were changed on day 1, and cages were changed on day 8. Alternatively, days 7 and 14 were considered to be the dirtiest because these were the days immediately preceding the cage and excreta pan change days, respectively. Samples were collected from the air streams of the supply plenum and the exhaust grille. For 2 wk during the normal cage change schedule, particulate, RSP, and carbon dioxide samples were collected daily. Endotoxin and ammonia concentrations were measured on 4 occasions during the 2-wk period: on study days 1 (day 7 of cage change cycle, day 1 of excreta pan use), 7 (day 14 of cage change, day 7 of excreta pan use), 8 (freshly changed cage and pan) and 14 (day 7 of cage and excreta pan use). New sampling devices were deployed between 0800 and 1000, were checked at 1600, and were changed at 0800 to 1000 on the following day. Each sample was collected for approximately 24 h. Exact start and stop times were recorded, and air sampling flow rates were checked at the start, midpoint, and end of the sampling by using a precalibrated rotameter (Gilian Instrument, West Cladwell, NJ).

To determine emission rates of the airborne contaminants, an experimental mass balance (EMB) model was used and is represented as equation 1:

$$S = \sum q_i C_i - \sum q_j C_j \qquad \text{Eq. 1}$$

where S is the source mass rate of emission (mass/time), q_i is the volumetric flow rate through the door and exhaust grille of the rabbit room (volume/time), C_i is the mass concentration of contaminants at the exhaust grille (mass/volume), q_j is the volumetric flow rate through the supply plenum (volume/time), and C_j is the mass concentration of contaminants at the supply plenum (mass/volume).

An emission factor is determined by using S and dividing by the number of animals. Applying this model requires measuring airflow and the concentrations of all contaminants at all entry and exit points for the space. The EMB model has been used to determine emission rates for offset printing, candy glazing, and a publication rotogravure press[58,60,61] and can be applied to animal facilities. The EMB requires measures of the contaminant entering and leaving the space. The difference between the rates of a contaminant leaving and entering the space is the emission rate into the space. The emission rates measured in this study were for a room containing 30 rabbits, and the emission factors are the emission rates normalized to the number of animals. Although the emission rate (mass of contaminant per time) is specific to the space in which it is measured, the normalized emission factor (mass of contaminant per animal) is transferable to other rooms with different numbers of animals or different ventilation rates.

To predict concentration in different animal housing paradigms, the completely mixed space (CMS) equation must be applied and is represented, at steady state, as equation 2:

$$C_{ss} = \frac{E}{kq} \times R \qquad \text{Eq. 2}$$

where C_{ss} is the steady state concentration (mass/volume), E is the emission factor (mass/time × rabbit), k is the mixing efficiency, q is the volumetric air-flow rate (volume/time), and R is the total number of rabbits.

The CMS model has been used to predict airborne concentrations in similar models.[11-13,58-62] A room's mixing efficiency is a unitless number in the range of 0 to 1. Mixing efficiency

is calculated by dividing the effective air-change rate by the mechanical air-change rate. The effective air-change rate was determined by releasing a tracer gas (carbon dioxide) in the rabbit room with cages and animals present and charting the decrease in tracer gas concentration to the baseline concentration,[3,18] whereas the mechanical air-change rate was determined by the rate of air flow at the supply plenum and exhaust grilles. Because rabbits have natural circadian rhythms and might emit contaminants at different rates at various times of the day, we applied the EMB and CMS models to concentrations that were a 24-h time-weighted average. In addition, integrated samples of dust, endotoxin, allergen, and ammonia were collected for 24 h to ensure sufficient mass for analysis. Rabbits were always present in the room; therefore they are used as a constant source of emission. When people entered the room for this study or for animal husbandry or experimental manipulation, these times points were recorded and excluded from the CO_2 data analysis to determine the concentration and emission attributed to rabbits. These models were applied with the assumptions that there was a constant source of emissions, thorough mixing of air throughout the room, a constant and measured room volume, and a measure of the air flow throughout the space. A sling psychrometer was used to determine dry and wet bulb temperatures daily at 1600. A psychrometric calculator was used to determine relative humidity.

Occupational exposure limits are designated by the Occupational Safety and Health Administration (OSHA) when establishing a permissible exposure limit or a legally binding limit to the concentration of compound (that is ammonia, particulates not otherwise regulated, and carbon dioxide) that can be in the air during an 8-h time-weighted period.[33] Alternatively, limits are established by the American Conference of Governmental Industrial Hygienists (ACGIH), which establishes threshold limit values based on the toxicity of different chemicals to humans or animals.[2]

Particulate collection. Total suspended particulate (hereafter, particulate) was collected daily on an open-face Teflon filter (diameter, 37 mm; pore size, 1.0 µm; SKC, Houston, TX) for 24 h at a nominal flow rate of 10 l/min by using a calibrated air-sampling pump. Open-face filters were used because they were unlikely to be damaged during sampling and because open-face sampling gives a more uniform distribution of particles on the filter, resulting in less opportunity for sample loss during handling. Field blanks were deployed on each sampling day. Each filter was desiccated for 72 h and weighed (Microbalance MC-5, Sartorius AG, Goettingen, Germany) prior to and after sampling. Each filter was weighed a minimum of 3 times on 2 separate dates to achieve a standard deviation less than 0.005 mg. The method used was a modification of NIOSH Method 0500.[37] The limit of quantification for these samples was 0.015 mg. After gravimetric analysis, filter samples were processed individually for RSP quantification.[5,41,50,63]

RSP quantification. Two rabbits were sedated with ketamine hydrochloride (30 mg/kg IM; 100 mg/ml Ketaset, Fort Dodge Animal Health, Madison, NJ) and received sterile-filtered USP-grade pilocarpine (Sigma–Aldrich, St Louis, MO) diluted with sterile water and injected at a dose of 2 mg/kg. Saliva was collected into sterile microfuge tubes as it dripped from the mouth. A standard curve was established by measuring the absorbance of RSP at various concentrations of saliva. Absorbance values that fell between 0.2 and 1.4 µg were within the linear section of the curve and were considered valid.

The RSP measured in room samples was specifically identified by antisalivary protein antibody made in guinea pigs. Guinea pigs were sedated with a 10:1 fentanyl:valium combination. Fentanyl (50 µg/ml, Sublimaze, Taylor Pharmaceuticals, Decatur, IL) and diazepam (5 mg/ml, Abbott Laboratories, North Chicago, IL) were mixed together immediately prior to SC injection at an anesthetic dose of approximately 0.6 to 1 mg/kg fentanyl and 6 to 10 mg/kg diazepam. Once sedated, the dorsum of the guinea pigs was clipped free of hair and prepped with repeated (3 times) povidone iodine and alcohol swabs. The first immunization consisted of 50 µg of 18,000-kDa RSP mixed with sterile water for injection (0.5 ml) and 0.5 ml Freund Complete Adjuvant. Subsequent immunizations consisted of the same concentration of RSP and incomplete Freund Adjuvant. The total volume injected was 0.5 ml ID per guinea pig in 0.025-ml quantities and a single 0.05-ml quantity injected SC in the flank. The interval between immunizations was 3 wk. Guinea pigs were checked daily for adverse reactions to the adjuvant, and none were noted. Seven days after the second immunization, guinea pigs were sedated again with the above anesthetic regimen, and bled (less than 5 ml) via the anterior vena cava. Serum was analyzed for the presence of anti-RSP antibody via a Western blot. Guinea pigs were immunized one more time to achieve a high titer and were bled terminally 7 d after the last immunization. The terminal bleed occurred via cardiocentesis under anesthesia with 60 mg/kg ketamine hydrochloride (Ketaset, Fort Dodge Animal Health) and 10 mg/kg xylazine hydrochloride (20 mg/ml, Rompun, Bayer Animal Health, Shawnee, KS). Death was confirmed through cessation of heartbeat and respiration after exsanguination.

The same filters used to determine particulate concentration were analyzed to quantify RSP.[5,41,50,63] After particulate concentration was determined, filters were cut into small pieces, placed in an microfuge tube with 1200 µl of 15 mM sodium carbonate buffer at pH 9.6, and placed in an ultrasonic cleaner for sonication (model 100005, Sper Scientific, Scottsdale, AZ) for 1 h. A 200- or 400-µl portion of the extract was removed and dried by vacuum evaporation. Extracted protein from the dust samples, a positive control saliva sample collected from a rabbit (described earlier; 0.5 µl, equivalent to approximately 0.2 µg of protein), and known quantities of bovine serum albumin were run on a 4% to 12% Bis Tris gel (Invitrogen Life Technologies, Carlsbad, CA) with 2-(N-morpholino)ethanesulfonic acid as the liquid phase in the chromatography. The gels were silver-stained, and the optical density of each sample was determined by comparison with samples of known concentration of saliva and bovine serum albumin by phosphoimaging (FX Pro Plus Multi-imager System, BioRad Laboratories, Hercules, CA).

Endotoxin. Endotoxin was sampled on polycarbonate membrane filters (diameter, 37 mm; pore size, 0.45 µm)[16] in endotoxin-free polystyrene cassettes (Zefon International, St Petersburg, FL). Endotoxin concentration was measured at the supply and exhaust locations on days 1, 7, 8, and 14. A field blank was deployed on each sampling day. Filter samples were extracted with 2.5 ml of 0.005% Tween 20 in pyrogen-free water and glassware, with shaking. Extracts were vortexed for several minutes. The quantity of endotoxin in a 50-µl sample was determined by using a chromogenic *Limulus* amebocyte lysate assay (Associates of Cape Cod, East Falmouth, MA).[16] Concentrations were reported in endotoxin units (EU) per volume of sampled air (EU/m^3) and converted to ng/m (10 ng/EU).[34]

Ammonia. Ammonia was measured by using passive samplers filled with reactive glass-fiber filters impregnated with citric acid which trap and determine NH_3 concentration (Ogawa and Company USA, Pomona Beach, FL).[53] Ammonia concentration was measured for 24 h on days 1, 7, 8, and 14. Field blanks

were deployed on each sampling day. Analysis was performed by using ion chromatography (RTI International, Research Triangle Park, NC).

Carbon dioxide. CO_2 concentration at supply and exhaust locations was monitored (Q-trak Monitor, TSI) every 10 s for the duration of the study. This monitor was calibrated immediately prior to study start and on the final day of the study by using 1000 ppm CO_2 (supplied by the manufacturer).

Data analysis. Contaminant concentrations and emission factors were determined for particulate, RSP, and CO_2 for 14 study days and for ammonia and endotoxin on 4 study days. Concentration and emission factors are expressed as mean ± SD. Statistical testing was conducted by using natural log-transformed values for all contaminants except CO_2. Two-tailed, parametric, paired *t* tests were used for comparing days considered to be cleanest (days 1 and 8) and days considered to be dirtiest (days 7 and 14) to determine whether there were statistically significant differences ($P \leq 0.05$) in concentration or emission at these time points. Regression analysis was applied by regressing contaminant concentration on days 1 through 7 and days 8 through 14 for a total of 7 d each week to test for statistically significant ($P \leq 0.05$) positive slope indicating increasing concentrations or emissions of particulate, RSP, or CO_2. SAS software (SAS Institute, Cary, NC) was used for all analyses.

Results

Animals. The same rabbits remained in the room throughout the 2-wk sampling period. Rabbit weight (mean ± SD) was 3.88 ± 0.326 kg before study and 3.78 ± 0.327 kg at study conclusion. Rabbit body weight did not change significantly over the course of the study.

Room and husbandry conditions. Cages were changed as described in the Methods, and ventilation measurements indicated that mean mechanical air-change rate was 30.3 air changes/h in the animal housing space; mean effective air-change rate was 19.13 air changes/h with a mixing efficiency (*k*) of 0.622. Average dry bulb temperature was 22 °C (range, 21.1 to 23.9 °C). Mean relative humidity was 45% (range, 34.5% to 62.6%).

Contaminant screening. ***Particulate.*** Daily particulate concentrations are shown in Figure 2; mean particulate concentration was 0.019 mg/m^3. Due to laboratory error, the filters on days 2 and 3 were not weighed after sampling; therefore those dates were excluded from particulate analysis. All weighed samples had measured particulate that exceeded the limit of quantification. The mean particulate emission factor was 7.77 µg/(min × rabbit) (range, 4.62 to 11.2 µg/[min × rabbit]). There were no statistically significant differences between clean (days 1 and 8) and dirty (days 7 and 14) days for particulate emission.

Occupational exposure limits for each of the contaminants of interest are shown in Table 1. There are no established occupational exposure limits for particulate matter containing allergens. Measured particulate matter concentrations were below the OSHA permissible exposure limits for particulates not otherwise regulated[33] and below the ACGIH threshold limit value for particulates not otherwise classified.[1]

RSP quantification. A gel showing the molecular weight of RSP in rabbit saliva and an example of a particulate sample are shown in Figure 3. The effect of the sonication method was determined by eluting a known amount of protein on filters, sonicating, running on a gel, silver-staining, and measuring optical densities by using a phosphoimager. Protein recovery was 97%. RSP concentrations are summarized in Figure 4. The mean RSP concentration was 34.9 ng/m^3 (range, 6.0 to 61.0 ng/m^3). The mean RSP emission factor was 21.0 ng/(min × rabbit) (range, 3.11 to 36.2 ng/[min × rabbit]). There were no statistically significant differences between clean (days 1 and 8) and dirty (days 7 and 14) days for RSP emission. Regression analysis revealed no statistically significant relationship between particulate and RSP levels throughout the study.

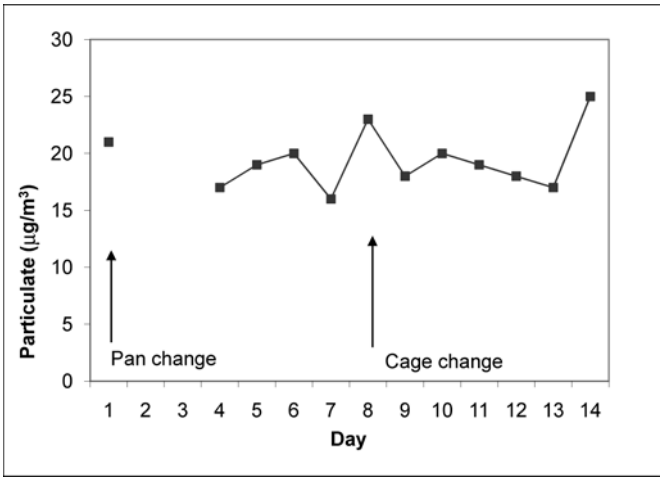

Figure 2. Particulate concentration over time.

Table 1. Occupational Safety and Health Administration permissible exposure limits[33] and American Conference of Governmental Industrial Hygienists threshold limit values[2] for measured airborne contaminants

Compound	Permissible exposure limit	Threshold limit value
Particulate	15 mg/m^3	10 mg/m^3
Ammonia	50 ppm	25 ppm
Carbon dioxide	5000 ppm	5000 ppm

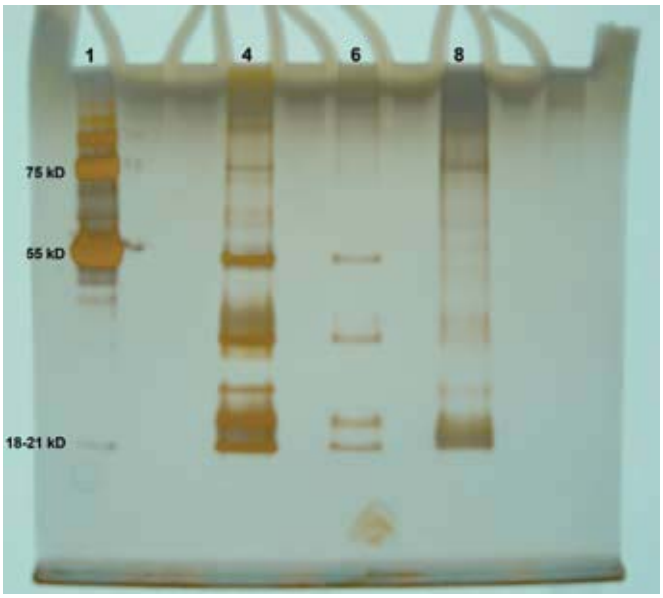

Figure 3. Silver-stained protein gel comparing rabbit saliva to dust samples obtained during study. Note rabbit salivary protein at 18 to 21 kDa in both samples. Lane 1, 10 µg bovine serum albumin; lane 4, 1 µg saliva; lane 6, 0.1 µg saliva; lane 8, 100 µg particulate sample 6.

Endotoxin. Endotoxin concentrations are presented in Figure 5. The mean endotoxin concentration was 0.47 ng/m^3 (range,

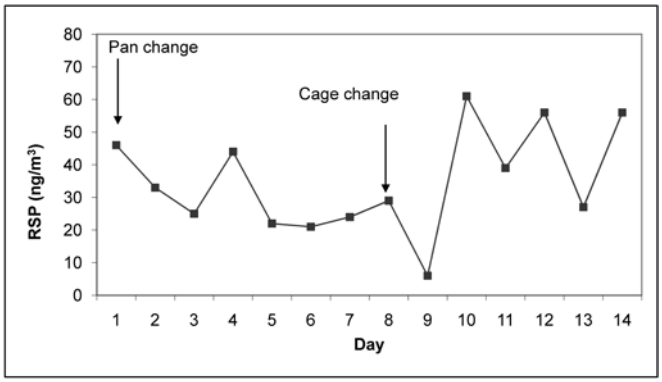

Figure 4. Rabbit salivary protein (RSP) concentration over time.

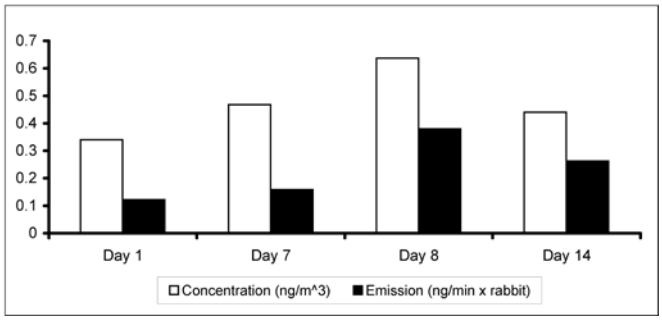

Figure 5. Endotoxin concentration and emission factors by day of study.

0.340 to 0.637 ng/m^3), and the mean emission factor was 0.231 ng/(min × rabbit) (range, 0.122 to 0.380 ng/[min × rabbit]). There were no statistically significant differences between clean (days 1 and 8) and dirty (days 7 and 14) days for endotoxin emission. OSHA and ACGIH have not set limits for endotoxin levels, but the Dutch Expert Committee on Occupational Standards recommends an occupational exposure limit of 50 EU/m^3 (5 ng/m^3) measured as 8-h time-weighted average.[22] Endotoxin concentrations measured in this study were below the Dutch standard (Figure 5).

Ammonia. Ammonia concentrations and emission factors are presented in Figure 6. The mean ammonia concentration was 1.64 ppm (range, 0.25 to 3.06 ppm), and the mean emission factor was 0.718 mg/(min × rabbit) (range, 0.107 to 1.350 mg/[min × rabbit]). Ammonia emission was significantly (P = 0.0161) higher on days considered dirty (days 7 and 14) versus days considered clean (days 1 and 8). Ammonia concentrations measured in this study were below OSHA and ACGIH limits (Table 1). However, ammonia has a detectable odor at concentrations well below the OSHA and ACGIH limits, at 0.04 to 53 ppm,[8] and ammonia levels in the current study exceeded odor detection thresholds.

Carbon dioxide. CO_2 concentrations are summarized in Figure 7. The mean CO_2 concentration was 915 mg/m^3 (508 ppm). A statistically insignificant, albeit detectable, increase in CO_2 concentrations in room supply and exhaust air occurred over the study period. The exhaust air concentration of CO_2 increased at a greater rate than did the supply air concentration during both weeks. The mean CO_2 emission factor was 86.4 mg/(min × rabbit) (range, 61.4 to 122.0 mg/[min × rabbit]). CO_2 emission did not differ significantly between clean and dirty days. Measured CO_2 concentrations were below OSHA and ACGIH limits (Table 1).

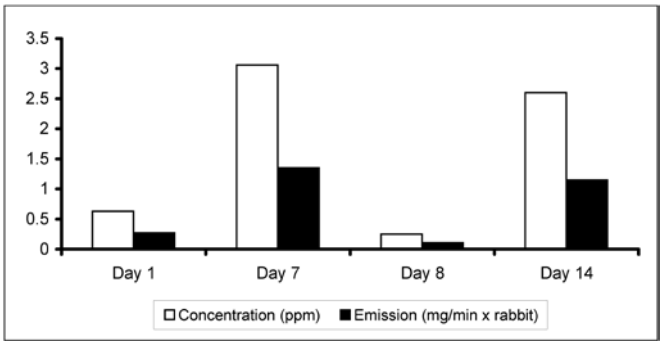

Figure 6. Ammonia concentration and emission factors by day of study.

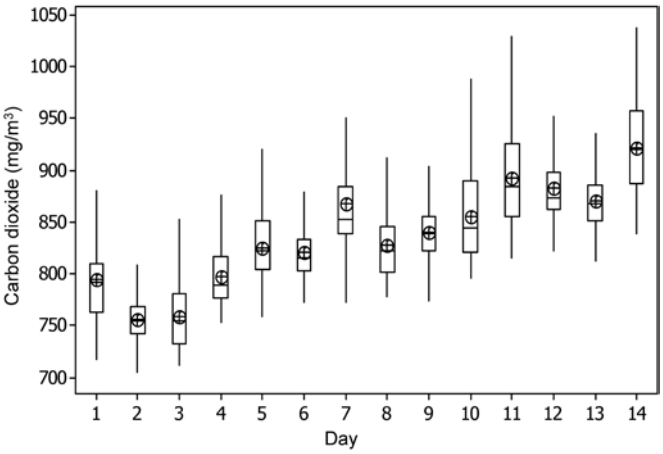

Figure 7. CO_2 concentration box plot. Box represents the interquartile range (IQR) of measured concentration over the course of a day, whiskers represent +/- 1.5 x IQR. The horizontal line within the box represents the median and the X enclosed with a circle represents the mean for each day of study.

Discussion

We successfully sampled and characterized the air in a rabbit room in which excreta pans and cages were changed at the same frequencies as used for other rooms in our facility. The airborne contaminants we sampled in the rabbit room were chosen in light of published evidence indicating that some of these contaminants are important occupational health concerns and because they are considered to be airborne contaminants that linger in the air of rooms where rabbits are housed (that is, ammonia and endotoxin). Some contaminants at high concentrations, like particulate, may help adsorb and concentrate the odors that are being produced, therefore magnifying both health and odor concerns.[7]

Allergen levels have been assessed and evaluated for various species housed in laboratory animal facilities, but such reports typically focus on mouse and rat rooms.[17,32,41,46,54] Our study assessed a prominent allergenic rabbit salivary protein, RSP, as a marker of rabbit allergen. This method proved to be simple and reliable. Our results found no relationship between time of the week and environmental exposure to RSP. RSP levels in the room increased over the 2-wk sampling period but not statistically significantly. Because our data were limited to the length of the sampling period, a useful follow-up would be to confirm the results in another 2-wk sampling period.

Increased allergen and particulate levels are risks for many reasons. Personnel sensitized to rabbit allergens may be at risk of developing allergic symptoms such as flu-like symptoms,

contact dermatitis, and occupational asthma, which can cause job absences or a change from working in the laboratory animal field.[10,64] The allergen level that presents a risk to personnel is difficult to predict and varies from person to person. HEPA-filtered ventilated cages for rabbit housing have recently been commercially developed, whereas mice and rats have been housed in filter-topped cages or individually ventilated cages for many decades. Therefore, rabbits housed conventionally may present a greater risk to personnel than do mice and rats housed in filter-topped caging.[17] In addition, particulate is an allergen risk because respirable particles can enter deep into a worker's respiratory tract. The ACGIH threshold limit values (Table 1) may not protect workers when the particulate matter contains allergens. The ACGIH specifically states the threshold limit value applies to "particles that do not have an applicable threshold limit value; are insoluble or poorly soluble in water… and have low toxicity (that is, are not cytotoxic, genotoxic, or otherwise chemically reactive with lung tissue, and do not emit ionizing radiation, cause immune sensitization, or cause toxic effects other than by inflammation or the mechanism of 'lung overload')."[1] Particulate generated from animals, bedding, feed, or waste is likely to be water-soluble and is known to cause immune sensitization, and these limits may not apply. Particulate matter has been speculated to exacerbate atopic asthma[20] as well as odor.[7] Therefore, particulate at high concentration would be both a health risk and a nuisance. Particulate matter concentrations in the current study were less than those detected in a similar study involving airborne contaminants in a rabbit room.[27] This difference may be attributed in part to the large air exchange rate in our rabbit room or to the use of crepe paper noncontact bedding, which differed from the wood chip bedding used in the previous study.[27,28] Although particulate levels did not vary over time, we only sampled over a single 2-wk period, and repeating the study would solidify our findings.

The endotoxin present in rabbit rooms necessitates special consideration by occupational health professionals because of its association with respiratory symptoms in nonmouse-sensitized research personnel exposed to laboratory mice.[42] Endotoxin was shown to increase airway hyperresponsiveness to histamine and lower lung function in sensitized farmers.[48,57] However, the role of endotoxin is controversial and may be protective for certain medical conditions.[47] Comparing the endotoxin concentrations we measured with others[42] is difficult because the previous study used personal samplers, whereas we measured overall room concentrations at the exhaust grille. Further, comparing our findings to those of other studies[27,28] is problematic because samples in the previous studies were obtained from 2 stationary sampling sites in locations other than the supply and exhaust plenums. Although difficult to directly compare our results with those in the literature, the endotoxin concentrations and rates subsequently calculated by using the EMB and CMS equations appear consistent with those of previous reports (data not shown).[27]

The ammonia concentrations we measured are similar to those reported in another study conducted in a rabbit room,[27] however in that study, excreta pans were changed twice weekly, cages were changed weekly, and ammonia concentrations were evaluated on days 1 and 2. Our day 7 sample demonstrated 10 times higher ammonia levels than did the cited day 2 samples.[27] In addition, ammonia was the sole compound in our study that was significantly higher on days that were considered dirty versus clean, which reflects increased production of ammonia over time as urease-positive bacteria proliferate in fecal matter. Ammonia concentrations detected in the current study are lower than those reported to have health effects for rodent species,[19,44,45,52] but our ammonia detection was limited to just 4 sampling times that were chosen because they were likely to represent the cleanest and the dirtiest time points in our rabbit room. A follow-up study would include more data points for both ammonia and endotoxin.

Exposure to high concentrations of carbon dioxide has been linked to adverse health effects in both people and rodents.[29,30] In this study, we measured an average CO_2 concentration of 930 mg/m^3 (517.8 ppm). Although we measured exhaust concentrations that were about 2 times higher than our supply concentrations, they were an order of magnitude below the applicable occupational exposure limits (Table 1). Figure 5 appears to show a trend of increasing CO_2 concentration over the study period, with a slight decrease after week 1 followed by a continued increase over week 2; however, this finding was not statistically significant. This result may reflect an atmospheric increase or instrument drift due to extensive usage over 14 d. The Q-trak Monitor (TSI) was calibrated before and after usage but may drift with prolonged usage. This theory is supported by the synchronous patterns of the daily average supply and exhaust CO_2 concentrations. Another theory for the observation is that increasing rabbit-generated CO_2 in room air was influencing the pattern of the observed supply air concentration, despite positioning of the supply air monitor before the air diffuser in the fresh-air supply duct.

In this study, we determined the concentrations of airborne contaminants (particulate, endotoxin, ammonia, carbon dioxide, and RSP) in a rabbit room during a 2-wk period. In addition, the concentrations of airborne contaminants at the supply plenum and exhaust grille of the rabbit room were used in a well-established experimental mass balance equation[11-13,58-62] to calculate an emission factor for the respective airborne contaminants. The report of emission factors of these contaminants in a rabbit room is novel, and a possible application of these emission factors is to extrapolate data on airborne contaminants under different housing conditions. To illustrate the usefulness of emission factors, we applied our measured ammonia emission factor to a room housing 30 rabbits that had decreasing air-change rates, to illustrate how expected room concentrations might be calculated by using equation 2 (Table 2). In the first example, we include our actual room conditions, and as the ventilation rate decreases and animal numbers remain constant, ammonia levels increase. In another scenario, we illustrate how equation 2 can be used to estimate emission concentrations for a room twice the size of ours with 15 air changes hourly and different rabbit housing densities (Table 3). Because we have doubled the room size and halved the air-change rate, 30 rabbits still result in a predicted ammonia concentration of 4.7 ppm, as was detected in our rabbit room. As is demonstrated in these examples, managers and occupational health specialists can use emission factors to manage housing density and husbandry practices in a rabbit facility and to determine the potential for staff exposure to airborne contaminants. We used ammonia for the examples because this was the compound that increased significantly on days considered dirty, but all of the compounds for which an emission factor was determined can be substituted for ammonia and applied in this model. When comparing calculated to actual concentrations, equation 2 overestimates actual ammonia exposure concentration because we are using the value calculated when ammonia was highest, day 7, just prior to cage change. When doing an occupational health risk assessment, overestimation, rather than underestimation, of potential exposure risk is preferable. However, the use of our calculated emission

Table 2. Example of using ammonia emission factor to predict concentration for various air change rates

Volumetric air flow rate (q; m^3/min)	Air change rate[a]	Predicted room concentration (ppm)
18.4	30.3 (study conditions)	4.7
12.1	20	7.1
9.1	15	9.4
6.1	10	14.1

For this example, the following parameters are constant: room volume, 36.37 m^3; mixing factor, 0.622; number of rabbits, 30; emission factor, 1.35 mg/(min × rabbit) (worst-case condition, determined on day 7 before cage or tray change).
[a]Calculated as volumetric air flow rate divided by room volume.

Table 3. Example of using ammonia emission factor to predict concentration for various rabbit housing densities

Total no. of rabbits	Predicted room concentration (ppm)
10	1.6
20	3.1
30	4.7
40	6.3
50	7.9
60	9.4

For this example, the following parameters are constant: room volume, 72.7 m^3; volumetric air flow rate, 18.19 m^3/min (15 air changes hourly); mixing factor, 0.622; emission factor, 1.35 mg/(min × rabbit) (worst-case condition, determined on day 7 before cage or tray change).

factors in these scenarios has not been validated by actual measurements taken under these specific room conditions. A useful follow-up study would be to validate these predictions. However, the current data introduce the concept of emission factors and their potential usefulness. The model can be adjusted for different volumetric air flows, mixing factors, volumes, or rabbit densities; however, the emission factors presented here are for rabbits that are housed in individual, open cages with noncontact bedding and are fed 130 g of high-fiber diet daily. In addition, humidity extremes can affect emission factors by increasing or decreasing contaminants such as particulate;[25] however, the mean humidity level during this sampling period was 45% which is nearly midrange of the *Guide*'s recommendations (30% to 70%).[38]

Few studies have investigated airborne contaminants in rabbit rooms in a laboratory animal environment,[27] and to our knowledge, no reports have thoroughly documented the room ventilation and airborne contaminant emission factors. We found that airborne contaminants were below the current environmental health guidelines and standards, including those set by OSHA and ACGIH, for particulates, endotoxin, ammonia, and carbon dioxide in a rabbit room at the given population density, husbandry, and ventilation conditions and the weekly pan change frequency. Our results show that with regard to minimizing allergen levels, cage change frequency is not as important as other engineering controls that are present in laboratory animal facilities, such as having an air handling system with a high air-change rate. Moreover, given the results of this study, we determined emission factors by using a simple mass balance equation, which can be used to approximate airborne contaminant levels in laboratory animal facilities housing rabbits under similar husbandry conditions. These findings likely will be useful in managing and designing facilities for the maintenance of rabbits as well as for ensuring that environmental exposure limits for specific airborne contaminants are not exceeded.

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