Characterization and Quantification of Microenvironmental Contaminants in Isolator Cages with a Variety of Contact Beddings

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Abstract | Microenvironmental contaminants were measured within isolator-type cages housing DBA/1J mice on 8 contact beddings. Each cage contained 850 cm^3 of bedding and 5 mice randomized by body weight. Seven cages with and 2 to 4 cages without mice were evaluated per bedding. Macroenvironmental conditions were defined and controlled. Macro- and microenvironmental temperatures, relative humidity, and carbon dioxide and ammonia concentrations were determined daily during each of 3 seven-day test periods. An air sampling pump and detector tubes were used to measure hydrogen gas, 2-butanol, acetone, ethanol, carbon monoxide, acetic acid, hydrogen sulfide, sulfur dioxide, and formaldehyde on the final day of each test period. In addition, gas chromatographic analysis was used on the seventh day to detect additional volatile alcohols and ketones. Ammonia concentrations ranged from 0 to 410 ppm, depending on the bedding type and day of measurement. On the basis of the mean microenvironmental ammonia concentration in the cages with mice, the beddings were ranked from highest to lowest in ammonia generated: aspen shavings, pine shavings, reclaimed wood pulp bedding, virgin pulp loose bedding, hardwood chip bedding, recycled paper bedding, virgin cellulose pelleted bedding, and corn cob bedding. The temperature, relative humidity, and carbon dioxide concentration were similar between beddings. No other contaminants were detected except acetic acid (mean = 0.86 ppm) in the cages with and without mice containing corn cob bedding. Sulfur dioxide (mean = 0.42 ppm) was only detected in cages with mice and corn cob bedding. In summary, the concentration of ammonia generated varied significantly in cages containing mice and different contact beddings.

Isolator caging systems provide individual animal microenvironments and aid in the development and maintenance of specific-pathogen-free rodents for use in research. These caging systems are cost effective and allow containment at the cage level without expensive equipment. Although the filter tops may protect rodents from contamination with adventitious microbes (1–5), intracage airflow may be restricted, thus allowing the accumulation of prohibitively high concentrations of waste gases and humidity (6–11). High humidity prevents fecal and urinary desiccation and provides the optimal milieu for bacterial proliferation and subsequent ammonia production. Ammonia has been detected at amounts documented to impact physiologic systems (10, 12–21). Temperature, relative humidity, carbon dioxide, and ammonia are routinely evaluated microenvironmental parameters. There are potentially numerous fermentation gases produced by bacterial flora in excrement that may accumulate within the cage. These products may reach concentrations that alter physiologic and immunologic function (15–18, 20, 21). The effects of the microenvironment on laboratory animals and on experimental results are, however, controversial, which has made it increasingly difficult to establish comprehensive environmental guidelines for housing rodents.

Contact beddings should provide a physiologically stable and stress-free environment for the animals. The selection of contact beddings should be based on optimal characteristics. Ideally, beddings should be chemically and biologically inert, highly absorptive, nontoxic, dust-free, compatible with the research study, and inexpensive.

The purpose of this study was to characterize and quantify microenvironmental contaminants in isolator cages and evaluate the effects of eight commercially available contact beddings on microenvironmental conditions.

Materials and Methods

Overall study design: Eight contact beddings were evaluated to determine their effect on microenvironmental conditions in isolator cages housing five mice of uniform body weight. Micro- and macroenvironmental temperature, relative humidity, and carbon dioxide and ammonia concentrations were determined on each day of a 7-day test period. On the seventh day hydrogen gas, 2-butanol, ethanol, acetone, carbon monoxide, acetic acid, hydrogen sulfide, sulfur dioxide, and formaldehyde concentrations were determined. In addition on the seventh day, mass air samples were obtained from each cage for gas chromatographic analyses for volatile gases and ketones. Selection of evaluated contaminants was based on the expected fermentation products generated by the gastrointestinal tract flora of the mouse. The analyses were conducted over 7-day test periods. The study was run in triplicate so that seven cages containing mice per bedding were evaluated. Additionally, 2 to 4 cages per bedding without mice were included to analyze for the presence of contaminants in the head space that may be off-gassed from the beddings. A total of three of the cages with mice and two of the cages without mice containing corncob bedding were further analyzed to confirm the presence of acetic acid and sulfur dioxide in the head space, using National Institute of Occupational Safety and Health (NIOSH)-approved methods #1603 and #6004, respectively.

Animals: Two-hundred female DBA/1J mice (*Mus musculus*) (Jackson Laboratory, Bar Harbor, Maine) were studied. Mice were specific-pathogen-free and had a conventional (undefined) gastrointestinal tract flora. Mice were randomized according to biomass and were rerandomized for each test period. Five mice were housed per experimental cage. Total biomass (mean ± SEM, 68.8 ± 1.46 g) per cage was below the standards for housing density recommended by the *Guide* (21). Statistical analysis verified that there was no significant difference among biomass means between cages. Mice were fed a commercial diet (Prolab 3000, Agway, Inc., Syracuse, N.Y.) and provided distilled water in water bottles ad libitum.

Macroenvironment: The animals were housed in a holding room measuring 13.50 x 13.50 x 7.92 feet in an AAALAC-accredited animal facility incorporating a clean/dirty corridor system. The HVAC system was a constant volume, terminal reheat type with direct steam humidification. Only animals and caging under study were housed in the holding room. Ventilation provided fifteen changes of 100% fresh air per h. Differential pressures provided directional airflow so

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