Evaluation of Cage Micro-Environment of Mice Housed on Various Types of Bedding Materials

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A variety of environmental factors can affect the outcomes of studies using laboratory rodents. One such factor is bedding. Several new bedding materials and processing methods have been introduced to the market in recent years, but there are few reports of their performance. In the studies reported here, we have assessed the cage micro-environment (in-cage ammonia levels, temperature, and humidity) of mice housed on various kinds of bedding and their combinations. We also compared results for bedding supplied as Nestpaks versus loose bedding. We studied C57BL/6J mice (commonly used) and NOD/LtJ mice (heavy soilers) that were maintained, except in one study, in static duplex cages. In general, we observed little effect of bedding type on in-cage temperature or humidity; however, there was considerable variation in ammonia concentrations. The lowest ammonia concentrations occurred in cages housing mice on hardwood bedding or a mixture of corncob and alpha cellulose. In one experiment comparing the micro-environments of NOD/LtJ male mice housed on woodpulp fiber bedding in static versus ventilated caging, we showed a statistically significant decrease in ammonia concentrations in ventilated cages. Therefore, our data show that bedding type affects the micro-environment in static cages and that effects may differ for ventilated cages, which are being used in vivaria with increasing frequency.

Outcomes of studies with laboratory animals can depend heavily on multiple environmental factors. In the case of rodents, one such factor is the type of bedding. Physiologic changes may occur after exposure to some types of bedding and could affect experimental results. Some bedding generates dust and particulates that might cause respiratory or ocular changes. Bedding that is very absorbent could reduce operating costs.

In 1980, Kraft (1) published a review of bedding available at that time for laboratory rodents. She listed several desirable characteristics of bedding—among other characteristics, it should be moisture absorbent, inedible, non-traumatic, nontoxic, readily available, relatively inexpensive, non-deleterious to cage washers, and free of dust and splinters. At the time the article was published, white pine shavings were the most commonly used rodent bedding. It was known at that time, however, that cedar and white pine shavings should not be used as bedding for rodents used in pharmacologic studies. Some other rather exotic bedding had been assessed: hay (edible) and peat moss, newsprint, and alfalfa (stained animals' coats). Pelleted peanut hulls had recently been introduced to the market but had not been fully evaluated. Their absence from today's market suggests that they were not satisfactory. Natural products are prone to variability and, perhaps, microbial or chemical contamination.

Dr. Kraft ended her review with the following questions: "Taking into account scientific, economic, humane, and legal aspects of laboratory animal bedding, is there agreement that there should be standards for bedding? And who will do the work in order to obtain the results on which the standards are to be based?" (1). Recently available products such as cellulose, corn cob, recycled paper, and Nestpak bedding are gaining popularity but may not have been fully evaluated. For example, variations in absorbency can affect both in-cage humidity and the microflora that convert urea into ammonia. Here we report an assessment of the environment—temperature, relative humidity, and ammonia concentrations—within cages housing NOD/LtJ or C57BL/6J mice on several bedding types or combinations thereof.

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Materials and Methods

Mice. We obtained eight-week-old NOD/LtJ (NOD) and C57BL/6J (B6) male mice as well as pairs of B6 breeder mice from JAX Research Systems (JRS; Bar Harbor, Maine). All JRS colonies are regularly monitored for and are free of 15 viruses (mouse hepatitis virus, two mouse parvoviruses, reovirus, Theiler's mouse encephalomyelitis virus, ectromelia virus, mouse rotavirus, thymic virus, pneumonia virus of mice, Sendai virus, murine cytomegalovirus, lactic dehydrogenase-elevating virus, K virus, mouse adenovirus, and polyoma virus), 17 bacterial species (including *Helicobacter* spp.), two *Mycoplasma* spp., external and intestinal parasites, and *Encephalitozoon cuniculi*. In addition, fecal samples from the mice were tested for *Proteus* species prior to study and were negative. Mice were housed in static polycarbonate duplex cages (floor space, 51.7 in^2) with loose-fitting Reemay filters (Reemay 2033, Thoren Caging Systems, Inc., Hazleton, Pa.). One study compared ammonia concentrations in static versus positively ventilated cages at an anemometer reading of 0.025 Pascals (0.0001 in. of water). The duplex cage (Thoren Caging Systems, Inc.) is divided into two pens with wire-rod tops to hold the water bottles and diet for each pen. In each duplex cage, one side held four B6 or NOD male mice or a litter of B6 mice with both parents. The mice were provided ad libitum with acidified water (pH 2.8 to 3.1, monitored continuously) and pelleted 5K52 (modified from the NIH 31M open formula; 6% fat) diet (PMI Nutrition International, Brentwood, Mo.) that was autoclaved at 100°C for 58 min. The room in which the mice were housed was supplied with HEPA-filtered air at 19 air changes per hour and was maintained at a temperature (mean ± standard error) of 22 ± 2°C, relative humidity of 35% ± 4%, and a 14:10-h light:dark cycle. Bedding was autoclaved and changed after 3 weeks, except where noted in the Results. Manipulation of cages occurred in a Maxi-Miser (Thoren Caging Systems, Inc.) mobile ventilated cage-changing station. The bedding types, sources, manufacturers and/or distributors, and amounts used per cage are given in Table 1. Except for pine shavings, the bedding amounts were based on manufacturers' recommendations.

Micro-environmental monitoring. For each experiment described below, in-cage temperature, relative humidity (RH), and ammonia concentrations were measured using an INNOVA multi-gas analyzer

