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Corey E. Becker [a] , Carolyn F. Mathur [a] & Bradley G. Rehnberg [a]

[a] Department of Biological Sciences , York College of Pennsylvania

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The Effects of Chronic Exposure to Common Bedding Materials on the Metabolic Rate and Overall Health of Male CD-1 Mice

Corey E. Becker, Carolyn F. Mathur, and Bradley G. Rehnberg
Department of Biological Sciences, York College of Pennsylvania

Anecdotes and personal Web pages claim that cedar and pine beddings cause respiratory distress in rodents, although no previous research could be found to support these claims. There have, however, been published studies of respiratory distress in cedar and pine mill workers. That research links exposure to wood dust to asthma and to bronchial and alveolar damage in humans. This study looks at the effects of 3 types of bedding (CareFRESH®Original, cedar, and pine) on the growth, food intake, oxygen consumption, IgE antibody concentrations, and general appearance and behavior in male CD-1 mice. Mice who were housed on these beddings for approximately 4 months did not show significant differences in any of these variables. This suggests that these 3 materials provide equally healthy substrates for long-term rearing of mice and possibly other rodents.

Anecdotes and personal Web pages claim that cedar and pine beddings cause respiratory distress in rodents such as hamsters, mice, guinea pigs, and rats. A national pet store chain has even stopped stocking cedar bedding. However, there are no published studies that have looked for a causal relationship between bedding and respiratory health in rodents. Until now, the most relevant research has found a link between respiratory disorders in humans and working in cedar and pine mills and other lumber industries.

Correspondence should be sent to Bradley G. Rehnberg, Department of Biological Sciences, York College of Pennsylvania, York, PA 17405–7199. Email: brehnber@ycp.edu

Occupational asthma is a common disease of sawmill employees working with western red cedar (*Thuja plicata;* Chan-Yeung, 1987, 1994; Frew, Chan, Lam, & Chan-Yeung, 1995). It is believed that the asthma is caused by a sensitization to plicatic acid, an aromatic hydrocarbon that makes up 40% of the nonvolatile components of this species of tree (Chan-Yeung, 1994; Frew et al., 1995). The asthma persists even after ceasing exposure to the dusts in the sawmills in at least 60% of patients (Frew et al., 1995).

Asthma caused by plicatic acid sensitization has also been shown to cause an increase in allergen-IgE antibodies (Chan-Yeung, 1994). Along with asthma, western red cedar dust has been linked with chronic bronchitis (Ayars, Altman, Frazier, & Chi, 1989). Ayars et al. filled the lungs of recently euthanized rats to capacity with plicatic acid solutions in vivo. They found that plicatic acid at 1 mg/mL causes destruction of the basal cell layer of the bronchial epithelium. They also observed $10.2 \pm 3.3\%$ lysis of rat lung cells at a concentration of 0.1 mg/mL of plicatic acid in vitro.

Ayars et al. (1989) also performed these two experiments with abietic acid, a constituent found in pinewood. This acid also causes lysis of rat lung cells, in vitro, as well as destruction of both bronchial epithelium and alveolar epithelium in vivo. However, it showed higher levels of lung injury, both in vitro and in vivo, than plicatic acid at similar concentrations. This shows that abietic acid is potentially more hazardous to lung tissue than plicatic acid.

Cartier et al. (1985) found that exposure to plicatic acid from wood dust can cause an increase in specific IgE antibody levels in humans. The immune system releases IgE antibodies in response to inflammation of the respiratory tract, which is a prerequisite for asthma (Randolph, Carruthers, Szabo, Murphy, & Chaplin, 1999). In addition, measurements of mouse IgE levels are used to assess sensitivity to various respiratory allergens (Sailstad et al., 1998).

Even though there have been no formal studies on the link between respiratory health and beddings, there have been several studies that show certain beddings may contain carcinogenic agents or have negative effects on the liver of rodents. Schoental (1973) discovered that spontaneous nasal tumors developed in mice and rats who were housed on red cedar or pine beddings. These tumors were linked to the phenolic aldehydes found in the bedding. Vesell (1967) found that mice, when kept on red cedar bedding, slept less and had increased levels of liver drug-metabolizing enzymes.

This study looks at the effects of cedar and pine beddings on the growth, food intake, oxygen consumption, and IgE antibody levels in mice and general appearance and behavior. CareFRESH®Original bedding, made of reclaimed paper, is an alternative to wood bedding materials and was similarly evaluated. The manufacturer of CareFRESH®Original claims it is healthier than other beddings and free from aromatic compounds that are found in cedar and pine beddings (CareFRESH®Original Pet Bedding, 2007). Because there have been

few direct in vivo evaluations of bedding on the respiratory well being of rodents, this study tested five null hypotheses. We propose that there will be no effect of bedding type on the following:

1. Rates of food intake,
2. Increases in body weight,
3. Rates of oxygen consumption,
4. Levels of IgE antibodies in blood, and
5. General appearance and behavior of the mice.

METHODS

This study examines possible effects of three types of bedding (CareFRESH® Original, cedar, and pine) on the growth, food intake, oxygen consumption, IgE antibody concentrations, and general appearance and behavior in male CD-1 mice. Thirty healthy, 35-day-old male CD-1 mice were purchased from Charles Rivers Laboratories and housed in the York College animal room. Care of nonhuman animals and their housing was in accordance with institutional guidelines. Upon arrival, each mouse was placed into its own plastic cage (19.1 cm × 29.2 cm × 12.7 cm) with a microbarrier lid (Allentown Caging Equipment Company, Inc.) to intensify exposure to volatiles and particulates by reducing air exchange with room air. Mice were fed LM Animal Farms Classic Mouse and Rat Food (www.petmountain.com). For the last 3 weeks of the study, however, they were fed Kaytee Forti-Diet for Mouse, Rat, & Hamster (www.kaytee.com). Food and water were given ad libitum. Mice were housed in a 12L:12D photoperiod, and room temperature was 24.93°C (SEM 0.27).

For the first 19 days, the mice were housed on newspaper bedding. During this time, the first metabolic rate readings were recorded, and the first blood collections were performed to obtain control levels of IgE antibodies. Sufficient blood was obtained from 13 of the 30 mice to serve as IgE controls. Monitoring of weight gain and food intake also began; these measurements continued to be monitored weekly throughout the experiment. On the 19th day, each mouse was randomly assigned to one of three permanent bedding conditions: CareFRESH®Original, cedar, or pine ($n = 10$ mice per condition). The cedar bedding was Premier Pet Aromatic Red Cedar Bedding; the pine was Premier Pet Natural Pine Bedding (www.premier.com). All beddings were changed weekly.

Metabolic rates were determined by measuring oxygen consumption using an open-flow gas exchange system manufactured by Qubit Systems. A mouse was placed in a sealed chamber for 6 to 10 min, depending on air flow speed and the size of the mouse. A probe measured the steady state level of oxygen

inside the chamber, which permitted calculation of oxygen consumption in mL O_2/g/min. Metabolic rates were measured periodically over 16 weeks.

Blood was collected from the tail just prior to being put into the bedding conditions (on the same day) and then 1, 2, and 4 months after being put into the bedding conditions. A razor blade was used to make a small vertical cut in a blood vessel on the distal end of the tail. The 1-month period between collections was adequate for the incisions to fully heal. The blood was collected with a heparinized tube and then emptied into a microcentrifuge tube. The blood was then stored frozen (−20°C) until testing.

The IgE antibody levels were determined with the Mouse IgE ELISA-Immunoperoxidase Assay for Determination of IgE in Mouse Sera purchased from Immunology Consultants Laboratory, Inc. Results were read by a Wallac 1420 Multilabel Counter and interpreted with Wallac 1420 Manager V 3.00 Software.

Statistics were performed with Graphpad Prism 5 software. Two-way ANOVAs with repeated measures were used to compare the bedding conditions over time for all variables measured.

RESULTS

The mice showed consistent growth across the three bedding conditions over 20 weeks (Figure 1). There were no significant differences in body weight based on bedding condition, $F(2, 513) = 0.20$, $p = .8174$, but there was a significant difference with respect to time, $F(19, 513) = 168.33$, $p < .0001$. The interaction between the bedding conditions and time was not significant, $F(38, 513) = 0.55$, $p = .9883$.

There were no significant differences in oxygen consumption based on bedding condition, $F(2, 216) = 0.29$, $p = .7494$, but there was a significant difference based on time, $F(8, 216) = 17.80$, $p < .0001$ (Figure 2). The control readings at the beginning of the study were the lowest for all three groups. Once mice were placed on their test bedding, weight-specific metabolic rates gradually increased to Week 16. The interaction between the bedding conditions and time was not significant, $F(16, 216) = 0.75$, $p = .7380$. After removing the Week 0 data from the ANOVA, there were still no significant differences based on bedding condition, $F(2, 189) = 0.32$, $p = .7324$, and there was still a significant difference seen over time, $F(7, 189) = 5.20$, $p < .0001$.

There was a gradual decline in the weight-specific consumption of food eaten over time until Week 16 (Figure 3). The clear increase in the amount of food eaten in Weeks 17 and 18 correlates with the change in the brand of food from LM Animal Farms Classic to Kaytee Forti-Diet. There were no significant differences seen between the bedding conditions, $F(2, 486) = 0.60$, $p = .5572$, but there was a significant difference seen with respect to time, $F(18, 486) =$

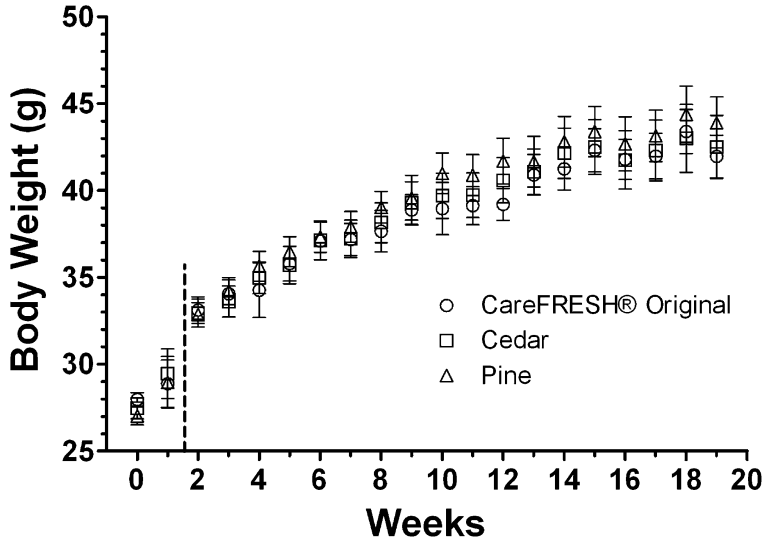

FIGURE 1 Mean weight (g) and standard error of the mean for mice in CareFRESH® Original ($n = 10$), cedar ($n = 10$), and pine ($n = 10$) beddings. Body weights at Weeks 0 and 1 were collected before the mice were put into the three bedding conditions. The vertical line separates before and after being put in the bedding conditions.

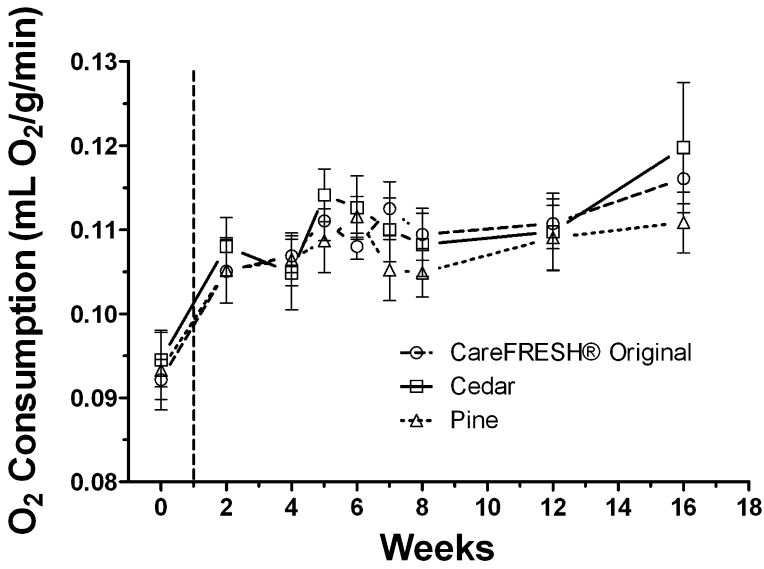

FIGURE 2 Mean ($n = 10$) oxygen consumption and standard error of the mean by mice in CareFRESH® Original, cedar, and pine beddings. Oxygen consumptions at Week 0 were collected before the mice were put into the three bedding conditions.

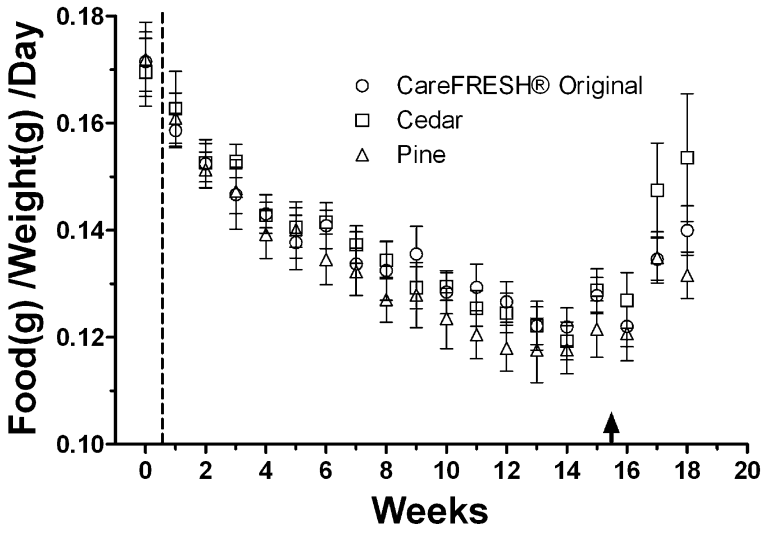

FIGURE 3 Mean grams of food eaten per gram of body weight per day and standard error of the mean for mice in CareFRESH® Original ($n = 10$), cedar ($n = 10$), and pine beddings ($n = 10$). Food consumption for Week 0 was calculated with data collected before the mice entered the bedding conditions. The arrow indicates when the food was switched from LM Animal Farms Classic Mouse and Rat Food to Kaytee Forti-Diet for Mouse, Rat, & Hamster food.

47.46, $p < .0001$. The interaction between the bedding conditions and time was not significant, $F(36, 486) = 0.91$, $p = .6297$.

Pine, cedar, and CareFRESH® Original beddings were evaluated over a period of 4 months for possible effects on IgE antibody levels. A two-way ANOVA of IgE data involved the three test beddings as one factor and time in months as the second factor. There were no significant differences in mean IgE levels between the bedding conditions, $F(2, 52) = 1.88$, $p = .1733$, and there were no significant differences based on time, $F(4, 52) = 1.18$, $p = .3293$ (Figure 4).

CareFRESH® Original and cedar data were quite variable over the 4-month period, whereas pine had the lowest and most consistent levels from month to month. The control condition involving shredded newspaper was used in housing all animals before the bedding comparisons began. The controls were not carried through the 4-month experimental period and were therefore not involved in the two-way ANOVA. However, separate one-way ANOVAs were used to compare the controls with each bedding condition over time. There were no significant differences between the control and CareFRESH® Original, $F(3, 39) = 0.33$, $p = .8009$, control and cedar; Kruskal-Wallis, KW = 4.77, $p = .1894$; and control and pine, Kruskal-Wallis, KW = 1.18, $p = .7569$.

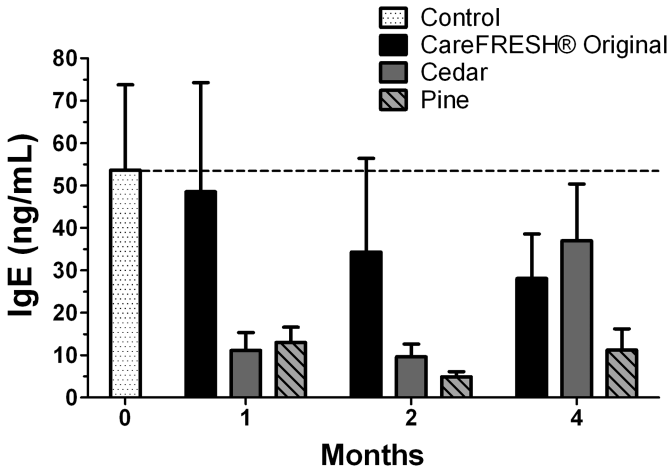

FIGURE 4 Mean IgE antibodies (ng/mL) and standard error of the mean for mice reared in CareFRESH®Original, cedar, and pine at 1, 2, and 4 months as well as for mice reared in the control bedding. All sample sizes (n) are 10 except for the control ($n = 13$) and pine ($n = 9$). The dashed line projects the control IgE antibody concentration.

There were no observed differences in the manner of use of the beddings for any of the conditions. All the mice slept on or in their bedding. Some of the mice would push their bedding into a large pile and burrow into it for a nest, whereas others would leave the bedding evenly spread in the cage. This, however, did not seem to be related to the type of bedding material. All mice in the three conditions used the same areas of their cages for their urinary and fecal waste. This area was located at the end of the cage where they had access to their water and food. All mice used the opposite end of the cage for their sleeping area.

Finally, respiratory distress was not observed for any of the groups of mice throughout the experiment. Mice were never seen sneezing or breathing irregularly. All the mice appeared to be healthy throughout the course of the experiment, which is consistent with the steady growth observed in all three groups.

DISCUSSION

The null hypotheses of this study could not be rejected. Bedding condition did not cause significant differences in body weight, food intake, metabolic rate, immune response, or general appearance. The significant increases in body

weight and the decreases in weight-specific food consumption during the 4-month study period were expected (Hill, Wyse, & Anderson, 2004). This shows that different bedding conditions do not cause mice to grow at different rates or eat different amounts of food. The increase in food consumption seen in all mice at the end of the study probably reflects either a preference for Kaytee Forti-Diet over LM Animal Farms Classic or is a temporary response to novel food.

The most apparent and consistent change in oxygen consumption occurred between the time the mice were kept on newspaper and when they were placed into their bedding conditions. After this point, there were no significant differences between the bedding conditions, although there was a significant difference seen over time. There is no clear explanation for a lower metabolic rate when living on newspaper bedding.

The newspaper was not very absorbent and needed to be changed every few days to prevent a strong buildup of ammonia, which is the product of urease-positive bacteria breaking down the urine and feces (Gamble & Clough, 1976). Perhaps living in suboptimal conditions caused the oxygen consumption of the mice to be low, and it increased when they were placed onto more sanitary beddings. The low oxygen consumption could also have been related to the conditions in which the mice were living before they were shipped or to their experiences during shipping, both of which are unknown.

IgE antibody levels also did not significantly differ between the bedding conditions or over time. The overall lack of statistical significance can be attributed to sample size and high data variability. The data for the CareFRESH® Original bedding was particularly variable. Sailstad et al. (1998) reported that individual mouse IgE levels in response to allergens can be highly variable. Although the results were not significant, the pine bedding had the lowest mean values throughout the experiment and appeared to be the least variable. Because the IgE antibody levels for the cedar and pine bedding were not higher than those of the CareFRESH® Original bedding, it is not likely that plicatic acid and abietic acid, as found in resinous beddings, were having an effect on the respiratory tracts of the mice. The highest values were seen from the control conditions when the mice were being housed on newspaper as bedding; IgG antibody levels may show a better relationship between plicatic acid and asthma than IgE antibody levels. Salari, Howard, Chan, Dryden, & Chan-Yeung (1994) found that guinea pigs showed IgG production in response to sensitization to plicatic acid.

Some research has shown that rodents actually have a preference for wood chip beddings over artificial beddings. A study by Lanteigne and Reebs (2006) found that hamsters preferred pine shavings over aspen shavings, corncob, and wood pellets. Iturrian & Fink (1968) found that pregnant mice will give birth on natural flakewood bedding versus commercial cellulose bedding if given the choice. Also, 39% of mice forced to give birth on the cellulose bedding later moved their nests to flakewood bedding.

Even though ammonia levels from urine were not measured in this study, personal observation through smell indicated that ammonia levels were highest when the mice were housed on the newspaper. It is possible that the apparently higher levels of ammonia were adversely affecting the respiratory tract of the mice and causing an allergen IgE immune response. Gamble and Clough (1976) found that ammonia causes damage to both the tracheal and bronchial epithelia. They also found that ammonia levels increase with high heat and humidity, both of which are optimal for bacterial growth. Crook et al. (1991) found that humans have an increase in IgE antibodies when exposed to pig urine. It might be that pine bedding was more able to absorb urine and neutralize ammonia than were cedar and CareFRESH®Original beddings, which resulted in lower mean IgE antibody levels. In future studies of bedding alternatives, humidity should be controlled and ammonia monitored when measuring oxygen consumption by mice.

CONCLUSION

Overall, this research shows that CareFRESH®Original, cedar, and pine are all equally healthy bedding materials for mice with respect to growth, food intake, oxygen consumption, IgE antibody levels, and overall well being for at least a 4-month period. It is probably safe to say that growth, food intake, and oxygen consumption would not begin to differ between bedding groups after a 4-month exposure. The same conclusion about IgE has less certainty because no true trends were observed, and the data were variable. It now appears that, when deciding among CareFRESH®Original, cedar, and pine beddings, choices for mice can be made based on availability, cost, or preference and not necessarily on health concerns. Extending this conclusion to other laboratory rodents will require similar research involving other species.

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