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Effects of Compressed Paper Bedding on Mouse Breeding Performance and Recognition of Animal Health Concerns

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The combination of bedding substrate and nesting material within the microenvironment of mice is an important consideration for animal care programs in regard to optimizing animal wellbeing. We used 3 general or breeding mouse colonies in our institution to evaluate the effects of bedding substrate on nest building, breeding performance, and recognition of animal health concerns. A scoring system was developed to assess the incorporation of bedding into the nest cup base and walls (nest base incorporation, NBI) in a controlled study with mice bedded on either compressed paper (CP) or corncob (CC) bedding. Compared with CC cages, CP cages had higher NBI scores. To determine the influence of bedding type on the recognition of animal health concerns in an animal facility, cages bedded with CC followed by CP were evaluated for the overall frequency of health-concern reports during a 2-mo time frame for each bedding type in a single-subject A-B study design. The frequency of animal health-concern reports was similar in cages using CC or CP bedding. The animal health condition, rather than bedding type, was associated with the severity of the health problem at the initial report. Breeding performance was compared for 6 mo in matched CC and CP cages containing one of 13 genetically modified mouse lines. NBI scores were higher for breeders housed on CP compared with CC bedding. Monogamous breeder pairs housed on CP had significantly higher indexes of breeding performance (measured as the number of pups per dam per week on study) than did CC cages. This report supports the use of CP bedding in the mouse microenvironment to improve general wellbeing by supporting nesting behavior and reproductive performance without hindering the detection of animal health concerns.

Abbreviations: CC, corn cob; CP, compressed paper; NBI, nest based incorporation

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Bedding substrate affects general health and wellbeing in many ways and is an important part of an animal's microenvironment. The basic functions of a bedding substrate include the maintenance of animal cleanliness and appropriate intracage air quality. Bedding should encourage animals to perform species-specific behaviors, such as nest building, thereby enabling animals to control their environment and engage in active thermoregulation.[6,8] Bedding substrates should be compatible with other husbandry-associated activities, including the identification of health concerns by animal care personnel.

Nest building is an important component of the normal behavioral repertoire of mice. Studies consistently have demonstrated that nesting material improves mouse welfare by supporting complex nest building and thermoregulation.[4,5,7] Previous studies focused on the addition of a second material into the cage to encourage nest building, but few studies have examined how the bedding material itself supports nest building. Mice have been shown to prefer a cage containing bedding material over one without bedding, although bedding was preferred less than were areas with nesting material.[21] In addition, increased bedding material size has been associated with better nest building in some mouse strains.[19]

Daily cageside visual inspection of mice by animal-care personnel is common[22] and a regulatory requirement.[8] Bedding substrate provides a visual backdrop for this evaluation to identify health concerns and varies greatly in appearance among materials. For example, corncob (CC) bedding has a mixed color palate, including shades of brown and black, compared with compressed paper (CP) bedding, which is white. The background contrast provided to each cage by the bedding substrate may influence animal assessment, but no studies evaluating this possibility have been published to our knowledge.

Taken together, optimizing the combination of bedding and nesting material within the cage is important to providing high-quality animal care and improving animal welfare. We previously demonstrated that, compared with CC bedding, CP bedding improves the microenvironment for mice in IVC by decreasing intracage ammonia and reduces the frequency at which cages require early changing.[16] Given this benefit, we sought to evaluate the effect of CP bedding on mouse nesting behavior and on the identification of health concerns in the general mouse population. We hypothesized that, compared with CC bedding, CP bedding would improve nesting behavior, improve breeding performance, and allow early identification of common health concerns on cageside examination.

Materials and Methods

Animal Details. All animal activities were approved by the IACUC (PRO00007149, PRO00007537, PRO00009435) at the University of Michigan, an AAALAC-accredited institution. Figure 1 represents all of the studies included and described hereafter. Mice were assessed for pathogen status by surveillance testing

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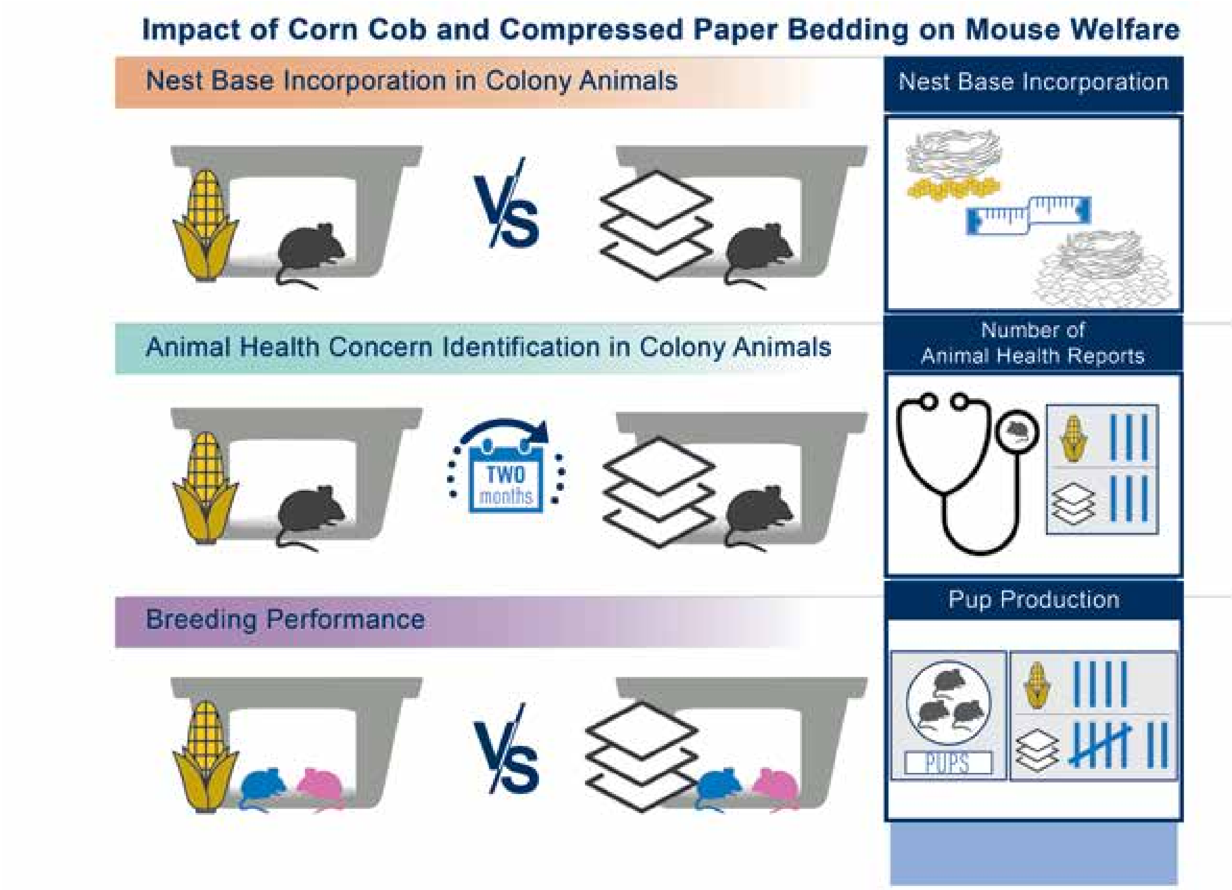

Figure 1. Pictorial representation of the study design and outcome for each of the 3 studies performed to evaluate the effects of bedding substrate, corncob or compressed paper, on mouse welfare.

using dirty bedding sentinels and exhaust air dust for the following pathogenic agents: lymphocytic choriomeningitis virus, mouse adenovirus, *Mycoplasma pulmonis*, Theiler murine encephalomyelitis virus, pneumonia virus of mice, reovirus, Sendai virus, mouse hepatitis virus, minute virus of mice, mouse parvovirus, mouse rotavirus, ectromelia virus, polyomavirus, pinworms, and fur mites. All animals were negative for the listed pathogens for the duration of the study.

Mice were housed in individually ventilated cage (IVC) racks (Allentown, Allentown, NJ) in rooms with ambient air temperatures that were consistent with the *Guide for the Care and Use of Laboratory Animal* (72.0 to 78.2 °F [22.2 to 25.7 to °C]).[8] Animals were fed a complete diet (5LOD or 5008 irradiated rodent chow, LabDiet, St Louis, MO) ad libitum with continuous water access (automated automatic watering system for IVC or glass water bottle for static cages). Water was either filtered or reverse-osmosis-treated prior to being made available to animals. Cages were bedded with 0.3 L of either a mix of 1/4-in. and 1/8-in. CC bedding (Bed-o'Cobs, Andersons, Maumee, OH) or CP bedding (Pure-o'Cel, Andersons). Both bedding substrates were available for routine husbandry at the University of Michigan. In addition, all cages received the standard provision of crinkle paper nesting material: a single, 6-g EnviroPAK (WF Fisher and Son, Sommerville, NJ). Cages were changed every 2 wk, or sooner if the cages were deemed to be more than 25% wet. If not excessively soiled, nest transfers were conducted at the time of cage change without the addition of a fresh EnviroPAK. Typically, cage changes occurred during the morning hours. Data regarding the rate of early cage changes for the various bedding types have been published.[16]

Nest base incorporation (NBI) score. The crinkle paper nesting material in the EnviroPAK allows robust and complex nest construction, including cup walls and a dome.[5] Given that feature and our goal to assess how bedding supports nest building, we developed the NBI score to evaluate how well mice incorporated the bedding into the cup base and walls of the nest structure. NBI scores were determined as follows: 1) no visible bedding incorporation into the base of the nest cup; 2) a bedding base is included in the nest cup but not in the cup-wall; 3) a bedding base is included in the nest cup, with cup-wall incorporation less than 3 cm in height; and 4) a bedding base is present in the nest cup, cup-wall incorporation of at least 3 cm in height. A ruler was held against the side of the cage to help determine when the nest cupping was greater than 3 cm. Figures 2 and 3 provide representative images of each of the NBI scores for CC- and CP-bedded cages, respectively. Each cage was removed from the rack, all aspects of the nest were visualized (including those not adjacent to the cage wall), and a score was determined in real time. A score was assigned based on the overall character of the nest, which was visualized for roughly 30 s. A single reviewer performed NBI evaluations for each study, including the colony animal assessment and the breeding performance studies. Persons who evaluated cages for NBI could not be blind to the type of bedding in the cage due to their differences in appearance. The presence or absence of pups in the nest at the time of NBI was not controlled. To determine interrater reliability, 3 raters scored the same set of 25 pictures of representative cages, and the results were compared across reviewers. For intrarater reliability, 3 reviewers rated the same 25 cages over 3 d, and scores were compared across all 3 d. Across 3 reviewers, intrarater

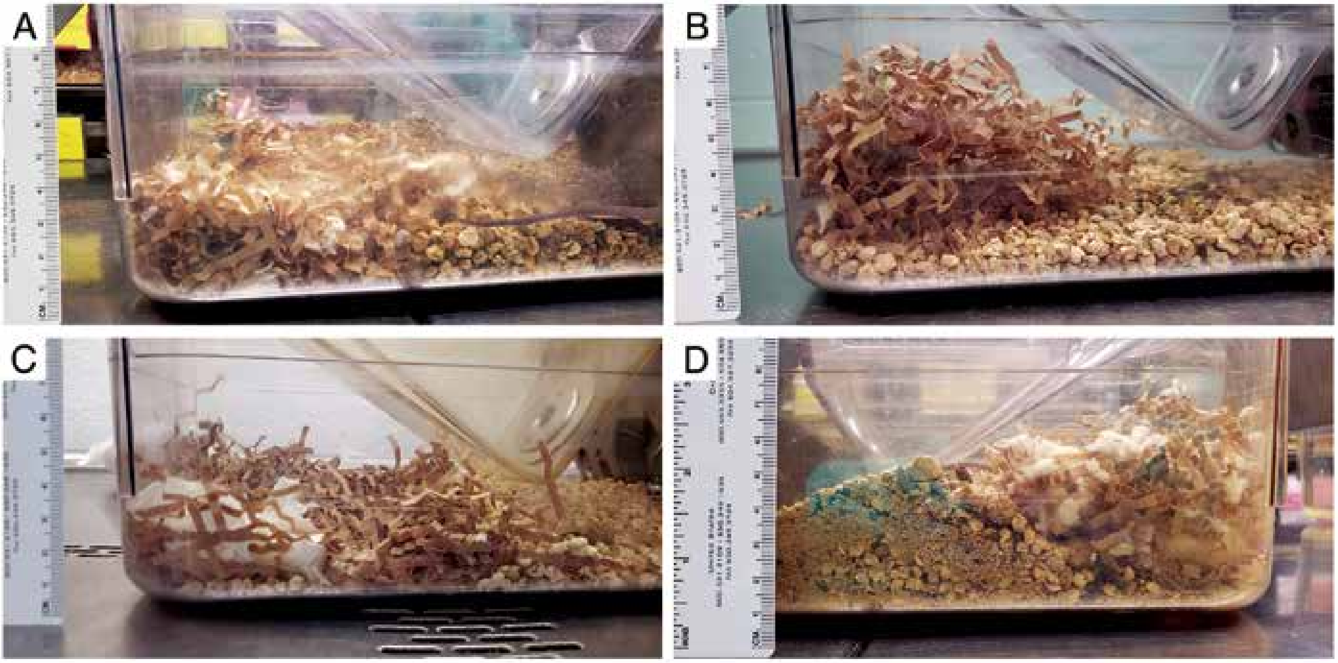

Figure 2. Representative images of nest base incorporation (NBI) scores for CC cages. Images include the area of the nest evaluated as well as the complex nesting material portion of the nest. (A) NBI = 1. (B) NBI = 2. (C) NBI = 3. (D) NBI = 4.

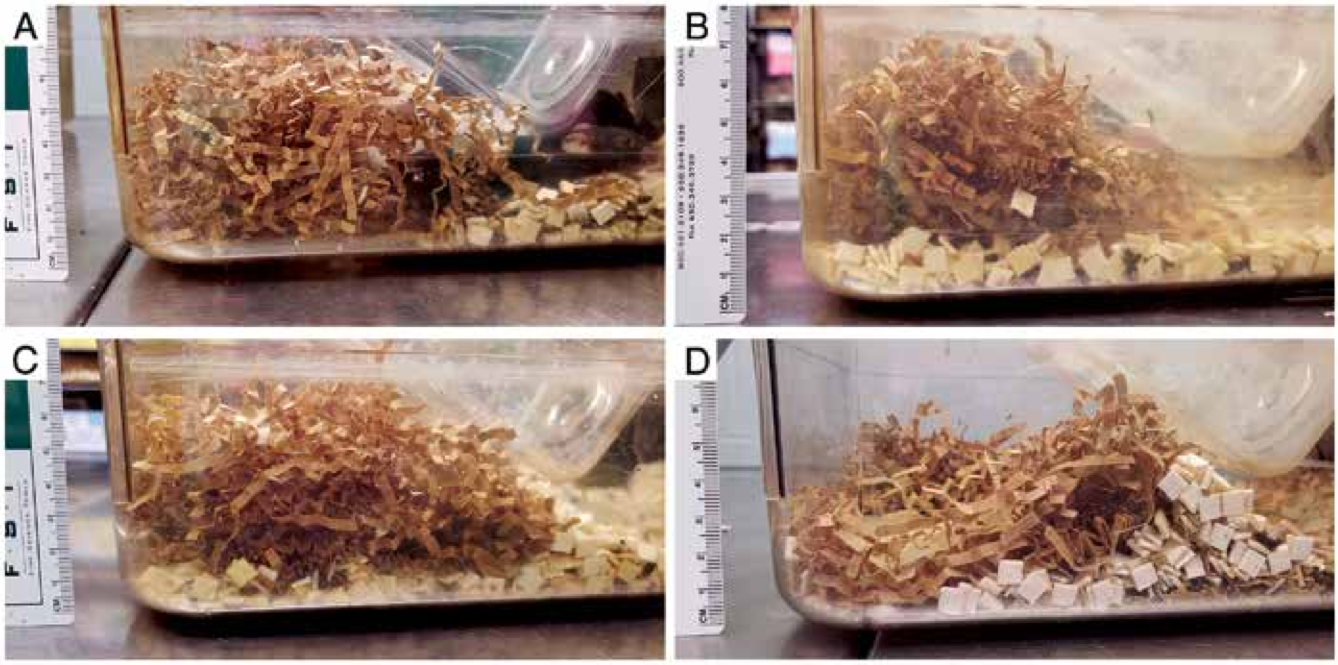

Figure 3. Representative images of nest base incorporation (NBI) scores for CP cages. Images include the area of the nest evaluated as well as the complex nesting material portion of the nest. (A) NBI = 1. (B) NBI = 2. (C) NBI = 3. (D) NBI = 4.

reliability for NBI was 92% and interrater agreement was 93%. An intraclass correlation coefficient was calculated to evaluate interrater reliability. The interclass coefficient was 0.926 with a P value of 2.15×10^{-24} and 95% CI of 0.863 to 0.964.

NBI of colony animals. Mice were bedded with either CC or CP (n = 215 total cages; CC, 107 cages; CP, 108 cages) to evaluate how bedding affected NBI scores. On rare occurrences, no nest structure could be identified within a cage; these cages were excluded from the study. To ensure equal representation from the various categories, evaluated cages were selected to control for the number of mice per cage, sex, relative age (younger than 4 mo or at least 4 mo), and breeding status (Table 1). Male and female mice (*Mus musculus*) of various ages, genotypes, and genetic backgrounds, including but not limited to CD1, BALB/c, C57BL/6 and 129, were used. We were unable to acquire complete genetic and strain information, given that these were colony animals. Regardless of bedding type, all cages were evaluated at 9 to 13 d after cage change to ensure that differences were not due to the amount of time since mice were placed in the cage. Cages were assessed during daylight hours, with both bedding types evaluated simultaneously. Cages were housed in 2 adjacent rooms with similar census numbers that were in

Table 1. Population demographics regarding cages evaluated in the *NBI in Colony Animals* study

No. of mice per cage (including pups)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Corncob	1	13	31	23	24	3	4	2	2	2	1	1	0	0	1
Compressed paper	1	13	31	23	24	3	4	2	2	2	1	1	1	0	0

	No. of cages containing		
	Breeders	Male mice	Female mice
Corncob	21	60	26
Compressed paper	32	47	29

the same vivarium, with both bedding types being used in the rooms. The majority of cages were assessed for NBI score only once; the few cages (*n* = 22) that were sampled repeatedly were controlled for in the statistical analysis.

Identification of animal health concerns. To evaluate the effect of bedding type on the reporting of common animal health concerns, a separate population of colony mice (*n* = 2 to 5 mice per cage) were housed on CC for a period of 2 mo followed by CP as a single-subject design across 34 housing rooms in a single vivarium. Data were collected for 2 mo on CC prior to the bedding transition, followed by an acclimation period; data then were again collected for 2 mo on CP bedding. Two months were provided to allow for acclimation to the new bedding. Animals received once-daily (between 0600 and 1500) cageside visual examinations by trained personnel to identify health concerns per standard procedures. Abnormal findings were reported to veterinary personnel on an animal treatment report that was evaluated within 24 h of the original report. The animal treatment reports contained checkboxes selected by personnel to disclose commonly recognized abnormalities at the time of initial reporting, such as 'fight wounds' and 'dystocia' as well as generalized categories of 'lesions' and 'other.' Census numbers were recorded biweekly by using an electronic barcoding system, and a census for each period was determined as the average of the housing area per month during the observation period (CC month 1, *n* = 5274; CC month 2, *n* = 5206 cages; CP month 1, *n* = 5152; CP month 2, 5074 cages).

A clinical condition scoring system has been developed at our institution to assist veterinary staff in objective assessment of the severity of spontaneous rodent health concerns. A clinical condition score (CCS) is assigned to each animal on presentation and on subsequent health monitoring visits: 1) a minor health concern to be monitored; 2) a health concern without systemic illness that might benefit from veterinary intervention; 3) systemic illness requiring veterinary intervention; 4) systemic illness portending a humane endpoint; and 5) moribund condition requiring immediate euthanasia. Thus, the higher the CCS, the more severe the condition.

During the data collection intervals, animal treatment reports for 3 of the most commonly reported spontaneous health concerns in the general mouse population at the University of Michigan (i.e., dystocia, fight wounds, and ocular abnormalities) were reviewed to evaluate the total number of reports and the severity (according to CCS) of the condition at presentation while housed on each bedding type. The total theoretical number of animal treatment reports possible during the experimental timeframes was determined as the total number of cages multiplied by the census days in a month. Total potential animal treatment reports were 319,668 for the CC period and 317,034 for the CP period.

Breeding performance. The Unit for Laboratory Animal Medicine Breeding Colony manages maintenance breeding of a variety of rodent strains for many investigators at the University of Michigan. All breeding performance assessments were conducted within this service by using cages from investigators that volunteered to participate and remain anonymous. Thirteen lines of genetically modified mice (Table 2) on backgrounds consisting of C57BL/6, B6:129, BALB/c, or FVB were randomized into the different bedding groups involved in the study and housed in 2 rooms within the same vivarium. The names of genotypes were provided by investigators, but we were unable to acquire complete genotype and strain information. Monogamous pair breeding cages were randomized into either CC (*n* = 29) or CP (*n* = 30) groups. NBI scores were assessed twice, separated by 14 d, for breeding cages on CC or CP bedding at 14 d after cage change. To control for historic pup production and to ensure equal distribution for each line, at least one cage of each line or strain was put onto each of the 2 bedding types to control the effect of line or strain variation on reproductive output. All breeding cages were then tracked for 6 mo to assess the numbers of litters and live pups in each litter. Each cage was examined daily, early in the light cycle, for the presence of pups, and the number of live pups was determined as soon as the presence of pups was identified by the care staff and was recorded for that date. Each cage represented an experimental unit. The care staff was blind to the overall purpose of the study but could not be blind to the type of bedding for each cage. The number of weaned pups was not assessed in this evaluation, given that pups from maintenance lines are typically culled prior to postnatal day 21. Breeders generally were retired after 4 or 5 litters and subsequently replaced with new cages using the same bedding type. The final number of cages for each genetic line on the 2 bedding types is presented in Table 2. The breeding performance index was calculated for each breeding female by dividing the total number of pups by the number of weeks on study for each female during the 6-mo time frame. We analyzed the number of litters for each breeder in the study to ensure an equal number of litters in cages bedded with the two bedding types.

Statistical analysis. Visual and statistical analysis was performed using R version 3.4.3 (CRAN) https://cran.r-project.org or GraphPad Prism version 8.3.0 (www.graphpad.com). Sample sizes were not calculated a priori. For all analyses, a *P* value of 0.05 was considered significant. In general, summary values were expressed as means for normal data, while for non-normal data, summary values were expressed as medians with interquartile ranges.

In the *NBI in Colony Animals* study, a cumulative linked mixed-effects model with a logit link function for ordinal regression by using clmm2 from the ordinal package was used to evaluate the effects of bedding and sex on NBI scores.[2,20] Fixed effects included bedding, breeding status, and sex, whereas random effects included cage ID to control for repeated sampling. Animal housing room was evaluated but had no effect on the statistical model.

For the *Identification of Animal Health Concerns* study, to compare differences in the number of animal treatment reports and CCS for mice in cages on CC and CP bedding, cumulative linked mixed-effects models with a logit link function were used. For the model to evaluate changes in total number of animal treatment reports, fixed effects included bedding and random effects included the month sampled. To examine room-level effects in a limited set of conditions, fixed effects included bedding, and random effects included the animal housing room sampled. To evaluate the effects of CC and CP bedding on specific conditions and room-level repeated sampling, fixed effects included animal treatment report condition and bedding, with a random effect of room sampled.

For analysis of NBI scores in the *Breeding Performance* study, a 2-tailed Wilcoxon rank-sum test was used to determine differences in average live pups per litter on the different bedding types. Breeding performance indexes and average pup numbers were compared by using a 2-tailed Student *t* test. For analysis of the average number of pups for each litter number, a 2-tailed Student *t* test was used to compare the average number of pups on each bedding type for each litter number. For litter numbers of 6, statistical analysis could not be performed due to insufficient sample size.

Results

NBI in colony animals. We used the NBI scoring system to evaluate the overall incorporation of bedding material into the nest cup base and wall by mice in our general colony that were housed on either CC or CP bedding. Compared with CC cages, CP-bedded cages had higher NBI scores (Figure 4, $P < 0.001$, $r = 2.9075$). This finding was independent of mouse breeding status, which did not significantly alter NBI ($P = 0.606$, $r = 0.214$).

Identification of animal health concerns. We next examined colony-wide animal treatment reports for mice housed in CC and CP cages. During the 2-mo CC phase, 799 animal treatment reports were submitted, whereas 862 were submitted during the CP phase, there were 862 animal treatment reports across the various potential conditions (Figure 5 A, $P = 0.304$). The actual rate of health reports compared with the potential number of health reports based on census days for the CC phase was 0.25% and for the CP phase was 0.27%. Of the health conditions, the general categorization of 'other' was reported most frequently. No consistent influence of bedding type was detected on the frequency of the health condition reported, with some conditions increasing and others decreasing depending on the bedding type (Figure 5 A). Using the CCS, we then examined the 3 most commonly recognized health concerns that might be managed through veterinary intervention: fight wounds, ocular abnormalities, and dystocia. When controlled for repeated sampling at the room level, bedding did not significantly influence the frequency of animal treatment reports for these selected conditions ($P = 0.090$). At the time of presentation, CCS was significantly different for mice with fight wounds or ocular abnormalities but not dystocia (Figure 5 B; $P = 0.001$, 0.021, and 0.414, respectively; $r = –3.628$, –2.618, and –0.935, respectively). However, CCS across these conditions was not significantly affected by the type of bedding ($P = 0.641$).

Breeding performance. Given the NBI score data from colony animals, we sought to evaluate the effect of bedding on breeder performance in a controlled setting. We first examined whether NBI scores were different in mouse breeding cages in which we could control multiple factors, including strain, similar to the colony mice data. Mice in CP-bedded cages again had significantly higher NBI scores compared with CC-bedded cages (Figure 6, $P < 0.001$; Wilcoxon test statistic = 929). Breeding performance index was significantly higher for breeders housed on CP bedding compared with CC bedding (Figure 7 A, $P = 0.033$, $t = –2.19$). In addition, the average number of pups per litter was significantly higher by 1.3 pups for breeder mice that were housed on CP bedding compared with CC ($P = 0.0317$, $t = –2.21$). Next, we analyzed the average number of pups per litter for each breeder to examine the effect of CP bedding on female mice with various numbers of litters. CP-bedded dams had a higher average number of pups in the first litter compared with CC-bedded cages (Figure 7 B, $P = 0.015$). No significant difference between CC- and CP-bedded cages was detected for litters 2 through 5 ($P = 0.375$, 0.734, 0.687, and 0.975, respectively).

Table 2. Basic genotype information volunteered by investigators in the Breeding Performance study and associated distribution regarding bedding type

	No. of cages	
Genotype	Corncob	Compressed paper
AR flox	2	3
AsCremTmG	2	2
Caspase1 KO	2	2
FVB/N	1	1
IL1F9 KO	1	3
IL27 KO	3	2
IL36R KO	3	3
IPAF KO	5	3
MMTV	2	2
Wisp3 Cross	1	1
TLR5 KO	1	4
TM1a KO	1	1
Wisp3 KO	2	1

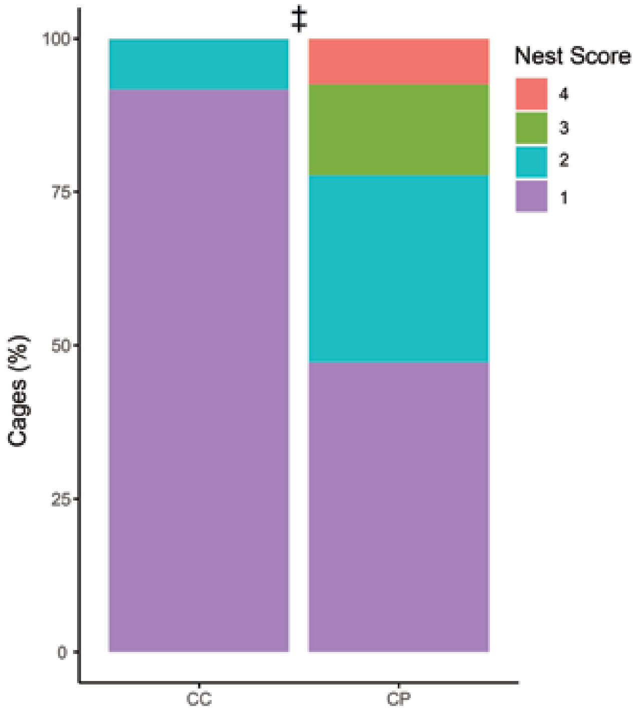

Figure 4. Mice in CP-bedded IVC have higher NBI scores than CC IVE. (A) NBI score for general colony cages bedded on either CC or CP (‡, $P < 0.001$; $r = 2.9075$; cumulative linked mixed-effects model).

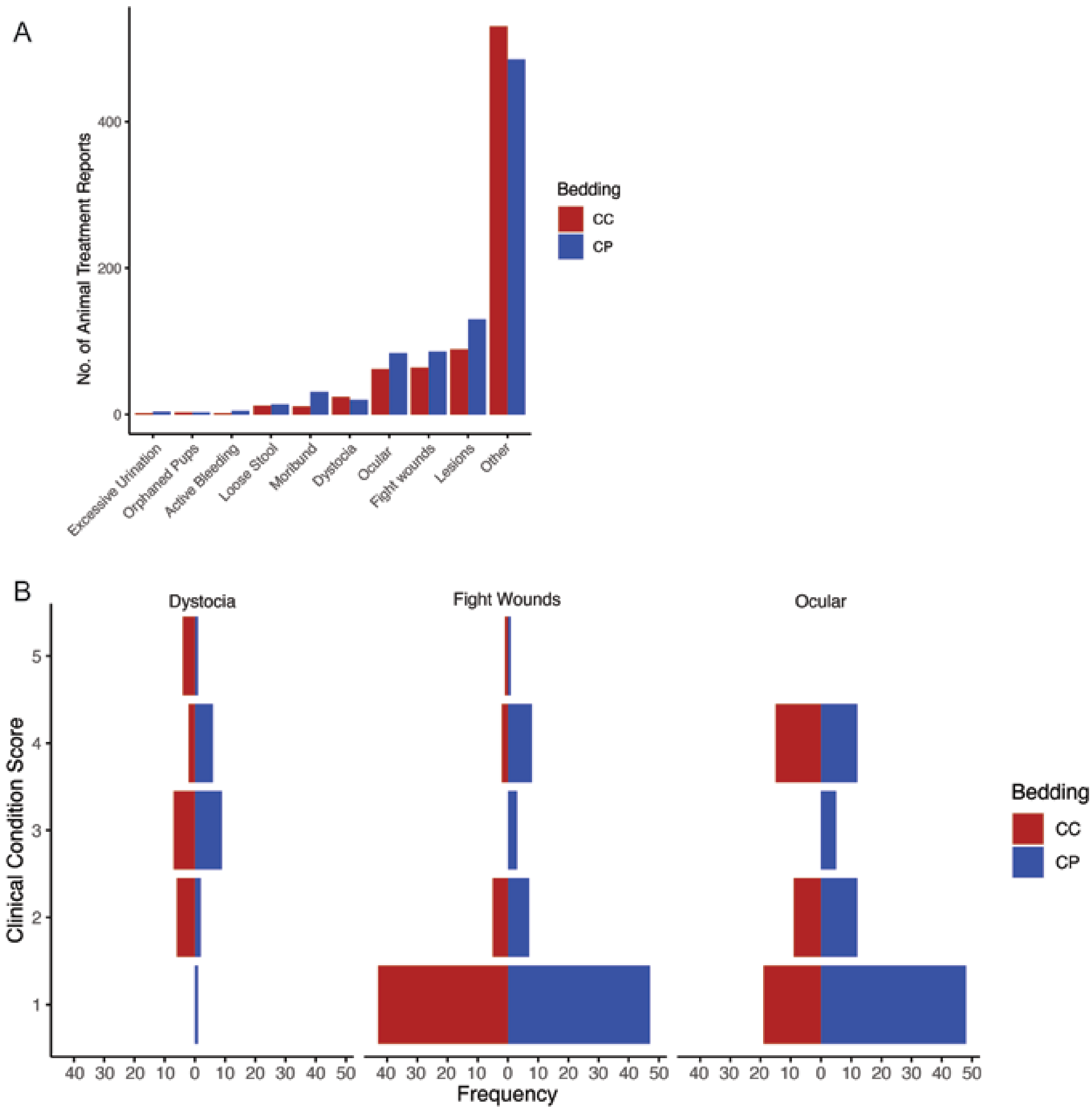

Figure 5. Bedding does not influence the number of animal treatment reports or clinical condition scores. (A) Overall incidence of animal health concerns reported for cages housed on CC bedding (red) or CP bedding (blue; $P = 0.304$). (B) Frequency of clinical condition scores for mice with dystocia, fight wounds, or ocular lesions when housed on CC or CP bedding. CCS differed for fight wounds and ocular abnormalities but not dystocia ($P = 0.001$, 0.021, and 0.414, respectively; $r = -3.628$, –2.618, and –0.935, respectively). CCS across these conditions was not significantly influenced by the type of bedding ($P = 0.641$). A cumulative linked mixed effects model was used for these comparisons.

Discussion

These studies were conducted both colony-wide and in targeted mouse populations. We hypothesized that CP bedding would improve the overall wellbeing of mice in IVC. We found that mice on CP had greater incorporation of bedding into the nest cup base and walls, supporting their species-specific behavior of nest building, and improved breeding performance. Also, CP-bedded cages did not influence the frequency or identification of health concerns.

Generally, increased enrichment in the murine microenvironment is associated with improved overall wellbeing.[1] Complex nesting material (e.g., EnviroPAK) is used as a standard enrichment for the mouse microenvironment at our institution. This material and its packaging provide a complete nest, hosting walls and a dome, with minimal manipulation by the animal. We initially attempted to perform nest scoring by using published methods,[4,7] in addition to NBI scores, but we were unable to recognize appreciable differences in overall nest scores. Regardless of sex and breeding status, bedding manipulation into a nest was greater in mice housed on CP bedding, as demonstrated by higher NBI scores, compared with mice housed on CC. The inherent form and shape of compressed paper bedding squares most likely provide a substrate more amenable to nest construction than small-diameter corncob bedding. Our findings suggest that CP bedding allowed more robust nesting behavior, despite the presence of an optimal nesting material. The combination of CP bedding and complex nesting material supported the species-specific behavior of nest building, increasing nest complexity with multiple substrates and potentially improving overall animal welfare.

The influence of bedding and enrichment on mice breeding performance has driven performance-based standards in animal care. Bedded cages with nesting enrichment have been documented increase overall pup survival rates[11] and mouse breeding index,[5] compared with bedded cages without nesting enrichment. The use of wood shavings compared with chip-processed bedding increases the average number of weaned mice, a difference postulated to result from better nest construction due to the incorporation of shavings into the cotton nesting material, providing tall and sturdy nest cup walls.[9] Consistent with this, we found that providing CP bedding, which resulted in higher NBI scores in the general colony, positively affected the 6-mo breeding performance index of breeding mice. Across 13 different genetically modified mouse lines, the number of pups per litter increased by 1.3 pups, on average, for breeders housed on CP compared with CC bedding. Mouse dams typically increase in breeding performance from the first to second parity, with litter sizes remaining stable over the next several litters, until dropping for subsequent litters.[3,10,15,18] In contrast, we observed a significant increase in the average number of pups in the first litter for monogamous pair breeders housed on CP bedding, suggesting that CP bedding improves first-litter breeding performance. Interaction between enrichment-based breeding optimization and mouse strain has been documented with BALB/c and 129/Sv mice[23] but our data suggest that the combined effects of CP bedding and complex nesting material on breeding performance are more broadly applicable. However, multiple factors are likely to affect breeding performance. We cannot rule out effects that differences in physical and biochemical characteristics of bedding types may have on study outcomes. For example, bedding type can influence murine thermoregulation,[6] and CC bedding may contain endotoxins and estrogens, which have been demonstrated to alter breeding performance in rats and mice.[12,13,17,24] The key effect of CP on first-litter production, with relative consistency in production between CC and CP thereafter, suggests that the bedding's physical characteristics warrant further investigation as an explanatory mechanism.

Daily visual health assessments of rodents can be helped or hindered by the presence or absence of visual barriers, such as a complete nest, and the background contrast of bedding. Overall frequencies of health concerns were similar to those previously reported.[14] No differences were observed in the total number of animal health concern reports for mice housed on either bedding type or under the specific conditions that we examined. Similarly, the severity of clinical disease according to CCS did not differ between cages on CC or CP bedding. CCS was influenced by which health condition was being assessed, an effect that can be expected given that health conditions can present with differing severity depending on how they progress. In the current studies, we used a single-subject research design in light of the practical realities of performing these studies in our colony, and we were limited in the information that we could collect pertaining to colony mice that lacked animal treatment reports. Given these constraints, some of the observations in the animal health reports were due to normal variation in the study population. Further studies are warranted to identify more definitively the effects of bedding on the reporting of animal health concerns.

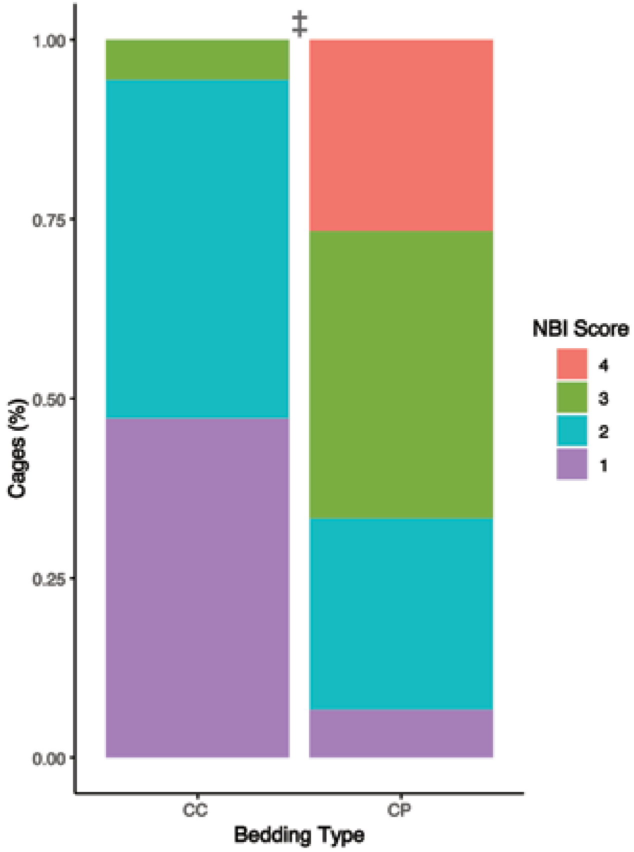

Figure 6. Breeding mice bedded with CP have increased NBI scores compared with CC cages. NBI was calculated for breeding mice bedded on either CC or CP in IVC cages (‡, $P < 0.001$; Wilcoxon rank-sum test).

Given that these current studies involved colony animals, access to genetic and strain information was not consistently available. Strain is an important factor in nest construction and breeding productivity, and bedding type might have strain-specific effects on these characteristics. Our study design attempted to control for this bias by using matched controls, randomization, and a large number of cages. More work with specific strains is warranted to determine how strain specific differences may influence our current results, particularly the breeding studies. Our colony-wide, general population studies to identify animal health concerns were done sequentially, so we cannot directly compare the effects of CP and CC bedding in these populations. We attempted to evaluate animal treatment reports in the targeted breeding performance study, but too few health concerns were reported to allow assessment. Future studies are needed to investigate the relationship between bedding substrate and the reporting of animal health concerns. We were not able to blind our reviewers, given the difference in the appearance of the bedding types. However, general end-user feedback for the CP bedding, which was novel for our institution, was typically neutral or negative, despite data demonstrating improved environmental parameters[16] and animal welfare, suggesting a bias against CP.

Overall, this report demonstrates that providing mice with CP bedding improves their nest building, promotes breeding optimization, and does not hinder the ability of personnel to identify animal health concerns.

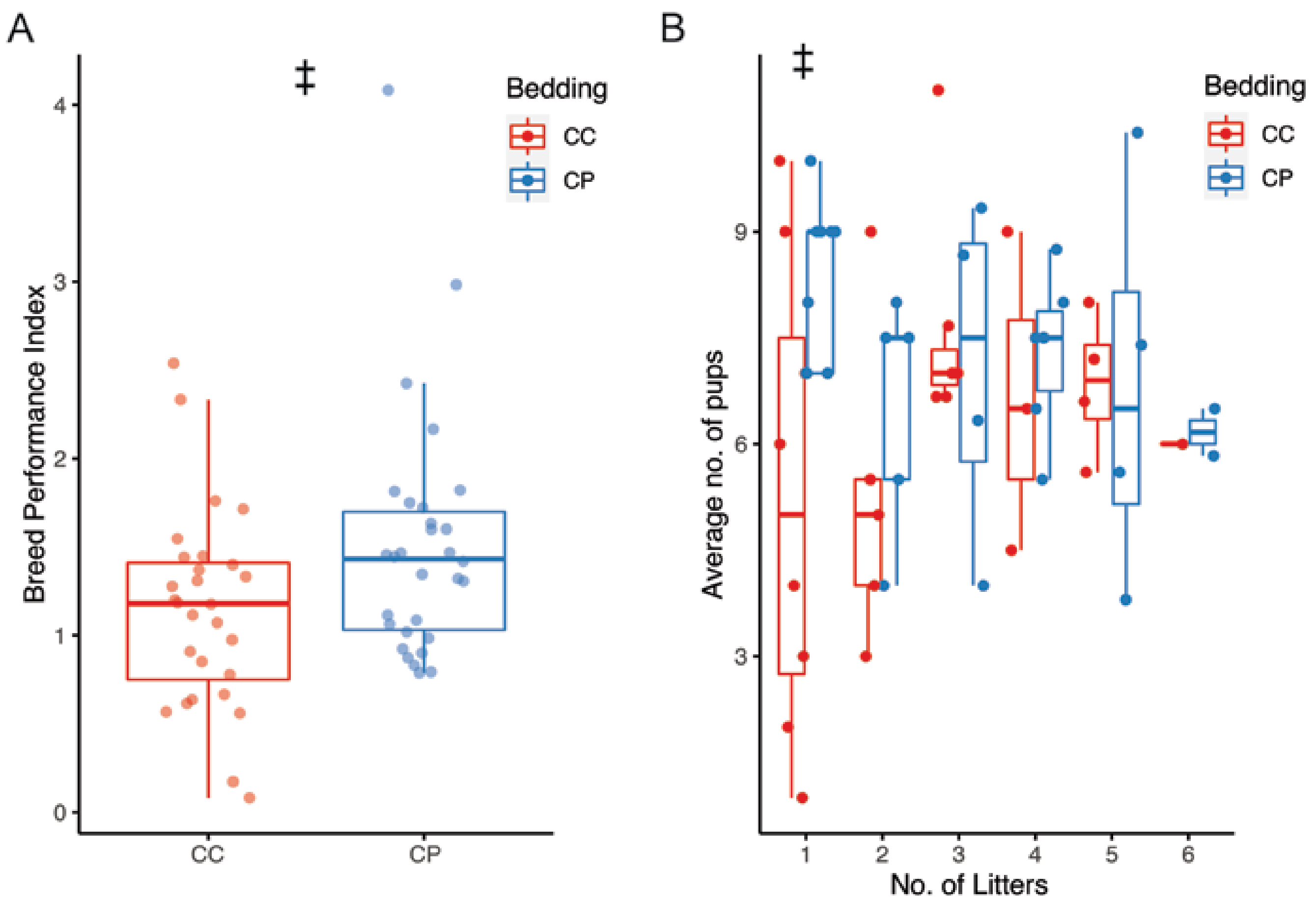

Figure 7. Mice in CP-bedded cages have increased reproductive performance. Pup production in breeding cages on either CC or CP bedding was measured for the first 3 litters of production. (A) 6-mo Breeding performance index (no. of pups/female/week) for breeders on either CC or CP bedding (*, $P = 0.033$). (B) Number of pups per litter stratified by the litter number. Pup production was compared by using a Student *t* test.

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