**Journal of the American Association for Laboratory Animal Science**

Volume 60, Number 1, January 2021

**ORIGINAL RESEARCH**

***Biology***

**Layssol-Lamour et al. Reference Values for Hematology, Plasma Biochemistry, Bone Marrow Cytology and Bone Histology of NOD.Cg-*PrkdcscidIl2rgtm1Wjl*/SzJ Immunodeficient Mice, pp. 4-17**

Domain 1

Primary Species: Mouse (*Mus musculus*)

SUMMARY:This article established references ranges for hematology, plasma biochemistry, bone marrow cytology, and bone histology in NOD.*Cg-PrkdcscidIl2rgtm1Wjl*/SzJ(NSG) mice (n=40 males and females, 9 weeks old, bred from breeders obtained from Charles River). Reported biological characteristics of this strain include low peripheral leukocyte counts, lack of mature T, B, and NK cells, severe aplasia of thymus and lymph nodes, and the absence of splenic white pulp. There are current mean values of hematology and serum biochemistry for male and female NSG mice available on the JAX website. However, they do not meet the recommended international criteria for establishing reference values because little information was available for preanalytics, analytics, and statistics.

Blood was analyzed using the IDEXX ProCyte Dx analyzer and SysmexXT-2000iV for RBC and WBC values and by manual cell count and a VetScan VS2 chemistry analyzer for serum biochemistry. Bone marrow cytology and histology from sections of mouse femur were evaluated by a clinical pathologist.

For hematology, their main findings include a statistically significant effect of the method (analytical compared with manual) on lymphocytes and monocytes, with mean counts being 10-fold lower and higher on blood smear evaluation, respectively. Additionally, lymphocyte and eosinophil counts were very low (10 and 20% of the C57BL/6J (B6) controls), but neutrophil counts were not significantly different. Females had slightly lower values except for WBC count (1.8-fold higher) and neutrophil counts (2-fold higher) compared to males.

For chemistry, their main findings include high glucose concentrations (80% higher) compared to B6 mice, which was attributed to non-fasting prior to sample collection.

For bone marrow evaluation, median and mean M:E ratio were 1.76 and 1.92 respectively, with no gender effects observed. Femoral bone marrow had normal architecture with 90-100% cellularity in the medullary space. In terms of cell distribution, megakaryocytes and adipocytes were found in the diaphysis and metaphysis, respectively. Bone marrow cell counts were lower in females, except for adipocytes.

Overall, the authors’ work was designed based on CLSI and ASVCP recommendations, providing researchers with a reliable baseline for these physiologic values for NSG mice, and stresses the importance of reporting analytic methods when establishing baseline (e.g. automated analyzers vs manual counts), due to the significant analytical bias that could result.

QUESTIONS

1. Which of the following is NOT a characteristic of NSG mice?
   1. Lack mature T, B, and natural killer cells
   2. Lack mature neutrophils
   3. Thymic aplasia
   4. Absence of splenic white pulp
2. Which of the following cell types are affected as a result of the *Il2rg*genemutation in NSG mice, when compared to NOD SCID mice?
   1. T-cells
   2. B-cells
   3. Natural killer cells
   4. Neutrophils
3. Free response. Why is important to do manual counts for blood smears in NSG mice?

ANSWERS

1. b
2. c
3. There is a difference in analytical detection in automated analyzers compared to manual counts for lymphocytes and monocytes (e.g. Lymphocyte and monocyte mean counts are 10-fold lower and higher on blood smear evaluation, respectively, compared to automated analyzer in the paper).

***Husbandry***

**Burlingame et al. Identification of Sick or Dead Mice (*Mus musculus*) Housed with 6 Grams of Crinkle Paper Nesting Material, pp. 18-27**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY:The authors conducted a 2-step prospective epidemiologic study to 1) evaluate whether 0, 2, or 6 grams of nesting material alters the ability to identify sick or dead mice, and 2) evaluate the number and severity of health concerns identified in the presence of 6 grams of crinkle paper nesting material at cage-side health check as compared with cage change.

For laboratory mice (*Mus musculus*), nesting material is perhaps one of only a few enrichments that meet all criteria.

Providing 8 grams of nesting material increases breeding performance, increasing the number of pups born to dams at the same level of food consumption and also increasing pup weaning weight.

Providing nesting material to a laboratory mouse gives it the ability to manipulate its microenvironment and perform goal-directed, species-specific behaviors. In addition, how the nesting material is used can provide an indicator of the animals’ wellbeing. Studies have shown that mice experiencing pain, distress, or discomfort do not participate in nest building.

Mice were considered to be poor models for some disease conditions, yet their suitability improved when home cage temperatures were raised to levels equivalent to those made possible by the provision of sufficient nesting material.

These include mouse models of Graft Versus Host Disease and western diet induced atherosclerosis. The immunosuppressive effects of cold stress on tumors is also well studied.Cold stress resulted in higher non-shivering thermogenesis and significantly increased chronic stress, as reflected by increased adrenal weight. These factors all contributed to reduce subcutaneous tumor metabolism in immunodeficient mice; an effect ameliorated by the provision of shelter. Nesting material has also been shown to reduce the occurrence of abnormal behaviors in mice, such as stereotypies.

Despite all the advantages that nesting material provides to the health and welfare of laboratory mice, it may compromise the ability of care staff to see mice during cage-side health checks.

A diverse array of strains/stocks, sexes, and ages typical of a large academic setting were included in this study, purchased from a variety of vendors. The study population included breeding trios and pairs and singly or group housed single-sex experimental mice, with a maximum density of 4 to 5 adults, depending on mouse weight, and ranging in age from 21 d up to 2 y of age. All mice were housed on ventilated racks in transparent polypropylene cages The rooms were maintained at 22 **°**C (**±** 2 **°**C) with 30% to 70% relative humidity and a 12:12 h light: dark cycle. Cage changes were performed by a husbandry technician every 14 d, or more frequently as needed.

Data was recorded for two years, 2014 and 2015. When clinical health concerns were noted during cage-side health checks or cage changes, an animal treatment report (ATR) was generated and given to the veterinary technicians. Upon initial evaluation, the veterinary technician would assess the clinical health of the animal and determine a clinical condition score. The clinical condition score was classified as a mild, moderate, or severe clinical health condition category (CHCC) to determine the degree of severity at which health concerns were being identified and reported. Each ATR was also categorized as either a success (correct identification of early-stage illness or at the specified humane endpoint) or a failure (the animal was found at a late-stage or end stage of illness.

A low percentage of the total mouse population (approximately 0.13% in 2014 and 0.16% in 2015) received ATRs, based on the estimated total number of animal observation opportunities during the 6 mo. data collection period (average number of mice per day \* number of days). In 2014, the daily average total census consisted of 462 mice per day, with approximately 154 mouse deaths and approximately. There were 28,336 total animal health observations in each nesting material group (0, 2, or 6 grams) over the 6 mo. study. In 2015, the daily average census consisted of approximately 511 mice per day, equaling approximately 94,024 total animal health observations, with all cages receiving 6 grams nesting material. In 2014, a total of 107 ATRs were reported. Of that total, 38 ATRs were from mice in the 0 gram group, 34 in the 2 grams group, and 35 in the 6 grams group. In 2015, a total of 148 ATRs were reported from mice given 6 grams of nesting material.

In 2014, mild CHCCs were numerically more frequent than moderate or severe. Our main question of whether nesting material inhibits the ability of husbandry staff to identify sick mice at the early stages of illness during cage-side health checks was tested with an interaction between observation activity (cage-side health check compared with cage change) and CHCC severity. The severity of the CHCC did not differ between daily health check or at biweekly cage change

Overall, mild CHCCs were documented statistically more often than moderate (Tukey; *P* **<** 0.05) or severe (Tukey; *P* **<** 0.05). Further, the type of study conducted significantly affected the number of ATRs produced (F3,15 = 32.03; *P* **<** 0.001). Oncology studies produced significantly more ATRs than any other study category (Tukey; *P* **<** 0.05). Physiology studies produced more ATRs than metabolism/ diabetes studies and the “other” research category (Tukey; *P* **<** 0.05) but there was no difference between metabolism/diabetes and other (Tukey; *P* **>** 0.05).

The analysis of the ATR success and failure is similar to the CCHCs. Overall, more ATRs were reported during daily health check (F1,9 = 7.3; P = 0.024). However, because the number of observations of the 2 activities was not equal (13 cage-side:1 cage change observation), more ATRs were reported at cage change due to the lower frequency of observations (χ2 = P < 0.001). Reporting rates were equivalent at daily cage-side exams and biweekly cage change for both successful identification and failure (see Table 5), (F1,9 = 0.95; P = 0.35). Thus, the risk of failure was not higher when animals were observed during daily cage-side health check as compared with biweekly cage change. Whether the mice were observed at cage-side or cage change, successful identifications were more common than failures (F1,9 = 30.70; P < 0.001). Study type was again significant (F3,9 = 32.03; P < 0.001), with oncology studies accounting for significantly more ATRs than the other areas (Tukey; P > 0.05).

In the 2014 study of the single oncology Principal Investigator’s mouse colony, we compared mouse health concerns reported over a 6 mo. period in each of the 3 groups: 0, 2, and 6 grams of nesting material. The overall occurrence of health conditions and the stage of

Typically, animal wellbeing is confirmed by visual inspection of the animals themselves, although other signs of health and wellness in the cage environment can also be used. Increasingly complex and complete nests make it harder to observe mice during cage-side exams, particularly while the lights are on and mice are naturally inactive.33 Thus, animal care programs must weigh the relative risks of providing optimal amounts and types of nesting material with the consequent decreased visibility of the animals at cage-side health check. This can potentially delay identification of health concerns until the cage is changed.

Illness when reported was assessed. During the 2014 study, we found a similar rate of ATRs across the 3 nesting conditions. Providing up to 6 grams of nesting material did not result in health conditions being identified at a later stage of illness. These results indicate that provision of nesting material did not prevent the ability to identify health concerns. In all 3 groups, more mild clinical condition scores were found than either moderate or severe. In addition, more mice with health concerns were successfully identified in all 3 groups. As a result these mice could be monitored and treated rather than euthanized. The mouse colony of the same oncology lab was studied in 2015, with all cages given 6 grams of nesting material. In this 2015 data set, the ATR data from cages with 6 grams of nesting material was consistent with the 2014 ATR data from all 3 nesting material groups. Again, mild clinical health condition scores were numerically more frequent than moderate or severe, confirming that mice displaying early signs of illness were successfully identified. These results support that 6 grams of nesting material does not hinder the ability of husbandry or veterinary staff to identify mice in need of veterinary intervention.

The study findings showed that, based on the frequency of observations at health checks compared with cage change, health conditions were identified at a higher rate during cage change. However, severe health concerns, or failures, were not higher at cage change as compared with health check. This finding confirms that the presence of 6 grams of nesting material did not increase the risk of more severe health outcomes for the mice. While this may imply that large nests are reducing the ability of staff to identify health concerns during daily observations, cage-side health checks overall have previously been shown to identify fewer health concerns than cage change. The authors found no significant difference in the number of mice identified for veterinary attention at cage change, compared with daily health checks in each of the 3 clinical health categories.

The presence of nesting material did not increase the number of animals reported for severe health issues, however, the type of research affected the type of CHCCs documented. Oncology research had the largest reported number of animal health concerns; but the vast majority were reported at the mild stage of clinical illness. Taken together, results indicate that husbandry staff can readily identify mice in need of veterinary care, regardless of the presence of nesting material, at early stages of disease across the wide variety of mouse models.

A peer academic institution with a similar size and diversity of rodent research types reported a roughly 10% spontaneous mortality rate of adult mice. The mortality rates in our study ranged from 6 to 8%. The comprehensive death log data from 2014 showed comparable deaths for mice with 0 and 2 grams of nesting material, with a small numeric increase in the number of mice found dead in cages with 6 grams of nesting material. These results may suggest that 6 grams of nesting material is contributing to more animals being found dead, but when the data was broken down by month, a decline was observed in the percentage of mice given 6 grams of nesting material and found dead as the study progressed. A larger number of deaths occurred during the first 3 mo. in cages with 6 grams of nesting material, but during the last 3 mo. deaths were similar in number between all 3 nesting groups. While many factors could lead to animals being found dead, these results imply that a transition period occurs when introducing nesting material. The 2015 death log data showed an overall higher likelihood of mice being found dead outside of the nest, suggesting the nests did not significantly hinder the discovery of dead animals. When the number of observations is factored in (χ2 analysis), the number of mice found dead at daily cage-side health check is less than the expected rate. Much like the identification rate of health conditions, this may be a result of increased examination and time spent to observe the condition of the cage and animals during cage cleaning.

The authors recognize that environmental enrichment may inhibit full visualization of rodents during daily cage-side health checks, so other factors such as nest building, cage organization, and movement within the nest are being used at their facility to inform the health assessments of the mice.

A limitation of the study is that additional factors are likely to affect the quality of nests, including background strain or stock or genetic manipulation of the mouse, sex, unique environmental factors in specific facilities and other unknown factors.

QUESTIONS

1. Which one of the following is not a characteristic of a truly enriching item or substrate?
   1. Biologically relevant to the specific species.
   2. Gives the animal some semblance of control over its environment.
   3. Shields the animal from perceived stress.
   4. Allows proper identification of the animals
2. T/F: The provision of nesting material to mice is now legally required in Europe
3. T/F: Both male and female mice readily build nests but only do so when reproductively active.
4. T/F: Identifying the latency at which nesting material is being manipulatedcan provide useful information when studying behavioral deficits in mice
5. T/F: The presence of fresh feces, urine, and an organized cage (a well-formed nest located away from the urine site) is indicative of healthy mice inside the cage.
6. The recommended amount of nesting material needed to achieve sufficient insulation to reduce cold stress is:
   1. 2-4 grams
   2. 4-6 grams
   3. 6-8 grams
   4. 8-10 grams

ANSWERS

1. d
2. T
3. F
4. T
5. T
6. d

**Allen et al. Effects of Compressed Paper Bedding on Mouse Breeding Performance and Recognition of Animal Health Concerns, pp. 28-36**

**Domain**4 **Primary Species:** Mouse (Mus musculus)

**SUMMARY:** Bedding substrate and nesting material are important components of an animal’s microenvironment. Bedding affects intracage air quality, animal cleanliness, and an animal’s ability to perform species-specific behaviors as nest building. This group previously demonstrated that compressed paper (CP) bedding decreases intracage ammonia and reduces early cage changing frequencies when compared to corncob (CC) bedding. This paper aims to show that compared with CC bedding, CP bedding would 1) improve nesting behavior, 2) improve breeding performance, and 3) allow early identification of common health concerns.

The nest base incorporation (NBI) scoring system was developed to evaluate how well bedding was incorporated into the cup base and walls of the nest structure. All aspects of each nest were visualized for roughly 30 seconds and the overall character of the nest was scored 1 to 4 with 1) no visible bedding incorporated into base of nest cup 2) bedding base in nest cup but no cup-wall 3) bedding base in nest cup with cup-wall less than 3 cm height 4) bedding base in nest cup with cup-wall greater than 3 cm height. 215 total cages (CC n=107; CP n=108) were evaluated at 9-13 d after cage change. To evaluate effect of bedding on reporting of common animal health concerns, a separate population of colony mice were housed on CC for 2 months and data collected then transitioned to CP and evaluated for 2 months after an acclimation period of 2 months. A clinical condition score (CCS) is assigned to each animal presenting with a health concern and again at monitoring visits. The lower CCS correspond to low severity such as 1) a minor health concern to be monitored whereas the higher CCS correspond to severe conditions 4) systemic illness portending a humane endpoint and 5) moribund condition requiring immediate euthanasia. Thirteen lines of genetically modified mice were utilized to evaluate bedding effects on breeding performance. Monogamous pairs were randomized into CP (n=30) or CC (n=29) bedding groups. NBI scores were assessed twice 14 d after cage changes and all breeding cages were tracked for 6 mo. for number of pups per dam per week on study.

CP bedded cages had higher NBI scores (p<0.001). Both CP and CC beddings had similar number of health reports and clinical condition scores (CCS). Compared to mice bedded in CC, mice in CP-bedded cages had significantly (p<0.001) higher nest base incorporation (NBI). Breeding performance index was significantly (p=0.033) higher for those on CP bedding. Also, the average number of pups per litter was significantly (p=0.0317) higher by 1.3 pups for those bedded on CP.

This paper supported the hypotheses that the CP bedding would improve nesting behavior and breeding performance compared to CC bedding. Further, the CP bedding improved first-litter breeding performance for the evaluated monogamous breeding pairs. CC bedding may contain endotoxins and estrogens which have been shown to alter breeding performance. Even though no significant differences were found in animal health reporting, more studies should be done evaluating this aspect because even though the CP bedding improved environmental parameters the general user feedback for the CP bedding was neutral or negative at this institution.

**QUESTIONS**

1. What are some functions of bedding?

a. Maintain animal cleanliness

b. Maintain intracage air quality

c. Allow species-specific behaviors

d. Allow husbandry to identify animal health concerns

e. All of the above

2. T/F: An NBI score of 3 indicated a bedding base is included in the nest cup, with cup-wall incorporation of at least 3 cm in height

3. Which bedding improved first-litter breeding performance in this study?

a. Corn cob

b. Compressed paper

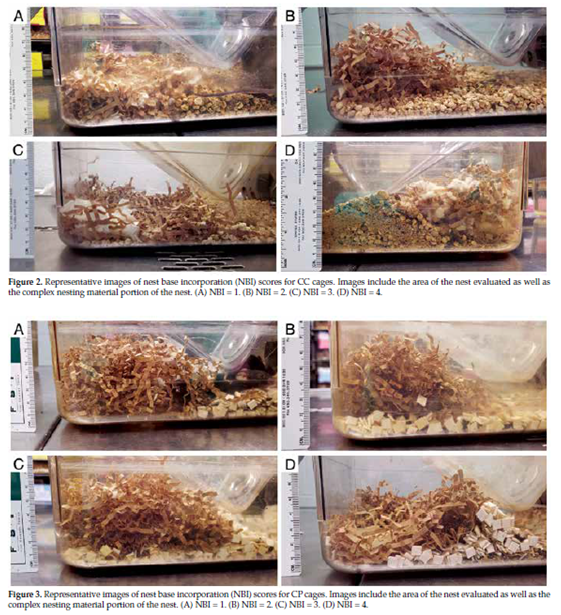
**ANSWERS**

1. e

2. False, less than 3 cm in height

3. b

Nest Base Incorporation Scoring System



**Tataryn et al. Comparison of Four Beddings for Ammonia Control in Individually Ventilated Mouse Cages, pp. 37-43**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Adult male outbred Crl:CD1(ICR) mice, housed in IVCs, and free of common pathogens as proven by sentinel health monitoring were used. A crossover design was used in which each group was exposed to each bedding type for a period of 2 wk, so a total of 20 cages with 4 mice each were used. Ammonia levels were measured on days 0, 2, 4, 7, 9, 11,and 14. Four bedding materials were evaluated in this study: kiln-dried aspen shavings; 1/4-in. corncob; 1/8-in. pelleted cellulose and a refined virgin diced cellulose product. Either pelleted virgin cellulose or refined virgin diced cellulose can be viable alternatives to corncob bedding when the use of corncob may interfere with research data, without the need for more frequent cage changing. In addition, these novel bedding types appear suitable for general use with regard to successfully controlling intracage ammonia levels during a 2-wk cage-change interval in IVC.

Results:Results showed that corncob, pelleted cellulose, and refined diced cellulose consistently provided better ammonia control in IVC than did aspen shavings. Ammonia concentrations for aspen bedding were significantly higher beginning at day 4 (days 4, 7, 9, 11, and 14).

The novel cellulose products performed better than corncob by the end of the 2-wk study period. Findings contrast with a prior report using a traditional cellulose product probably because of the use of novel cellulose beddings designed to provide better ammonia control, including a pelleted cellulose with porous pellets and a diced cellulose made using a refined pulping process. The ammonia levels in cages containing corncob bedding were significantly higher than those containing diced cellulose beginning at day 11 and continuing to the end of the cage change cycle (day 14). On day 14, cages containing corncob bedding had significantly higher ammonia levels than those containing pelleted cellulose. Differences between the two cellulose products were of low magnitude and did not support an overall conclusion in favor of either product.

The 50-ppm threshold was exceeded in 85% of aspen cages, 10% of corncob and pelleted cellulose cages, and 5% of diced cellulose cages. This value was used as a welfare endpoint, and mice in these cages were removed from the study. By the end of the study, the 25-ppm threshold was exceeded in 90% of aspen cages, 40% of corncob cages, and 10% of pelleted cellulose and diced cellulose cages.

Aspen and corncob cages showed urine spots when viewed from the bottom of the cage a fact that might help when “as needed spot changes” of bedding are required. Cellulose products showed primarily fecal accumulation, and diced cellulose showed also yellow discoloration – so soil level cannot be judged the same way but “spot changes” will not be necessary with the same frequency.

Ammonia/Bedding Information:

*Intracage Ammonia Levels Affec*t: cage air quality, needed frequency of bedding change, mouse respiratory physiology, immune function, hepatic microsomal enzyme activity and severity of *Mycoplasma pulmonis* infection.

*Hardwood Beddings* (e.g. shaved aspen)

* Pros: Natural material, light, soft, allows for burrowing and nest building, thus aiding in thermoregulation
* Cons: Poor fluid absorbency and ammonia control.

*Pelleted Corncob*

* Pros: Availability, high absorbency, ability to minimize ammonia levels (recommended for use in static cages or with extended cage-change intervals), and low cost
* Cons: Potential biologic effects, standard pellet processing does not eliminate microorganisms (irradiation or autoclaving are needed), contains high levels of estrogenic compounds which may induce endocrine disruption with implications for breast and prostatic cancer models or alter rodents’ behavior, mice may ingest corncob bedding, which contains digestible material and can affect feed-conversion efficiency in high-fat diet studies

*Processed Cellulose Products*

* Pros: Softer than corncob, good absorbency compared with wood, not digestible material or estrogenic, standard processing usually involves high temperatures and chemical extractants that result in reduced endotoxin content and coliform counts compared with natural bedding products.
* Cons: Poor long-term ammonia control (vs corncob)à new products are developed and their ammonia control is examined in this paper!

*Methods To Assess Intracage Ammonia Levels*: colorimetric paper, color-based reagents, and photoionization devices (PID). Reagent tubes gave precise, reliable measurements and were the authors’ preferred method.

QUESTIONS

1. Which bedding material contains high levels of estrogenic compounds?

a. Aspen shavings

b. Corncob

c. Pelleted cellulose

d. Diced cellulose

2. Which of the following is an advantage of the corncob compared to aspen wood bedding?

a. High fluid absorbency

b. Softness

c. Good for burrowing

d. High ammonia levels

3. True/False: Refined virgin diced cellulose mouse bedding results to higher ammonia levels at the end of a 2-week interval, when compared to corncob bedding.

4. Which is NOT true for pelleted cellulose and refined virgin diced cellulose mouse bedding products:

a. Provide better ammonia control than corncob at 2 weeks

b. Have good absorbency when compared with wood

c. Are processed in low temperatures resulting in high endotoxin and coliform levels

d. They are softer than corncob

ANSWERS

1. b

2. a

3. False

4. c

**Oatess et al. Effects of Acrylic Tunnel Enrichment on Anxiety-Like Behavior, Neurogenesis, and Physiology of C57BL/6J Mice, pp. 44-53**

Domain 4  
Primary Species: Mouse (M*us musculus*)

SUMMARY: Mice are highly social, nocturnal, burrowing animals that have several environmental needs that may not be addressed in the laboratory environment. Psychological stress and physical stress can impact research by altering an animal’s biology. Previous studies have shown increased aggression from structure placement in cages including nest boxes, igloos, and platforms. The objective of the study was to address concerns of structural enrichment in the form of a single red acrylic mouse tunnel. The authors hypothesized that that the addition of simple structural enrichment into the murine housing environment would significantly alter study parameters as compared with those of mice housed with standard enrichment (social housing and nesting material). Mice given an acrylic tunnel in addition to standard enrichment were found to exhibit:

1) Modest differences in open field testing suggestive of decreased anxiety by traveling 9.6% farther and depositing 25% fewer fecal boli than standard housed mice. C57/B6 mice are less anxious after provision of tunnel enrichment.

2) Neurogenesis in the dentate gyri of enriched mice were not significantly altered after 12wks of tunnel enrichment compared to standard environmental enrichment animals.

3) There was an increased gain in body weight particularly by male mice compared to animals without the tunnel.  There was no difference among female weights between groups.

4) The plasma corticosterone concentration measurement and neutrophil: lymphocyte ratio was used to compare physiologic stress between treatments at a single time point at the end of the study. The results indicate that acrylic tunnel placement did not have significant effect on physiologic stress measurements. Additionally, females had heavier adrenal gland weight and higher corticosterone levels than male mice.

5) Animals housed in groups of 3 at 4 week of age, acrylic tunnel enrichment did not display increased aggression beyond what is typically expected. The authors described only one case of male-male aggression over a 10-week period.

The results demonstrated that adding a single red acrylic tunnel as environmental enrichment is unlikely to induce significant confounding effects on anxiety, neurogenesis, body weight or physiologic parameters in mice. The authors conclude by recommending the inclusion of “simple structural enrichment” in addition to social housing and nesting material as part of a standard rodent enrichment plan.

QUESTIONS

1. T/F: Mice are a negative thigmotactic prey species.

2. T/F: Mice are highly social, nocturnal, burrowing, and nesting mammals.

3. Increased aggression in mice has primarily been associated with:

* + 1. Igloos
    2. Nesting material
    3. Elevated platforms
    4. All the above
    5. None of the above

ANSWERS

1. False

2. True

3. d. All of the above

***Management***

**Randall et al. Mental Wellbeing in Laboratory Animal Professionals: A Cross-Sectional Study of Compassion Fatigue, Contributing Factors, and Coping Mechanisms, pp. 54-63**

Domain 5  
  
SUMMARY:Compassion fatigue (CF) is a profound state of emotional and physical exhaustion that caregivers can develop when they are unable to refuel and regenerate because of the nature of their work. The objective of this paper was to examine the prevalence of CF in laboratory animal professionals and how it is affected by personal and work-related factors, coping mechanisms, and support programs. Data were compared between general laboratory animal professionals and those working in large multinational contract research organizations (CROs).

The population examined included individuals over 18 years old working with laboratory animals in Canada or the US. A voluntary, anonymous questionnaire was distributed that consisted of questions on demographics, CF, nature of work, solutions/coping mechanisms, and Ten-Item Personality Index (TIPI). Data were collected over a one month period in 2019.

There were no major differences in the responses of the general population vs. CRO participants. The majority of respondents reported having experienced CF at some point in their career (66-69%), although 30% were uncertain suggesting a lack of awareness. CF was characterized by exhaustion, apathy, sadness, depression, anxiety, frustration, and guilt. Higher emotional stability, openness, and extraversion were personality traits associated with respondents that did not report CF. Factors influencing CF included: understaffing, relationships with animals, lack of coping resources, poor relationships with superiors, and lack of compassion fatigue training and awareness. Coping mechanisms utilized included: talking to someone, getting away from work, self-care, physical activity, and owning a pet.

Few respondents (12-29%) indicated the presence of an institutional CF program or receiving training on self-care or resiliency. Those that did have a program indicated value in physical activity, having a quiet place to reflect, and access to self-care training. Programs allowed for peer support and strengthening of relationships with coworkers.

QUESTIONS

1. True/False: 'Compassion fatigue' and 'burnout' can be used interchangeably.

2. Which of the following is/are impacted by compassion fatigue?

a. The individual's quality of life

b. Departmental/institution function

c. The economics of the department/institution

d. All of the above

ANSWERS

1. False: Compassion fatigue is seen in caregivers and is caused by wanting to help those who are suffering. Burn out is not specific to caregivers.

2. d. - All of the above. In addition to affecting the individual's quality of life, CF can also impact departmental/institutional function and economics due to absenteeism, compensation costs, turnover, friction between staff, and reduced ability to complete tasks and meet deadlines. 

***Anesthesia***

**Kendall et al. Pharmacokinetics and Efficacy of a Long-lasting, Highly Concentrated Buprenorphine Solution in Mice, pp. 64-71**

Domain 4

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Buprenorphine is a partial µ-agonist used to relieve pain in many laboratory animal models. Standard buprenorphine-HCl (0.3 mg/ml is typically administered every 8-12 hours. Studies have shown though that in some strains of mice the efficacy may be as short as 4 hours. Sustained release forms of buprenorphine are available as compounded medications and may offer up to 48 hours of analgesia after administration. However, state laws may make it difficult for veterinarians to obtain these compounded formulations of controlled substances.

Bup-LHC (Simbadol, 1.8 mg/ml) is an FDA approved veterinary drugs for use in cats for 24 hours of analgesia. This study tested the pharmacokinetics of Bup-LHC at 0.9 mg/kg in male C57BL/6J and female CD1 mice and the clinical analgesic efficacy in an experimental laparotomy model in female CD1 mice.

Results showed that Bup-LHC at 0.9 mg/kg SC reached plasma levels to provide analgesia to male B6 mice for at least 12 hours.  The plasma level in female CD1 mice was below therapeutic levels at 8 hours. In the efficacy test of ventral midline ovariectomy in CD1 female mice, this dose provided clinically relevant analgesia for at least 6 hours. Bup-LHC appears to offer long analgesia then Bup-HCl, and had no side effects such as heavy sedation, hyperactivity, pica or injection site lesions (possible with Bup-SR).

QUESTIONS

1. True/False: General activity can be an objective evaluation of mouse pain after a procedure and can be quickly measured.

2. All of the following can be used to assess pain in mice except:

a.  Activity level

b.  Grooming

c.  Facial grimace

d.  Nesting activity

e.  Fecal output

ANSWERS

1. True

2. e

***Experimental Use***

**Hickman. Wellbeing of Mice Euthanized with Carbon Dioxide in Their Home Cage as Compared with an Induction Chamber, pp. 72-76**

Domains 2 and 5

Primary Species: Mouse (*Mus musculus*)

SUMMARY: According to AVMA guidelines on euthanasia, home cages should be used for euthanasia using carbon dioxide (CO2)/inhalant gas anesthetics to minimize distress. This is based on the research that demonstrates transient increases in blood pressure, heart rate, and corticosterone in mice after a cage change. However, this is not always feasible when a select member(s) of the cage cohort that need euthanasia or from personnel safety point of view, it may be beneficial to use smaller induction chamber to minimize personal exposure to anesthetic gases. Author of the current study investigated distress associated with CO2 euthanasia using induction chamber or home cage using two strains of mice (ICR and SJL male and female mice). Fasting blood samples were collected to assess physiological parameter of distress (e.g., blood glucose, serum corticosterone and noradrenaline), and behavioral indicators of distress (e.g., rearing, jumping, sniffing at the gas inlet, and grooming) were evaluated using video recordings of mice from the time of introduction of gas (in home cage or induction chamber) to the estimated time to loss of consciousness. Although there were significant differences among the strains, there were no significant differences in the parameters evaluated between home cage and induction chamber groups. Thus, author concluded that there is no significant improvement in the wellbeing of mice when the home cage is used instead of an induction chamber during induction of anesthesia or euthanasia using CO2. Therefore, either the home cage or an induction chamber can be used for induction of anesthesia or CO2 euthanasia in mice.

QUESTIONS

1.  T/F: The recommended volume displacement rate of 30% per minute for the CO2 euthanasia in mice is to minimize the potential pain and distress by creating longer induction phase.

2. General recommendations for the process of euthanizing rodents used in research includes;

a. Maintenance of stable groups

b.  Reduction of transport prior to the euthanasia process

c.   Selection of euthanasia methods that minimize pain and distress

d.  All the above

ANSWERS

1. True

2. d

**Schuster and Pang. Assessment of an Electronic Mechanical Sensory Threshold Testing Device (RatMet) in Wistar Rats (*Rattus norvegicus*), pp. 77-84**

Domain 3: Research

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: von Frey (vF) monofilaments remain the “gold standard” for quantifying mechanical nociception in animal pain models despite criticisms for inconsistencies in testing methods, filament properties, and nonlinearity.  The authors used a carrageenan inflammatory model (Wistar rats) to compare withdrawal threshold measurements of vF monofilaments with measurements of a novel mechanical threshold testing device (RatMet).  The findings were as follows: all RatMet probe sizes and vF monofilaments identified a reduction in withdrawal threshold after treatment; linear relationships were observed with the RatMet probes whereas exponential relationships were observed with the vF monofilaments; RatMet probe accuracy did not differ when comparing a single test with the averages of multiple tests per timepoint.  The authors conclude that the novel RatMet device produces comparable data to the vF monofilaments and may also decrease the testing period without compromising data quality.

QUESTIONS

1. Drawbacks of vF monofilaments include which of the following?
2. Nonuniform surface area when applied
3. Estimated withdrawal thresholds
4. Inconsistency in testing methodology
5. Interrater inconsistency
6. All of the above
7. vF monofilaments are also correctly termed \_\_\_\_\_\_\_\_\_\_\_\_\_.
8. Von-Weinstein filaments
9. Frey-Weinstein filaments
10. Semmes-Weinstein filaments
11. Semmes-Frey filaments
12. True or False: Repeated testing with vF monofilaments can increase withdrawal thresholds in healthy uninjured rats.

ANSWERS

1. e; also augmented hypersensitivity of the animal due to a training effect or tissue damage and sensitivity to operator hand tremor
2. c
3. False; decrease

**Nolan et al. Evaluation of the Sterility of Reynolds Wrap Aluminum Foil for Use During Rodent Surgery, pp. 85-90**

Domain 3; K14. Aseptic requirements for performing surgery

**SUMMARY**: Strict adherence to correct aseptic technique is critical to ensure successful surgical outcomes. The Guide states that failure to maintain appropriate asepsis may result in physiologic and behavioral effects that negatively influence animal wellbeing and research outcomes.  Breaks in asepsis can lead to both clinical and subclinical infections and may result in physiologic changes in parameters such as fibrinogen levels, glucose concentrations, leukocyte counts, and histology, which can have negative impacts on pain and distress, alter experimental results and delay postoperative recovery. In rodent surgery during biomedical research, the surgeon often has multiple roles and is responsible for the surgery (including maintaining asepsis) as well as patient monitoring and maintaining anesthetic depth. Handling anesthetic equipment and use of certain equipment (such as stereotaxic apparatus or microscopes) may involve handling knobs or dials, thus making maintenance of asepsis challenging during surgery. Application of as sterile barrier material to manipulate equipment during surgery allows the surgeon to maintain sterility. A recent study validated the use of cling film directly from the box as a sterile rodent drape, but the sterility of aluminum foil has not been previously assessed. Ten boxes of food-grade aluminum foil from several manufacturer lot numbers were tested.  While the exterior of the packaging was handled with non-gloved hands or non-sterile gloved hands, the foil itself was only manipulated with sterile technique.  A combination of ATP swabs and RODAC plates were used to assess for organic material and bacterial contamination.  For each microbiologic testing time point, investigators donned a disposable gown, surgical face mask, and bouffant cap. The foil had no RLU at any time point (0, 14, 28 days and 6 months) after opening compared to uncovered surfaces. Foil from 2/10 boxes had bacterial growth (1 cfu) on RODAC plates (Bacillus spp.) compared to positive controls that yielded 15 cfu.  Of the 130 RODAC plate samples that were collected from aluminum foil over the 6 m period, 3.8% of those had minimal bacterial growth. Most Bacillus species (other than B. anthracis and B. cereus) are considered to be clinically insignificant.  The authors speculate that the contamination occurred during the manufacturing process since they were handled aseptically for the study. The authors recommend the use of foil from the box for use during aseptic rodent surgery for up to 6 m after opening.

**QUESTIONS**

1.   What does RODAC stand for (as in RODAC plate)?

a.  Replicates of Data and Colonies

b.   Replicate Organism Detection and Counting

c.   Regional Organism Dirty Algorithm Counting

d.  Replicate of Data Auditing Clones

e.  Reproduction of Data Apprehending Clones



2.  In the graphic above, someone is testing a surface for organic matter.  What are they actually measuring for and what units are used to assess the results?

a.  Bacterial and viral DNA; base pairs per microliter

b. Sterility assurance level (bacterial colonies); colony forming units/centimeter squared

c.  ATP; relative light units

d.   Bacterial and viral growth; colony forming units per paddle

e.   ATP; colony forming units/centimeter squared

**ANSWER**S

1. b

2.  c

**Chae et al. Effects of General Anesthesia on Intraocular Pressure in Rabbits, pp. 91-95**

Domain 1

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY:  Intraocular pressure (IOP) in rabbits is an essential measurement parameter for these animals in a research setting as they are often used in glaucoma studies, toxicity tests, and other preclinical work.  Ensuring that differences seen in IOP are attributable to studies rather than anesthesia is therefore critical.  In other lab animal species (mice and rats) the IOP decreases with Isoflurane inhalation.  In this paper the authors compare baseline IOP in NZW rabbits (captured 60 times over 1 week without anesthesia and averaged) with animals in three treatment groups (Iso, Ket+Xy, Ket+Xy+Iso).  IOPs were measured with a tonometer which was calibrated for use with rabbit eyes (ex vivo).  Unexpectedly the authors found a significant increase in IOP when rabbits were under Isoflurane alone.  This pressure quickly returned to normal when the animal was exposed to oxygen only.  The IOP in the Ket+Xy group decreased as expected.  The use of Ket-Xy as a premedication for Isoflurane appeared to rescue the high IOP of Isoflurane alone.  This research is important because it demonstrates the effects of anesthesia on IOP so that it can be avoided or accounted for in rabbit ophthalmic models.

QUESTIONS

1. What does ARVO stand for?
   1. Association for Rabbit Vision and Ophthalmology
   2. Association for Retina, Vision, and Ophthalmology
   3. Association for Research in Vision and Ophthalmology
   4. Association for Recordings of Vision in Oryctolagus cuniculus
2. What is the expected effect of Isoflurane on the IOP on mice and rats?
   1. Increases IOP
   2. Decreases IOP
   3. Increases for mice and decreases for rats
   4. No change in either species
3. What expected effect does Isoflurane have on IOP in NZW rabbits?
   1. Increases IOP
   2. Decreases IOP
   3. No effect on IOP
   4. Depends on the sex of the rabbit

ANSWERS

1. c
2. b
3. a

**Hershey et al. Clinical Indicators of Moribundity in Swine Experimentally Inoculated with African Swine Fever Virus, pp. 96-102**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Pig (*Sus scrofa)*

SUMMARY: African swine fever virus (ASFV) infection is a disease commonly asymptomatic in the common warthog but can be transmitted to domestic swine via tick vector or direct contact. Infection in domestic swine can cause high rates of morbidity and mortality, with death within 2-14 days. The purpose of this study was to delineate clinical indicators of ASFV that may predict the onset of moribundity in swine. Health record data from 103 pigs inoculated with ASFV were utilized and information of incubation period, survival period, daily rectal temperatures, if the animal was found dead or euthanized, and ASFV strain type were analyzed. The findings were as follows: 1) pigs were more likely to be euthanized if infected with the Lisbon ASFV strain, 2) the greatest attention to moribundity is recommended during the first 2-3 days of illness, because death due to disease is more likely in that period 3) swine infected with the Lisbon strain lived longer after inoculation than the Georgia strain and were more likely to be euthanized than found dead. Overall, the results indicated that rectal temperature may have a potential utility as a marker for predicting moribundity and viral strain and duration of survival after inoculation were important factors for predicting death due to disease rather than euthanasia.

QUESTIONS

1.   What genus of tick allows for the transmission of African Swine Fever virus?

* 1. *Ixodes sp.*
  2. *Ornithodoros sp.*
  3. *Hyalomma sp.*
  4. *Amblyomma sp.*

2.  Which organization classified ASFV as a reportable transboundary animal disease?

* 1. World Organization of Animal Health
  2. Animal Health Institute
  3. World Trade Organization
  4. USDA APHIS

ANSWERS

1. b. *Ornithodoros sp.*

2. a. World Organization of Animal Health

***Case Study***

**Bailey et al. Use of Introduction Enclosures to Integrate Multimale Cohorts into Groups of Female Rhesus Macaques (*Macaca mulatta*), pp. 103-111**

Domain 4: Animal Care

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: Limited standard procedures and guidance are available on practical approaches for maintaining genetic diversity in large captive NHP breeding colonies. Only set “rule” is to avoid inbreeding by means of rotating adult males from one set of females to another. Introductions of males are socially disruptive, may result in aggressive behavior and constraints related to simultaneously housing, breeding, and providing ongoing veterinary care for thousands of animals with a highly complex social structure creates unique challenges for genetic management in these colonies. Additionally, increasingly skewed sex ratios (fewer adult males per female) results in group stability.  In this study, authors designed and constructed 3 unique “introduction enclosures” to develop a novel introduction process that might help to integrate larger numbers of adult males into groups successfully. Further, authors compared this new method to traditionally used introduction methods and received feedback from various teams (colony managers, veterinary staff, animal care staff). Authors reported that although this novel introductory method took more time than the traditional method, the integration via the new method was more successful and benefited both macaques and the facility personnel.

QUESTIONS

1.  What is the median age (in years) of male “transfer out” from their natal groups?

a. 3

b. 3.5

c. 4

d. 4.5

2.  In a captive setting, managing breeding groups can be accomplished by?

a. Removing related group members before breeding can occur

b. Cross-fostering of infant males into other groups near birth

c. Disbanding groups before female offspring of males are old enough to reproduce

d. Rotate adult males from one group of females to another every few years so that those males cannot breed with their maturing daughters

e. All of the above

3.  The long-term goals of conducting male introductions are?

a. Efficient and safe integration of males into groups of females

b. Create socially stable breeding groups

c. Produces a satisfactory number of offspring

d. Maintains genetic diversity

e. All of the above

4.  What are the limits of the amount of time that males can interact with the female group in traditional method of introduction?

a. About 7 h daily (Maximum of 12 h)

b. About 5 h daily (Maximum of 7 h)

c. About 5 h daily (Maximum of 12 h)

d. About 10 h daily (Maximum of 14 h)

5.  The 2 newly constructed enclosures were built a year apart, so authors tested the first model and made adjustments before the second was built. From below options what change was not incorporated?

a. Dayton infrared gas tube heaters

b. Removable hanging polypropylene panels that served as wind blocks

c. An extra animal door was added to the compound to give direct access from the enclosure to the compound

d. An extra door was added to the compound to give direct access to animal care staff and vet staff for medication

e. The second enclosure was designed to be larger than the first in order to accommodate larger groups of males

6.  In the new introduction method, how long a male had protected contact interaction with females per day?

a. 5 hrs

b. 7 hrs

c. 24 hrs

d. 12 hrs

e. 10 hrs

7.  Entire duration of introductions in traditional method ranges?

a. 6 to 36 days

b. 9 to 41 days

c. 10 to 128 days

d. 10 to 41 days

8.  Entire duration of introductions in new method ranged?

a. 6 to 36 days

b. 9 to 41 days

c. 10 to 128 days

d. 10 to 41 days

9. Of the 12 traditional multimale introductions evaluated?

a. One was fully successful (8%), and 4 were partially successful (33%)

b. Three were fully successful (25%), and 9 were partially successful (75%)

c. Six were fully successful (50%), and 6 were partially successful (50%)

d. Nine were fully successful (75%), and 3 were partially successful (25%)

10. Of the 4 new multimale introductions using new introduction enclosures evaluated?

a. All 4 were fully successful (100%)

b. Three were fully successful (75%), and 1 were partially successful (25%)

c. Two were fully successful (50%), and 2 were partially successful (50%)

d. One was fully successful (25%), and 3 were partially successful (75%)

ANSWERS

1. d

2. e

3. e

4. b

5. d

6. c

7. a

8. c

9. a

10. b