Journal of the American Association for Laboratory Animal Science
Copyright 2024
by the American Association for Laboratory Animal Science

Vol 63, No 3
May 2024
Pages 251–256

Ammonia Accumulation as a Proxy to Determine Cage-change Frequency in Antelope Ground Squirrels (*Ammospermophilus leucurus*)

Megan M Ellis, MS, DVM,[1,*] Jourdan E Brune, DVM, PhD,[1] Karuna Patil, MS, MBA, DVM,[2] and Thea L Brabb, DVM, PhD[1]

Due to a lack of evidence-based standards for cage-change intervals for antelope ground squirrels (AGS, *Ammospermophilus leucurus*), we evaluated cage ammonia accumulation in our colony of adult, wild-caught AGS and identified factors that influenced ammonia levels. Intracage ammonia was measured daily in singly housed AGS in static caging that contained a running wheel and 1/2, 3/4, 1, or 2 quart (qt) of corncob bedding. Cages were changed when ammonia levels reached greater than 50 ppm, our upper acceptable limit for ammonia based on mouse studies of ammonia aversion and toxicity. We also measured average daily water consumption over 2 wk to examine any correlation between water use and ammonia accumulation. We hypothesized that the desert-dwelling AGS would not reach intracage ammonia levels of greater than 50 ppm in a 2-wk interval at any bedding volume. Our data showed that intracage ammonia was highly variable among individuals and was significantly associated with water consumption and bedding volumes. Seventeen percent of AGS on 1/2 qt of bedding and 18% on 3/4 qt of bedding reached greater than 50 ppm ammonia before 7 d. All AGS on 1 and 2 qt of bedding remained below 50 ppm ammonia for 1 wk. Even when maintained on 2 qt of bedding, not all AGS remained below 50 ppm ammonia for 2 wk. Therefore, we concluded that the most appropriate option was weekly cage change for singly housed AGS on 1 qt of bedding in static caging.

Abbreviation: AGS, antelope ground squirrels

DOI: 10.30802/AALAS-JAALAS-23-000047

Introduction

Antelope ground squirrels (AGS; *Ammospermophilus leucurus*) are small rodents that show great promise for research of circadian rhythms and sleep. They are one of only a few diurnal rodent species and therefore provide a unique advantage over crepuscular mice and nocturnal rats for translational studies of circadian rhythms.[18,19] However, limited published information is available on AGS husbandry. In their natural environments, AGS are a desert-adapted species; therefore, the husbandry standards for mice may not be appropriate. Mice require 160 to 240 mL/kg/d of water,[1] but free-ranging AGS can require as little as 85 mL/kg/d in dry seasons.[12] Species such as the Mongolian gerbil are theorized to be a more appropriate comparison for AGS husbandry and housing needs, as they are also desert-adapted and have lower water requirements of 88 mL/kg/d and less urine production than do mice.[3] AGS are not yet domesticated, are more challenging to handle than gerbils, may be more prone to stress associated with vivarium housing, and, as they are wild caught, are of unknown, likely varied, ages. Therefore, due to a lack of well-established husbandry standards for AGS, studies are needed to provide evidence-based standards for the species.

Cage changing frequency must fulfill the need to remove waste products from the cage microenvironment while avoiding the stress associated with more frequent cage changes. In mice, high-frequency cage changes have been correlated to increased aggression and anxiety.[2,22,23] On the other hand, changing cages too infrequently allows the accumulation of waste products such as ammonia. High ammonia concentration in the home cage has been correlated with nasal mucosal lesions and irritation to the skin, eyes, and lungs in rats and mice.[17,24] Maximum ammonia exposure limits are not currently cited by the *Guide for the Care and Use of Laboratory Animals*.[4] Therefore, 50 ppm, the standard for maximum human exposure per OSHA,[16] has sometimes been used as a guideline for maximum acceptable ammonia levels in rodent cages. Mice do not show evidence of aversion to ammonia levels up to 110 ppm,[10] and pathology is not often seen before 50 ppm in adult mice.[8,11,15,24] These standards help inform ideal cage-change intervals, which often are every 1 to 2 wk in mice. In desert-adapted gerbils, studies have shown the rate of intracage ammonia accumulation can be much lower, remaining under 6 ppm over 6 wk for pair-housed gerbils in IVC.[14] These studies have been used to justify an extended cage-change interval of up to every 4 wk. This reduction in cage-change frequency is a refinement.

The rate of ammonia buildup is not only species-specific and related to the rate of urine production, but also varies with the bedding and type of caging. One of the most important factors is the ventilation of the cage. Cages matched for mouse sex, age, and stocking density reached an average of 250 ppm of ammonia after only 7 d under static conditions as compared with 10 ppm after 14 d in individually ventilated cages.[15] High intracage humidity levels may also increase ammonia levels, as desiccation of the waste products is slowed, and bacterial

Submitted: 5 Jun 2023. Revision requested: 27 Jun 2023. Accepted: 13 Feb 2024.
[1]Department of Comparative Medicine, University of Washington, Seattle, Washington; and [2]Seattle Children's Research Institute, Seattle, Washington
**Corresponding author. Email: ellis68@purdue.edu*
This article contains supplemental materials online.

growth rates increase.[21] Sex can be a factor in ammonia accumulation, which is higher in male mice with high stocking density.[22] However, the association is less clear for singly housed males.[22] Even the amount of bedding can affect the ammonia levels; as ammonia rises more slowly in mice cages with larger amounts of bedding.[11,20]

Because too-frequent cage changes can cause animal stress due to scent disruption and because of the unknown consequences of high levels of ammonia accumulation in AGS, we measured ammonia concentrations in AGS cages. We hypothesized that our singly housed AGS would have intracage ammonia concentration of less than 50 ppm for a 2-wk interval whether housed with on 1/2, 3/4, 1, or 2 quart (qt) of corn-cob bedding. We studied multiple bedding volumes to identify a volume that would prolong the cage-change interval while still allowing the AGS full access to running wheels needed for future studies. We also predicted that females would take longer than males to reach this concentration. We measured intracage ammonia levels daily over a period of 2 mo in cages provided with ½, 3/4, 1, and 2 qt of bedding. To avoid health or welfare concerns, cages were changed when the concentration reached greater than 50 ppm. We also tracked average daily water consumption for 2 1-wk periods to correlate water usage with ammonia accumulation. The results of our studies support a weekly cage-change interval for AGS housed in static cages containing a running wheel and 1 qt of corncob bedding.

Materials and Methods

Animals. Healthy adult AGS of both sexes were used in this study. Females weighed 104 ± 13 g and males weighed 10 ± 8 g at baseline. AGS were wild-caught on the same weekend in early April 2021; all procedures and protocols for this study were approved by the University of Washington IACUC. Researchers involved in collection had a collecting permit through the Oregon Department of Fish and Wildlife. The ages of all AGS are unknown, but they were all adults (based on body weight), and their reproductive status was verified by physical exam. All females were of breeding age, but none were pregnant, lactating, or had recently weaned pups. All AGS were healthy based on their physical exam on intake. No pathogen surveillance was performed, and no prophylactic treatments were administered upon arrival at the vivarium; therefore, all AGS had an unknown pathogen status. Research, husbandry, and veterinary services staff handled AGS only completing all handling of SPF animals due to their unknown pathogen status. AGS were observed daily by animal care staff for overall health and well-being. All AGS were maintained in accordance with standards described by the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*.

Housing and husbandry. All AGS used in this study were housed singly in filter-topped polycarbonate static rodent caging (Alternative Design Manufacturing and Supply; part no. PC88D-PC) and filter-top lids (Alternative Design; part no. FT8XL-PC) with a floor space of approximately 220 in.^{2} (20 × 11 × 9 in.). Cages were placed on a rack holding a maximum of 25 cages per rack and were equipped with 500-mL polycarbonate water bottles with silicone plugs and stainless-steel tubes. Rodent chow (Laboratory Autoclavable Rodent Diet, 5010; LabDiet, St. Louis, MO) and autoclaved, acidified municipal water were provided ad libitum. Each cage contained a 6-in.-diameter stainless-steel wheel with rods spaced approximately 0.47 in. apart; these were hung from the food hopper near the back of the cage and were used both for enrichment and for the research group's experimental use. AGS were housed singly partially due to concerns of aggression in captive settings and partially so that wheel running could later be quantified for each animal. The environmental parameters of the housing rooms were maintained at temperatures of 19.9 to 22.0 °C (67.8 to 71.6 °F), relative humidity of 35 to 64%, and 12:12 h light:dark cycle (lights on 0800 to 2000).

Cages contained autoclaved corncob bedding (diameter, 1/4 in.; Bed-O'Cobs) and a cotton square (Ancare) for nest building. All cage changes in the study were full changes, including the cage bottom, running wheel, wire-top feeder, and filter-top. AGS had been acclimated to these conditions for 4 wk before this study. The study was performed in May through July 2021. No other studies were being performed on the AGS at the time of this study.

Ammonia measurements. Colorimetric ammonia indicators (Small Animal Ammonia Sensor; Pacific Sentry, Redmond, WA) were placed in a 50-mL conical tube with at least 10-mm drilled holes to allow air circulation. The tubes were placed on top of the wire-top feeder, approximately 7 in. from the cage floor. Sensors were placed during the first half of the light cycle (0800 to 1000). After a period of at least 1 h but no more than 2 h, the tubes were removed, and readings were taken. Between readings, sensors were removed from the room and allowed to off-gas for the remainder of the day, consistent with manufacturer recommendations. Sensors were randomized for placement in individual cages each day. The lid was removed for a maximum of 5 s before being replaced to minimize falsely lower ammonia levels in the cage. This methodology was validated based on quantitative ammonia measurement with Drager tubes (Supplementary Figure S1).

Ammonia data for each cage were recorded based on the color of the disc's central reading window, as per manufacturer instructions. A yellow sensor color indicated less than 1 ppm ammonia, yellow-green indicated 1 to 25 ppm, blue-green indicated 25 to 50 ppm, and dark blue indicated >50 ppm. Any cage with a result of greater than 50 ppm ammonia was immediately changed by the research staff. The days spent at each ammonia level and the cumulative days until cage change were recorded.

Bedding volume and ammonia accumulation. Our initial studies tested 3 bedding volumes in all 20 AGS, and ammonia measurements were taken daily. Twelve AGS (n = 8 males, n = 4 females) received 1/2 qt of bedding for a trial period of a maximum of 27 d. Eleven AGS (n = 8 males, n = 3 females) received 3/4 qt of bedding for a maximum of 20 d. Twenty AGS (n = 14 males, n = 6 females) received 2 qt of bedding for a maximum of 56 d. Bedding for each cage was not changed until that cage reached greater than 50 ppm on the ammonia sensor. In all cases, bedding was not changed until a cage reached greater than 50 ppm on the ammonia sensor. Before the study, the decision was made to exclude any cages that required an early cage change (that is cage floods). However, no cages were excluded from this study.

1-qt Bedding trial. All 20 AGS (n = 14 males n = 6 females) received 1 qt of bedding for 14 d. Ammonia measurements were taken daily. The days spent at each ammonia level and the cumulative days until cage change were recorded. The bedding was changed every 7 d, regardless of the ammonia level reading on the day of cage change. Before the study, the decision was made to exclude any cages that required an early cage change (i.e., cage floods). However, no cages were excluded from this study.

Water consumption. For 2 1-wk periods during the 2 qt bedding trials, water bottles were weighed before being placed in the cage

and after they had been in the cage for 1 wk. Two 1-wk periods were chosen to encompass the amount of time between cage changes, with one replication in case of any variation from week to week. The average daily water consumption was determined for each 1-wk period for each AGS by weighing water bottles at the beginning and end of the week and averaging the weight lost. When cages and bottles were changed during the week-long period, both water bottles had weight loss calculated, and these values were added together. This approach provides an only estimate of water consumption because some water loss may occur due to a dripping bottle or evaporation. AGS were weighed weekly during these periods to determine weight-affected water consumption.

Statistical analysis. The data for the bedding volume and ammonia accumulation experiment and for the assessment of the water loss experiment were analyzed and found to be non-normal per the Shapiro–Wilk normality test ($P < 0.05$). Therefore, the nonparametric Kruskal–Wallis test was used to analyze the significance of the effect of sex and bedding volume on days greater than 50 ppm ammonia.

Spearman correlation coefficients were used to evaluate the relationship between the time to reach greater than 50 ppm of ammonia accumulation and water loss from the bottles. If the correlation coefficient was between −0.7 and −1.0, a strong negative association existed between the variables. If the correlation coefficient was between −0.3 and -0.7, the association between the variables was weak. A correlation coefficient between −0.3 and 0.3 indicated no association between the variables.

All statistical analyses were performed by using RStudio statistical software version 2022.07.01, and data visualization was performed in GraphPad Prism 9.5.1. Post hoc power analysis was performed using G*Power 3.1.9.7.[7]

Results

Bedding volume and ammonia accumulation. The average number of days to reach greater than 50 ppm ammonia varied by bedding volume and between individual animals; variation by sex could not be analyzed due to insufficient statistical power. During the 1/2-qt bedding trial ($n = 12$), 17% of the AGS reached greater than 50 ppm ammonia before 7 d, 67% reached greater than 50 ppm between 7 d and 14 d, and 17% took longer than 2 wk to reach greater than 50 ppm (Figure 1A). Group values ranged between 5 and 27 d to reach greater than 50 ppm ammonia. Therefore, 1/2 qt of bedding was deemed inadequate for a cage-change interval of 1 wk due to the accumulation of ammonia above 50 ppm in 2 individuals.

During the 3/4-qt bedding trial ($n = 11$), 18% of the AGS reached greater than 50 ppm ammonia before 7 d, 73% reached greater than 50 ppm between 7 and 14 d, and 9% reached greater

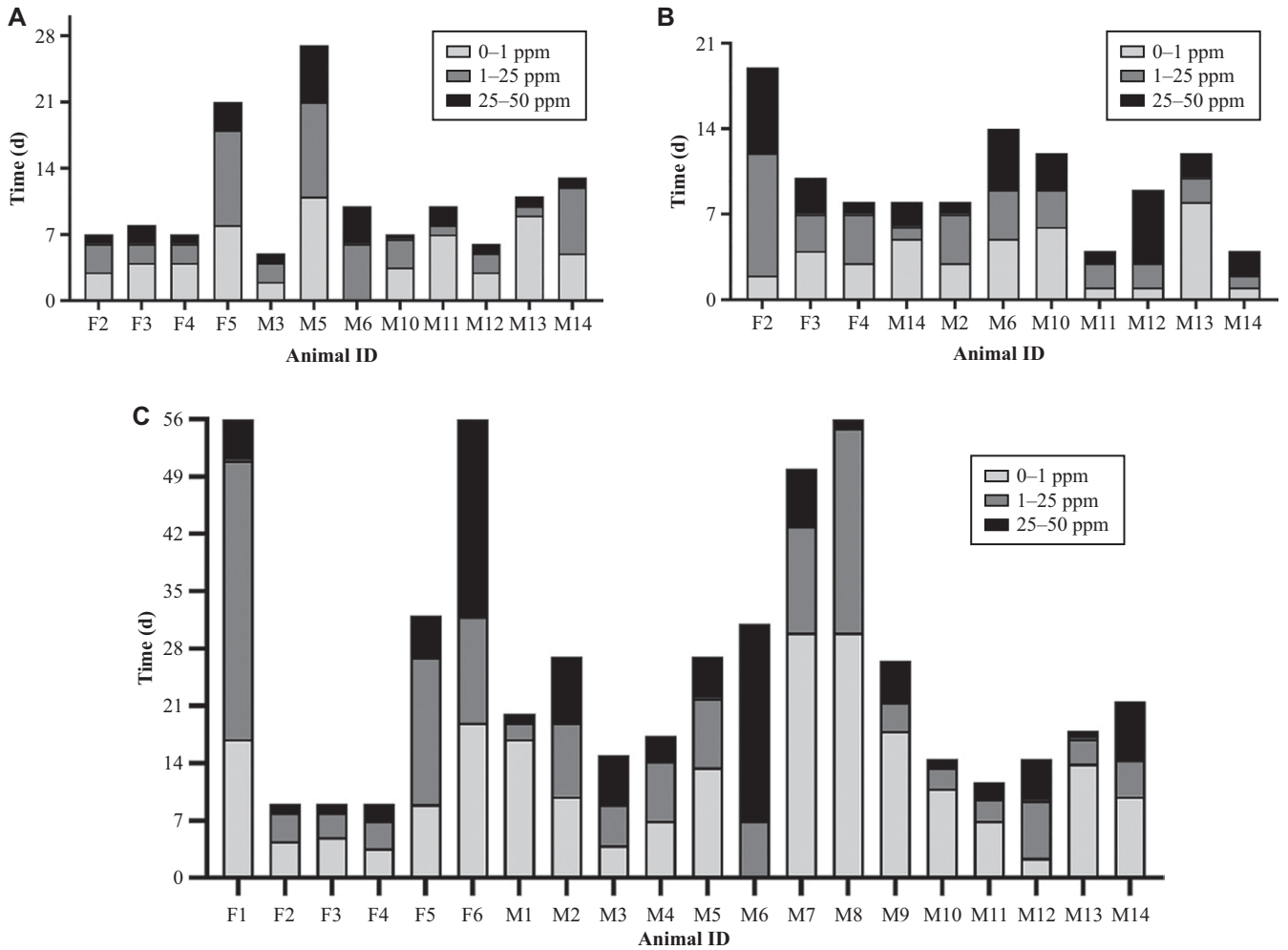

Figure 1. Ammonia sensors were read daily until the cage change. Cages were changed when sensors reached >50 ppm ammonia. (A) Cages housing one adult AGS on 1/2 qt of bedding ($n = 12$). (B) Cages housing one adult AGS on 3/4 qt of bedding ($n = 11$). (C) Cages housing one adult AGS on 2 qt of bedding ($n = 20$). Animals undergoing multiple trials had all trial data averaged. All animals lettered M are males; all animals lettered F are females.

than 50 ppm after longer than 2 wk (Figure 1B). These data were slightly less variable than in the 1/2-qt trial. Group values ranged between 4 and 19 d to reach greater than 50 ppm ammonia. Therefore, the 3/4-qt bedding was deemed inadequate for a cage-change interval of 1 wk due to the accumulation of ammonia above 50 ppm in 2 individuals.

During the 2-qt bedding trial (n = 20), none of the animals reached greater than 50 ppm ammonia before 7 d, 20% reached greater than 50 ppm between 7 and 14 d, 35% reached greater than 50 ppm between 14 and 21 d, 15% reached greater than 50 ppm between 21 and 28 d, 15% reached greater than 50 ppm between 28 and 35 d, and 15% did not reach greater than 50 ppm during the 56 d they remained on 2 qt of bedding (Figure 1C). Some AGS groups required a minimum of 9 d to reach greater than 50 ppm ammonia; others had not reached greater than 50 ppm ammonia after 56 d. This amount of bedding was determined to be adequate for a cage-change interval of 1 wk. Because 20% of AGS reached greater than 50 ppm ammonia between 7 and 14 d, a cage-change interval of 2 wk would be adequate for preventing intracage ammonia levels from reaching >50 ppm for all animals.

Overall, an average of 26 d was required for AGS on 2 qt of bedding to reach greater than 50 ppm ammonia, which was significantly longer than 10 d on 3/4 qt of bedding ($P < 0.05$) and 11 d on 1/2 qt of bedding ($P < 0.01$) (Figure 2). The amount of time needed to reach greater than 50 ppm ammonia on 3/4 qt of bedding was not significantly better than 1/2 qt of bedding. Post hoc power analysis indicated that power was inadequate for detecting significant differences between the amount of time required for male and female AGS to reach greater than 50 ppm with 1/2 qt and 3/4 qt of bedding ($\beta > 0.5$).

1-qt Bedding trials. During 2 1-wk-long trials on 1 qt of bedding, none of the AGS reached ammonia levels greater than 50 ppm (n = 20). Ten percent of the AGS reached ammonia levels of 25 to 50 ppm in the first 7-d trial, whereas 15% of the AGS reached this level during the second 7-d trial. Fifty percent of the AGS had ammonia readings of 0 to 1 ppm for the entire duration of both 7-d trials (Figure 3).

Assessment of water loss from bottles. A strong negative correlation (Spearman, $\rho = -0.7882$ [$P < 0.001$]) was detected between average daily water loss from the bottles and days to greater than 50 ppm ammonia on 2 qt bedding was found (Figure 4). Animal weight and days to greater than 50 ppm were not significantly correlated ($P = 0.806$). The correlation between the daily water loss from the bottles and days to greater than 50 ppm ammonia was significant when daily water loss from the bottles was standardized to the individual animal's body weight ($P < 0.005$, data not shown).

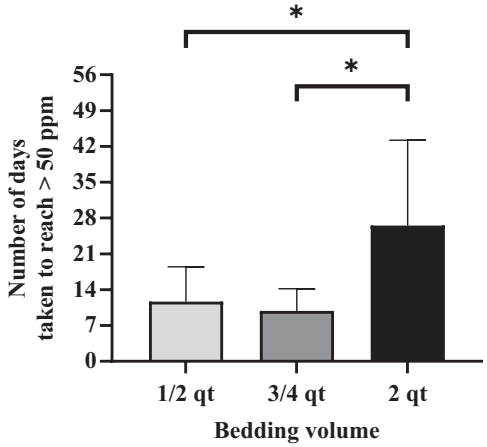

Figure 2. Average number of days taken to >50 ppm ammonia as a function of bedding volume. Cages with 2 qt of bedding took significantly longer to reach >50 ppm on 2 qt of bedding than did cages with 3/4 qt (*, $P < 0.01$) or 1/2 qt (*, $P < 0.01$) of bedding. Three AGS went for 56 d at 2 qt of bedding without reaching >50 ppm. None of the other AGS did so at the other bedding volumes.

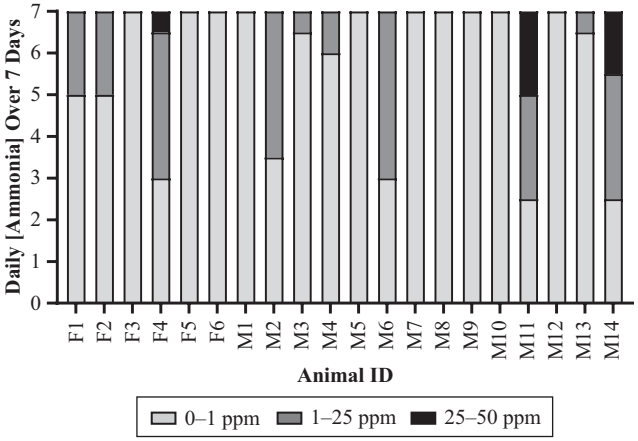

Figure 3. Daily ammonia sensor readings in cages housing one adult AGS on 1 qt of bedding (n = 20). Data are averaged from 2 1-wk-long trials and show the average number of days each cage spends at a given ammonia level for 1 wk after cage change.

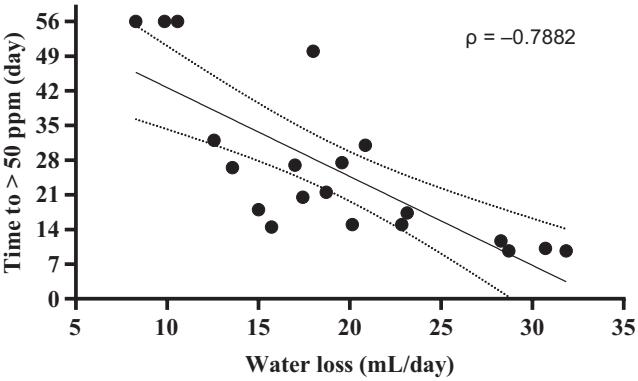

Figure 4. Data show a significant correlation between daily water lost from water bottles and the number of days until the cage reached >50 ppm of ammonia (n = 20). $P < 0.001$.

Discussion

Despite considerable variation with regard to intracage ammonia accumulation for individual AGSs, we validated a standardized cage-change interval of 1 wk for our ASGs housed with 1 qt of corncob bedding. Our data showed that despite considerable variation across AGS, ammonia did not accumulate above 50 ppm in any cages that were bedded with 1 qt of corncob bedding and changed weekly. Different bedding volumes were tested. Individual AGS housed on 1/2 qt and 3/4 qt of bedding reached levels greater than 50 ppm before a week had passed, so these volumes were not tested further. While ammonia accumulation was significantly slower at 2 qt bedding volumes, this bedding volume did keep ammonia under 50 ppm in all AGS for over 9 d and was not selected as an ideal volume because it occasionally blocked running wheel access. Ammonia accumulation correlated with water consumption. Statistical power was not sufficient for assessing the effect of AGS on ammonia levels.

Overall, our final cage-change frequency every week is consistent with the current standards for mice in static caging.

The short cage-change interval is likely heavily influenced by the use of static cages, which have also been implicated in a faster rate of ammonia accumulation in mice.[8] Our hypothesis that AGS intracage ammonia accumulation may more closely resemble that of desert-dwelling species such as gerbils was not supported by comparisons with the literature. Published literature is currently not available on intracage ammonia levels for gerbils housed in static caging. An internal assessment at our institution showed that singly housed gerbils can be maintained for up to 4 wk in static caging without ammonia accumulating above 25 ppm (data not shown), but because this assessment was performed in cages that contained 4 qt of corncob bedding, those data are not directly comparable to this study.

Our water use experiment found that higher water loss (proxy for water consumption) is significantly negatively correlated with days to greater than 50 ppm ammonia accumulation. Due to the study design, we cannot rule out the possibility of water loss due to leaking bottles or sipper play, although we did not note any flooded or wet-appearing cages during the study. Our AGS used between 77 and 306 mL/kg/d (average of 179 mL/kg/d); this range includes values that are higher than field findings of 85 to 238 mL/kg/d (average of 162 mL/kg/d).[10] The field studies show fluctuation in water consumption by season, availability, and the water content of food, whereas caged AGS may have no need to conserve water and concentrate urine because water is freely available. This is hypothesized to be done by captive gerbils but not yet studied.[14] Any loss due to a leaking or sipper play would increase the overall cage moisture and humidity, and humidity and ammonia levels are positively correlated in mouse cages.[18] Even though we cannot clearly know how much water was consumed as compared with wasted, the relationship is still clear. Studies using metabolic cage studies or other methods of measuring water intake and urine excretion would be necessary to better understand the drinking behavior of captive AGS.

Our findings on the relationship between bedding volume and ammonia accumulation in AGS echo findings in mice: intracage ammonia levels rise more slowly with a greater volume of bedding.[9,20] However, in our study, an increase in bedding from 1/2 to 3/4 qt did not significantly change the rate of ammonia accumulation. Similarly, a recent study in mice showed that providing 150% of the normal amount of bedding in IVC cages does not significantly affect ammonia accumulation.[6] Because our highest-volume group (2 qt) had 400% of the bedding of the lowest-volume group, perhaps a threshold must be reached to have significant effects on ammonia accumulation. Overall, in larger static cages, more bedding may absorb more urine, preventing the volatilization of ammonia compounds and contributing to lower humidity.

Our initial studies did not include a 1-qt ammonia accumulation arm, which unfortunately eliminates our ability to compare that volume statistically to the other volumes investigated. Instead, we performed week-long trials of the 1-qt bedding volume and found that none of the AGS reached ammonia levels of greater than 50 ppm in either of 2 trials, and only 3 AGS reached levels of 25 to 50 ppm on 1 or 2 d. We opted to use 1 qt of bedding for the second study due to its compatibility with the research group's experimental equipment (running wheels) and because the prior trials at 1/2 and 3/4 qt failed to stay below greater than 50 ppm in all animals for 1 wk. Even at the tested bedding volumes, significantly different rates of ammonia accumulation would probably occur if different bedding types were used. A study using mice in static cages found that those bedded with reclaimed wood pulp had more ammonia accumulation than did those with corncob, aspen wood chip, or recycled newspaper bedding.[8] Due to these constraints and the unique wild-caught population, our findings may not be applicable to all AGS housing paradigms, and internal investigation should be used when needed.

This study did not have the necessary statistical power for definitive conclusions on the effect of sex on intracage ammonia. In well-powered mouse studies of ammonia accumulation, high-density cages of male mice reach cage-change criteria faster than high-density cages of female mice.[25] Studies in *Mus domesticus* show higher rates of urine excretion in male mice as compared with females[5]; however, a study in CD1 male and female mice found a difference between males and females only when housed by sex at a density of 5 mice per cage, but not when housed at of one or 3 mice per cage.[24] A larger number of AGS would be necessary to detect an effect in this highly variable population.

A unique and species-specific aspect of this study is the husbandry of a wild-caught population of unknown age, genetic relationship, and health status. Some subclinical physiologic differences could influence ammonia accumulation or urine production, as in diabetic mouse models,[13] because we found significant variation among individuals in the rate of ammonia accumulation. Although specific individuals of each group consistently had higher or lower ammonia levels, we believe this variation represents the true variability of a wild-caught, outbred population and is not due to sampling error, measurement error, or poor sampling. Therefore, none of the AGS were excluded. The variation among individuals in turn influenced the statistical power of the research. This study demonstrates the challenges of housing wild-caught species in standard rodent housing.

In summary, we validated a 1-wk cage-change interval in singly housed AGS housed in static microisolation cages containing 1 qt of corncob bedding. Although some individuals could have a longer cage-change interval, standardizing the interval allows us to minimize the room disruption of cage change and creates a routine for both the animals and the personnel. Our data show that bedding volume and water usage were predictors of ammonia accumulation in our population. Larger amounts of bedding amounts could both decrease the rate of ammonia accumulation and allow for the enriching activities of burrowing and digging. Further study of ammonia accumulation in AGS, the effects of sex, macro- and microenvironmental effects on intracage ammonia levels, and the effects of ammonia on AGS health would be useful. In addition to evaluating sex effects by using adequate numbers of male and female AGS, metabolism studies could be performed to quantify water consumption and urine excretion. The actual upper tolerance limit of AGS for ammonia is currently unknown. Although we used 50 ppm in this study, preference tests and wild burrows could be studied to reveal aversive levels. These studies could be used to better define ideal husbandry conditions for captive AGS to refine the use of this valuable model.

Supplementary Materials

Figure S1. A short validation study was performed using cages spiked with 1 mL of an ammonium hydroxide solution at the ratios shown. The colorimetric sensors showed agreement with the Drager tubes in 92% of the solutions tested.

Acknowledgments

We thank George James Kenagy for his expertise on the species of interest. We also thank Victor Zhang and Horacio de la Iglesia for their support with these animals.

Conflict of Interest

The authors have no conflicting interests to declare.

Funding

This paper was supported in part by National Institute of General Medical Sciences (NIGMS) grant A164009.

References

1. **Bachmanov AA, Reed DR, Beauchamp GK, Tordoff MG.** 2002. Food intake, water intake, and drinking spout side preference of 28 mouse strains. Behav Genet **32**:435–443. https://doi.org/10.1023/A:1020884312053.
2. **Balcombe JP, Barnard ND, Sandusky C.** 2004. Laboratory routines cause animal stress. Contemp Top Lab Anim Sci **43**:42–51.
3. **Boice R, Arledge JG.** 1968. Water requirements of gerbils and kangaroo rats in the laboratory. Psychol Rep **23**:1063–1069. https://doi.org/10.2466/pr0.1968.23.3f.1063.
4. **Council NR.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): The National Academies Press.
5. **Drickamer LC.** 1995. Rates of urine excretion by house mouse (*Mus domesticus*): Differences by age, sex, social status, and reproductive condition. J Chem Ecol **21**:1481–1493. https://doi.org/10.1007/BF02035147.
6. **Eskandarani MA, Hau J, Kalliokoski O.** 2023. Rapid ammonia build-up in small individually ventilated mouse cages cannot be overcome by adjusting the amount of bedding. Lab Anim **52**:130–135. https://doi.org/10.1038/s41684-023-01179-0.
7. **Faul F, Erdfelder E, Buchner A, Lang A-G.** 2009. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav Res Methods **41**:1149–1160. https://doi.org/10.3758/BRM.41.4.1149.
8. **Ferrecchia CE, Jensen K, Van Andel R.** 2014. Intracage ammonia levels in static and individually ventilated cages housing C57BL/6 mice on 4 bedding substrates. J Am Assoc Lab Anim Sci **53**:146–151.
9. **Freymann J, Tsai PP, Stelzer HD, Mischke R, Hackbarth H.** 2017. Impact of bedding volume on physiological and behavioural parameters in laboratory mice. Lab Anim **51**:601–612. https://doi.org/10.1177/0023677217694400.
10. **Green AR, Wathes CM, Demmers TG, Clark JM, Xin H.** 2008. Development and application of a novel environmental preference chamber for assessing responses of laboratory mice to atmospheric ammonia. J Am Assoc Lab Anim Sci **47**:49–56.
11. **Horn MJ, Hudson SV, Bostrom LA, Cooper DM.** 2012. Effects of cage density, sanitation frequency, and bedding type on animal wellbeing and health and cage environment in mice and rats. J Am Assoc Lab Anim Sci **51**:781–788.
12. **Karasov WH.** 1983. Water flux and water requirement in free-living antelope ground squirrels *Ammospermophilus leucurus*. Physiol Zool **56**:94–105. https://doi.org/10.1086/physzool.56.1.30159970.
13. **Kim AK, Hamadani C, Zeidel ML, Hill WG.** 2020. Urological complications of obesity and diabetes in males and females of three mouse models: Temporal manifestations. Am J Physiol Renal Physiol **318**:F160–F174. https://doi.org/10.1152/ajprenal.00207.2019.
14. **McCullagh EA, McCullagh P, Klug A, Leszczynski JK, Fong DL.** 2017. Effects of an extended cage-change interval on ammonia levels and reproduction in mongolian gerbils (*Meriones unguiculatus*). J Am Assoc Lab Anim Sci **56**:713–717.
15. **Mexas AM, Brice AK, Caro AC, Hillanbrand TS, Gaertner DJ.** 2015. Nasal histopathology and intracage ammonia levels in female groups and breeding mice housed in static isolation cages. J Am Assoc Lab Anim Sci **54**:478–486.
16. **OSHA.** OSHA occupational chemical database: Ammonia. Washington (DC): Occupational Safety and Health Administration.
17. **Perkins SE, Lipman NS.** 1995. Characterization and quantification of microenvironmental contaminants in isolator cages with a variety of contact beddings. Contemp Top Lab Anim Sci **34**:93–98.
18. **Refinetti R, Kenagy GJ.** 2018. Circadian rhythms of body temperature and locomotor activity in the antelope ground squirrel, *Ammospermophilus leucurus*. J Therm Biol **72**:67–72. https://doi.org/10.1016/j.jtherbio.2018.01.001.
19. **Refinetti R, Kenagy GJ.** 2018. Diurnally active rodents for laboratory research. Lab Anim **52**:577–587. https://doi.org/10.1177/0023677218771720.
20. **Rosenbaum MD, VandeWoude S, Johnson TE.** 2009. Effects of cage-change frequency and bedding volume on mice and their microenvironment. J Am Assoc Lab Anim Sci **48**:763–773.
21. **Rosenbaum MD, VandeWoude S, Volckens J, Johnson T.** 2010. Disparities in ammonia, temperature, humidity, and airborne particulate matter between the micro-and macroenvironments of mice in individually ventilated caging. J Am Assoc Lab Anim Sci **49**:177–183.
22. **Van Loo PL, Van der Meer E, Kruitwagen CL, Koolhaas JM, Van Zutphen LF, Baumans V.** 2004. Long-term effects of husbandry procedures on stress-related parameters in male mice of two strains. Lab Anim **38**:169–177. https://doi.org/10.1258/002367704322968858.
23. **Van Loo PL, Van Zutphen LF, Baumans V.** 2003. Male management: Coping with aggression problems in male laboratory mice. Lab Anim **37**:300–313. https://doi.org/10.1258/002367703322389870.
24. **Vogelweid CM, Zapien KA, Honigford MJ, Li L, Li H, Marshall H.** 2011. Effects of a 28-day cage-change interval on intracage ammonia levels, nasal histology, and perceived welfare of CD1 mice. J Am Assoc Lab Anim Sci **50**:868–878.
25. **Washington IM, Payton ME.** 2016. Ammonia levels and urine-spot characteristics as cage-change indicators for high-density individually ventilated mouse cages. J Am Assoc Lab Anim Sci **55**:260–267.