Preventing annoyance from odors in spaceflight: a method for evaluating the sensory impact of rodent housing

P. Dalton,1 M. Gould,1 B. Girten,2 L. S. Stodieck,3 and T. A. Bateman3,4
1Monell Chemical Senses Center, Philadelphia, Pennsylvania 19104; 2National Aeronautics and Space Administration Ames Research Center, Moffett Field, California 94035; 3BioServe Space Technologies, Aerospace Engineering Sciences Department, University of Colorado, Boulder, Colorado 80309; and 4Bioengineering Department, Clemson University, Clemson, South Carolina 29634

Submitted 22 April 2003; accepted in final form 24 July 2003

Daltón, P., M. Gould, B. Girten, L. S. Stodieck, and T. A. Bateman. Preventing annoyance from odors in spaceflight: a method for evaluating the sensory impact of rodent housing. J Appl Physiol 95: 2113–2121, 2003; 10.1152/japplphysiol.00399.2003.—For the scientific community, the ability to fly mice under weightless conditions in space offers several advantages over the use of rats. These advantages include the option of testing a range of transgenic animals, the ability to increase the number of animals that can be flown, and reduced demands on shuttle resources (food, water, animal mass) and crew time (for water refill). Mice have been flown in animal enclosure module (AEM) hardware only once (Space Shuttle Transport System (STS)-90) and were dissected early in the mission, whereas rats have been flown in the AEM on >20 missions. This has been due, in part, to concerns that strong and annoying odors from mouse urine (vs. rat urine) will interfere with crew performance in the shuttle middeck. To screen and approve mice for flight, a method was developed to evaluate the odor containment performance of AEMs housing female C57BL/6J mice compared with AEMs housing Sprague-Dawley rats across a 21-day test period. Based on the results of this test, consensus was reached that mice could fly in the AEM hardware for up to 17 days (including prelaunch and contingency) and that the AEM hardware would likely contain odors beyond this duration. Human sensory and electronic nose analysis of the AEMs postflight demonstrated their success in containing odors from mice for the mission duration of STS-108 (13 days). Although this paper focuses specifically on odor evaluations for the space shuttle, the concern is applicable to any confined, closed-system environment for human habitation.

animal habitat; sensory evaluation

ANIMAL EXPERIMENTATION IS FUNDAMENTAL to biomedical research relevant to human health. Similarly, subjecting laboratory animals to spaceflight is important to supplementing data collected from astronauts to characterize the effects of microgravity on physiological systems. Historically, rats have been the primary species for these examinations and have flown in space on many occasions: more than 20 space shuttle flights have included rats. Mice have flown much less often and only once on the space shuttle (Space Shuttle Transport System (STS)-90), where fetuses were dissected in-flight on flight days 3 and 6 (11).

The use of rats rather than mice is largely a function of historical preference, with the earliest Russian Cosmos biosatellite missions examining rats (15, 12). However, the last decade has seen an increased interest in spaceflight experiments using mice. The primary scientific benefit of using mice is the ability to examine the role specific genes play in the physiological adaptation to spaceflight, either by overexpressing or deleting a particular gene, a technique that is nearly exclusively available in mice. With the completion of the mapping of the mouse genome and the remarkable similarity between the mouse and human genome (10), the benefits of spaceflight research on wild-type and transgenic mice assumes even greater importance.

In addition to the scientific benefits of having mice as an option for microgravity research, there are very practical resource-related reasons that mice may be a beneficial biomedical specimen. At approximately ~10% the size of rats, mice simply require less volume, up and down mass (body weight, food, water, waste), oxygen consumption, and carbon dioxide removal. Although, relative to humans, resource requirements for mice or rats are relatively small, when multiplied over dozens of subjects (the number needed to make multiple scientific examinations), the difference is not trivial. Furthermore, on the basis of the volume available in the animal enclosure module (AEM), the primary spaceflight habitat currently available for rodent research, more mice can be accommodated than rats and with far less crowding. This can reduce the potential confounding influence of stress, hypokinesia, and altered musculoskeletal loading that can occur under conditions of overcrowding with rats.

Because the volume on any space shuttle experiment platform is restricted (Space Lab, Space Hab, or Middeck) and generally includes the living and working quarters for the astronauts, great care is...
taken by National Aeronautics and Space Administration (NASA) to preserve these environments and minimize any negative impact on the well-being of the crew. Specifically, one facet of concern is environmental odors, particularly malodors. Concern regarding environmental odors is not simply an issue of crew comfort, but also one of safety, with many signs of environmental emergencies initiating through detection of an odorous gas. The presence of malodors could mask the detection of such emergencies. Although this paper focuses specifically on odor containment for animal habitats on the space shuttle, the concern is applicable to any confined, closed-system environment for human habitation, including submarines and the International Space Station.

Mouse urine is often perceived as having a higher odor impact, compared with the urine odor of other rodents. For this reason, concerns about exposing astronauts to the more pungent and potentially offensive waste odors of mice have prevented them from becoming regular scientific subjects on the space shuttle. This paper describes the results from a comprehensive odor test to determine the ability of the AEM (NASA-Ames Research Center, Moffett Field, CA) habitat and its exhaust filters to contain the waste odors from mice. Based on the outcome from the most recent test, mice flew in the AEM hardware on STS-108 for 12 days plus 1 day prelaunch in December 2001 (16). An evaluation of odor characteristics that was made between the flight and ground control AEM units, confirming the success of the AEMs odor-containment ability, is also presented.

A comprehensive odor test protocol was developed and first conducted in June, 2000 at the NASA-Ames Research Center to evaluate the odor-containment abilities of AEMs housing two different densities (9 vs. 12 mice/AEM) of C57BL/6 (B6) female mice. (This test is referred to in this paper as the “June 2000 test.”) Qualified volunteers, who were screened for good health and olfactory sensitivity, sampled the air effluent from the filtration system on six AEMs housing either 9 or 12 mice and rated odor intensity, quality, and annoyance throughout a 21-day test duration. Two noteworthy findings emerged from this test. Differences in waste deposition patterns of mice (as compared with previous tests using rats) produced liquid waste leakage from the interior of the AEMs. This resulted in detectable odor breakthrough in the highest density AEMs on day 7 of the test and led to minor hardware modifications, which were implemented in the present study. Additionally, we made the novel observation that a small proportion of human volunteers seemed unusually able to detect odors they described as moderately strong and “urinous” from AEMs containing mice, whereas the majority of volunteers detected no odor. Although such variation in olfactory acuity is not uncommon, we sought to further quantify the distribution of this enhanced sensitivity characteristic among participants in the present study.

METHODS

Human Subjects

Twenty-two volunteers (6 women/16 men, mean age = 38.6 yr) were recruited for the 21-day evaluation by using advertisements placed in local newspapers in the Philadelphia metropolitan area. The gender distribution was intended to approximate the typical composition of shuttle crews. One male participant withdrew because of scheduling difficulties after five sessions; all other participants completed all test sessions. An additional 48 individuals (26 women, 22 men, aged 22–44 yr) were recruited to provide a one-time evaluation to determine a better estimate of the population distribution of sensitivity to rodent urine odors. Potentially eligible volunteers were screened for the presence of any health conditions that might impact their olfactory sensitivity (e.g., sinusitis, allergies, head injuries, chemical exposures) and for possessing normal olfactory acuity. All qualified volunteers were nonsmokers and had no nasal abnormalities as assessed by a physical exam before the start of the study.

All volunteers provided informed consent and were paid for their participation. The study protocol and consent forms were approved in advance by the NASA Ames Human Research Institutional Review Board and the University of Pennsylvania Institutional Review Board for the Use of Human Subjects. Volunteers were told that the purpose of the test was to evaluate potential odors emanating from sources that were present on manned spacecraft, including volatiles from foods, equipment, humans, and animals. To eliminate known biases that might alter the perceived odor reports, however, all AEMs were shrouded so that volunteers were blinded as to the source of the odors they were evaluating.

Each volunteer provided odor evaluations every other day for the first 18 days and every day for the final 3 days of the study for a total of 12 test sessions. The additional group of 48 volunteers made a single session evaluation on day 18 of the 21-day study.

Electronic Nose

As an adjunct to the evaluations made by the volunteers, an electronic vapor analysis instrument (electronic nose) was used to determine the potential utility of such a device for evaluating animal habitat odors for future hardware modifications. The instrument used in this test was a Cyranose 320 (Cyrano Sciences), a handheld, portable unit with an internal sampling pump that pulls air at a predetermined rate across a sensor array for analysis. The sensor technology consists of individual thin-film carbon-black polymer composite chemiresistors configured into an array. The collective output of the array is used to identify an unknown analyte by using standard data analysis techniques (8). Similar instruments have been used to discriminate between mouse strains from individual urine odors (14).

For the present study’s purposes, it was necessary to train the Cyranose to detect and differentiate between B6 female mouse urine (collected from the B6 mouse colony established at Monell) and the standard background odors that would be found in the air recirculated through an empty AEM supplied with food. Because the effect of concentration on sensor response was unknown for a complex mixture such as mouse urine, the Cyranose was trained on two concentrations of mouse urine: full strength and diluted to 1% with deionized water. The two samples were easily discriminable by human noses and, after eight training sessions on each stimulus, by the electronic nose. The instrument was also trained to...
identify the blank AEM and to identify room air in the test rooms.

Animals and Habitat

The AEM hardware has flown >20 times in the space shuttle middeck. It provides animals with food and water ad libitum and maintains a 12:12-h light-dark cycle. Food is provided in the form of a specially designed bar (foodbar) that is intended to be consumed with less crumbling and waste than standard laboratory chow (Teklad no. TD 97071, American Institute of Baking, Manhattan, KS), and is affixed to the wall of the habitat compartment and water box for consumption. The foodbar is formulated with higher water content than standard chow (~25% compared with 5%) and extruded.

On the space shuttle, an AEM occupies one middeck locker and has a habitat compartment that provides ~110 in.² of floor space and 1 ft.³ of volume. As shown in Fig. 1, air is circulated (1 pass) through the animal habitat at a sufficient rate (~12 ft.³/min, 0.25 ft./s) to force animal waste toward the exhaust filter in microgravity conditions. The internal structure of the AEM, where either mice or rats are housed is a wire mesh cage (~½-in.² spacing between the mesh for rats and ~¼-in.² spacing for mice). For rats, the habitat is a single unit. For mice, the wire cage habitat is divided in half to form two housing units.

The AEM exhaust filter consists of 13 layers bonded (silicone-based adhesive) within an aluminum box and sealed to the AEM with closed cell foam. The top layer, closest to the mice, is a polypropylene mesh treated with phosphoric acid to react with the gaseous ammonia constituent of urine to produce an ammonium phosphate precipitate. The balance of the filter consists of redundant layers for liquid absorption and dispersion, molecular adsorption (carbon and zeolite), particulate trapping (HEPA filter) and structural containment (wire mesh). The filter is ~6.4 cm thick (2.5 in.) and covers the floor area just below the wire-mesh cage.

Six 45-day-old male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were housed in each of three AEMs for the odor test and averaged 190 ± 17 g at the start of the study. At the end of the 21-day study, the rats averaged 314 ± 16 g. This strain, sex, and age was chosen to represent a configuration flown on multiple occasions (3, 21, 22). Eight 20-wk-old female C57BL/6 (B6) mice (Taconic Farms) were housed in each of three AEMs, with four mice loaded into each side of the AEM (8 mice/AEM); each mouse averaged 22.1 ± 1.0 g at the start of the study. At the end of the 21-day test, the mice averaged 22.3 ± 1.5 g. C57BL/6 mice were selected to mimic the strain chosen to fly on STS-108 (December 2001), a strain often used for models of skeletal unloading (2, 13, 18, 19). Female mice were chosen because their urine is less malodorous than that of male mice. The particular mice were chosen to represent the oldest age and largest weight under consideration for the STS-108 experiment (commercial biomedical testing module-01). The protocol for the care and use of these animals was approved by the Institutional Animal Care and Use Committees of both Monell Chemical Senses Center and NASA-Ames Research Center.

In addition to the three AEMs housing mice and the three housing rats, two AEMs were left empty but filled with food to serve as negative controls, whereas two AEMs were spiked with a filter cartridge containing 0.5% amyl acetate (banana) or 1% toluene on alternating days to serve as positive controls. AEM sensory evaluations were conducted in four rooms (each measuring ~10 ft. × 10 ft.) containing either two or three AEMs each. One positive or one negative control AEM was placed in each room along with one or two AEMs containing either rats or mice. The placement of AEMs was rotated both within and across rooms for each test day on a predetermined schedule. Daily odor screening evaluations for each subject were carried out in a separate room. To simulate ambient conditions on the middeck of the space shuttle (where the AEMs would be housed in flight), temperature and humidity were maintained at 26 ± 2°C and 50 ± 10% relative humidity throughout the test duration. These conditions also provided the most rigorous conditions for evaluating the generation and dispersion of detectable odors (17).

Olfactory Performance Screening

To establish minimum olfactory performance levels, each volunteer’s ability to detect a set of seven standard odorants was evaluated at the beginning of each testing session. Standards adapted from the NASA Odor Screening Document 80-60 consisted of isoamyl acetate, phenylethyl alcohol, methyl disulfide, 15-hydroxypentadecanoic acid lactone, menthone, acetic acid, and 1,8 cineole (Sigma Chemical, St. Louis, MO). Odorants were diluted to the appropriate concentration with Millipore brand ultra pure water (resistivity > 10 MQ·cm) and placed into 280-ml glass bottles with custom-designed Teflon nosepieces. Each bottle contained 10 ml of the diluted odorant or water only. Stock solutions of the standard odors were prepared weekly, and new sample bottles were prepared from the stock solutions on each test day.
On each trial, volunteers were presented with two bottles, a blank bottle (water only) and a stimulus bottle (diluted odorant in water). Volunteers sniffed consecutively from each bottle through one nostril while holding the other nostril closed. Choice of nostril was determined by the participant, with the requirement that they use the same nostril for all evaluations in a single test session. They were then asked to identify the bottle containing the odor, rate the intensity of the odor, and describe the odor by using a list of provided descriptors. Odor intensity was rated on a six-point category scale with the following descriptors: none = 0, barely detectable = 1, weak = 2, moderate = 3, strong = 4, very strong = 5.

The list of descriptors contained the recognized odor qualities of the seven screening odorants (solvent, eucalyptol, musk, floral, mint, vinegar-like, putrid, fruity). Each odorant was presented once during the screening evaluations for a total of seven trials. An individual was considered to have failed the odor screening if they failed to identify at least six of the seven screening odorants. If the volunteer failed the odor screening, they completed the referent odor and AEM evaluations that day, but their data were not included in the analysis. Data from two participants were excluded on only one occasion throughout the 21-day test.

Referent Odor Evaluations

To further standardize the sensory ratings over time, volunteers were asked to sniff and rate the odor intensity and annoyance of four referent odorants during each evaluation session by using the six-point category scale described above. Both intensity and annoyance were evaluated against the six-point category scale described above. Referent odors included rodent foodbar, B6 female mouse urine, 3-methyl-2-hexanoic acid, one of the main odorous components of human axillary secretions (24), and vanillin. Volunteers were also asked to provide quality descriptions of each odor by selecting one or more words from a list of 20 descriptors (see Appendix). Each referent odor stimulus was placed into a 280-ml glass bottle with a specially designed Teflon nosepiece. The bottles were wrapped with foil to avoid visual cues from the stimuli within. These referent odors were intended to provide an anchoring context to control for changes in intensity ratings of AEM odors over time.

AEM Evaluations

At each test session, volunteers evaluated the odor intensity and annoyance experienced from sniffing the exhaust air of each AEM at two distances: 12 and 6 in. from the front of the AEM. The AEM evaluations were made according to the following procedure. Volunteers entered the testing room and were asked to sit on a rolling chair in the center of the testing room. When prompted by the experimenter, the volunteer moved their chair to be 12 in. from the AEM. After 5 s, the volunteer rated odor intensity and annoyance by using the six-point category scale. In addition, they were asked, if they in fact smelled an odor, to try to describe it by selecting one or more words from the same list of 20 descriptors used with the referent odors. Then they were instructed to move forward and place their chin in a chin rest for 5 s. The chin rest was positioned directly in front of the AEM to sample odors emanating from the exhaust fan. The chin rest was positioned such that no volunteer's nose would be >6 in. from the front of the AEM (see Fig. 1 for details of placement). At that time, subjects again made ratings of odor intensity, annoyance, and odor quality. All ratings were entered directly by the experimenter into a data-collection program on a laptop computer in each test room. After the first AEM was evaluated, the volunteers repeated the rating procedure for the other AEMs within that room, separated by a 1-min interval. Each subsequent room was evaluated in the same fashion.

In addition to the regular evaluations made by the core sensory panel across the 21-day assessment, a separate group of 48 volunteers provided a one-time evaluation of AEMs containing rats and mice to gather data to better characterize the distribution of sensitivity to rodent urine odors among the normal population. Initially, day 16 was scheduled for these additional evaluations because observations from the prior test in June 2000 indicated that, by that day, most 9- and 12-animal-density AEMs were emitting odors that were clearly detectable to sensitive individuals. However, the lack of detectable odor on day 13 in the present test prompted a delay in this single-day evaluation. Alternatively, the 48 volunteers made their evaluations on day 18. During this one-time evaluation, volunteers evaluated a total of six AEMs: two AEMs containing six rats each, two AEMs containing eight mice each, and their respective blank control AEMs (2).

Statistical Analysis

The data were analyzed in two ways: 1) with the use of a modified $\chi^2$ test (20) that compared the performance of AEMs containing animals to those of empty AEMs and 2) a parametric analysis (mixed-model, repeated-measures analysis of variance) on the odor and annoyance ratings given to AEMs in each occupancy condition across the 12 test sessions.

For the nonparametric analysis, the odor category ratings (none to very strong) were coded from 0 to 5, and the resulting odor scores were divided into two score classes, 0–2 and 3–5. Classed odor score frequencies were determined by counting the number of scores in each score class for each test day. For each test day, a two × two test of independence using the adjusted G test (20) compared the classed odor score frequencies of the occupied AEMs to those of the unoccupied AEMs. The probability of type I error for the entire 21-day test was set between 0.05 and 0.10. This was achieved by setting $\gamma$ for any 1 day at 0.05 and for an individual test at 0.01.

For the parametric analysis, separate mixed model repeated-measures ANOVAs were conducted on the odor and annoyance ratings for the referent odor ratings and for the AEM odor ratings, with odorant type or AEM condition as the between-groups variable, respectively, and test session as the within-groups variable. The alpha level was set at 0.05 for all comparisons.

RESULTS

Parametric Analysis on Odor and Annoyance Intensity

Referent odor intensity. Table 1 presents the average ratings of the four referent odors presented in glass bottles before the AEM evaluations at each test session. There were significant differences between the ratings of odor and annoyance intensity among the four referent odors, with the odors of the foodbars and the undiluted mouse urine being rated as the most intense and annoying of the four odors. Axillary odor was rated as weakly intense and annoying. The vanilla solution was rated as a moderately intense odor with a barely detectable annoyance. On average, ratings of odor and annoyance intensity for the four referent odors did not change across the 3-wk test period ($P > 0.1$), thus
Table 1. Referent odors

<table>
<thead>
<tr>
<th>Rating Condition</th>
<th>Odor</th>
<th>Axillary odor</th>
<th>Vanilla</th>
<th>Foodbar</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>2.41 ± 1.40</td>
<td>3.50 ± 0.91</td>
<td>4.00 ± 0.82</td>
<td>4.00 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>2.00 ± 1.23</td>
<td>3.05 ± 1.33</td>
<td>4.14 ± 0.77</td>
<td>3.68 ± 1.04</td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>2.24 ± 1.09</td>
<td>2.90 ± 1.00</td>
<td>4.00 ± 0.71</td>
<td>3.33 ± 1.24</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>2.19 ± 1.29</td>
<td>3.19 ± 0.93</td>
<td>4.14 ± 0.65</td>
<td>2.95 ± 1.43</td>
<td></td>
</tr>
<tr>
<td>Day 9</td>
<td>3.09 ± 1.38</td>
<td>2.95 ± 1.05</td>
<td>3.95 ± 0.84</td>
<td>4.09 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>Day 11</td>
<td>2.29 ± 1.19</td>
<td>3.38 ± 0.74</td>
<td>3.90 ± 1.04</td>
<td>4.14 ± 0.85</td>
<td></td>
</tr>
<tr>
<td>Day 13</td>
<td>2.48 ± 1.50</td>
<td>3.05 ± 1.28</td>
<td>4.14 ± 0.73</td>
<td>3.71 ± 0.90</td>
<td></td>
</tr>
<tr>
<td>Day 15</td>
<td>2.08 ± 1.48</td>
<td>3.27 ± 0.88</td>
<td>4.00 ± 0.87</td>
<td>3.64 ± 1.14</td>
<td></td>
</tr>
<tr>
<td>Day 17</td>
<td>2.29 ± 1.27</td>
<td>3.38 ± 1.02</td>
<td>3.95 ± 0.92</td>
<td>3.33 ± 1.20</td>
<td></td>
</tr>
<tr>
<td>Day 19</td>
<td>2.52 ± 1.54</td>
<td>3.05 ± 1.07</td>
<td>3.86 ± 0.91</td>
<td>3.48 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>Day 20</td>
<td>2.18 ± 1.47</td>
<td>3.27 ± 0.88</td>
<td>3.77 ± 0.61</td>
<td>3.77 ± 1.19</td>
<td></td>
</tr>
<tr>
<td>Day 21</td>
<td>2.48 ± 1.47</td>
<td>3.19 ± 1.03</td>
<td>3.76 ± 1.04</td>
<td>3.86 ± 1.01</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD.

providing a stable baseline against which to assess any changes in the ratings of the AEMs across the test duration.

**AEM odor evaluation.** The mean odor and annoyance ratings made at a distance of 6 in. for AEMs containing mice and rats are depicted in Fig. 2. An overall ANOVA (employing Greenhouse-Geisser corrections for sphericity) on the odor and annoyance ratings revealed that the ratings of intensity and annoyance for the AEMs containing mice did not differ significantly from the ratings given to the AEMs containing rats (*P > 0.1*). At a distance of 12 in., none of the participants detected any odors from AEMs containing mice or rats, and these ratings did not differ from those given to the empty AEMs.

For the purpose of obtaining a better estimate of the population sensitivity to breakthrough odors from AEM-housed mice, the present test included an single test session evaluation on day 18 by a much larger sample of observers (*n* = 48), none of whom were involved in the 21-day test. The percentage of subjects from this group that appeared sensitive to the odor of mouse urine (i.e., who rated the odor intensity and annoyance as ≥3) was ~14%. Although volunteers rated the odor of AEMs containing mice higher than AEMs containing rats, there were no differences in perceived annoyance to the odors from the mice, rats, or blank controls (mean = 0.46 vs. 0.41; mouse vs. rat, respectively). Thus the data from this larger sample confirmed the earlier observation that some individuals appear to exhibit heightened sensitivity to the odor of mouse urine, but the percentage of individuals in the population with this trait is quite small.

Nonparametric Analysis of AEM Odor and Annoyance Intensity

Table 2 shows the mean odor and annoyance ratings for the occupied AEMs (rat and mouse) and the uncoupled AEMs (008 and 009) for each test day. Results of the adjusted *G*-statistic tests for AEMs containing mice and rats when individually compared with their re-

---

Fig. 2. Mean ± SE ratings of odor intensity (A and B) and annoyance intensity (C and D) from AEMs containing rats (A and C) compared with ratings from AEMs containing mice (B and D). Referent odor ratings to foodbars (Fd Bar) and BL-6J mouse urine are provided for comparison. Neg Cont, negative control; Barely Detect, barely detectable.
Table 2. Odor and annoyance ratings for occupied and unoccupied AEMs

<table>
<thead>
<tr>
<th>Day</th>
<th>Rat 003</th>
<th>Rat 004</th>
<th>Rat 007</th>
<th>Mouse 001</th>
<th>Mouse 002</th>
<th>Mouse 010</th>
<th>Mouse 008</th>
<th>Mouse 009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>1</td>
<td>0.82</td>
<td>0.22</td>
<td>1.00</td>
<td>0.27</td>
<td>0.91</td>
<td>0.23</td>
<td>0.95</td>
<td>0.16</td>
</tr>
<tr>
<td>3</td>
<td>0.59</td>
<td>0.13</td>
<td>0.55</td>
<td>0.17</td>
<td>0.45</td>
<td>0.25</td>
<td>0.55</td>
<td>0.21</td>
</tr>
<tr>
<td>5</td>
<td>0.67</td>
<td>0.20</td>
<td>0.71</td>
<td>0.17</td>
<td>0.43</td>
<td>0.13</td>
<td>0.71</td>
<td>0.20</td>
</tr>
<tr>
<td>7</td>
<td>0.40</td>
<td>0.16</td>
<td>0.30</td>
<td>0.12</td>
<td>0.40</td>
<td>0.20</td>
<td>0.45</td>
<td>0.20</td>
</tr>
<tr>
<td>9</td>
<td>0.64</td>
<td>0.29</td>
<td>0.55</td>
<td>0.18</td>
<td>0.36</td>
<td>0.19</td>
<td>0.41</td>
<td>0.25</td>
</tr>
<tr>
<td>11</td>
<td>0.40</td>
<td>0.22</td>
<td>0.70</td>
<td>0.20</td>
<td>0.40</td>
<td>0.16</td>
<td>0.65</td>
<td>0.17</td>
</tr>
<tr>
<td>13</td>
<td>0.55</td>
<td>0.20</td>
<td>0.45</td>
<td>0.18</td>
<td>0.40</td>
<td>0.16</td>
<td>0.85</td>
<td>0.25</td>
</tr>
<tr>
<td>15</td>
<td>0.38</td>
<td>0.17</td>
<td>0.48</td>
<td>0.19</td>
<td>0.38</td>
<td>0.17</td>
<td>0.81</td>
<td>0.33</td>
</tr>
<tr>
<td>17</td>
<td>0.33</td>
<td>0.20</td>
<td>0.48</td>
<td>0.19</td>
<td>0.33</td>
<td>0.17</td>
<td>0.33</td>
<td>0.16</td>
</tr>
<tr>
<td>19</td>
<td>0.50</td>
<td>0.21</td>
<td>0.65</td>
<td>0.22</td>
<td>0.20</td>
<td>0.15</td>
<td>0.60</td>
<td>0.21</td>
</tr>
<tr>
<td>21</td>
<td>0.48</td>
<td>0.18</td>
<td>0.24</td>
<td>0.12</td>
<td>0.38</td>
<td>0.15</td>
<td>0.62</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Table 3. Adjusted G statistic for AEM comparisons

| Test Day | n  | R-003  | R-004  | R-007  | M-001  | M-002  | M-103  | R-003  | R-004  | R-007  | M-001  | M-002  | M-103  |
|----------|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1        | 22 | 0.84   | 0.25   | 0.84   | 0.84   | 0.18   | 0.84   | 0.00   | 0.25   | 0.00   | 0.00   | 0.00   | 0.00   | 0.44   |
| 3        | 21 | 0.84   | 0.25   | 0.84   | 0.84   | 0.18   | 0.84   | 0.00   | 0.25   | 0.00   | 0.00   | 0.00   | 0.00   | 0.44   |
| 5        | 20 | 0.84   | 0.25   | 0.84   | 0.84   | 0.18   | 0.84   | 0.00   | 0.25   | 0.00   | 0.00   | 0.00   | 0.00   | 0.44   |
| 7        | 21 | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.00   | 0.44   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| 9        | 20 | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.00   | 0.44   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| 11       | 21 | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.00   | 0.44   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| 13       | 20 | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.00   | 0.44   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| 15       | 21 | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.00   | 0.44   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| 17       | 20 | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.00   | 0.44   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| 19       | 20 | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.00   | 0.44   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| 21       | 20 | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.44   | 0.00   | 0.44   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00   |

R, rat; M, mouse.
possible after the units were returned and before they were opened for removal of the mice. For the postflight electronic nose evaluation, none of the six AEMs in either flight or ground control configuration were identified as either full-strength or diluted mouse urine. Rather, all six AEMs were characterized by the electronic nose as blank or room air, confirming the ability of the AEM to contain all traces of mouse odor for a full 2 wk.

STS-108 flight: evaluation of flight and ground AEMs via electronic nose and dissection of the exhaust filters. The electronic nose was used in the same manner described to evaluate the three flight and three ground AEMs after the completion of the STS-108 spaceflight mission.

The flight and ground control filters from the STS-108 flight were also dissected to examine differences in urine distribution and penetration into the filter. The layers designed to absorb and disperse the urine (layers 2 and 4) show the presence of urine very clearly under black light. The zeolite (layer 3) and carbon (layers 5, 6, 7, and 8) layers also show the presence of fluorescing urine, but not as clearly. For two of the three flight filters, no urine was present on layer 2, indicating that all of the urine deposited on layer 1 evaporated before it penetrated the filter. The third flight filter had evidence of urine on layer 2 in small quantities. All of the ground had evidence of urine on layer 2, with one of the filters having urine penetrate to layer 5.

DISCUSSION

Evaluation of the mean ratings of odor and annoyance and the rating frequencies given to the two AEMs containing mice and the two AEMs containing rats confirmed their ability to successfully contain odors emanating from housed rodents. On average, none of the AEM odors were rated above weak, and throughout the first 19 days the average ratings for AEMs containing rats or mice hovered between no odor and barely detectable. However, similar to results from the June 2000 study, there were a few individuals, beginning on day 13, who appeared capable of detecting weak to moderate odors from AEMs containing mice.

The electronic nose proved to be a useful adjunct to the human panelist evaluation in that it appeared to be capable of discriminating mouse urine at two concentrations (full strength and dilute) from the blank AEM fitted with foodbars and from room air alone. Moreover, it identified the sample taken from one of the AEMs containing mice as diluted mouse urine on the same day as two of the most sensitive volunteers began to detect an odor from that AEM that they described as urine. Although assessments that depend heavily on the hedonic response to an airborne volatile may necessarily continue to rely on human evaluations for final determinations of acceptability, the adjunctive use of a trained electronic detector can prove very useful, particularly for interim evaluations of filter modifications and related hardware design changes.

Individual Variation in Olfactory Sensitivity

By day 10, it was evident that the AEMs in this test were performing at a superior level to those in the June 2000 test in their primary function of containing odors from mice (and rats). Although the vast majority of the subjects did not find the odors from the AEMs containing mice to be easily detectable or objectionable, there were 3 individuals among the 21 volunteers who appeared to exhibit sensitivity to odors from mouse occupants. Although none of these three volunteers was aware of the source of the odors they were being asked to evaluate, they readily identified the odor quality as urine. The percentage of subjects among the core sensory panel who were able to identify urine odors in this study (~14%) was approximately equivalent to the estimate from the larger one-session sample collected on day 18 (~15%).

Variation in perceived intensity and hedonic value was not evident in the volunteers’ reactions to all experimental odors. For example, there was substantial agreement in the ratings of odor intensity and annoyance to the foodbar and undiluted mouse urine that were used as referent odors. After the formal odor
evaluation, a small study was conducted to determine whether differences in odor thresholds for B6 mouse urine (i.e., the lowest concentration that could be discriminated from clean air) would predict sensitivity to the breakthrough components that were the source of complaint by a subset of individuals in the formal panel evaluations. Sixteen individuals were selected from either the panel or the day 18 one-time evaluation; eight subjects who rated the mouse-occupied AEMs as having a moderate or above odor and eight subjects who rated the mouse-occupied AEMs as having none or barely detectable odor. Although there was considerable variability in odor thresholds, individuals who rated the AEMs as odorous or objectionable were not able to detect mouse urine at lower concentrations than nonsensitive individuals (average detection threshold: 0.01% for sensitive subjects vs. 0.005% for nonsensitive subjects).

It is not uncommon to find extreme variation in olfactory response among a group of individuals with normal olfactory acuity. The human ability to detect specific odors is determined by the expression of receptors in the olfactory epithelium that are coded by a large, multigene family containing between 500 and 1,000 members (4). This extreme diversity in genetic coding can lead to enormous variation in individual sensitivity to specific volatiles. For example, it is estimated that every individual has a unique inability to smell one or more chemicals at concentrations that are readily detectable to the majority of people, a phenomenon termed specific anosmia (1). Certain types of specific anosmias appear to be much more widely genetically distributed. For example, the steroid musk androstenone, which is found in the saliva of pigs and the underarm secretions of certain men, cannot be smelled by ~50% of the world’s population, but, for those who can smell it, it produces a strongly unpleasant odor sensation, most frequently described as sweaty or urinous (23).

Recent evidence suggests that musk compounds, perhaps due to their large molecular size, elicit extreme variation in sensitivity across a range of normal individuals. Gilbert and Kemp (9), for example, found multiple specific hyperosmias (heightened sensitivity) as well as anosmias (insensitivity) to various natural and synthetic musk compounds. Although precise chemical identification of the volatiles that were breaking through the filtration system in the AEMs and leading to the reports of urinous odors among some individuals is lacking at present, it is not inconceivable that the breakthrough products of mouse urine could comprise one or more musk compounds. Our present data would suggest that as many as 85–90% of individuals may be relatively insensitive to these breakthrough components and thus would not find working or living around these odors objectionable. However, for the small subset of individuals who appear to exhibit heightened sensitivity to these compounds, close proximity to these AEMs may result in detection of an odor. This sensitivity was mitigated when the subjects rated the AEMs at a distance of 12 in.

It is likely that these results provide a conservative test of the potential odor impact of rodents housed in closed habitats, such as the space shuttle. The masking effect provided by the presence of additional background odors (e.g., from flame retardants, foods, human bodies, toilet), coupled with the rapid downregulation of olfactory sensitivity (i.e., adaptation) to odors that gradually increase in concentration (5), are likely to minimize the detectability and impact from rodents housed in spaceflight. Perhaps of even greater importance, there is significant potential for olfactory acuity to be attenuated in microgravity, due in part to cephalic fluid shifts that may alter the passage of odorous molecules to the olfactory receptors, a possibility that is often suggested by astronaut reports of the blandness of foods eaten in spaceflight.

Summary

Although by day 13 a few individuals reported the presence of a detectable odor, the AEMs clearly contained objectionable odors for the vast majority of participants through the 21-day test period. Indeed, in this test and the previous one conducted in June 2000, odor intensity appeared to peak and then diminish by the final days of the test. Because ratings were made to the referent odors on each day that AEMs were evaluated, it is unlikely that exposure-induced habituation played a role in the observed reduction in perceived odor intensity. Instead, this reduction may reflect some as yet undiscovered performance feature of the filtration system. On the basis of the formal evaluation, it appears that the AEMs are capable of containing odors from both rats and mice (observable when the AEMs were finally opened) for at least 3 wk and perhaps beyond. Moreover, previous olfactory research (6, 7) suggests that the stringent test conditions in which subjects rate the AEMs with minimal opportunity for adaptation and the careful exclusion of other background odors are indeed a very conservative test of the ability of the AEMs to control odors from animal occupants.

Based on the results from these tests, a consensus was reached to fly 24 mice (8 mice/AEM) on a 13-day mission (12-day flight plus 1 day prelaunch) on STS-108 in December 2001. Consistent with the findings from the 21-day test, electronic nose sensory analysis at 3 h postlanding confirmed the absence of any detectable odors emanating from the airstream of the recirculating filter, although such odors were easily detected when the AEMs were opened. Of equal importance, the crewmembers reported no detectable odors throughout the 13-day mission, where the AEMs were housed in the middeck. Thus, on the basis of odor containment, there is no reason to prevent continued and more frequent use of mice as subjects in physiological-based studies in spaceflight.

APPENDIX

Volunteers were also asked to provide quality descriptions of each odor by selecting 1 or more words from a list of the
following 20 descriptors: 1) fruity, 2) cool/cooling, 3) minty, 4) perfumey, 5) medicinal, 6) eucalyptus, 7) musk, 8) floral, 9) sweet, 10) solvent, 11) vinegar-like, 12) urine, 13) pungent, 14) sickening, 15) paint, 16) putrid, 17) sweaty, 18) banana, 19) camphor, and 20) chemical.

The authors thank Nadine Doolittle and Kimberly Leicester (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with animal monitoring and care, Mon-Gy Chen (Amgen) for assistance with setting up the equipment, and Anne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection, Donna Kupniewski and Maryanne Curran (Monell) for assistance with data collection.

REFERENCES