The Rodent Research Animal Holding Facility as a Barrier to Environmental Contamination

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ABSTRACT

The first step in verifying the design of the rodent Research Animal Holding Facility (RAHF) as a barrier to environmental contaminants was successfully completed at NASA Ames Research Center (ARC) during a 12-day biocompatibility test. Environmental contaminants considered were solid particulates, microorganisms, ammonia, and odor-producing organics. The 12-day test at ARC was conducted in August 1988, and was designed to verify that the rodent RAHF system would adequately support and maintain animal specimens during normal system operations. Additional objectives of this test were to demonstrate that: 1) typical particulate debris produced by the animal, i.e., feces and food bar crumbs, would be captured by the system; 2) microorganisms would be contained; and 3) the passage of odor-producing organics and ammonia generated by the animals was adequately controlled. In addition, the amount of carbon dioxide exhausted by the RAHF system was to be quantified. Of primary importance during the test was the demonstration that the RAHF would contain particles greater than 150 μm. This was done by analyzing collection plates placed underneath exhaust air ducts and underneath rodent cages during cage maintenance operations, e.g., waste tray and feeder changeouts. No particles larger than 150 μm were found in the collection plates that could be traced to the RAHF. Microbiological testing was performed using standard Rodac plates and a centrifugal air sampler with standard trypticase soy agar strips. No additional organisms were found in the test environment that could be traced to the RAHF. Odor containment was demonstrated to be less than "barely detectable" when samples of RAHF exhaust air were sniffed by a "blind" panel of "qualified" ARC civil service personnel. Ammonia could not be detected in the exhaust air from the RAHF system. Carbon dioxide levels were verified with a standard infrared analyzer to be less than 0.35%.

THE RODENT RESEARCH ANIMAL HOLDING FACILITY (RAHF) is a general-use facility for housing rodents to be used in life sciences experiments conducted aboard the Spacelab. The rodent RAHF will provide environmental control, food, water, illumination, and waste management for up to 24 rodents.

The RAHF was originally developed for NASA by the Lockheed Missiles and Space Company (LMSC) and was flown for an in-flight engineering evaluation on the Spacelab-3 (SL-3) mission in May 1985. The results from that mission indicated that the initial RAHF system design was a successful barrier to microbial contamination, but did not provide an adequate barrier to environmental contaminants such as solid particulates and odor-producing organics (1).

Subsequent to the SL-3 mission a RAHF system redesign effort was initiated by the Ames Research Center (ARC) Space Life Sciences Payloads Office and LMSC. This redesign focused on establishing the cage as the primary mechanical barrier to solid particulates and the RAHF Environmental Control System (ECS) as a secondary barrier to particulates as well as a primary barrier to microbial contaminants, gases, and odors. A Single-Pass Auxiliary Fan (SPAF) was added as part of the redesign to assist the RAHF ECS in containing solid particulates, odors, and microbial contaminants during cage-servicing operations. Figure 1 depicts the rodent RAHF system following its redesign.

Supporting equipment such as a General-Purpose Transfer Unit was developed to provide a secondary level of containment during cage removal and transfer. Secondary containment bags are also provided for stowing used waste trays and feeders.

RAHF SYSTEM CONTAINMENT REQUIREMENTS

Before the RAHF redesign effort began, considerable emphasis was placed upon developing practical and achievable containment criteria for the system. The requirements developed for the RAHF system focused on four key areas: solid particulates, odor, gases, and microbes.

1. Solid particulates. The cage was to contain particles greater than 150 μm. This size range was selected based upon an analysis of debris collected from the rodent cages and from the Spacelab module following the SL-3 mission. In addition, all exhaust air from the RAHF was to be filtered to

*Numbers in parentheses designate references at end of paper.
RAHF SYSTEM DESIGN

The rodent RAHF system is composed of seven basic subsystems: cage, cage module, ECS, water delivery, power, data, and control systems. System redesign to enhance the containment of environmental contaminants focused upon three key subsystems: the cage, the cage module, and the ECS.

The cage module system houses 12 cages and provides structural support, circulating air, lights, water system interfaces, temperature and humidity sensors, and power and data interfaces. The cage consists of a basic structure for housing two rodents in separate compartments, a waste-management subsystem, ammonia and odor control, a feeder (containing two food bars), an activity monitor, and a water lixit. Figure 2 depicts the rodent cage. When installed in the cage module, each cage front mates with the module through an O-ring gasket seal, which forms an impenetrable barrier to gas and particle escape. The feeder and waste tray doors are sealed against the cage front in a similar manner. In the normal recirculating airflow mode, the cage module operates at a negative pressure of approximately 0.5 inch H2O with respect to the surrounding environment. The module negative pressure ensures an inward flow of air to the cage module to contain particles, gases, and odors during normal airflow mode configuration.

The cage waste tray, shown in Fig. 3, is composed of several layers and has a top layer of porous Bondina polyester padding treated with phosphoric acid. The phosphoric acid reduces the pH of the rodent urine, thus inhibiting its decomposition into ammonia as a byproduct. Below the Bondina wicking is a layer of charcoal-impregnated random fiber pad for odor control. The next layer is Filtrete, a thin, electrically charged, woven fiber pad supported by 150-μm stainless steel mesh. Entrained urine and feces are dried by the recirculating airflow through the waste tray to inhibit decomposition and the resulting condensate is collected by the RAHF ECS.

Waste tray and feeder changeouts can be performed in flight to accommodate experimental objectives and to support basic animal maintenance functions. The changeouts require the insertion of closeout covers into the waste tray and cage bottom or into the feeder, depending upon which is being removed. These covers seal each respective assembly before it is removed. In addition, a secondary containment bag is used to enclose the used waste tray or feeder before it is stowed for postflight analysis and disposal.

The RAHF ECS provides the basic functions of air circulation, temperature control, humidity control, and oxygen and carbon dioxide exchange (O2 and CO2 partial pressures are regulated by Spacelab). The RAHF ECS is shown in Fig. 4. Air circulation through the cage module is approximately 80 cfm and is provided by a cluster of four fans. In addition, two centrifugal blowers force 35 cfm of air through the cold side of a thermoelectric cooling unit where the air is cooled to control temperature and remove moisture. The cooled, dehumidified air is mixed with the circulating air before it is returned to the cages. Two electric heaters are provided in line in the circulating air (one upstream of the cage module and one downstream of the cage module) to warm the air as required.

Carbon dioxide removal and oxygen replenishment for RAHF is accomplished by bleed air fans exchanging a minimum of 2 cfm of Spacelab cabin air between the cage module and the cabin. Two redundant fans in line with the bleed air exhaust force the outlet bleed air through a 0.3-μm HEPA filter and a charcoal bed (for odor control). The high-efficiency particulate air (HEPA) filter is a bacteriological barrier between animals and crewpersons.

During all waste tray and feeder changeouts and manipulations requiring cage removal from the cage module, the SPAF is activated from a control panel located below the
The SPAF is a high-flow centrifugal blower which provides a vigorous suction on the cage module when any of the cage seals (feeder, waste tray, cage latch/cage front) are penetrated. SPAF airflow increases the negative pressure within the cage module (from 0.5 inch H2O to 5.0 inches H2O, relative to ambient Spacelab internal pressure) and further ensures that all airflow is inward. This additional inward airflow is sufficient to entrain and retain all particulates within the cage module. SPAF discharge air is directed through a charcoal bed (for odor control) and a 0.3-μm HEPA filter (for particulate and microbiological containment) before entering the Spacelab cabin environment. A standby redundant SPAF also will be available in flight.

The RAHF water delivery system stores 9.5 liters of water in a butyl rubber bladder within a water tank. Figure 5 depicts the RAHF drinking water system. The bladder, pressurized with nitrogen to 55 psig and pretreated with iodine, discharges the water through an iodine-treated resin bed. The drinking water then flows through a pressure regulator that maintains downstream pressure at 11 psig as the water flows toward the cages. Two manifolds downstream from the water tank are connected in parallel, each with 12 three-way solenoid valves. Each cage compartment is fitted with lixit valves that deliver water to the animal at 8 psig when it displaces an internal stem. Approximately 0.5 cc of water is delivered each time the lixit is actuated. The number of actuations and time of actuation are entered into the RAHF data-acquisition system as a means of monitoring water consumption.

The RAHF power, data, and control systems are contained primarily in the Upper Electronics Box (UEB) and
Fig. 4. RAHF Environmental Control System.

Fig. 5. RAHF Water Delivery System.
Lower Electronics Box (LEB). A control panel on the front of the UEB provides the following functions:

1. Cage module light control (for simulated day/night cycles)
2. Drinking water dispenser malfunction indicators and on/off switches
3. Cage temperature monitor and control
4. Electrical power switches
5. Switches for alternative power during ascent and descent, for memory storage, cooling water, air/water separator, analog tape recorder, and run functions

System power distribution is provided by the LEB, which is located above the cage module and below the UEB.

RAHF BIOCOMPATIBILITY TESTING—OBJECTIVES AND REQUIREMENTS

The verification of the rodent RAHF system design as an effective barrier to environmental contamination was performed during a 12-day biocompatibility test. This test was conducted August 9-21, 1988, at ARC.

Initial test objectives were to demonstrate that:

1. The rodent RAHF system would adequately maintain rodent specimens in a habitable, nonstressful environment for a 12-day period.
2. Particulate debris produced by the animal, such as feces and food bar crumbs, would be contained by the system’s primary and secondary containment mechanisms in a 1-g environment.
3. Microorganisms in the animal cage or in other sites within the RAHF system would not be passed from the system and into the surrounding environment in a 1-g environment.
4. Odor-producing organics and gases such as ammonia, which are generated by the animals when they are housed in the RAHF in a 1-g environment, would be controlled.

Twenty-four flight-typical rodents were used during the test, which was conducted within one of the laboratories in the Space Life Sciences Payloads Project facility. Each rodent was certified as specific pathogen free (SPF) as defined in Appendix 7 of the Human Research Policy and Procedures for Space Flight Investigations, JSC-20483.

The laboratory selected as the site of the biocompatibility test met the requirement of having a total volume approximating that of the Spacelab module (77 m³). Additional requirements were that the lab be maintained between 68-72 °F and 20-30% relative humidity. Air exchange between the lab and the outside was set at a minimum rate of 10 volume changes per hour (the minimum required for animal holding facilities).

To control the cleanliness of the lab during test operations and provide a benign background as possible for microbiological and particulate sampling, all test support equipment was located outside the test lab. Connections to the RAHF were made through cabling and ducting fed through the lab access doors. In addition, interior surfaces of the test lab were cleansed with 70% ethanol and made visibly clean of particulate matter. Personnel within the test lab were required to wear typical animal clean-room attire, i.e., clean-room suits, caps, etc.

To further reduce external contamination, RAHF system data were monitored remotely. Figures 6 and 7 depict the RAHF parameters monitored during the test. The “RF1” display shown in Fig. 6 includes system performance data such as cage module temperatures and relative humidity, cooling loop water temperature, and circulating air fan pressure. The “RF2” display shown in Fig. 7 includes rodent data such as water counts (corresponding to lixit activation) and activity counts, generated each time a rodent passed through an infrared light beam within each cage.

Fig. 6. Rodent RAHF RF1 data display.

Fig. 7. Rodent RAHF RF2 data display.
RAHF BIOCOMPATIBILITY TESTING—RESULTS

The design of the test chamber met the test requirements except for room humidity. The humidity of the test chamber was never below approximately 50%, and ranged from 50-60%. An attempt to dry out the room with a dehumidifier was unsuccessful. Room humidity did not, however, significantly affect testing. The net result of the higher ambient humidity was an increase in the volume of water removed from the RAHF cage module circulating air and deposited in a condensate collection bag. Approximately 3 liters of water were removed from the cage module circulating air during the test.

Particulate containment (150 μm and larger) was verified by using 24 x 24 inch collection plates lined with double-sided white adhesive tape. Other types of particle counters were considered, but they were unsuitable because they only measured particles 10 μm and smaller.

The collection plates were placed directly below RAHF air exhaust ducts (bleed air and SPAF) and below cages, waste trays, and feeders when associated changeouts or other manipulations were taking place. The collection plates were used on the third, seventh, ninth, and twelfth days of the test. The collection plates provided a repository for any fallout that bypassed the exhaust air filters or was not entrained and retained within the cage module by the inward airflow created by the SPAF. If any particles escaped, they would be retained by the adhesive tape for post-test analysis and characterization (size, weight, and origin). Control plates were also placed within the test chamber, away from the RAHF, in an effort to document background particle contamination.

Visual inspection of all collection plates post-test did not identify any particles larger than 150 μm which could be traced to the RAHF. Comparison with control plates revealed the same types of debris normally found within the test chamber—dust, dirt, and test personnel debris (hairs, clothing fibers, etc.).

Microbiological containment testing verified that air outflow from the RAHF during animal habitation did not contribute to the microbial bioburden of the immediate RAHF external environment. In addition, the buildup of microbial bioburden in the RAHF during the 12 days of animal habitation was assessed. Finally, the microbiological testing program verified that the animals used in the test met and maintained the SPF criteria. Detailed results of the test are reported in ref. 3.

To verify the SPF status of the animals, oral swabs and fecal pellets were sampled. In all cases, no proscribed organisms were found in any of the test animals. The microbial content of the RAHF air outflow was measured by using standard Rodac plates with trypticase soy agar and lecithin, and with a centrifugal air sampler with trypticase soy agar strips. Results indicate that the microorganisms isolated from the RAHF bleed air port and from the SPAF exhaust port did not exceed in number nor differ significantly in identity and distribution from those in the ambient room air (3).

During the course of the test, there was an obvious buildup of microbial bioburden in the RAHF. This was expected to occur as the rats contributed their flora to the RAHF surfaces and the microorganisms in the immediate vicinity enter the RAHF through normal air balance intake, especially during SPAF operations. No proscribed microorganisms were found at any time (3).

A significant result from the microbiological testing during the biocompatibility test was the discovery of mold growth on the radiation-sterilized food bars. Consequently, research into mold-inhibiting additives or coatings for the food bars began. A potassium sorbate (0.15% concentration by weight) coating was applied to food bars used during the Spacelab Life Sciences-1 (SLS-1) Experiment Verification Test (EVT), conducted in March 1989 and proved to be successful in inhibiting mold growth.

Circulating air within the cage module and the RAHF exhaust air were tested for the presence of ammonia using a Gastec Model 800 injection detection pump with low-range detector tubes. Sampling was performed on the first, tenth, eleventh, and twelfth days of the test at two sites: 1) a test port midway down the module front on the left side, and 2) the RAHF bleed air exhaust port.

In each case, no ammonia was detected in the air exhausted to the surrounding environment. Approximately 0.25 parts per million of ammonia was measured in the cage module. In contrast, samples of the air surrounding the ground control animals housed in standard vivarium cages showed significant levels of ammonia (5-6 parts per million).

Carbon dioxide and oxygen levels within the cage module and in the exhaust air from the RAHF were monitored for the duration of the test. Carbon dioxide was measured with a LIRA-MSA Model 303 infrared analyzer. Oxygen was measured with an Applied Electrochemistry Model 22598 Oxygen Analyzer. Table 1 presents oxygen and carbon dioxide data. Air exiting the bleed air duct and module air pulled from the same sample port as used for ammonia detection were passed through a standard electrochemical oxygen analyzer and infrared carbon dioxide analyzer. In all cases, the levels of oxygen and carbon dioxide within the cage module were maintained within the required range.

Odor levels in the air surrounding the RAHF system were assessed by a test panel of 10 qualified ARC civil service personnel. Samples taken on the first, fourth, eighth, and twelfth days of the test were obtained by drawing approximately 3-4 liters each of bleed air and SPAF exhaust into sterile, 4.5-liter Teflon gas sampling bags. The bags were then transferred to an off-line, odor-free area for assessment by panel members. This was done to reduce the masking of the test sample by normal environmental odors and to ensure a completely "blind" assessment by the panel. In all cases the average rating by the 10-person panel never exceeded a score of 1, indicating "barely detectable." The initial design requirement for odor containment was that the averaged score should not exceed 1.5.

ADDITIONAL DESIGN VERIFICATION TESTING PLANNED

Following the successful completion of the rodent RAHF biocompatibility test, additional tests were planned to further demonstrate the performance of the system as a barrier to environmental contaminants. This testing includes an EVT...
Table 1. Percent Oxygen and Carbon Dioxide Measured at RAHF BleedAir and Cage Module Sample Port During Biocompatibility Test; August, 1988

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*Note: During SPAF operations, carbon dioxide and oxygen in the cage module approach ambient concentrations (21% O2, 0.03% CO2).
for the SLS-1 ARC Payload, conducted in March 1989, and an in-flight Particulate Containment Demonstration Test (PCDT) of the rodent RAHF as part of the SLS-1 ARC Payload.

During the SLS-1 EVT, the RAHF was loaded with SPF rodents and was operated according to planned flight timelines for the SLS-1 mission. The RAHF system was operated for 9 days (an 8-day mission duration plus a 24-hour pre-launch powerup to support the late access loading of animals). Observations were made as to how well the RAHF contained odor and particulates. Odor, microbiological, and particle containment results duplicated those obtained during the biocompatibility test. The use of a potassium sorbate coating as a mold inhibitor for the food bars was also evaluated. Mold was absent from food bars because of the sorbate coating.

The culmination of the RAHF testing to verify the containment of environmental contaminants will be the SLS-1 PCDT, to be carried out in-flight during SLS-1 mission operations in June 1990. The PCDT operations will evaluate particulate control during feeder and waste tray changeout and during the transfer of a cage from the RAHF system to the General-Purpose Work Station. Secondary containment mechanisms such as the General-Purpose Transfer Unit will be used during these operations. Moreover, 2 of the 12 cages flown in the cage module will not contain animals, but will instead be loaded with a representative 10-day animal debris load; consisting of simulated feces, flight food bar crumbs, and rodent hair. The particulate load will be color-coded to provide traceability and to enhance postflight analysis. A modified RCS Biotest centrifugal air sampler will be used to sample air from around the waste tray and feeder openings during changeout and around the cage opening during transfer to the GPWS. Modifications to the sampler will permit the capture and retention of particles in the size range of 74-300 μm.

CONCLUSIONS

Results from the rodent RAHF biocompatibility test have demonstrated that the redesign of the RAHF system has adequately resolved the issues raised during its initial flight on SL-3 regarding particle and odor containment. In addition, microbial and gas (ammonia) containment, as well as the control of carbon dioxide within the cage module, was reverified.

The improved design of the rodent cage, the addition of a Single-Pass Auxiliary Fan to increase the cage module negative pressure during cage-servicing operations, and the overall improvement in the integrity of system sealing have led to a renewed confidence in RAHF system performance and an opportunity to demonstrate this performance in flight on Spacelab Life Sciences-1 (SLS-1). A successful flight demonstration of the redesigned rodent RAHF system on SLS-1 will lead to future mission operations such as those to be performed during SLS-2, where even more complex and challenging research requiring the RAHF will be conducted.

REFERENCES

**Abstract**

The rodent Research Animal Holding Facility (RAHF), developed by NASA Ames Research Center (ARC) to separately house rodents in a Spacelab, was verified as a barrier to environmental contaminants during a 12-day biocompatibility test. Environmental contaminants considered were solid particulates, microorganisms, ammonia, and typical animal odors.

The 12-day test conducted in August 1988 was designed to verify that the rodent RAHF system would adequately support and maintain animal specimens during normal system operations. Additional objectives of this test were to demonstrate that: 1) the system would capture typical particulate debris produced by the animal; 2) microorganisms would be contained; and 3) the passage of animal odors was adequately controlled. In addition, the amount of carbon dioxide exhausted by the RAHF system was to be quantified.

Of primary importance during the test was the demonstration that the RAHF would contain particles greater than 150 µm. This was verified after analyzing collection plates placed under exhaust air ducts and rodent cages during cage maintenance operations, e.g., waste tray and feeder changeouts.

Microbiological testing identified no additional organisms in the test environment that could be traced to the RAHF. Odor containment was demonstrated to be less than "barely detectable." Ammonia could not be detected in the exhaust air from the RAHF system. Carbon dioxide levels were verified to be less than 0.35%.

**Key Words**

Research animal holding facility, Containment, Environment, Contamination, Habitat

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