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Rat 60 day acoustic exposure : 8, 16 and 32 kHz octave bands

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RAT 60 DAY ACOUSTIC EXPOSURE:

8, 16 AND 32 kHz Octave Bands

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In partial Fulfillment

of the Requirements for the Degree

Master of Sciences

By

Gary D. Mele

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ABSTRACT

RAT 60 DAY ACOUSTIC EXPOSURE: 8, 16 AND 32 kHz Octave Bands

Acute and chronic effects of continuous sound exposure (74 to 79 dB, SPL) on body weight, food and water intake, organ weights, hematology, hearing, and behavior of adult male Sprague-Dawley rats were studied. A sequence of three tests exposed groups of 9 rats to white noise filtered with either an 8 kHz, 16 kHz, or 32 kHz octave band filter for 5, 14, 30, or 60 days. Equal numbers of identically housed control rats were exposed concurrently to ambient sound (62 dB, SPL). Test rats used 5% more water than control rats in the 8 kHz and 32 kHz tests. For all frequencies, 5 day test rats had 6% larger spleens and 17% lower total leukocytes counts. For all frequencies and exposure times, test rats had 44% lower plasma corticosterone concentrations.

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INTRODUCTION

Rodent enclosures are being developed at NASA Ames Research Center by the Space Station Biological Research Project (SSBRP) for use on the International Space Station. Extensive testing is being conducted as part of a risk reduction effort to ensure the research community that a suitable habitat can be provided. Work conducted under this cooperative agreement has defined rat habitat noise limits, and verified a portion of those limits.

The Centrifuge Facility Project is keenly aware of the implications of sound energy to animal physiology and well-being (reference: ARC/CF-11212 para. 4.1.12.2.1 and recent working group meetings to discuss sound limits, chaired by Kristine Guerra, for Code SCS, July 1993). In addition, NASA acoustic requirements are specified in document NSTS 08080-1, 1972, revised 1994, and in NASA Technical Memorandum 108811, 1994. Though several general reviews have been published dealing with the effects of sound on animals (Busnel, 1963; Welch and Welch, 1970) including rats (Nitschke, 1982), the literature is inadequate to specifically set sound (noise) restrictions in the SSBRP rodent habitat. Peterson in 1980 reviewed the issue of background noise and laboratory animals and concluded that too little was known of the effects of noise to recommend imposition of governmental legislation. However, he also indicated that "regulation" of noise in animal facilities "remains an urgent priority". Interestingly, a number of authors have recently expressed concern over the inadequate control of sound as an important environmental variable in animal

vivariums, and have implicated this inadequate control as a confounding variable in the study of animal physiology and behavior (Besch, 1985; Milligan, et al, 1993).

The early Centrifuge Facility flight system specification for acoustic noise levels were based on research performed on humans (e.g., 73 dBA specification). The specification would therefore, not be appropriate for the rat since the auditory threshold curve for the rat is considerably different than the human with the rat hearing well into the "ultrasound" range (to approx. 100 kHz; Gourevitch and Hack, 1965; Kelley and Masterson, 1977; and Nitschke, 1982). It would be appropriate to develop a SSBRP noise specification that was specific for the rat with noise level maxima specified at various frequencies over the auditory range. This would be similar in principle to the human noise level curves developed to assure normal effective conversation over various distances, e.g., the SIL curves and the NC curves (Beranek, 1960).

On July 14, 1993 a group composed of D.C. Holley and G. Mele from SJSU and T. Castellano, M. Steele, K. Guerra, and L. Salerno of NASA met to propose maximum allowable habitat noise standards for rats. Previous standards were derived from human noise level curves, e.g., the SIL curves and the NC curves (Beranek, 1960). The noise standard was specific for the rat with maximum noise level specified for standard octave bands spanning the rat's auditory range. The derived "dB(r)" curve also took into consideration data indicating that audiogenic seizure in lab animals occurs at about 90-134 dB in the

frequency band 4 -80 kHz depending on the species (Lehmann and Busnel, 1963). The group agreed by consensus to the following values which define the dB(r) curve of maximum allowable noise in SSBRP enclosures. The group also agreed that the most important parts of the sound spectrum for a rat were the 8 kHz, 16 kHz and 32 kHz octave bands. These three octave bands span the section of the acoustic spectrum (5.26 kHz - 44.8 kHz) where rat hearing is the most sensitive (Kelly and Masterson, 1977). Several important rat vocalizations occur within these frequencies. Vocalizations made during mating, 22 kHz or 50 kHz, and agonistic encounters, 22-30Khz or 40-70 kHz fall mostly within this span (Nitschke, 1982).

Table 1. Proposed maximum chronic sound pressure level (SPL) for rats housed in the Centrifuge Facility Specimen Chamber and other animal habitats. The dB(r) curve.

Nominal center frequency, Hz	Octave Pass Band, Hz	Maximum band Sound dB(SPL)
31.5	22.4-44.7	100
63	44.7-89.1	100
125	89.1-178	100
250	178-355	100
500	355-708	95
1000	708-1410	90
2000	1410-2820	85
4000	2820-5620	80
8000	5620-11200	75
16000	11200-22400	75
32000	22400-44800	75
64000	44800-89600	80
128000	89600-179200	85

The verification of this noise standard required the development of methods for measuring the health and well being of rats. Research of the literature and consultation with Dr. G.P. Moberg of the University of California at

Davis (editor of Animal Stress, American Physiological Society, 1985), resulted in a set of physical and behavioral parameters which provided quantification of rodent health and well being. The set was designated the "Stress Assessment Battery (SAB)". The development and verification of the SAB are described in Holley, *et al.*, 1996: Appendix A.

METHODS

(A) Experimental Design

These *noise standard* verification experiments used the Stress Assessment Battery (SAB) to validate the "dB(r)" specification (Table 1) for the 8 kHz, 16 kHz and 32 kHz octave bands. A sequence of three experiments was performed. Each one exposed 4 groups of 9 white laboratory rats to chronic broadband noise within one octave band at the specified maximum sound pressure level for up to 60 days. Five groups of 9 rats housed under identical conditions received no exposure to experimentally produced noise. Food and water use and nocturnal behavior, via video taping, were monitored throughout each test. Also compared were the body weight, organ weights, selected blood chemistry, and hematology of control groups with 5, 14, 30, or 60 days of no noise exposure to test groups with 5, 14, 30, or 60 days of constant noise exposure. One control group was euthanized on day 0 of each test.

(B) Animals

Each octave band sound exposure test used 81 male Sprague Dawley rats (Simonson Laboratories, Gilroy, CA) for a total of 243 rats. Upon arrival each rat was weighed and randomly assigned to one of nine groups. Each group contained 9 rats. Rats for the 8 kHz, 16 kHz, and 32 kHz experiment experiments initially weighed 171.6 ± 0.6, 164.1 ± 0.7, 179.8 ± 0.7 grams (mean ± SEM) respectively. The 5 control groups were labeled: day 0 control, day 5 control, day 14 control, day 30 control, and day 60 control. The 4 test groups were labeled: day 5 test, day 14 test, day 30 test, and day 60 test. Animal group names describe the length of exposure time for the group and whether they received any exposure to the experimental noise. Test group rats were exposed to the experimental noise. Control group rats received no exposure to the experimental noise.

The locations of rat cages in each experiment followed the same pattern. The rats were initially weighed and put into shoe box vivarium cages. Each cage held 3 rats. Eight rat groups, 24 cages, were immediately placed in the four acoustic cabinets. One additional group went into the one of the temperature controlled environmental chambers containing the acoustic cabinets. Rats remained in this configuration for 1 to 2 weeks until the experiment started on test day 0. On test day 0, the day 60 control group was transferred into the control acoustic cabinets after the day 0 control group was removed. Sound exposure of the test rats began on test day 0 at approximately 1000h.

(C) Housing

The rats were maintained within three layers of containment. Two temperature controlled environmental chambers each held two acoustic cabinets. The four acoustic cabinets each held 6 shoe box vivarium cages. The thirty-three shoe box vivarium cages each held 3 rats. The environmental chambers provided a temperature controlled environment. The acoustic cabinets provided air circulation, controlled lighting and sound attenuation. Shoe box vivarium cages provided food, water, and living space.

1. Environmental Chambers

All experiments were conducted within two Environmental Chambers, DH-739A (test) and DH-739B (control) (Figure 1). Each chamber's temperature was controlled by a separate air conditioning/heating unit. Controls were adjusted so that the test and control cabinet internal temperatures were equal. Average temperatures within the test acoustic cabinets were 24.0 °C, 23.0 °C, and 23.9 °C for the 8 kHz, 16 kHz and 32 kHz experiments, respectively. Average temperatures within the control acoustic cabinets were 23.5 °C, 22.2 °C, and 22.2 °C for the 8 kHz, 16 kHz and 32 kHz experiments, respectively.

2. Acoustic Cabinets

Acoustic cabinets were constructed of 3/4" medium density fiberboard. Cabinets measured 43 1/2 x 33 x 27 3/4 inches internally. Each cabinet's external surface was covered with a 1/8 inch thick layer of sound insulating high density vinyl (PSP-8, Prospec non-reinforced barrier, West General Associates,

Inc., San Jose, CA). All inside surfaces were coated with shellac. The inside walls and ceiling were also covered with a single layer of 12" by 12" by 2" cardboard egg flats. The egg flats reduced sound reverberation and added a small amount of sound insulation.

Acoustic cabinets allowed for control of light and air circulation, and for some attenuation of external sounds. Two ceiling mounted 14 watt Vita-Lite fluorescent bulbs (Duro-Test Corp., North Bergen, NJ) provided illumination to an intensity of approximately 40 lux. Light level was measured inside each cage approximately 2 inches above the floor. A Model DT1 digital programmable lamp timer (Intermatic Inc., Spring Grove, Ill) set the lights to a 12L:12D light cycle with lights coming on at 0700 h. One single inlet blower (#G2S-097-DB61-08, EDM Industries) per cabinet pulled air through a 4 inch diameter hole at a rate of 29 CFM. This rate provided approximately 75 cabinet air exchanges per hour. Air entered the cabinet through two 4 inch diameter holes on the opposite side of the cabinet.

Figure 2 contains a diagram of an acoustic cabinet used for test rats. Acoustic cabinets for control rats did not have speakers installed.

3. Shoe Box Vivarium Cages

The rats were housed in 27 plastic shoe box vivarium cages (9"x19"x7"). Each cage held 3 rats. Metal floor grids prevented the rats from burrowing into the bedding material to avoid the sound. Wire tops completed the cage. Hanging feeders at the end of each cage held food. A lixit spout bolted to the

wire cage top provided water. This arrangement resulted in less interference with the applied noise than the standard placement of food and water in these cages. Food and water are usually placed on top of the wire cage tops of standard vivarium shoe box cages. Cages with bedding material and floor grids were exchanged for clean cages every 3 or 4 days.

(D) Food and Water

Food and water systems were designed to minimize interference with the applied sound. Teklad rodent diet (Harlan Teklad, Madison, WI) was available *ad lib* from a wedge type hanging feeder (#F601BRT, Allentown Caging and Equipment, Allentown, NJ). The feeder hung from the side of the cage nearest to the cabinet door. Distilled water came from a cage top lixit (#01-0060, S.E. Lab Group, Napa, CA). Water for each lixit came from a 250 ml polymethylpentene graduated cylinder with a hose fitting threaded into the base. A short length of 1/4 inch i.d., 3/8 inch o.d. Tygon tubing connected the cylinder to the lixit. The reservoir was filled approximately every second day.

(E) Generation of the Experimental Noise

Applied sound within each of the two test cabinets was produced by a custom multi-component system (Figure 3). A Brüel and Kjær type 1405 noise generator (Brüel and Kjær Instruments, Inc., Nærum, Denmark) created broad band (100 kHz bandwidth setting) white noise. The white noise was filtered by a Brüel and Kjær type 1617 octave band filter (Brüel and Kjær Instruments, Inc., Nærum, Denmark). This was set to "direct" output and full octave band filtering.

A custom volume control split the filtered white noise into two signals and regulated each one's amplitude. Each signal was then amplified by one half of a Bryston Model 4B stereo power amplifier (Bryston Ltd., Rexdale, Ontario). Bryston modified the amplifier to make its high frequency response flat up to 100 kHz. The output of each amplifier channel was connected to 12 Panasonic leaf tweeters (#EAS-10TH400, Matsushita Electric Corporation of America, Secaucus, New Jersey). The 12 speakers for a channel were in the same test cabinet. Two speakers, side by side, were positioned above the center of each shoe box vivarium cage. Speakers were hung within an inch of the cabinet top by 4 lengths of 12 pound test nylon monofilament line.

(F) Sound Measurement System

The custom sound measurement system can be divided into three sections. The *first section* consisted of a B & K type 4135, 1/4 inch microphone attached to a B & K type 2639 preamp and wired to a B & K type 5935 preamp/power supply (Brüel and Kjær Instruments, Inc., Nærum, Denmark). These were connected to *section two*, a National Instruments NB-A2000 12 bit A/D converter in an Apple Quadra 840AV computer. *Section three*, Labview, Version 3.0 (National Instruments, Austin, TX), provided the control program used to convert analog voltages into digital form and to perform spectrum analysis and display.

The calibration of the custom measurement system was validated by comparing its results to those obtained with a B & K Type 3550 multichannel

analyzer (provided courtesy of Mr. Richard Craig, B & K Western Regional Office, Orange, CA). At the time of the validation, both instruments were connected to one microphone using a BNC T connector. Both narrow and broadband tests were conducted. Measurements using the system were within 0.2% of those made with the B & K system.

(G) Acoustic Cabinet Measurements

1. Sound

Sound quality and quantity at each cage were measured every second or third day throughout each experiment with the computer based sound spectrum analyzer described above. The sound spectrum analyzer computed the average spectrum of thirty sound samples to produce one spectrum. Spectra were recorded on the computer's disk.

Recorded spectra were used to compute the average spectra and octave band sound pressure levels for each experiment for each acoustic cabinet.

2. Temperature

Temperature was measured with standard glass thermometers attached to the inside wall of each acoustic cabinet.

3. Humidity

Humidity was measured with a digital hygrometer placed on the top of a cage. The same hygrometer was rotated among cabinets throughout the experiments.

(H) Shoe Box Cage Measurements

1. Food

Food was weighed with a Sartorius Kilomat balance (#2116, 1000g/0.1g). During the first experiment, 16 kHz, food was weighed by transferring the remaining food from the hanging feeder into a 100 ml glass beaker for weighing. Food was added to the beaker and this was put back into the feeder. During the second and third experiments, 32 kHz experiment and 8 kHz, the hanging feeder was removed from the cage and weighed. Food was added to the feeder. The feeder was reweighed before being returned to the cage.

The mean daily food use per rat and the standard deviation of daily food use per rat were calculated for each cage of rats for the period prior to its termination day. For example, food data from test days -7 through 0 were used for rats euthanized on test day 0. Food data from test days 1 through 5 were used for rats euthanized on test day 5. This procedure produced two sets of 3 values for each test group and two sets of 3 values for each control group. One set of 3 values was the mean daily food use values. The second set of 3 values was the daily food use standard deviation values. Control and test group means and standard errors were calculated using the sets of 3 values.

2. Water

Water level within each water reservoir was recorded daily. Water use was calculated by subtracting one day's level from the previous day's level. Water was added to the reservoir every second day.

The mean daily water use per rat and the standard deviation of daily water use per rat were calculated for each cage of rats for the period prior to its termination day. For example, water data from test days -7 through 0 were used for rats euthanized on test day 0. Water data from test days 1 through 5 were used for rats euthanized on test day 5. This procedure produced two sets of 3 values for each test group and two sets of 3 values for each control group. One set of 3 values was the mean daily water use values. The second set of 3 values was the daily water use standard deviation values. Control and test group means and standard errors were calculated using the sets of 3 values.

3. Total Body Weight

Total body weight per cage was calculated by adding the three individual final body weights for the cage.

The standard deviation of the three final body weights from one cage was used as a measure of body weight variation within that cage. Increased variation could be the result of competition for food and water.

4. Video Behavior Analysis

Approximately twenty-three times per experiment, rats in a shoe box cage were videotaped during the dark portion of the daily light/dark cycle. On selected nights from 1855h to 2155h, a black and white CCD camera equipped with a 6 mm lens(#V-1070 and #V-4906 respectively, Marshall Electronics, Culver City, CA) was placed in one of the acoustic cabinets. This supplied video of one cage of rats to a Magnavox VCR. Infrared illumination came from a 7.5 watt

incandescent bulb equipped with a glass infrared filter (#M60,033, Edmund Scientific, Barrington, NJ).

Videotapes were scored by observing and noting the behavior of each rat as one of four or six classes. Behavior classes for the 16 kHz experiment were sleeping or still, eating or drinking, moving or grooming, and social interaction. Behavior classes for the 8 kHz and 32 kHz experiments were sleeping, awake and motionless, eating or drinking, moving about, grooming, interacting socially. Observations of each videotape were made at 10 minutes intervals for the first two hours of the videotape.

(I) Animal Termination Procedure and Measurements

1. Termination Procedure

The dissection team processed one group of 9 test rats after 5, 14, 30, and 60 days of exposure to the experimental sound, and one group of 9 control rats after 0, 5, 14, 30, and 60 days of exposure to ambient sound. Three cages were processed on test day 0. Six cages were processed on all other days. On days where both control and test rats were processed, day 5, day 14, day 30, and day 60, cages came alternately from control and test groups. Processing of rats began by 0730h PST and ended by 1330h PST. The following procedure was used for all groups.

This termination procedure was designed to minimize the acute corticosterone response of rats to handling. A 14" x 20" plastic cylinder filled with carbon dioxide was placed outside one of the environmental chambers. Two

team members entered the chamber, started a timer, quickly removed one shoe box cage from an acoustic cabinet, carried it outside the environmental chamber, placed the three rats into the CO_2 filled cylinder, carried the cylinder into the dissection room, and reconnected the CO_2 feed. When the timer showed that 2 minutes had passed, the unconscious rats were removed from the cylinder. Approximately 1-4 ml of blood was removed from each by heart puncture. Rats were then returned to the CO_2 filled cylinder until they died. The volume of drawn blood, time of day, and the time from the initial cage disturbance in the environmental chamber to the end of blood withdrawal for each rat were recorded.

The rats were subsequently weighed, then dissected to remove the stomach, heart, spleen, adrenals, kidneys, thymus, and testes. Adrenals were cleared of extraneous tissue and weighed immediately after they were removed to prevent desiccation. The stomach was placed into a buffered formalin solution for later histological analysis, as described below. All other organs were cleared of excess fat and weighed with as little delay as possible.

2. Body and Organ Weights

Rat bodies and organs were weighed using a Fisher Scientific Model 400D digital scale. Rat bodies were weighed to the nearest 0.1 gram. Organs were weighed to the nearest 0.001 gram.

3. Plasma Corticosterone

Plasma corticosterone concentration was determined using an Immunochem™ ^{125}I Corticosterone RIA kit (#07-120103, ICN Biomedicals, Inc., Irvine, CA). This double antibody radioimmunoassay is designed specifically for use with laboratory rats and mice.

4. Plasma Protein

Total plasma protein concentration was determined via the Lowry method using a diagnostic kit (#690A, Sigma Chemical Co., St. Louis, MO).

5. Total and Differential Leukocyte Counts

Total white blood cell count was determined using the Unopette method (#5853, Becton-Dickinson, Rutherford, NJ). These counts were done within hours of the termination of the rats. Blood smears were stained with Diff-quik (#B4132-1, Scientific Products, McGaw Park, IL). Differential leukocyte counts were determined by Mr. Wayne Pinard, AHT, (Veterinary Lab Technician, Adobe Animal Hospital, Los Altos, CA).

6. Stomach Histology

The stomachs were gently washed with buffered 10% formalin and then stored in formalin-filled jars for later histopathological analysis by pathologists at Consolidated Veterinary Diagnostics, Inc. (CVD, Sacramento, CA).

(J) Data Analysis

1. Introduction

The three octave band tests produced a large amount of data. Within each test were nine groups of nine rats. Each rat had 14 different parameters to be measured plus 2 daily and 1 terminal parameter for each cage of three rats. This gave a total of 3x9x9x14 + 3x3x9x2x67 + 3x3x9x1 = 14,337 measured values to be analyzed.

Data can be divided into three general classes. Class 1 contains data where each value represents a measurement from a single acoustic cabinet holding 6 cages. Acoustic cabinet measurements includes temperature, humidity, and sound level. Data class 2 contains data where each value represents a measurement from one shoe box cage of three rats. Shoe box measurements includes food and water daily measurements and their standard deviations, total rat weight per cage and its standard deviation, and behavior frequency distributions. Class 3 contains data where one value represents a measurement from one rat. Rat measurements include body and organ weights, plasma protein, plasma corticosterone, total leukocyte count, differential leukocyte counts, and stomach histology.

2. Acoustic Cabinet Measurements

Data from control and test acoustic cabinets were compared to ensure similar environmental conditions across cabinets. No statistical analysis was performed.

3. Shoe Box Measurements

Shoe box data included both continuous, e.g. food and water, and nominal, behavior frequencies, measurements. Continuous measurement data were analyzed using the 3 way analysis of variance (ANOVA) procedure described below. The ANOVA results show whether the sound frequency, sound exposure duration, or the presence of the experimental sound produced significant differences among the groups of rats for each parameter.

4. Rat Measurements

Rat measurement data included both continuous, e.g., weights, and ordinal, e.g., total leukocyte counts. Continuous measurement data were analyzed using the 3 way analysis of variance (ANOVA) procedure described below. The ANOVA results show whether the sound frequency, sound exposure duration, or the presence of the experimental sound produced significant differences among the rat groups for each parameter.

5. Analysis of Variance (ANOVA)

A multi-step procedure was used to analyze continuous shoe box data. Data analysis began with checks for normality and equal group variances. Data failing the quality testing were transformed, using either a square root or logarithmic transform, and tested again. Differential leukocyte proportions were transformed using the angular transform, arcsin(square root(data)). Data passing the quality checks were tested using a three way factorial ANOVA (Sokal

and Rohlf, 1981). Data failing the quality checks were tested using non-parametric methods.

A three way factorial ANOVA was used with continuous data which passed the data integrity tests. The three factors were sound exposure time, and sound frequency, and the presence or absence of the experimental sound. Significant differences due to sound exposure time, sound frequency, or any interactions including these factors required further analysis to determine which groups differed. Planned comparisons for sound exposure time compared each group to the next longer sound exposure time group. Planned comparisons for sound frequency compared the 8 kHz and 16 kHz experiments to the 32 kHz experiment, and compared the 8 kHz experiment to the 16 kHz experiment. Planned comparisons for interactions of the main factors compared control groups to test groups for all interaction subgroups.

RESULTS

(A) Cabinet measurements

1. Sound

Table 2 contains the average total sound pressure levels inside each test acoustic enclosure. The total experimental sound amplitude averaged 76.9 dB (SPL), 74.9 dB (SPL), and 79.1 dB (SPL) for the 8 kHz, 16 kHz, and 32 kHz experiments, respectively. Figure 4 through Figure 7 show the experimental

sound spectra for test acoustic cabinet T1 and the ambient sound spectrum for control acoustic cabinet C1

2. Temperature

Table 3 contains the group means ± SEM for all octave frequencies tested.

3. Humidity

Table 4 contains the group means ± SEM for all octave frequencies tested.

(B) Cage measurements

Table 5 through Table 10 contain the group means ± SEM for all octave band frequencies tested. Figure 8 through Figure 11 show the mean ± SEM values. Table 27 contains a summary of the results of statistical tests

1. Food Use

Mean daily food use, Table 5 and Figure 8, ranged from 21.1 g/rat/day for the 8 kHz experiment, day 5 test rats to 26.4 g/rat/day for the 32 kHz experiment, day 5 test rats. The largest difference between corresponding control and test groups was 1.4 g/rat/day for the 16 kHz experiment, day 14 rats and the 32 kHz experiment, day 60 rats. The overall mean values for control and test rats differed by 0.5 g/rat/day.

Analysis of the data found statistically significant differences due to sound frequency, sound exposure time, and several interactions of the main factors. No

statistically significant differences in mean daily food use could be attributed to the presence of the experimental sound.

Mean daily food use standard deviation, Table 6 and Figure 9, ranged from 1.2 g/rat/day for the 8 kHz experiment, day 30 control and test rats, the 32 kHz experiment, day 14 control and test rats, and the 32 kHz experiment control rats to 4.8 g/rat/day for the 16 kHz experiment, day 60 control rats. The largest difference between corresponding control and test groups was 1.7 g/rat/day for the 16 kHz experiment, day 60 rats. The overall mean values for control and test rats differed by 0.1 g/rat/day.

Analysis of the data found statistically significant differences due to sound frequency, sound exposure time, and all interactions of the main factors. Two statistically significant differences in mean daily food use standard deviation could be attributed to the presence of the experimental sound. Both differences occurred in the 16 kHz experiment. The 16 kHz experiment means and the 16 kHz experiment, day 5 means differed. The mean value for the 16 kHz experiment test rats, 4.0 g/rat/day, was 25% larger than the mean value for the 16 kHz experiment control rats, 3.2 g/rat/day. The mean value for the 16 kHz experiment, day 5 test rats, 3.0 g/rat/day, was 130% larger than the mean value for the 16 kHz experiment, day 5 control rats, 1.3 g/rat/day.

2. Water Use

Mean daily water use, Table 7 and Figure 10, ranged from 24.9 ml/rat/day for the 8 kHz experiment, day 30 control rats to 31.3 ml/rat/day for the 32 kHz

experiment, day 14 test rats. The largest difference between corresponding control and test groups was 3.0 ml/rat/day for the 32 kHz experiment, day 30 rats. The overall mean values for control and test rats differed by 1.1 ml/rat/day.

Analysis of the data found statistically significant differences due to sound frequency, sound exposure time, the presence of sound, and several interactions of the main factors. Several statistically significant differences in mean daily water use could be attributed to the presence of the experimental sound. Significant differences were found in the overall means, two experiment means, and the means for test day 30. The overall mean for test rats, 28.4 ml/rat/day, was 5% larger than the overall mean for control rats, 27.3 ml/rat/day. The mean values for the 8 kHz experiment test rats, 27.6 ml/rat/day, and the 32 kHz experiment test rats, 29.8 ml/rat/day, were 5% and 4% larger than the mean values for the 8 kHz experiment control rats, 26.4 ml/rat/day, and the 32 kHz experiment control rats, 28.6 ml/rat/day. The mean value for day 30 test rats, 27.8 ml/rat/day, was 5% larger than the mean value for day 30 control rats, 26.4 ml/rat/day.

Daily water use standard deviation, Table 8 and Figure 11, ranged from 1.0 ml/rat/day for the 8 kHz experiment, day 5 control rats to 6.3 ml/rat/day for the 16 kHz experiment, day 60 test rats. The largest difference between corresponding control and test groups was 4.4 ml/rat/day for the 16 kHz experiment, day 60 rats. The overall mean values for control and test rats differed by 0.2 ml/rat/day.

Analysis of the data found statistically significant differences due to applied sound frequency, sound exposure time, and several interactions of the main factors. Several statistically significant differences in the daily water use standard deviation which could be attributed to the presence of the experimental noise were found. Significant differences were found in the 16 kHz experiment means, day 60 means, the 16 kHz experiment, day 60 means, and the 32 kHz experiment, day 5 means. The mean value for the 16 kHz experiment test rats, 3.4 ml/rat/day, was 42% larger than the mean value for the 16 kHz experiment control rats, 2.4 ml/rat/day. The mean value for the day 60 test rats, 3.7 ml/rat/day, was 85% larger than the mean value for the day 60 control rats, 2.0 ml/rat/day. The mean value for the 16 kHz experiment, day 60 test rats was 232% larger than the mean value for the 16 kHz experiment, day 60 control rats. The mean value for the 32 kHz experiment, day 5 test rats was 57% smaller than the mean value for the 32 kHz experiment, day 5 control rats.

3. Total Body Weight per Cage

Mean total body weight per cage, Table 9, varied from 674 grams for the 16 kHz experiment, day 5 control rats to 1209 grams for the 32 kHz experiment, day 60 control rats. The largest difference between corresponding control and test groups was 50 grams for the 8 kHz experiment, day 60 rats. The overall mean values for control and test rats differed by 1.4 grams.

Analysis of the data found statistically significant differences due to the applied sound frequency, sound exposure time, and several interactions of the

main factors. No statistically significant differences in mean total rat weight per cage could be attributed to the presence of the experimental noise.

Mean body weight standard deviation per cage, Table 10, varied from 3.0 grams for the 8 kHz experiment, day 5 control rats to 35.5 grams for the 16 kHz experiment, day 60 test rats. The largest difference between corresponding control and test groups was 20.3 grams for the 16 kHz experiment, day 60 rats. The overall mean values for control and test rats differed by 1.1 grams.

Analysis of the data found statistically significant differences due to the sound exposure time and several interactions of the main factors. No statistically significant differences in mean body weight standard deviation per cage could be attributed to the presence of the experimental noise.

4. Video behavior analysis

Table 11 contains a summary of the video scoring.

(C) Rat Termination Measurements

1. Body Weight

Mean group body weight, Table 12 and Figure 12, varied from 225 grams for the 16 kHz experiment, day 5 control rats to 403 grams for the 32 kHz experiment, day 60 control rats. The largest difference between corresponding control and test groups was 16 grams for the 8 kHz experiment, day 60 rats. The overall mean values for control and test rats differed by 0.5 grams.

Analysis of the data found statistically significant differences due to the applied sound frequency, sound exposure time, and several interactions of the

main factors. No statistically significant differences in body weight could be attributed to the presence of the experimental noise.

2. Heart Weight

Mean group heart weight, Table 13 and Figure 13, varied from 0.807 grams for the 16 kHz experiment, day 5 control rats to 1.275 grams for the 32 kHz experiment, day 60 control rats. The largest difference between corresponding control and test groups was 0.078 grams for the 16 kHz experiment, day 30 rats. The overall mean values for control and test rats differed by 0.010 grams.

Analysis of the data found statistically significant differences due to the applied sound frequency, sound exposure time, and several interactions of the main factors. No statistically significant differences in heart weight could be attributed to the presence of the experimental noise.

3. Kidney Weight

Mean group kidney weight, Table 14 and Figure 14, varied from 1.971 grams for the 8 kHz experiment, day 5 test rats to 2.883 grams for the 16 kHz experiment, day 60 control rats. The largest difference between corresponding control and test groups was 0.174 grams for the 32 kHz experiment, day 5 rats. The overall mean values for control and test rats differed by 0.012 grams.

Analysis of the data found statistically significant differences due to the applied sound frequency, sound exposure time, and several interactions of the

main factors. No statistically significant differences in kidney weight could be attributed to the presence of the experimental noise.

4. Spleen Weight

Mean group spleen weight, Table 15 and Figure 15, varied from 0.604 grams for the 16 kHz experiment, day 5 test rats to 0.794 grams for the 8 kHz experiment, day 60 control rats. The largest difference between corresponding control and test group means was 0.076 grams for the 32 kHz experiment day 5 and the 8 kHz experiment, day 60 rats. The overall mean values for control and test rats differed by 0.011 grams.

Analysis of the data found statistically significant differences due to the applied sound frequency, sound exposure time, and all interactions of the main factors. One statistically significant difference in spleen weight could be attributed to the presence of the experimental noise. A significant difference was found in the day 5 means. The mean value for day 5 test rats, 0.692 grams, was 6% larger than the mean value for day 5 control rats, 0.654 grams.

5. Adrenal Weight

Mean group adrenal weight, Table 16 and Figure 16, varied from 16.8 milligrams for the 16 kHz experiment, day 5 test rats to 32 milligrams for the 16 kHz experiment, day 60 test rats. The largest difference between corresponding control and test group means was 4 milligrams for the 16 kHz experiment, day 5 rats. The overall mean values for control and test rats were both 0.0246 grams.

Analysis of the data found statistically significant differences due to the sound exposure time, and one interaction of the main factors. No statistically significant differences in adrenal weight could be attributed to the presence of the experimental noise.

6. Testes Weight

Mean group testes weight, Table 17 and Figure 17, varied from 2.565 grams for the 16 kHz experiment, day 5 test rats to 3.450 grams for the 32 kHz experiment, day 60 control rats. The largest difference between corresponding control and test groups was 0.044 grams for the 32 kHz experiment, day 14 rats. The overall mean values for control and test rats differed by 0.025 grams.

Analysis of the data found statistically significant differences due to the applied sound frequency, sound exposure time, and one interaction of the main factors. No statistically significant differences in testes weight could be attributed to the presence of the experimental noise.

7. Thymus Weight

Mean group thymus weight, Table 18 and Figure 18, varied from 0.302 grams for the 32 kHz experiment, day 60 control rats to 0.621 grams for the 8 kHz experiment, day 5 control and test rats. The largest difference between corresponding control and test groups was 0.064 grams for the 8 kHz experiment, day 14 rats. The overall mean values for control and test rats differed by 0.005 grams.

Analysis of the data found statistically significant differences due to the applied sound frequency, sound exposure time, and several interactions of the main factors. No statistically significant differences in thymus weight could be attributed to the presence of the experimental noise.

8. Plasma Corticosterone

Mean group plasma corticosterone level, Table 19 and Figure 19, varied from 0.90 μg/dl for the 32 kHz experiment, day 14 test rats to 7.26 μg/dl for the 8 kHz experiment, day 30 control rats. The largest difference between corresponding control and test group means was 4.04 μg/dl for the 16 kHz experiment day 60 rats. The overall mean values for control and test rats differed by 1.90 μg/dl.

Analysis of the data found statistically significant differences due to the sound exposure time and the presence of sound. One statistically significant difference in plasma corticosterone levels could be attributed to the presence of the experimental noise. A significant difference between control and test rats was found for the overall plasma corticosterone levels. The mean value for test rats, 2.38 μg/dl, was 56% of the mean value for control rats, 4.28 μg/dl.

9. Plasma Protein

Mean group plasma protein level, Table 20, and Figure 20, varied from 6.39 g/dl for the 8 kHz experiment, day 5 control rats to 10.27 g/dl for the 32 kHz experiment, day 60 test rats. The largest difference between corresponding

control and test group means was 1.10 g/dl for the 32 kHz experiment, day 60 rats. The overall mean values for control and test rats differed by 0.07 g/dl.

Analysis of the data found a statistically significant difference due to the applied sound frequency. No statistically significant differences in plasma protein level could be attributed to the presence of the experimental noise.

10. Total Leukocyte Counts

Mean group total leukocyte counts, Table 21 and Figure 21, varied from 9125 cells/μl for the 8 kHz experiment, day 60 control rats to 15347 cells/μl for 32 kHz experiment, day 5 control rats. The largest difference between corresponding control and test group means was 3653 cells/μl for the 32 kHz experiment day 5 rats. The overall mean values for control and test rats differed by 224 cells/μl.

Analysis of the data found statistically significant differences due to the sound exposure time and several interactions of the main factors. One statistically significant difference in total leukocyte counts could be attributed to the presence of the experimental noise. A significant difference between control and test rats was found for the day 5 rats. The mean value for day 5 test rats, 12101 cells/μl, was 83% of the mean value for day 5 control rats, 14648 cells/μl.

11. Lymphocyte Proportion

Mean group lymphocyte proportion, Table 22 and Figure 22, varied from 87.2 % for the 16 kHz experiment, day 14 control rats to 95.1 % for 32 kHz experiment, day 60 test rats. The largest difference between corresponding

control and test group means was 2.8 % for the 16 kHz experiment, day 14 rats. The overall mean values for control and test rats differed by 0.2 %.

Analysis of the data found statistically significant differences due to the sound exposure time and the sound frequency. No statistically significant differences in lymphocyte proportion could be attributed to the presence of the experimental noise.

12. Monocyte Proportion

Mean group monocyte proportion, Table 23 and Figure 23, varied from 0.1 % for the 8 kHz experiment, day 60 control rats to 2.2 % for the 16 kHz experiment, day 14 control rats. The largest difference between corresponding control and test group means was 0.8 % for the 16 kHz experiment, day 30 rats. The overall mean values for control and test rats differed by 0.2 %.

Analysis of the data found statistically significant differences due to the sound frequency and one interaction of the main factors. No statistically significant differences in monocyte proportion could be attributed to the presence of the experimental noise.

13. Neutrophil Proportion

Mean group neutrophil proportion, Table 24 and Figure 26, varied from 4.5 % for 32 kHz experiment, day 60 test rats to 10.7 % for the 16 kHz experiment, day 5 test rats. The largest difference between corresponding control and test group means was 1.7 % for the 8 kHz experiment day 14 rats. The overall mean values for control and test rats differed by 0.1 %.

Analysis of the data found statistically significant differences due to the sound frequency and the sound exposure time. No statistically significant differences in neutrophil proportion could be attributed to the presence of the experimental noise.

14. Eosinophil Proportion

Mean group eosinophil proportion, Table 25 and Figure 25, varied from 0.0 % for the 16 kHz experiment, day 14 test rats to 1.2 % for the 16 kHz experiment, day 14 control rats. The largest difference between corresponding control and test group means was 1.2 % for the 16 kHz experiment, day 14 rats. The overall mean values for control and test rats differed by 0.2 %.

Analysis of the data found a statistically significant difference due to the presence of sound. One significant difference in eosinophil proportion could be attributed to the presence of the experimental noise. A significant difference between control and test rats was found for the overall mean values. The mean value for test rats, 0.4%, was 67% of the mean value for control rats, 0.6%.

15. Neutrophil/Lymphocyte ratio

Mean group neutrophil/lymphocyte ratio, Table 26 and Figure 26, varied from 0.048 for 32 kHz experiment, day 60 test rats to 0.123 for the 16 kHz experiment, day 5 control rats. The largest difference between corresponding control and test group means was 0.019 for the 8 kHz experiment day 14 rats. The overall mean values for control and test rats differed by 0.001.

Analysis of the data found statistically significant differences due to the sound frequency and the sound exposure time. No statistically significant differences in neutrophil/lymphocyte ratio could be attributed to the presence of the experimental noise.

16. Stomach histology

Holley, *et al.*, 1996: Appendix B contains the histological report summaries submitted by the clinical laboratory performing the analysis (CVD, Sacramento, CA). CVD report numbers are; 8 kHz study - CVD No.X5005805; 16 kHz study - CVD Nos. X5000291 and X4007181; 32 kHz experiment study - CVD No. X500257.

For all three exposure experiments (8, 16, 32 kHz experiment) the changes observed by the pathologists were considered to be incidental. The summary for the 8 kHz exposure experiment indicated that:

> "...there was no evidence of erosion or ulceration in either the glandular or nonglandular mucosa. The sections were well-fixed and often had not only the luminal epithelium, but the mucous layer over the glandular mucosa still intact. The minimal inflammatory infiltrates observed are considered to be incidental and of no clinical significance. The vacuolar change seen in individual cells of the glandular mucosa could be an early degenerative change related to stress or this may be a normal aging change."

Similar findings appeared in control and sound treatment groups, thus indicating that the treatment had no effect on the stomach histology.

Holley, *et al.*, 1996: Appendix B also includes the Final Report of Laboratory Examination from the University of Missouri, College of Veterinary Medicine Research Animal Diagnostic and Investigative Laboratory.

DISCUSSION AND CONCLUSIONS

The major objective of this Cooperative Agreement was to develop a noise level specification for laboratory rats in the Centrifuge Facility Specimen Chambers (Space Station Biological Research Project), and to validate the specification for 3 noise octave bands: center frequencies 8 kHz, 16, kHz, and 32 kHz. This has been accomplished. Objective measures were used to verify that the chronic noise exposure was not harmful to the animals from physiological and behavioral perspectives. These measures were defined in the Stress Assessment Battery Validation for the Rat Acoustic Tolerance Test (Holley, *et al.*, 1996: Appendix A).

(A) Stress Assessment Battery (SAB) Measures

Table 27 shows the results of the analysis of variance performed on the cage and termination data. The three way factorial ANOVA compared control vs. test animals for all frequency ranges and exposure times.

1. Food and Water Use

Test rats used 5% more water than control rats. In the 8 kHz and 32 kHz experiments this amount was statistically significant ($P < .05$). Previous experiments have shown a high correlation between food and water use. In this study, the small difference in water use cannot be explained by greater food use of test rats. Test rats used 2% less food than control rats. This difference was not significant for any sound frequency, test day or pair of control and test rat groups. The food and water use here is consistent with that of rats in the stress

assessment battery validation test (Holley, *et al.*, 1996: Appendix A). In that experiment the restrained rats used more water than unrestrained rats but, did not use more food. The current finding may be an indication of a small reaction to the constant applied sound.

2. Body and Organ Weights

Across all test frequencies, day 5 test rats had 6% larger spleens than control rats. *No other body or organ weight differences were found to be significant with respect to the application of sound.* This spleen effect may be a transient process related to adaptation to the constant applied noise.

3. Blood Chemistry

Sound exposed test rats exhibited 44% lower plasma corticosterone concentrations than did control rats (see Table 19). Note that the plasma corticosterone concentration was lower in the sound exposed test animals than the control animals in every instance (frequency exposure and number of days exposed). *If the animals were being "stressed" by the applied sound exposure we would expect increased plasma corticosterone levels (*Holley, *et al.*, 1996: Appendix A). To the contrary, in this study the test animals had lower plasma corticosterone. It should also be noted that the absolute concentration difference is small, mean for all controls was 4.3 μg/dl and the mean for all test animals was 2.4 μg/dl. These values are in the range of normal rat plasma corticosterone concentrations (D'Agostino, *et al.*, 1982). No literature indicating a negative plasma corticosterone effect in response to a stressor could be found. There is

some indication that a negative adrenal response may occur in humans under some conditions of "psychological stress", but this has never been established in animal models (Dr. G.P. Moberg, University of Calif., Davis, personal communication). *Given the role of the glucocorticosteroids and the small differences found in the test animals vs. the control animals, the decreased plasma corticosterone finding is not of major physiological significance.* It is possible that the constant background of applied white noise in the cages of the test animals served as a "masking effect" blocking external sounds that may tend to cause animal arousal with concomitant small increases in plasma corticosterone. It is also possible that the sound produced a slight phase shift in the plasma corticosterone circadian rhythm. At the time of day that these animals were sacrificed the plasma corticosterone concentration were at or near a circadian low. Therefore, a phase shift in the corticosterone circadian secretion profile might result in slight differences in one group compared to another.

No statistically significant differences in plasma protein level could be attributed to the presence of the test noise.

4. Hematology

Across all test frequencies, only day 5 test rats had 17% fewer total leukocytes than day 5 control rats. The physiological significance of this is unknown.

The 16 kHz, day 14 test rats had a lower proportion of eosinophils than the 16 kHz, day 14 control rats. This result is suspect since, the 16 kHz, day 5 test group is the only rat group in which no eosinophils were found.

5. Stomach Histology

It is well established that chronic stress can lead to stomach ulceration. Accordingly, stomach histopathological examination was performed on each animal for all experimental groups. *Changes observed by the pathologists were considered to be incidental for all three sound frequency tests.*

With this type of microscopic histological analysis indefinite findings and artifacts from the tissue preparation and staining are common. The histologists observed what they initially interpreted to be a peculiar mineralization of the tunica muscularis in a number of samples examined. This led to speculation that the mineralization may have been abnormal. Since the effect was noted with about the same frequency in both control and sound treatment groups, it was concluded that this was not due to the effect of the sound exposure. The probable artifactual nature of the suspected mineralization was confirmed by consultations with the following: 1) Dr. Sig Rich, D.V.M., SJSU, ACUC consulting veterinarian; 2) Dr. Russell, D.V.M., consulting veterinarian for Simonsen Laboratories, the supplier of the rats; 3) Dr. DePauli, senior pathology consultant at CVD (see transcription of telephone conversation with Dr. Funk dated 2/1/95, in Holley, *et al.*, 1996: Appendix B). Never-the-less, to ensure that the rats used in this study were normal and healthy: 1) four rats from the SJSU vivarium were

euthanized and their stomachs sent to CVD for histopathology (CVD No. X5000676); 2) Dr. Russell from Simonsen Laboratories sent tissue samples from 3 rats directly to CVD from the breeding facility in Gilroy, CA, these tissues included stomach, kidney, thyroid, and parathyroid glands (CVD No. X5000907); and 3) 2 live rats from this study were sent directly to the Research Animal Diagnostic and Investigative Laboratory, University of Missouri, College of Veterinary Medicine, for a complete histological, parasitical and microbiological analysis (Holley, *et al.*, 1996: Appendix B).

After review of the laboratory results listed above, and consultation with the veterinarians listed above, it was concluded that the animals used for this study were normal and in excellent health. The mineralization reported in animals of both control and sound treatment groups was probably artifactual and of no consequence to this study.

Sound treatment did not appear to result in abnormal stomach histology

6. Statistical Differences not Attributable to the Presence of Sound

Many statistically significant differences could be attributed to the sound frequency, the sound exposure duration, or the interaction of these factors (Table 27). These results, which did not depend on the presence or absence of the experimental sound, were not explained above. Statistically significant differences due to sound frequency can be attributed to the greater starting weight of rats in the 32 kHz experiment. Statistically significant differences due

to sound exposure time are expected since this also represents the effects of age.

Overall Conclusions

Taken collectively the SAB data indicates that 74 to 79 dB (SPL) chronic noise exposure when applied in octave bands with center frequencies of 8 kHz, 16 kHz, or 32 kHz for up to 60 days does not produce deleterious effects in male white laboratory rats. It is felt that the dB(r) curve establishing noise limits for animal habitats housing rats (Table 1) is valid in the octave bands with center frequencies of 8, 16, and 32 kHz. These findings should not be extrapolated to other animal species, e.g. mice.

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Table 2. Average pressure sound level for each test acoustic cabinet, dB SPL. Test T1 and test T2 are test acoustic cabinets T1 and T2, respectively.

8 kHz		16 kHz		32 kHz experiment	
Test T1	Test T2	Test T1	Test T2	Test T1	Test T2
76.5	77.3	75.6	74.1	79.1	79.0

Table 3. Temperature means ± S.E.M, °C.

8 kHz		16 kHz		32 kHz experiment	
Control	Test	Control	Test	Control	Test
23.5 ± 0.1	24.0 ± 0.2	22.2 ± 0.1	23.0 ± 0.1	22.2 ± 0.2	23.9 ± 0.1

Table 4. Relative humidity means ± S.E.M, %.

8 kHz		16 kHz		32 kHz experiment	
Control	Test	Control	Test	Control	Test
51 ± 2	47 ± 1	N/D	N/D	38 ± 2	45 ± 4

Table 5. Daily food use per rat means ± S.E.M, grams per rat per day, with 3 rats per cage.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	19.5 ± 0.7	N/D	21.2 ± 0.5	N/D	23.6 ± 0.5	N/D
Day 5	22.0 ± 0.5	21.1 ± 0.7	23.1 ± 0.4	23.6 ± 0.3	25.6 ± 1.5	26.4 ± 1.3
Day 14	21.8 ± 0.4	22.3 ± 0.7	24.5 ± 0.4	23.1 ± 0.9	25.6 ± 0.4	24.8 ± 0.3
Day 30	22.4 ± 0.2	21.8 ± 0.5	24.6 ± 0.1	24.0 ± 0.3	25.5 ± 0.3	24.5 ± 0.3
Day 60	21.6 ± 0.3	21.8 ± 0.6	23.5 ± 0.7	23.2 ± 0.8	23.8 ± 0.3	22.4 ± 0.4

Table 6. Daily food use per rat standard deviation ± S.E.M, grams per rat per day, with 3 rats per cage.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	0.9 ± 0.2	N/D	1.9 ± 0.1	N/D	5.1 ± 0.6	N/D
Day 5	1.9 ± 0.7	1.3 ± 0.3	1.4 ± 0.2	0.7 ± 0.1	1.3 ± 0.2	3.0 ± 0.6
Day 14	1.2 ± 0.0	1.2 ± 0.3	1.8 ± 0.2	1.6 ± 0.2	3.7 ± 0.2	4.5 ± 0.3
Day 30	1.8 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	1.2 ± 0.2	3.3 ± 0.6	3.9 ± 0.1
Day 60	1.2 ± 0.2	1.6 ± 0.0	1.6 ± 0.1	1.3 ± 0.2	4.8 ± 0.4	4.5 ± 0.1

Table 7. Daily water use per rat mean ± S.E.M, ml per rat per day, with 3 rats per cage.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	24.6 ± 0.4	N/D	24.3 ± 0.7	N/D	27.7 ± 0.3	N/D
Day 5	27.2 ± 1.0	28.0 ± 0.2	26.3 ± 0.7	27.7 ± 0.1	30.2 ± 0.9	30.6 ± 0.6
Day 14	26.8 ± 0.9	27.8 ± 0.8	27.7 ± 0.5	27.6 ± 0.5	29.5 ± 0.2	31.3 ± 0.7
Day 30	24.9 ± 0.6	26.9 ± 0.5	27.5 ± 0.9	26.7 ± 0.7	26.8 ± 1.2	29.8 ± 0.5
Day 60	26.7 ± 0.7	27.7 ± 1.6	25.9 ± 0.2	28.9 ± 1.7	27.8 ± 0.9	27.5 ± 0.6

Table 8. Daily water use per rat standard deviation ± S.E.M, ml per cage per day with 3 rats per cage.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	3.1 ± 0.4	N/D	1.6 ± 0.2	N/D	1.1 ± 0.2	N/D
Day 5	1.1 ± 0.2	1.0 ± 0.2	1.6 ± 0.2	1.9 ± 0.8	2.8 ± 0.6	1.2 ± 0.3
Day 14	2.1 ± 0.4	1.7 ± 0.2	1.7 ± 0.4	1.5 ± 0.1	1.5 ± 0.4	1.1 ± 0.2
Day 30	1.5 ± 0.3	1.4 ± 0.3	4.3 ± 0.4	3.9 ± 0.10	1.5 ± 0.1	1.8 ± 0.3
Day 60	2.2 ± 0.1	2.5 ± 0.3	1.9 ± 0.2	6.3 ± 0.4	1.8 ± 0.3	2.1 ± 0.2

Table 9. Total weight mean ± SEM of rats per cage, grams, with 3 rats per cage.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	627.5 ± 9.3	N/D	605.5 ± 5.3	N/D	806.7 ± 5.0	N/D
Day 5	745.5 ± 4.4	739.5 ± 7.2	674.0 ± 15.2	691.1 ± 2.5	873.5 ± 2.4	905.7 ± 5.3
Day 14	877.0 ± 21.1	882.9 ± 10.0	890.6 ± 12.6	882.0 ± 26.8	1000.7 ± 25.0	979.9 ± 7.1
Day 30	995.6 ± 17.0	988.2 ± 6.8	1052.8 ± 12.6	1029.9 ± 8.8	1084.1 ± 14.6	1099.7 ± 1.4
Day 60	1108.5 ± 6.6	1158.0 ± 21.0	1169.3 ± 50.5	1150.4 ± 18.3	1209.1 ± 17.5	1190.0 ± 7.2

Table 10. Total weight standard deviations per cage, grams, with 3 rats per cage.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	4.9 ± 0.6	N/D	5.2 ± 1.6	N/D	5.8 ± 2.0	N/D
Day 5	3.0 ± 1.1	7.3 ± 0.8	10.9 ± 2.4	3.5 ± 1.6	13.2 ± 1.3	11.6 ± 2.5
Day 14	15.4 ± 3.9	6.1 ± 1.3	9.5 ± 2.3	9.0 ± 6.2	19.5 ± 9.4	9.5 ± 2.8
Day 30	23.0 ± 2.5	20.0 ± 9.2	18.1 ± 6.4	16.1 ± 6.6	16.2 ± 3.1	21.0 ± 6.8
Day 60	10.5 ± 1.6	13.5 ± 2.6	15.2 ± 4.3	35.5 ± 10.7	32.3 ± 4.6	20.4 ± 4.3

Table 11. Behavior frequency scoring of video tapes. Values represent the mean ± SEM number of behavioral events observed in the two hour scoring period.

8 kHz experiment			16 kHz experiment			32 kHz experiment		
Day 5	Control	Test	Day 5	Control	Test	Day 5	Control	Test
sleep	N/D	69 ± 0	sleep/sniff	N/D	170 ± 0	sleep	84 ± 0	70 ± 0
still	N/D	52 ± 0	eat/sniff	N/D	11 ± 0	still	55 ± 0	85 ± 0
eat/drink	N/D	18 ± 0	move/groom	N/D	31 ± 0	eat/drink	22 ± 0	16 ± 0
move	N/D	18 ± 0	social	N/D	7 ± 0	move	4 ± 0	3 ± 0
groom	N/D	44 ± 0				groom	29 ± 0	33 ± 0
social	N/D	18 ± 0				social	24 ± 0	12 ± 0
Day 14	Control	Test	Day 14	Control	Test	Day 14	Control	Test
sleep	64 ± 2	78 ± 5	sleep/sniff	161 ± 0	157 ± 0	sleep	61 ± 2	64 ± 0
still	62 ± 6	51 ± 3	eat/sniff	12 ± 0	15 ± 0	still	60 ± 6	75 ± 0
eat/drink	21 ± 3	21 ± 3	move/groom	34 ± 0	35 ± 0	eat/drink	23 ± 1	18 ± 0
move	5 ± 2	9 ± 2	social	12 ± 0	13 ± 0	move	7 ± 3	5 ± 0
groom	38 ± 2	36 ± 1				groom	44 ± 4	44 ± 0
social	29 ± 6	25 ± 8				social	25 ± 1	13 ± 0
Day 30	Control	Test	Day 30	Control	Test	Day 30	Control	Test
sleep	N/D	N/D	sleep/sniff	160 ± 2	159 ± 8	sleep	63 ±10	91 ± 5
still	N/D	N/D	eat/sniff	16 ± 1	11 ± 0	still	69 ± 0	46 ± 3
eat/drink	N/D	N/D	move/groom	32 ± 3	38 ± 8	eat/drink	19 ± 5	21 ± 1
move	N/D	N/D	social	11 ± 4	10 ± 1	move	10 ± 4	6 ± 2
groom	N/D	N/D				groom	43 ± 2	38 ± 4
social	N/D	N/D				social	15 ± 3	16 ± 4
Day 60	Control	Test	Day 60	Control	Test	Day 60	Control	Test
sleep	96 ± 6	98 ± 6	sleep/sniff	164 ± 4	156 ± 16	sleep	67 ± 0	96 ± 4
still	47 ± 2	44 ± 3	eat/sniff	12 ± 4	10 ± 3	still	68 ± 0	54 ± 9
eat/drink	19 ± 3	17 ± 3	move/groom	35 ± 3	44 ± 9	eat/drink	23 ± 0	22 ± 1
move	9 ± 2	9 ± 1	social	8 ± 2	8 ± 5	move	8 ± 0	8 ± 3
groom	34 ± 3	37 ± 3				groom	38 ± 0	34 ± 8
social	14 ± 1	15 ± 3				social	15 ± 0	6 ± 2

Table 12. Body weight means ± S.E.M, grams, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	209.2 ± 2.1	N/D	201.8 ± 1.9	N/D	268.9 ± 2.0	N/D
Day 5	248.5 ± 1.2	246.5 ± 2.4	224.7 ± 4.2	230.4 ± 1.3	291.2 ± 3.9	301.9 ± 3.6
Day 14	292.3 ± 5.9	294.3 ± 2.5	296.9 ± 3.6	294.0 ± 5.7	333.6 ± 8.0	326.6 ± 3.2
Day 30	331.9 ± 7.3	329.4 ± 7.0	350.9 ± 6.2	343.3 ± 5.6	361.4 ± 5.4	366.6 ± 6.7
Day 60	369.5 ± 3.3	386.0 ± 5.4	389.8 ± 9.7	383.5 ± 11.5	403.0 ± 9.9	396.7 ± 6.3

Table 13. Heart weight means ± S.E.M, grams, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	0.764 ± 0.012	N/D	0.754 ± 0.021	N/D	0.958 ± 0.018	N/D
Day 5	0.887 ± 0.009	0.860 ± 0.010	0.807 ± 0.016	0.808 ± 0.014	0.983 ± 0.025	0.960 ± 0.019
Day 14	0.994 ± 0.022	0.974 ± 0.037	1.072 ± 0.024	1.083 ± 0.029	1.090 ± 0.030	1.065 ± 0.023
Day 30	1.062 ± 0.025	1.057 ± 0.028	1.173 ± 0.028	1.095 ± 0.026	1.145 ± 0.013	1.178 ± 0.027
Day 60	1.147 ± 0.020	1.205 ± 0.027	1.239 ± 0.035	1.228 ± 0.031	1.275 ± 0.032	1.245 ± 0.021

Table 14. Kidney weight means ± S.E.M, grams, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	1.753 ± 0.030	N/D	1.804 ± 0.067	N/D	2.402 ± 0.059	N/D
Day 5	2.051 ± 0.034	1.971 ± 0.051	1.975 ± 0.063	2.025 ± 0.066	2.314 ± 0.055	2.488 ± 0.102
Day 14	2.230 ± 0.057	2.192 ± 0.041	2.363 ± 0.076	2.478 ± 0.103	2.690 ± 0.055	2.582 ± 0.066
Day 30	2.440 ± 0.053	2.449 ± 0.055	2.777 ± 0.072	2.678 ± 0.044	2.766 ± 0.079	2.738 ± 0.047
Day 60	2.590 ± 0.068	2.622 ± 0.122	2.883 ± 0.105	2.749 ± 0.114	2.814 ± 0.066	2.773 ± 0.057

Table 15. Spleen weight means ± S.E.M, grams, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	0.615 ± 0.019	N/D	0.555 ± 0.014	N/D	0.671 ± 0.017	N/D
Day 5	0.688 ± 0.013	0.674 ± 0.016	0.604 ± 0.018	0.658 ± 0.014	0.669 ± 0.019	0.745 ± 0.017
Day 14	0.683 ± 0.021	0.706 ± 0.021	0.698 ± 0.031	0.682 ± 0.021	0.724 ± 0.020	0.715 ± 0.020
Day 30	0.712 ± 0.022	0.693 ± 0.027	0.776 ± 0.018	0.708 ± 0.018	0.723 ± 0.031	0.747 ± 0.024
Day 60	0.718 ± 0.035	0.794 ± 0.022	0.755 ± 0.028	0.764 ± 0.023	0.776 ± 0.028	0.761 ± 0.025

Table 16. Adrenal weight means ± S.E.M, mg, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	18.7 ± 0.7	N/D	17.0 ± 2.8	N/D	33.4 ± 1.4	N/D
Day 5	23.2 ± 1.3	21.6 ± 1.2	20.8 ± 1.7	16.8 ± 1.6	18.7 ± 1.4	19.8 ± 1.6
Day 14	20.3 ± 1.3	22.2 ± 1.6	23.4 ± 2.0	23.0 ± 1.8	25.7 ± 2.0	24.0 ± 2.0
Day 30	25.1 ± 0.9	24.6 ± 1.5	25.0 ± 1.7	28.7 ± 2.4	27.8 ± 1.4	29.3 ± 0.6
Day 60	26.3 ± 1.7	26.0 ± 1.4	31.7 ± 1.3	32.1 ± 2.3	27.6 ± 2.0	27.8 ± 1.4

Table 17. Testes weight means ± S.E.M, grams, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	2.324 ± 0.039	N/D	2.273 ± 0.043	N/D	2.817 ± 0.087	N/D
Day 5	2.798 ± 0.039	2.687 ± 0.041	2.630 ± 0.062	2.565 ± 0.029	3.117 ± 0.066	3.142 ± 0.035
Day 14	3.051 ± 0.049	3.150 ± 0.025	3.135 ± 0.050	3.101 ± 0.064	3.303 ± 0.078	3.147 ± 0.096
Day 30	3.290 ± 0.075	3.132 ± 0.106	3.292 ± 0.036	3.324 ± 0.040	3.308 ± 0.081	3.424 ± 0.043
Day 60	3.327 ± 0.076	3.384 ± 0.039	3.362 ± 0.100	3.312 ± 0.071	3.450 ± 0.061	3.329 ± 0.036

Table 18. Thymus weight means ± S.E.M, grams, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	0.558 ± 0.022	N/D	0.538 ± 0.009	N/D	0.511 ± 0.026	N/D
Day 5	0.621 ± 0.022	0.621 ± 0.037	0.551 ± 0.018	0.560 ± 0.024	0.511 ± 0.014	0.571 ± 0.019
Day 14	0.565 ± 0.023	0.501 ± 0.013	0.560 ± 0.030	0.556 ± 0.022	0.479 ± 0.035	0.444 ± 0.019
Day 30	0.422 ± 0.016	0.462 ± 0.022	0.474 ± 0.019	0.424 ± 0.011	0.375 ± 0.020	0.419 ± 0.030
Day 60	0.330 ± 0.012	0.370 ± 0.017	0.335 ± 0.026	0.306 ± 0.014	0.302 ± 0.014	0.349 ± 0.025

Table 19. Plasma corticosterone means ± S.E.M, µg/dl, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	3.14 ± 0.24	N/D	0.16 ± 0.25	N/D	2.15 ± 0.35	N/D
Day 5	2.98 ± 0.37	2.31 ± 0.49	3.30 ± 0.58	1.36 ± 0.33	5.22 ± 1.34	2.01 ± 0.41
Day 14	4.12 ± 0.87	1.55 ± 0.16	2.51 ± 0.44	1.79 ± 1.34	2.63 ± 0.29	0.90 ± 0.38
Day 30	7.26 ± 1.35	3.47 ± 1.37	3.30 ± 0.89	2.68 ± 0.86	4.68 ± 0.71	1.52 ± 0.40
Day 60	4.54 ± 0.41	3.98 ± 1.10	6.61 ± 1.63	2.57 ± 0.85	4.79 ± 0.72	3.98 ± 0.92

Table 20. Plasma protein means ± S.E.M, g/dl, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	7.62 ± 0.75	N/D	8.46 ± 0.36	N/D	8.55 ± 0.49	N/D
Day 5	6.39 ± 0.49	6.90 ± 0.35	8.11 ± 0.54	7.65 ± 0.77	9.08 ± 0.32	8.49 ± 0.42
Day 14	6.79 ± 0.19	7.15 ± 0.97	7.66 ± 0.47	6.85 ± 0.66	9.92 ± 1.22	9.57 ± 0.49
Day 30	7.38 ± 0.35	7.81 ± 0.50	8.21 ± 0.53	8.69 ± 0.55	8.89 ± 0.72	9.06 ± 0.27
Day 60	7.58 ± 0.33	7.16 ± 0.31	7.97 ± 0.39	8.07 ± 0.39	9.17 ± 0.42	10.27 ± 1.00

Table 21. Leukocyte means ± S.E.M, cells/μl, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	14821 ± 3301	N/D	10458 ± 1268	N/D	14083 ± 1337	N/D
Day 5	14792 ± 776	13028 ± 1675	13806 ± 1439	11583 ± 1442	15347 ± 894	11694 ± 1795
Day 14	12750 ± 1175	11403 ± 1269	11000 ± 1488	13056 ± 1090	14042 ± 1336	14750 ± 1103
Day 30	14167 ± 805	12792 ± 888	11903 ± 1100	12542 ± 1522	11583 ± 1509	12500 ± 1540
Day 60	9125 ± 796	11722 ± 1358	12153 ± 869	11681 ± 1326	11028 ± 1067	12250 ± 1061

Table 22. Lymphocytes means ± S.E.M, % total leukocytes, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	94.2 ± 2.8	N/D	89.9 ± 1.0	N/D	91.8 ± 0.9	N/D
Day 5	92.2 ± 0.6	91.3 ± 1.1	88.0 ± 1.4	88.3 ± 1.9	90.3 ± 1.2	89.0 ± 1.8
Day 14	91.7 ± 1.2	90.2 ± 1.1	87.2 ± 1.3	90.0 ± 0.9	91.6 ± 0.8	92.4 ± 1.0
Day 30	89.9 ± 0.8	90.7 ± 1.3	90.8 ± 1.3	90.0 ± 1.7	93.2 ± 0.8	91.8 ± 0.7
Day 60	91.7 ± 1.1	92.7 ± 1.2	91.8 ± 0.6	92.5 ± 0.8	93.1 ± 1.6	95.1 ± 0.7

Table 23. Monocytes means ± S.E.M, % total leukocytes, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	0.10 ± 0.10	N/D	0.67 ± 0.29	N/D	0.51 ± 0.14	N/D
Day 5	0.27 ± 0.11	0.64 ± 0.15	0.78 ± 0.32	1.22 ± 0.46	0.83 ± 0.19	0.68 ± 0.26
Day 14	0.21 ± 0.11	0.19 ± 0.13	2.22 ± 0.60	2.00 ± 0.53	0.39 ± 0.11	0.32 ± 0.17
Day 30	0.01 ± 0.06	0.24 ± 0.08	0.78 ± 0.22	1.56 ± 0.44	0.33 ± 0.12	0.23 ± 0.11
Day 60	0.13 ± 0.09	0.81 ± 0.54	0.72 ± 0.17	1.17 ± 0.29	0.59 ± 0.23	0.17 ± 0.08

Table 24. Neutrophils means ± S.E.M, % total leukocytes, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	5.53 ± 2.67	N/D	9.22 ± 1.00	N/D	7.42 ± 0.97	N/D
Day 5	6.99 ± 0.39	7.69 ± 1.06	10.67 ± 1.32	9.89 ± 1.46	8.80 ± 1.34	9.60 ± 1.58
Day 14	7.74 ± 1.09	9.41 ± 1.00	9.33 ± 1.05	8.00 ± 0.91	7.31 ± 0.82	6.58 ± 0.84
Day 30	9.13 ± 0.76	8.54 ± 1.21	8.11 ± 1.25	7.89 ± 1.49	5.94 ± 0.86	7.37 ± 0.72
Day 60	7.22 ± 1.05	6.20 ± 1.33	6.69 ± 0.49	6.00 ± 0.74	5.87 ± 1.43	4.50 ± 0.65

Table 25. Eosinophils means ± S.E.M, % total leukocytes, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	0.20 ± 0.20	N/D	0.22 ± 0.15	N/D	0.27 ± 0.07	N/D
Day 5	0.48 ± 0.27	0.38 ± 0.24	0.56 ± 0.29	0.56 ± 0.24	0.64 ± 0.19	0.73 ± 0.27
Day 14	0.36 ± 0.14	0.19 ± 0.09	1.22 ± 0.43	0.00 ± 0.00	0.67 ± 0.28	0.68 ± 0.24
Day 30	0.92 ± 0.28	0.54 ± 0.15	0.33 ± 0.17	0.56 ± 0.24	0.51 ± 0.18	0.61 ± 0.25
Day 60	0.71 ± 0.18	0.26 ± 0.10	0.70 ± 0.13	0.33 ± 0.12	0.31 ± 0.12	0.22 ± 0.12

Table 26. Neutrophil/Lymphocyte ratio means ± S.E.M, no units, 9 rats per group.

	8 kHz		16 kHz		32 kHz experiment	
	Control	Test	Control	Test	Control	Test
Day 0	0.062 ± 0.031	N/D	0.104 ± 0.012	N/D	0.082 ± 0.012	N/D
Day 5	0.076 ± 0.005	0.085 ± 0.013	0.123 ± 0.017	0.115 ± 0.019	0.099 ± 0.016	0.111 ± 0.020
Day 14	0.086 ± 0.013	0.105 ± 0.012	0.108 ± 0.014	0.090 ± 0.011	0.080 ± 0.001	0.072 ± 0.010
Day 30	0.102 ± 0.009	0.096 ± 0.015	0.091 ± 0.015	0.090 ± 0.018	0.064 ± 0.001	0.081 ± 0.008
Day 60	0.080 ± 0.013	0.068 ± 0.016	0.073 ± 0.006	0.065 ± 0.009	0.066 ± 0.018	0.048 ± 0.007

Table 27. Statistical Results. An asterisk, *, indicates a significant difference found. A blank, , indicates no significance differences found. F = effects due to octave frequency, T = effects due to exposure time, S = effects due to the presence or absence of sound.

Variable	Test	F	T	S	FxT	FxS	TxS	FxTxS
body weight	ANOVA	*	*		*	*	*	*
heart weight	ANOVA	*	*		*	*	*	*
kidneys weight	ANOVA	*	*		*	*	*	*
spleen weight	ANOVA	*	*		*	*	*	*
adrenals weight	ANOVA		*		*			
testes weight	ANOVA	*	*		*			
thymus weight	ANOVA	*	*		*		*	*
neutrophil/lymphocyte	ANOVA	*	*					
leukocyte	ANOVA		*		*		*	*
lymphocyte %	ANOVA	*	*					
neutrophil %	ANOVA	*	*					
monocyte %	ANOVA	*			*			
eosinophil %	ANOVA			*				
plasma corticosterone	ANOVA		*	*				
plasma protein	ANOVA	*						
Body Wt/cage	ANOVA	*	*		*	*	*	
Body Wt SDev/cage	ANOVA		*		*	*	*	
Food/cage	ANOVA	*	*		*	*	*	
Food SDev/cage	ANOVA	*	*		*	*	*	*
Water/cage	ANOVA	*	*	*	*	*	*	
Water SDev/cage	ANOVA	*	*		*	*	*	*

Figure 1. Diagram of the environmental chambers, acoustic cabinets, and shoe box cages.

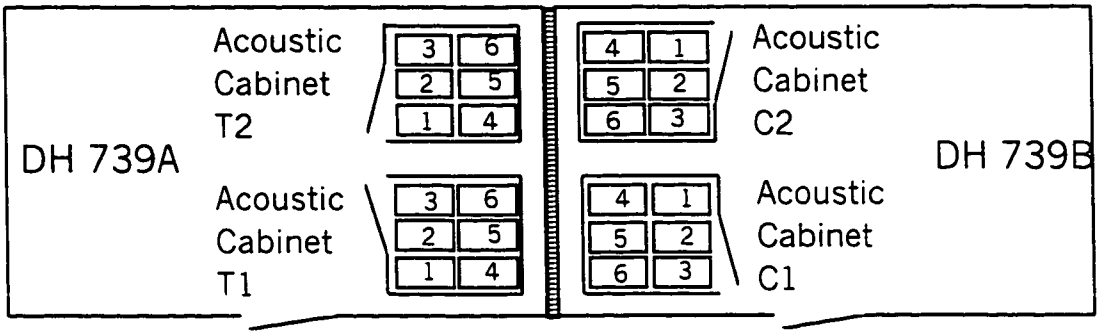

Figure 2. Diagram of a test group acoustic cabinet. Control acoustic cabinets had no speakers installed.

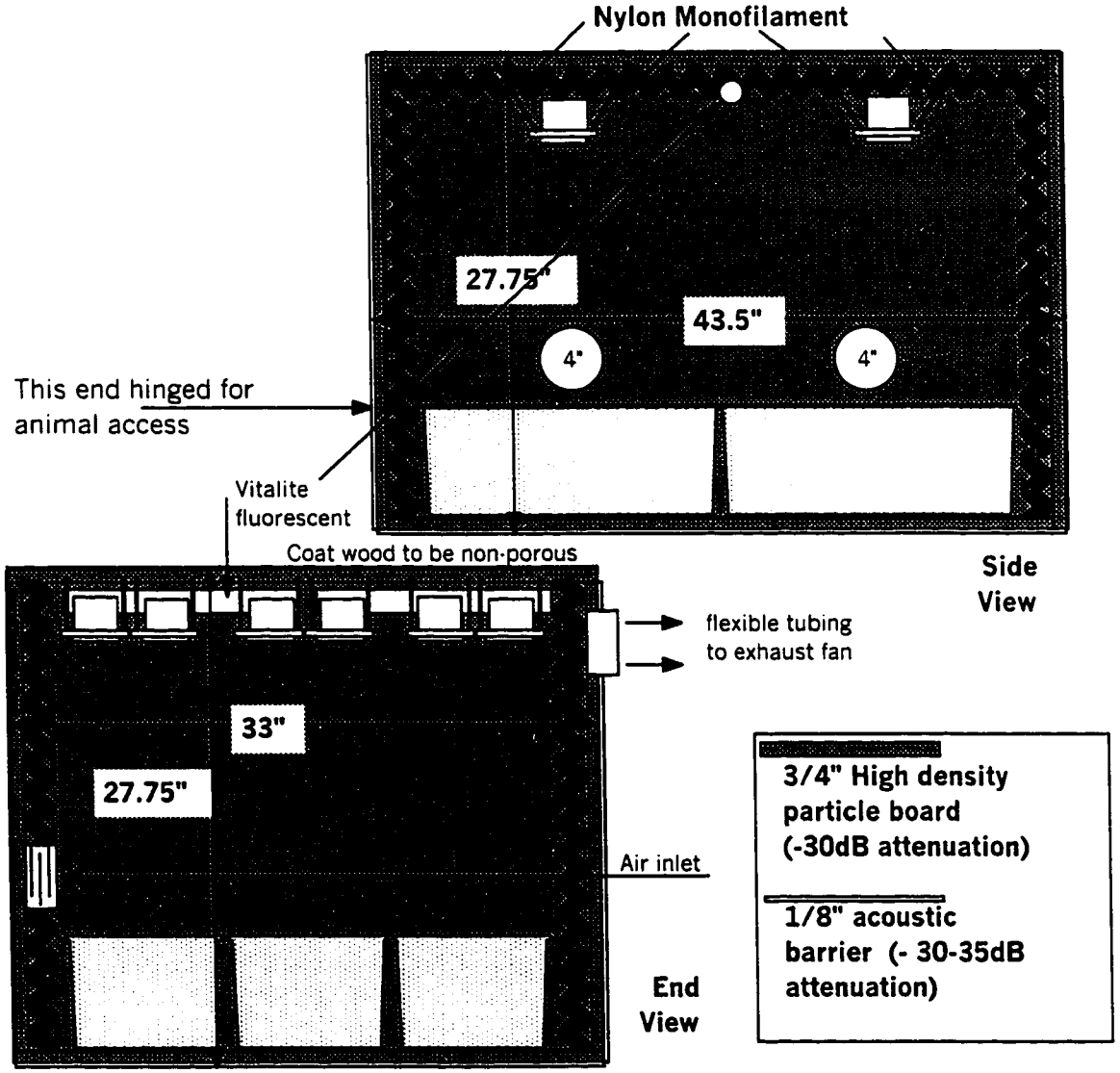

Figure 3. Block diagram of the sound generation equipment.

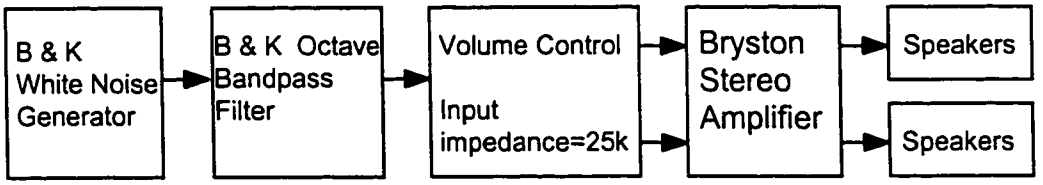

Figure 4. Acoustic cabinet T1 sound spectrum for the 8 kHz octave band test

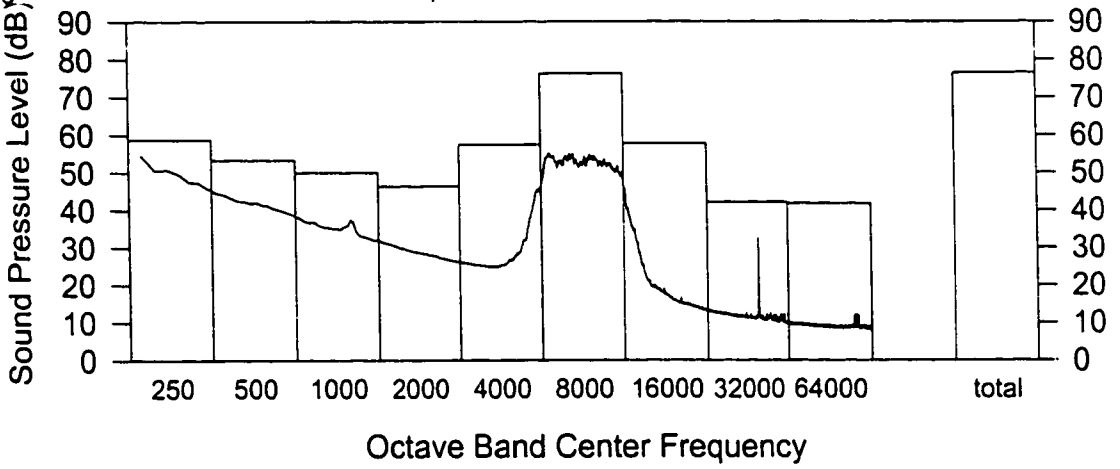

Figure 5. Acoustic cabinet T1 sound spectrum for the 16 kHz octave band test.

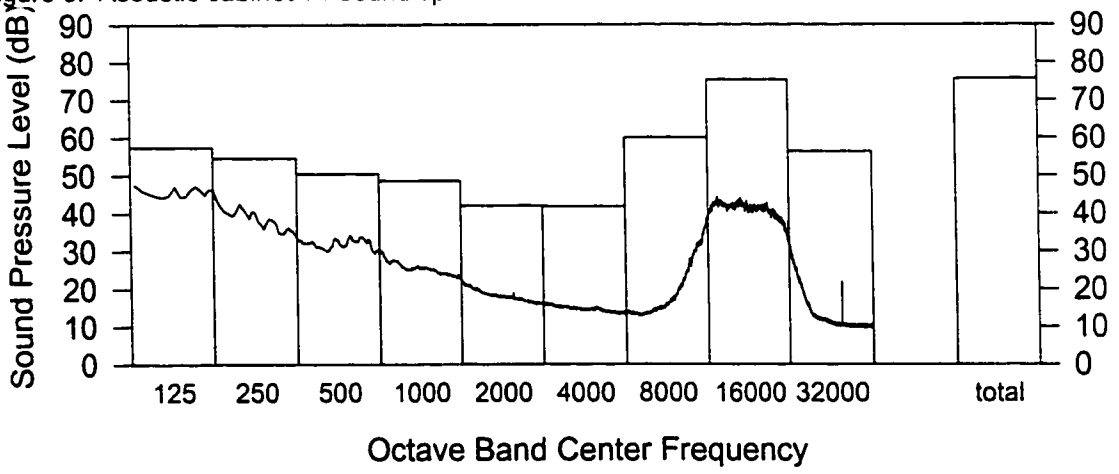

Figure 6. Acoustic cabinet T1 sound spectrum for the 32 kHz experiment octave band test.

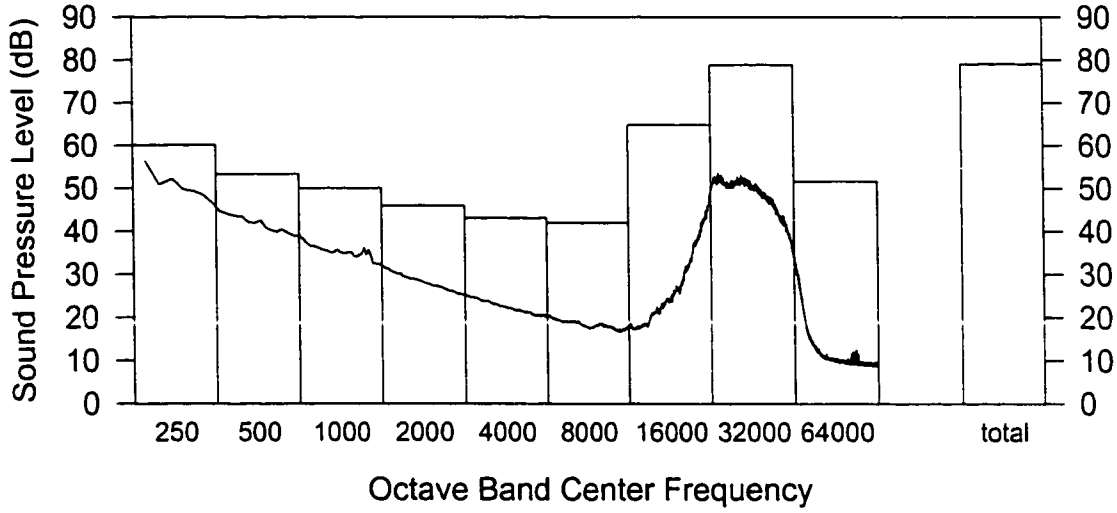

Figure 7. Control acoustic cabinet C1 sound spectrum for the 8 kHz experiment octave band test.

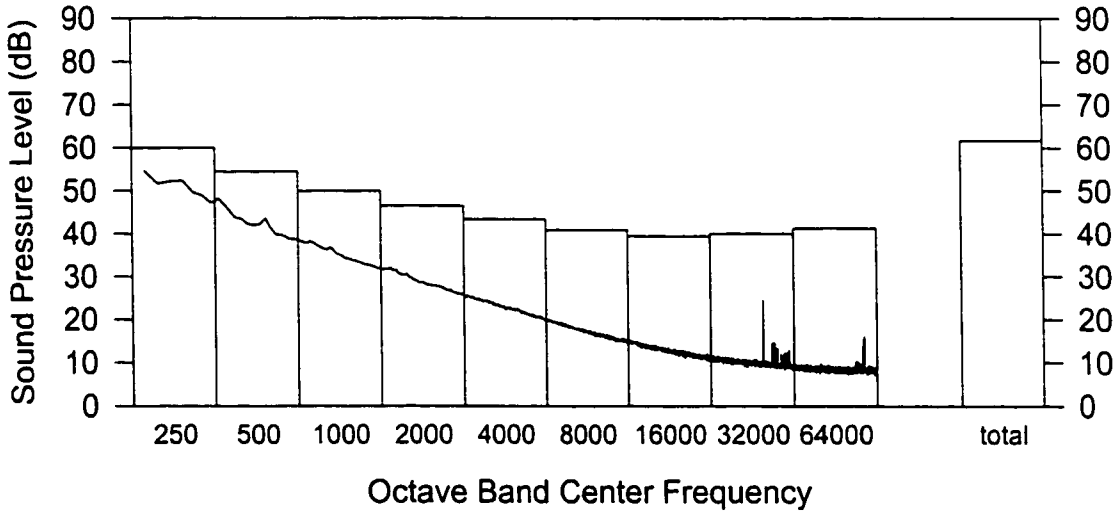

Figure 8. Food use means ± S.E.M, grams per rat per day, with 3 rats per cage.

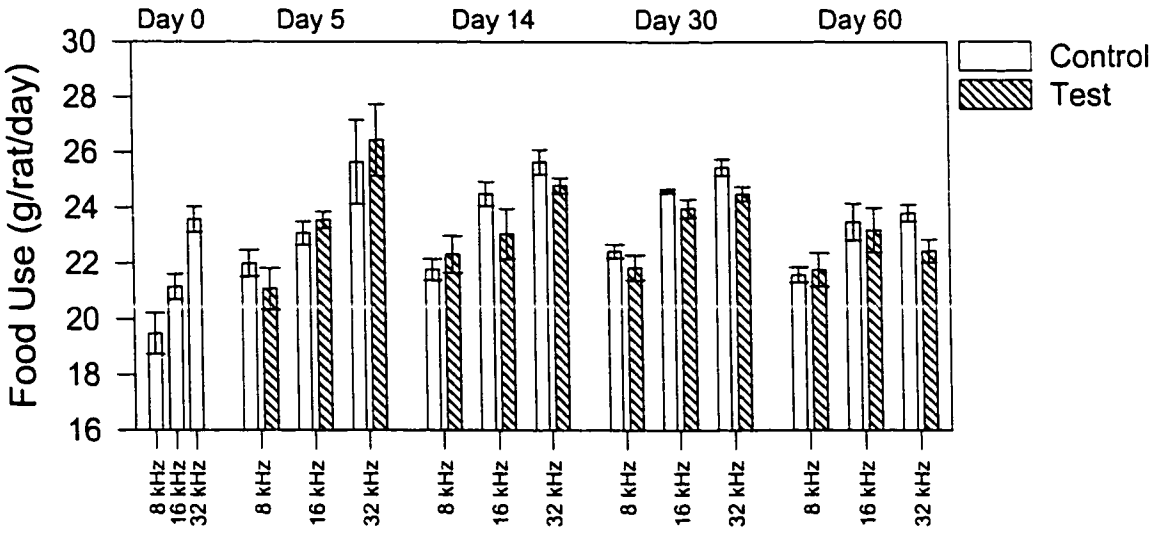

Figure 9. Food use standard deviation means ± S.E.M, grams per rat per day

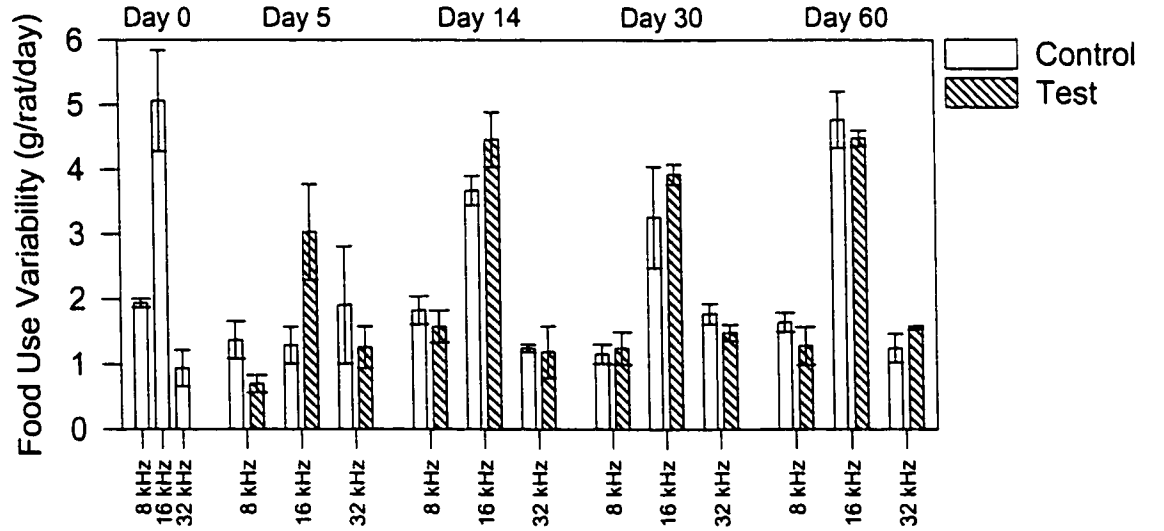

Figure 10. Water use means ± S.E.M, ml per rat per day

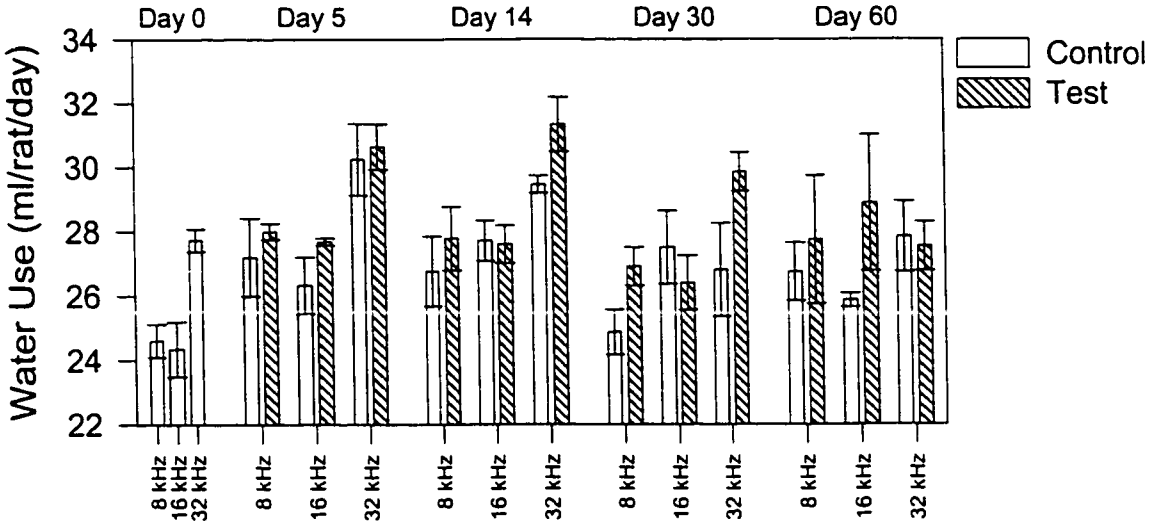

Figure 11. Water use means ± S.E.M, ml per rat per day

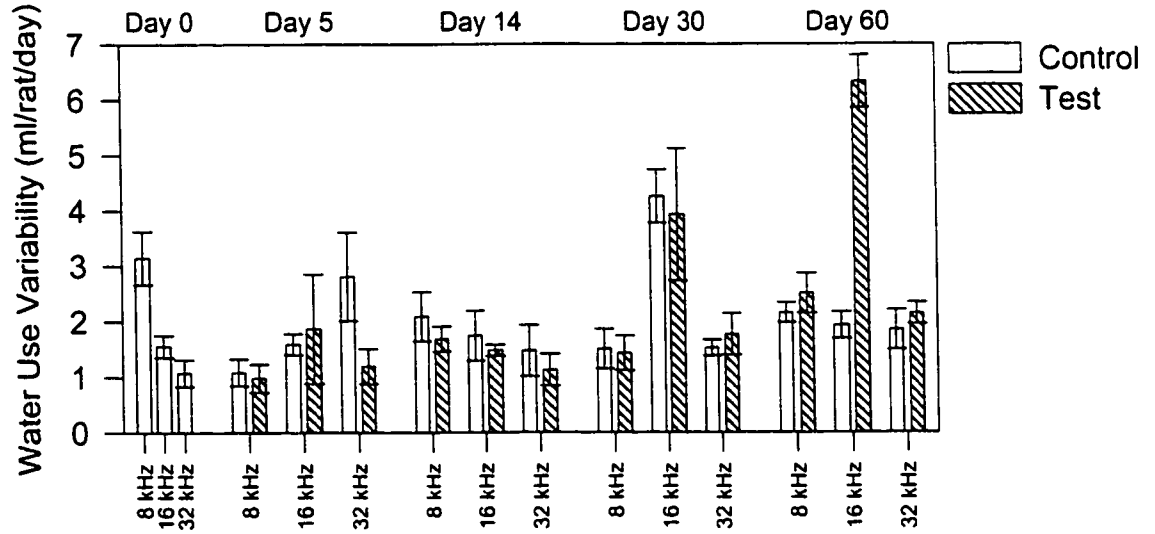

Figure 12. Body weight means ± S.E.M, grams.

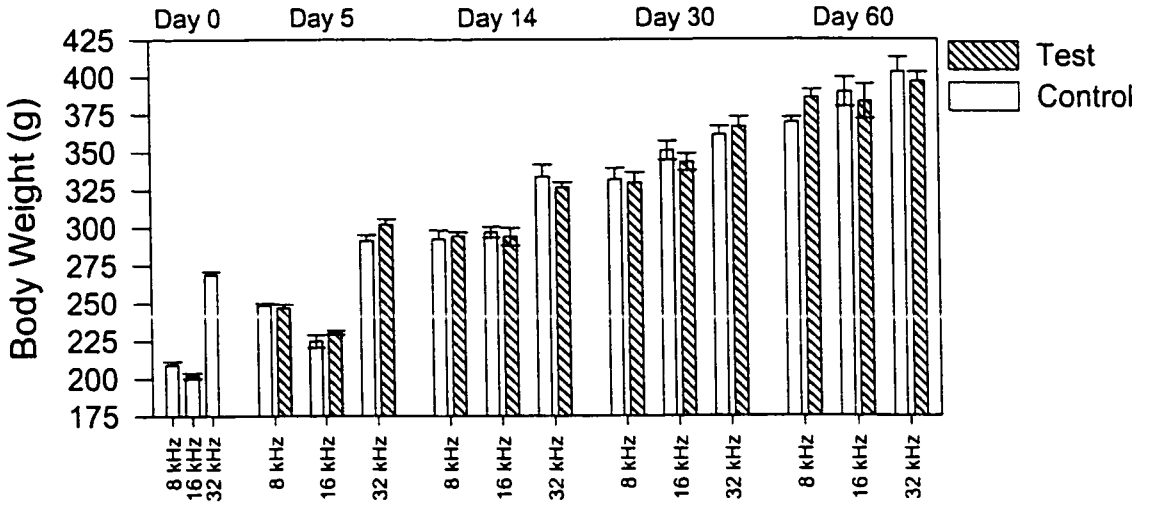

Figure 13. Heart weight means ± S.E.M., grams.

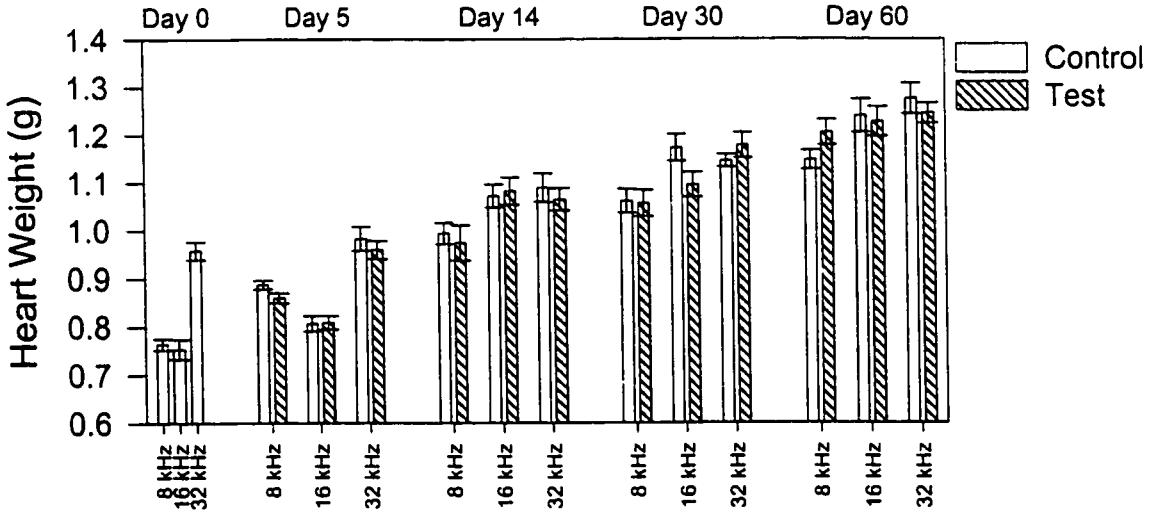

Figure 14. Kidney weight means ± S.E.M., grams.

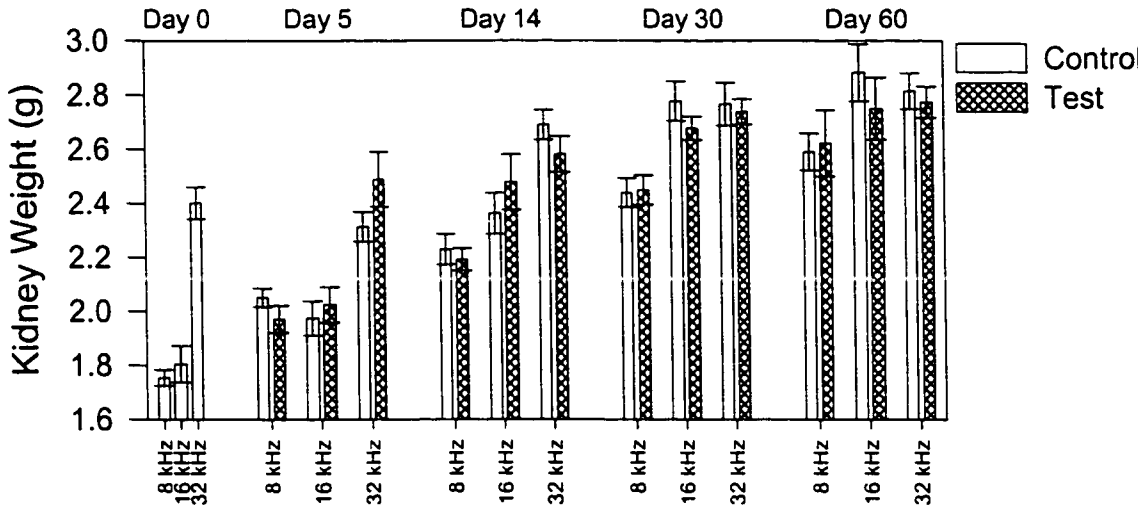

Figure 15. Spleen weight means ± S.E.M, grams.

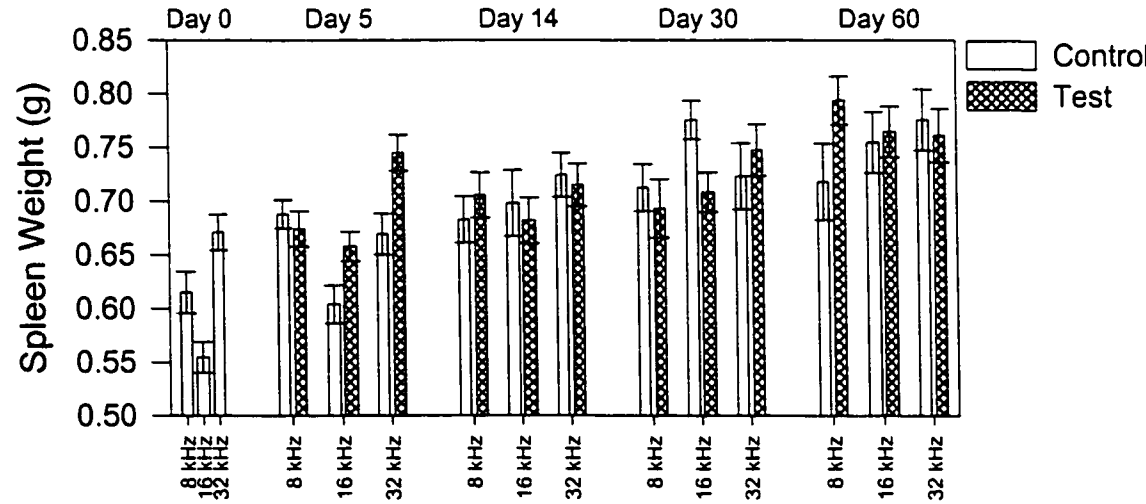

Figure 16. Adrenal weight means ± S.E.M, mg.

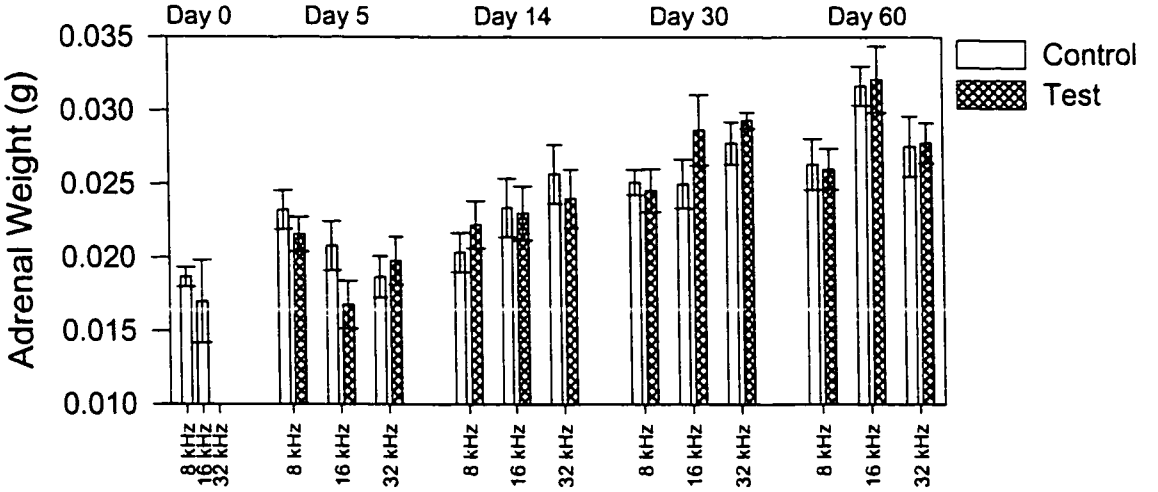

Figure 17. Testes weight means ± S.E.M, grams.

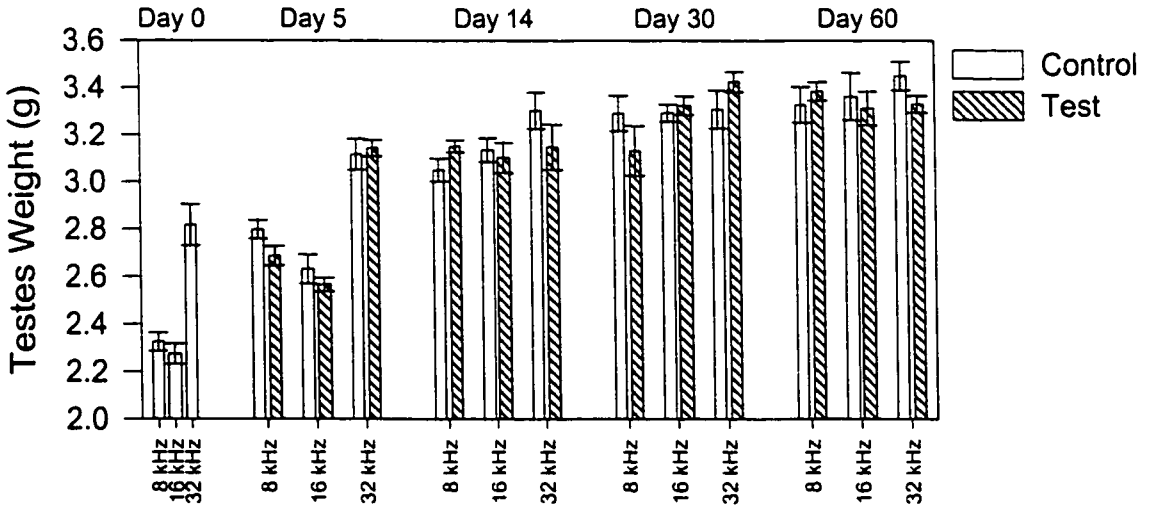

Figure 18. Thymus weight means ± S.E.M, grams

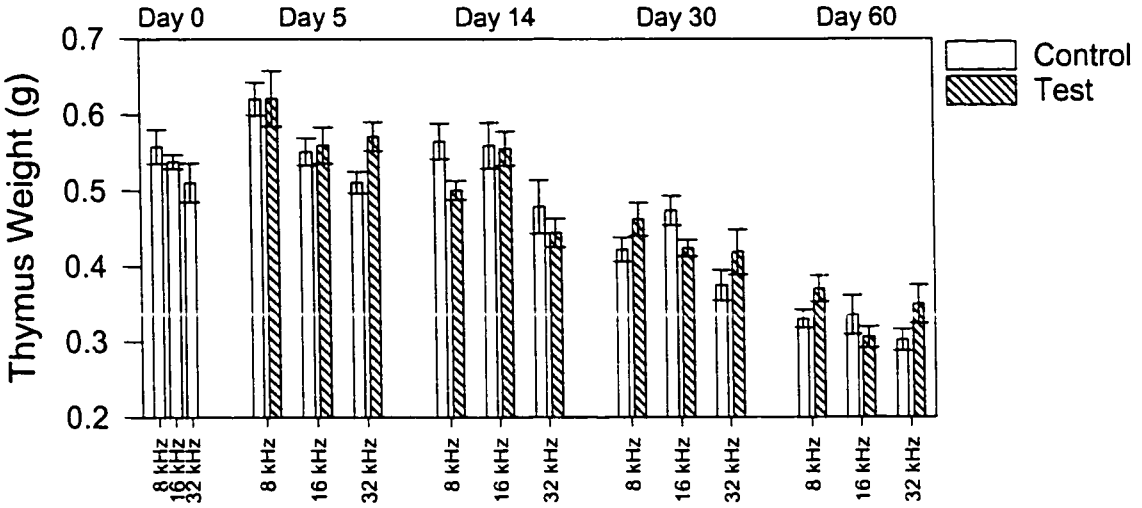

Figure 19. Plasma corticosterone means ± S.E.M, µg/dl.

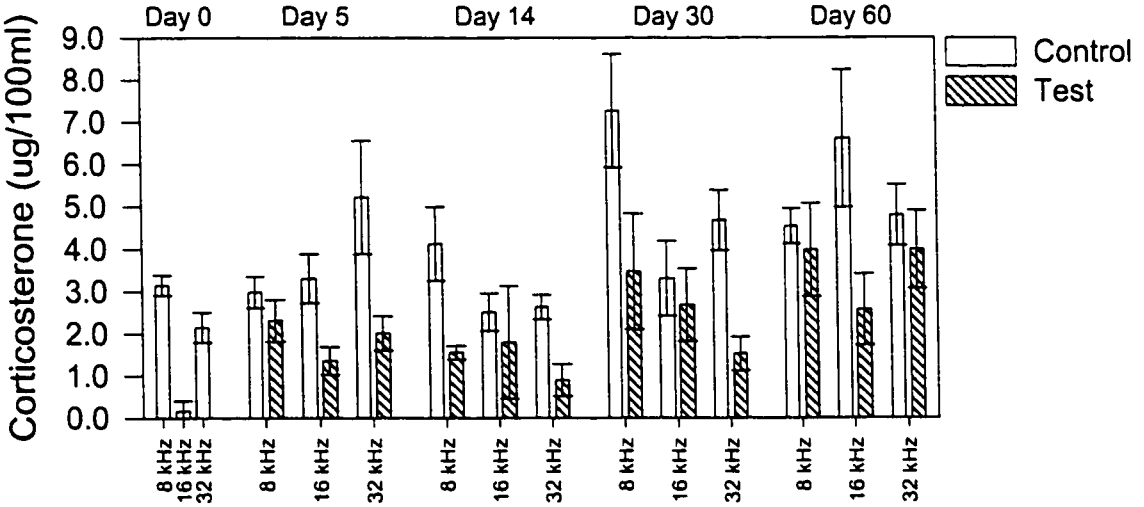

Figure 20. Plasma protein means ± S.E.M, g/dl.

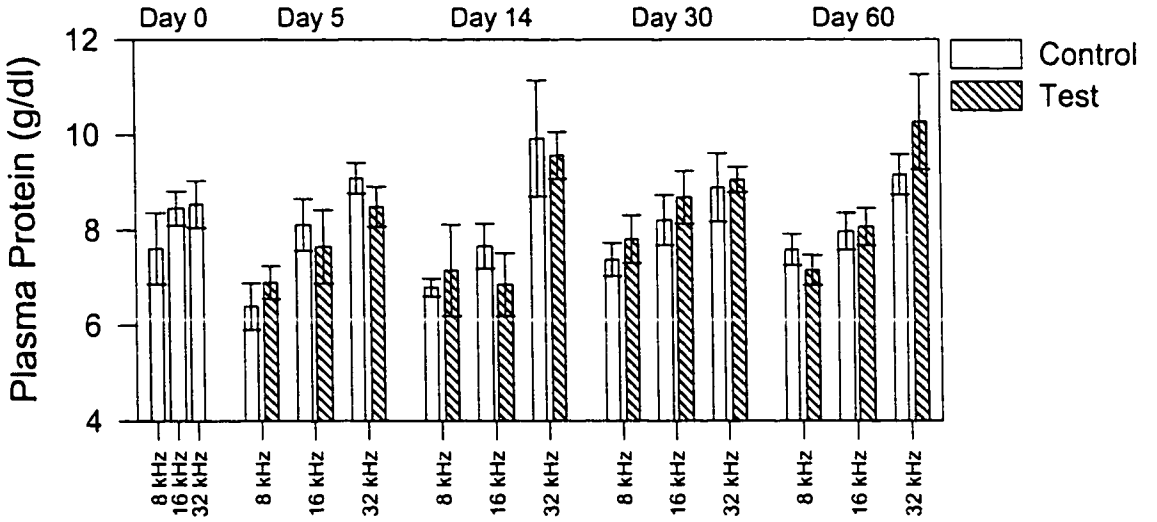

Figure 21. Leukocyte means ± S.E.M, cells/µl

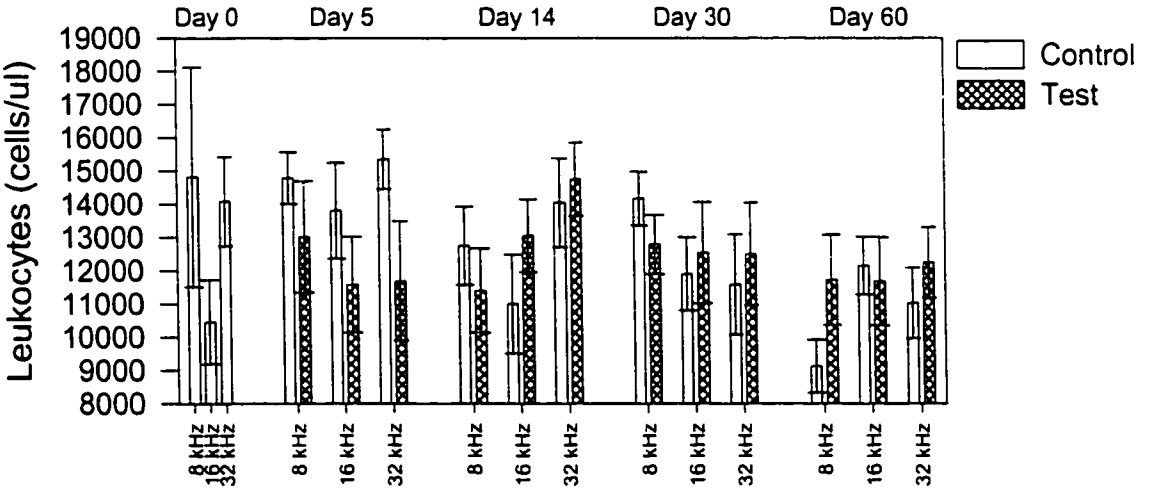

Figure 22. Lymphocytes means ± S.E.M. % of total leukocytes.

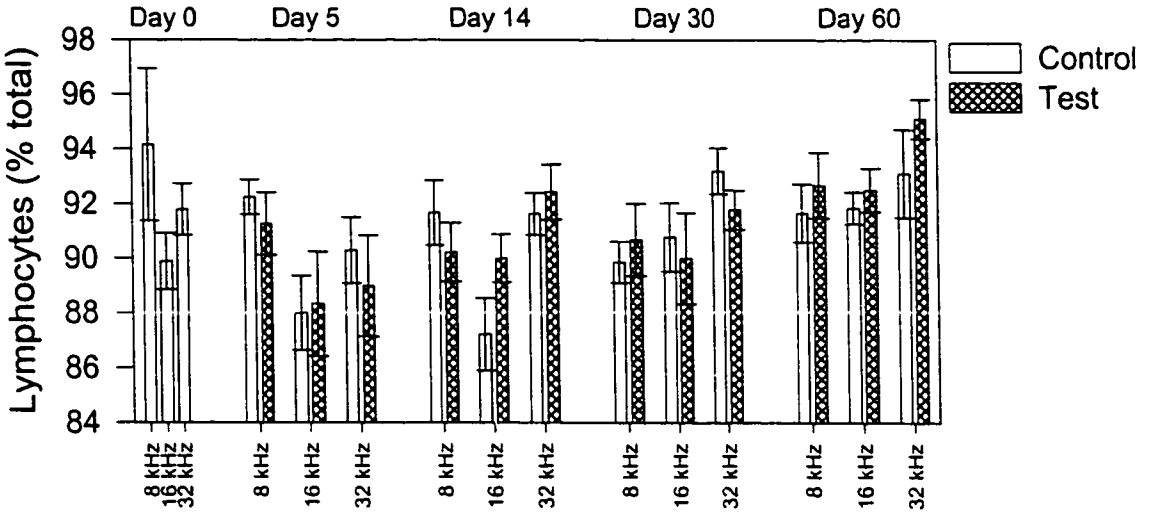

Figure 23. Monocyte means ± S.E.M. % of total leukocytes.

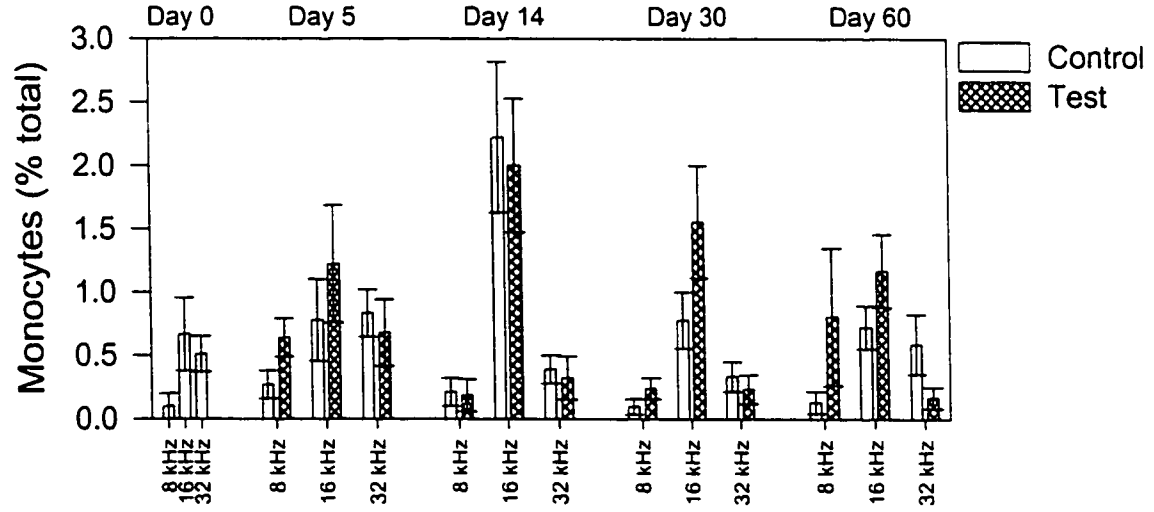

Figure 24. Neutrophil means ± S.E.M. % of total leukocytes.

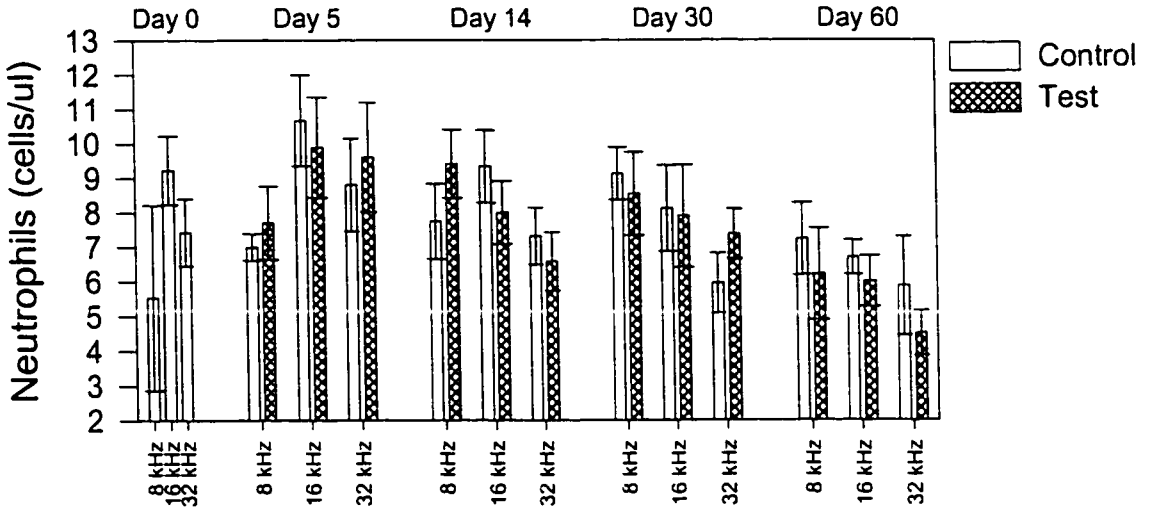

Figure 25. Eosinophil means ± S.E.M. % of total leukocytes.

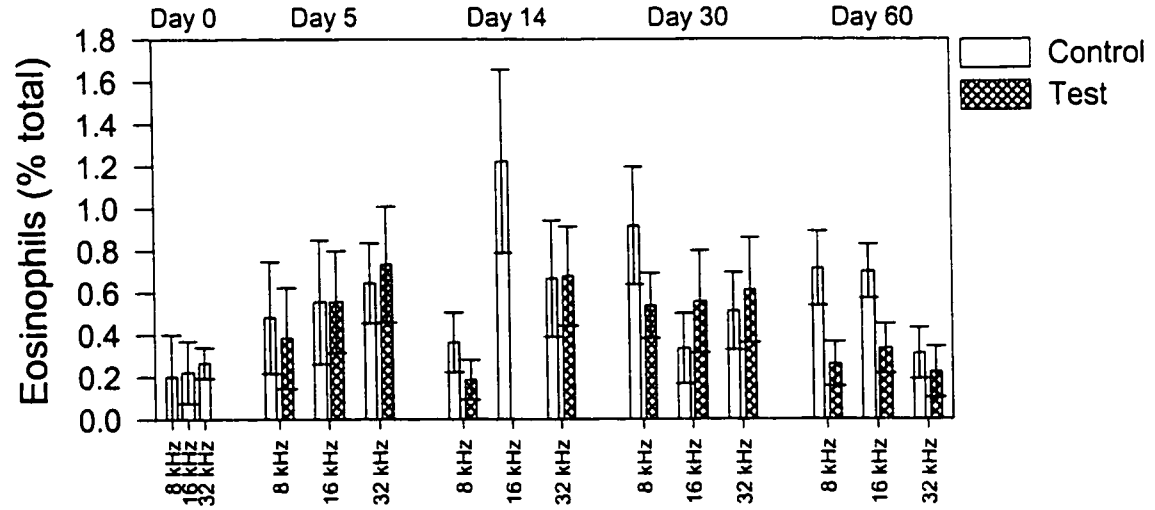

Figure 26. Neutrophil/Lymphocyte means ± S.E.M. % of total leukocytes.