3-10-2010

Development of a Novel Noise Delivery System for JP-8 Ototoxicity Studies

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DEVELOPMENT OF A NOVEL NOISE DELIVERY SYSTEM FOR JP-8 OTOTOXICITY STUDIES

THESIS

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THESIS

Presented to the Faculty

Department of Systems and Engineering Management

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Industrial Hygiene

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March 2010

AFIT/GIH/ENV/10-M04

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Abstract

Numerous chemicals with ototoxic properties may cause hearing loss directly, potentiate noise-induced hearing loss, or produce additive effects. Of interest to the US Air Force are studies showing ototoxic effects of JP-8 jet fuel and its hydrocarbon constituents. The Naval Health Research Center (NHRC) at Wright-Patterson AFB, Ohio, in conjunction with the USAF, is studying the ototoxic effects of JP-8 in rats. The study requires a white noise source that is one octave band wide, centered at 8 kHz frequency, delivered from outside of exposure chambers. Sound pressure levels must be within +/- 2 dB at all exposure points within each chamber and within +/- 2 dB over a 6-hour run. Electrodynamic shakers were successfully used to produce the required input noise in three exposure chambers by inducing vibration in chamber plenums. Distribution of sound pressure levels across chamber exposure points were well controlled within a +/- 1.5 dB prediction interval ($\alpha = 0.05$) or better. Stability at a central reference point was well controlled over 6-hour runs within a +/- 1 dB prediction interval ($\alpha = 0.05$) or better. The final system solution gives the NHRC a unique capability to deliver noise and whole-body JP-8 aerosol exposures simultaneously.

To the Fisher rats that dedicate their ears to science for the betterment of man.

Acknowledgements

I would like to thank my wife for her strength in not only supporting me throughout my graduate education, but also simultaneously enduring full-time employment, the birth of our beautiful daughter, and the start of a second pregnancy. She is an inspiration and without her this thesis would not be complete.

I would also like to thank my daughter for bringing so much joy to my life. Watching her grow into a beautiful little girl brought many smiles to my face and erased much of the stress of academic life.

Additionally, I would like to thank my academic and thesis advisor, Major Jeremy Slagley, for his amazing ability to seemingly know something about every conceivable subject. His expert guidance and willingness to set aside anything he is working on to listen to student concerns goes unmatched.

Finally, I would like to thank the Naval Health Research Center (NHRC) for trusting in me to deliver a product so pivotal to the success of their research initiative. This thesis effort was fully supported by the NHRC. To the NHRC personnel I was so fortunate to work with, thank you for the many hours of hard work and laughs that we shared.

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DEVELOPMENT OF A NOVEL NOISE DELIVERY SYSTEM FOR JP-8 OTOTOXICITY STUDIES

1. Introduction

Background

Noise-induced hearing loss (NIHL) is a significant concern for both the US Department of Defense (DoD) and private industry. The most recent data from the US Department of Veterans' Affairs indicates that over $8 billion was paid to veterans for hearing loss disabilities over the past three decades, 1977-2006. More than $900 million of that total was paid in 2006 alone and data indicate an exponential increase in cost in the most recent decade (Figure 1). (CHPPM, 2006)

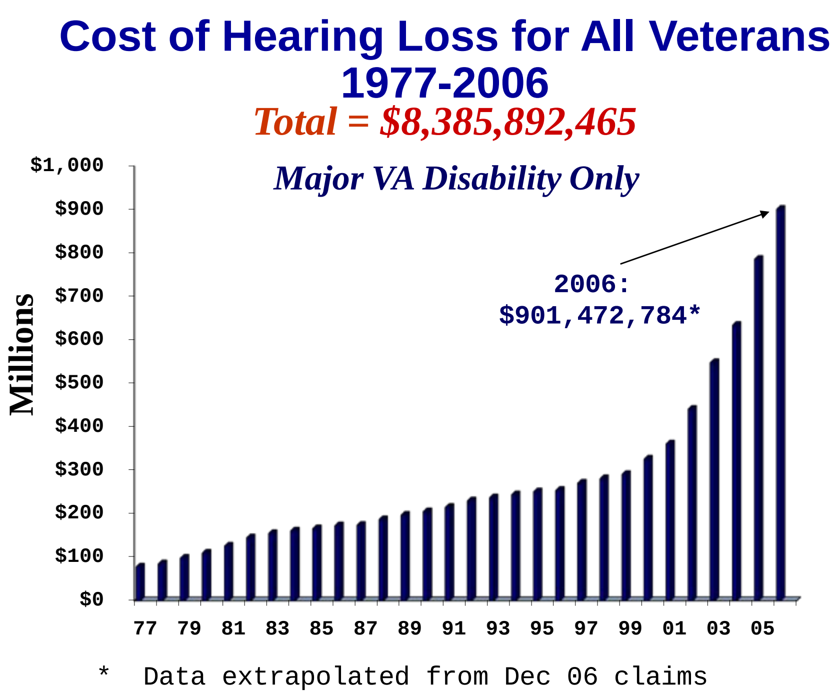


Figure 1. Exponential increase in hearing loss claims (CHPPM, 2009)

Occupational noise exposure standards are set based on exposure to noise alone. However, there are numerous chemicals with ototoxic properties that may cause hearing loss directly, may potentiate noise-induced hearing loss, or may produce additive effects (Śliwinska-Kowalska, et al., 2007). The National Institute for Occupational Safety and Health (NIOSH) estimates that over 22 million workers are occupationally exposed to hazardous noise and that an additional nine million are exposed to substances that are potentially ototoxic. However, that data is extrapolated from small sample populations due to the lack of a national occupational hearing loss and noise exposure surveillance system in the United States. (NIOSH, 2009) Actual figures could be much higher.

Though exposures to hazardous noise are easily identified and can be prevented through engineering controls, administrative controls, or personal protective equipment, ototoxins present a level of complexity that is not currently well understood. The NIOSH Hearing Loss Research Program has recognized the potential significance of ototoxins and defined "Outputs and Transfer - Research Goal 4.6: Prevent hearing loss from exposure to ototoxic chemicals alone or in combination with noise" (NIOSH, 2009). Additionally, the US Army Center for Health Promotion and Preventive Medicine (CHPPM) has recognized the significance of ototoxins and notes that audiometric monitoring is necessary to evaluate whether exposure to an ototoxic substance is affecting the hearing of exposed workers since exposure thresholds for ototoxicity are unknown. CHPPM highly recommends that annual audiograms be performed on any worker whose airborne exposure to a known or suspected ototoxin is at 50% or more of the occupational exposure limit (OEL), regardless of noise levels. Yearly audiograms are also recommended for dermal exposures to toluene, xylene, n-hexane, organic tin, carbon disulfide, mercury, organic lead, hydrogen cyanide, diesel fuel, kerosene fuel, jet fuel, JP-8 fuel, organophosphate pesticides, or chemical warfare nerve agents, where the exposure may result in a systemic dose equivalent to 50% or more of the OEL. (CHPPM, 2003) The US Army has recognized the significance and adopted CHPPM guidance in US Army Pamphlet 40-501, *Hearing Conservation Program*. It states that personnel will be enrolled in a comprehensive HCP when they are exposed to known or suspected ototoxins. (US Army, 1999)

Currently, the US Air Force (USAF) has no equivalent policy on ototoxins (US Air Force, 2006). However, exposure to JP-8 jet fuel and the potential for ototoxicity may be of great interest to the USAF. Aromatic hydrocarbons in JP-8 jet fuel, such as toluene and ethylbenzene, have been researched individually and have known otoxicity in laboratory animals (Fechter L. D., et al., 2007) (Gagnaire & Langlais, 2005). Based on allowances in the current detail specification for JP-8 jet fuel, MIL-DTL-83133F, the JP-8 formulation may contain up to 25% aromatics by volume (Defense Logistics Agency, 2009). Additionally, JP-8 formulations may consist of a mixture of up to 300 different aromatic hydrocarbons.

Recent emphasis has been placed on studying the ototoxic effects of exposure to JP-8 in its finished product form. This is of particular interest to the DoD due to high operations tempos and prevalent exposure of service members to JP-8 and similar jet fuels, primarily in the USAF. Additionally, a retrospective epidemiology study with a relatively small sample size compared USAF personnel who worked with jet fuel in a hazardous noise environment to personnel not exposed to jet fuel, but who were exposed to similar noise levels. The study found that personnel exposed to jet fuel and hazardous noise had a significant odds ratio for greater hearing loss when compared to those exposed to noise alone. (Kaufman, LeMasters, Olsen, & Succop, 2005)

Problem Statement

The Naval Health Research Center (NHRC) Environmental Health Effects Laboratory located at Wright-Patterson AFB, Ohio, in conjunction with the USAF, is conducting a study on the ototoxic effects of JP-8 in rats. In the initial phase of the study, four groups of rats will be observed in separate chambers, with one chamber being a control group and the other three chambers being exposure groups at 75, 85, and 95 decibels (dB). The study requires a very specific white noise source that is one octave band wide, centered at 8 kilohertz (kHz) frequency. The average sound pressure level (SPL) must be within +/- 2 dB at all exposure points within each chamber and within +/- 2 dB over the course of a 6-hour run. A system is needed to deliver the noise source to each of the three exposure chambers at the respective dB amplitude. Additionally, the system must be capable of providing real-time monitoring of noise levels inside of all four chambers and continuously log the data over each six-hour exposure day. The system design is complicated by the potential aggressive/hazardous nature of JP-8 aerosol inside the chamber and noise sources would ideally be transmitted from the exterior of the chamber. The NHRC lacks in-house acoustics expertise for developing the necessary system.

Research Focus

The focus of this research is to design, procure, and install a noise delivery and real-time analysis system for use in the NHRC JP-8 ototoxicity study. The end goal is the delivery of a fully operational system that meets all requirements of the NHRC study protocol. The final solution will represent the first known facility in the United States with the capability to deliver whole-body JP-8 aerosol and noise exposures simultaneously. The following questions will be used to assess system effectiveness in meeting NHRC protocol requirements:

1. Can a system be designed to deliver NHRC protocol noise requirements in existing aerosol exposure chambers?

2. Can the system deliver +/- 2 dB distribution at all exposure points within each chamber?

3. Can the system maintain a +/- 2 dB distribution over 6-hour runs?

4. Is the system robust enough to withstand repeated 6-hour runs without performance degradation?

5. Can the system provide real-time monitoring and continuous data logging over 6-hour runs?

Table 1 provides an overview of specific hypotheses that will be tested.

Table 1. Outline of hypotheses to be tested

Test Performed	Null Hypothesis (H_O)	Alternative Hypothesis (H_A)	Analysis Method
Stinger versus tapping	A stinger affixed to the plenum surface will not produce a higher SPL at 8kHz than a stinger tapping against the plenum	A stinger affixed to the plenum surface will produce a higher SPL at 8kHz than a stinger tapping against the plenum	Compare SPL from SLM measurements
Temperature effect on shaker performance	Shaker temperature increase will not affect overall SPL and frequency distribution	Shaker temperature increase will affect overall SPL and cause frequency distribution	Compare external shaker temperature to SLM measurements over time
Crossover insertion	Use of a crossover will not produce a better defined 8 kHz peak than use of audio filtering software alone	Use of a crossover will produce a better defined 8 kHz peak than use of audio filtering software alone	Comparison of SLM frequency spectrums
10-point chamber characterization	Distribution of average 8 kHz SPL across 10 randomly selected measurement points will not be within +/- 2 dB in each chamber	Distribution of average 8 kHz SPL across 10 randomly selected measurement points will be within +/- 2 dB in each chamber	SLM measurement and, if practical, statistical distribution analysis
32-point chamber characterization	Distribution of average 8 kHz SPL across all 32 chamber measurement points will not be within +/- 2 dB in each chamber	Distribution of average 8 kHz SPL across all 32 chamber measurement points will be within +/- 2 dB in each chamber	SLM measurement and, if practical, statistical distribution analysis
Endurance test	Distribution of 8 kHz SPL measured at a central reference point will not be within +/- 2 dB over a 6-hour run	Distribution of 8 kHz SPL measured at a central reference point will be within +/- 2 dB over a 6-hour run	Statistical distribution analysis
Compressor engineering controls	Modifying existing acoustical enclosures over air compressors will not reduce the sound pressure level near the control chamber	Modifying existing acoustical enclosures over air compressors will reduce the sound pressure level near the control chamber	Comparison of frequency spectra before and after modification

Methodology

Various acoustic sources, including speakers and electrodynamic shakers, will be tested to see if they are capable of producing the required protocol levels of 75, 85, and 95 dB within the chambers. Supporting acoustic equipment (amplifiers, crossovers, audio editing software, etc) will also be tested for capability of shaping the input noise source to the required octave band and amplitude conditions. A sound level meter will be used to characterize the chambers at operating conditions to insure that +/- 2 dB over space and time is achieved. Demonstration equipment will be borrowed from vendors for pilot testing. A final solution including a real-time analysis system will be designed, specifications will be documented, and the requirements will be coordinated through the USAF acquisitions process for procurement. Upon delivery of all equipment, the system will be installed, fully tested, and ready for turn-key operation by NHRC lab personnel.

2. Literature Review

Human Epidemiology

NIOSH notes that ototoxicity gained attention and further research interest following the publication of “Occupational exposure to organic solvents and noise: effects on hearing” by Morata et al in 1993 (NIOSH, 2009). In that publication, the authors presented significant evidence of greater effects of combined exposure to solvents and noise on hearing loss in a group of printing and paint manufacturing workers. Workers were categorized into four exposure groups: unexposed (N = 50), workers exposed to noise only (N = 50), workers exposed to noise and toluene (N = 51), and workers exposed to an organic solvent mixture and no noise (N = 39). The risk of hearing loss was statistically significantly greater for all exposed groups as compared to the unexposed group. The combined toluene and noise exposure group stood out with an 11 times greater relative risk ratio for hearing loss when compared to the unexposed group. Relative risk for the noise only group was four times greater and five times greater for the solvent mixture group. Results of the study suggested that exposure to the solvent mixture had a direct toxic effect on the auditory system and combined exposure to noise and toluene appeared to have an additive effect. (Morata, Dunn, Kretschmer, LeMasters, & Keith, 1993)

In a second epidemiology study, researchers examined the effects of solvent exposure on hearing without exposure to hazardous noise. Exposure groups were selected from a reinforced fabric manufacturing facility. Processes in the factory involved application of polyurethane coating to a variety of fabrics. Significant quantities of toluene and methyl ethyl ketone were used in the plant, but numerous additional solvents including trichloroethylene, acetone, n-methyl pyrrolidone, dimethylformamide, chlorobenzene, and isopropyl alcohol were also used in lesser quantities. Mixtures of varying solvent concentrations were used depending on the fabric treatment being applied, leading to workers being exposed to different mixtures of solvents over time. An industrial hygienist categorized the workers into three groups based on job performed and the anticipated exposure level, with category 1 being minimal exposure and category 3 being maximal exposure. Category 1 (N = 20) included workers performing office and administrative functions with minimal solvent exposure. Category 2 (N = 18) included maintenance personnel, floor supervisors, and jobs handling finished fabric with moderate solvent exposure. Category 3 (N = 72) included workers with high exposures to solvents including coating machine operators, helpers, mixers, and hazardous waste handlers. Worker hearing status was measured with a three-part test battery including pure-tone hearing thresholds (0.5 – 8 kHz), high-frequency hearing thresholds (12 and 16 kHz), and a dichotic digits test. Significant associations were found between solvent exposure and results of the three hearing tests. However, covariates such as age, gender, race, and ethnicity were also found to be significant with regard to test outcomes. The authors concluded that occupational exposure to solvents may have a direct effect on both peripheral and central auditory function. (Fuente, et al., 2009)

Another study evaluated the effects of occupational exposure to styrene, toluene, and noise on hearing. The exposure group (N = 290) consisted of yacht yard and plastic factory workers, with exposures to: styrene only (N = 194); styrene and toluene (N = 26); styrene and hazardous noise (N = 56); or styrene, toluene, and hazardous noise (N = 14).

A reference group was established from white-collar workers exposed to neither solvents nor noise (N = 157) and factory workers exposed to hazardous noise only (N = 66). Study subjects were given detailed questionnaires and audiometric examinations. Results showed an increased odds ratio for developing hearing loss in all exposure groups: noise only (OR = 3.3); styrene only (OR = 5.2); styrene and toluene (OR = 13.1); styrene and hazardous noise (OR = 10.9); or styrene, toluene, and hazardous noise (OR = 21.5). Mean hearing thresholds were significantly higher in solvent exposed groups as compared to unexposed reference groups at all frequencies tested. The study provided additional evidence that occupational exposure to solvents is associated with increased risk of hearing loss and that combined exposures to noise and solvents appear to be more ototoxic than noise exposure alone. (Śliwinska-Kowalska, et al., 2003)

An epidemiology study related to JP-4 and JP-8 jet fuel exposure in USAF personnel is one of several driving factors behind the NHRC protocol. Kaufman et al evaluated the effects of occupational exposure to jet fuel on hearing in military workers at Hill AFB, Utah. Subjects selected for the study were aircraft maintenance or other personnel required to have at least three years of exposure to hazardous noise. Fuel exposed personnel were also required to have a minimum of three years fuel exposure. A total of 138 subjects met eligibility requirements and were included in the study. Noise dosimetry records and solvent exposure data was collected from base bioenvironmental engineering records for the potential exposure groups of all study participants. Audiometric evaluations were conducted on all subjects and data was collected by questionnaire on work histories, recreational exposures, personal protective equipment, medical histories, alcohol, smoking, and demographics. Fuel exposure estimates for JP-4 ranged from 0 – 33% of the OEL and 0.5 – 11% of the OEL for JP-8. The study found that personnel with three years of exposure to jet fuel and noise had an adjusted odds ratio for hearing loss of 1.7. Personnel with 12 years of jet fuel and noise exposure had an even higher adjusted odds ratio of 2.41. The authors concluded that the findings suggest that jet fuel is ototoxic and has the potential to cause greater hearing damage than noise alone. (Kaufman, LeMasters, Olsen, & Succop, 2005)

Animal Studies

A study in France investigated the interaction of simultaneous exposure to noise and toluene in adult Long-Evans rats. The rats were divided into four groups: control (N = 21), noise only (N = 22), toluene only (N = 24), and noise plus toluene (N = 23). The exposure groups were placed in exposure chambers six hours per day, five days per week, for four weeks. The toluene and toluene plus noise groups were exposed to a concentration of 2000 ppm toluene. The noise and toluene plus noise groups were exposed to 92 dB octave band noise centered at 8 kHz. No information was provided as to the type of noise (white, pink or otherwise) and no details were provided with regard to frequency energy distribution that would aid in discerning the type of noise. Background noise inside the exposure chambers did not exceed 66 dB. The authors state that “the animals were housed alone in individual cages for a 4-week exposure with a speaker above the cages.” No additional information was provided with regard to the design of the noise delivery system, generation and filtering of the noise source, characterization of noise distribution in chambers prior to the study, or method of measuring sound pressure levels during the study. An adaptation of the frequency spectrum the authors reported can be seen in Figure 2. The 8 kHz octave band does not appear to be flat and leans to the left with an approximately 6 dB differential between 6.3 kHz and 10 kHz. The authors indicated that at the time of the article they were not aware of any other studies that investigated simultaneous exposure to noise and toluene, only sequential exposures. Results of the study indicated that hearing impairment caused by combined exposure to toluene and noise exceeded the additive impairment caused by toluene or noise alone. The authors also note that the cochlear damage induced by toluene and noise differ, with noise alone inducing stereocilia damage and toluene alone inducing outer hair cell loss. (Lataye & Campo, 1997)

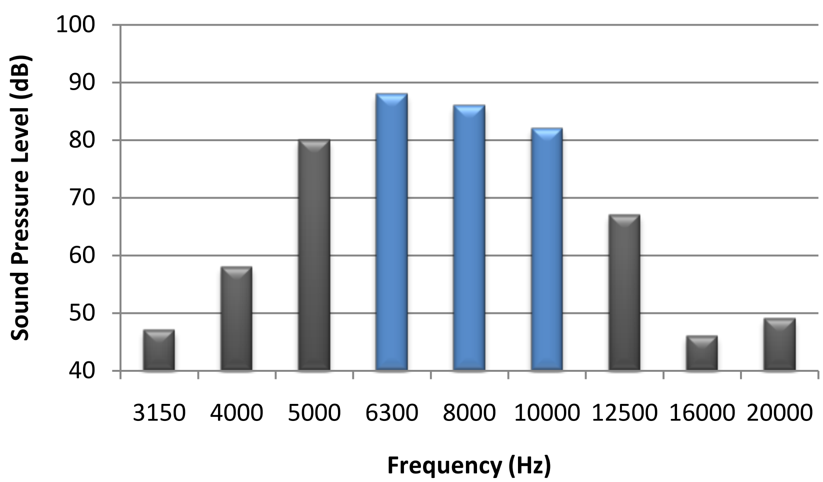


Figure 2. 8 kHz octave band noise with neighboring bands (adapted from Lataye & Campo, 1997)

A separate study investigating the combined effects of noise and toluene exposure was conducted in Denmark. The first phase of the study investigated the effects of long-term exposure to low levels of toluene and noise in rats. Four groups of 12 rats were exposed simultaneously to 4 – 20 kHz wide-band white noise at 90 dB and either 0 ppm, 100 ppm, 200 ppm and 500 ppm toluene. A fifth control group of 12 rats was exposed to neither toluene nor noise. Exposures to toluene were six hours per day, five days per week for 90 days and exposures to noise were four hours per day, five days per week for 90 days. A second phase of the study investigated interactions between higher levels of toluene and either wide-band noise or impulse noise. Eight groups of 12 rats were exposed simultaneously six hours per day for 10 days to 0 ppm, 500 ppm, 1000 ppm, or 1500 ppm toluene and 4 – 24 kHz noise at 92 dB delivered as either continuous wide-band noise or impulse wide-band noise. Impulse noise reached a peak of just over 130 dB. However, the authors do not mention the frequency or duration of impulse injection. The noise in both phases was generated using a computer with a 16-bit digital-to-analog converter and amplified by powered audio amplifiers. The amplifiers drove dome tweeters located above each cage. Sound pressure levels were measured at the floor level of the cages with a 1.27 cm (0.5 inch) condenser microphone and spectrum analyzer. (It should be noted that a 1.27 cm (0.5 inch) microphone may not be ideal for impulse measurements depending on the frequency and duration of the impulse generation.) The authors note that rats were in wire mesh cages within the exposure chambers; however, they fail to mention whether the rats were housed in individual cages or community cages and how many speakers and measurement points were used. Background noise levels in the exposure chambers were 35 dB in the 2 – 48 kHz frequency range. Rats exposed to noise and toluene at 500 ppm or less for 90 days did not show increased hearing impairment compared to rats exposed to noise only. In the 10-day exposures to higher levels of toluene, a synergistic interaction was noted in the groups exposed to 1500 ppm toluene and both wide-band continuous noise and wide-band impulse noise. However, hearing impairment was much greater in the impulse noise group. The authors conclude, “ototoxicity of organic solvents may be a hazard to human hearing due to the exacerbation of hearing loss by a possible co-exposure to especially harmful noise, such as impulse noise.” (Lund & Kristiansen, 2008)

A team in Sweden conducted a third study investigating the combined effects of toluene and noise exposure in rats. Previous studies had investigated the effects of simultaneous exposure to noise and toluene or sequential exposure to toluene followed by noise. Researchers in this study investigated exposures in the reverse order with the interaction of sequential noise exposure followed by toluene exposure. Five groups of male Sprague-Dawley rats were used. The first group (N = 10) was a control group exposed to neither toluene nor noise. The second group (N = 10) was exposed to noise only for four weeks. The third group (N = 10) was exposed to toluene only for two weeks. The fourth group (N = 10) was exposed to noise for four weeks followed by toluene for two weeks. The fifth group (N = 10) was exposed to noise for four weeks, followed by four weeks rest, then two weeks toluene exposure. In all cases, toluene exposures were 1000 ppm for 16 hours per day, seven days per week and noise exposures were 100 dB for 10 hours per day, seven days per week. The authors state that the rats were exposed to noise in a sound-insulated exposure chamber, but do not provide further description of the chamber. The noise source was described as “a 2 kHz wide noise band sweeping from 3 to 30 kHz at a frequency of 0.5 Hz. It was chopped at a frequency of 0.5 Hz with a duty cycle of 50%. Modulation and chopping created a continuously varying signal, with a maximum sound level of 105 dB SPL within the frequency range 5 – 15 kHz." Sound levels were measured using an integrating sound level meter. The authors do not mention either at what positions and how many positions the sound levels were measured or how the sound was delivered to the chamber. However, they do state that cages were systematically repositioned within the chamber to minimize variation in individual noise exposure levels. Some level of hearing loss was observed after all exposures. Impairment after exposure to noise followed by toluene was greater than exposure to noise alone or toluene alone, but was not greater than the additive loss caused by noise alone and toluene alone. The authors conclude that reversing the order of exposure to noise followed by toluene contrasts with results of exposure in the reverse order and that the "exposure sequence can determine the extent of auditory impairment." (Johnson, Nylen, Borg, & Hoglund, 1990)

A fourth study investigated the interaction of exposures to noise and acrylonitrile. Researchers hypothesized that "moderate noise exposure, that does not produce permanent hearing loss by itself, could initiate oxidative stress and that acrylonitrile could render the inner ear more sensitive to noise by disrupting intrinsic antioxidant defenses." Adult Long-Evans rats were divided into six groups: controls (N = 9); acrylonitrile 50 mg/kg only (N = 11); 97 dB noise only (N = 8); 95 dB noise only (N = 6); acrylonitrile 50 mg/kg plus 97 dB noise (N = 8); and acrylonitrile 50 mg/kg plus 95 dB noise (N = 6). Acrylonitrile was administered via subcutaneous injection 30 minutes prior to daily noise exposure. Noise exposures were administered in a reverberant 40 L glass cylinder. Applying a band pass filter to broadband noise generated by a function-generator produced octave band white noise with 8 kHz center frequency. The band pass filter system used 48 dB per octave roll-off. The shaped noise source was amplified and fed to speakers located approximately five cm above wire-cloth enclosures housing the rats. Sound levels were measured at the approximate height of the rats' ears by a sound level meter with 1/1 octave filter set. Sound levels in the exposure chamber were maximal between 6.3 and 10 kHz, approximately 7 dB lower at 5 and 12.5 kHz, and 20 dB lower at 4 and 16 kHz (Figure 3). In the groups of rats exposed to either acrylonitrile or noise alone, permanent hearing or hair cell loss was not observed. However, in groups exposed to both acrylonitrile and noise, permanent hearing and outer hair cell loss was observed. The authors conclude that acrylonitrile "can potentiate NIHL at noise levels that are realistic in terms of human exposure, and that the outer hair cells are the main target of toxicity." (Pouyatos, Gearhart, & Fechter, 2004)

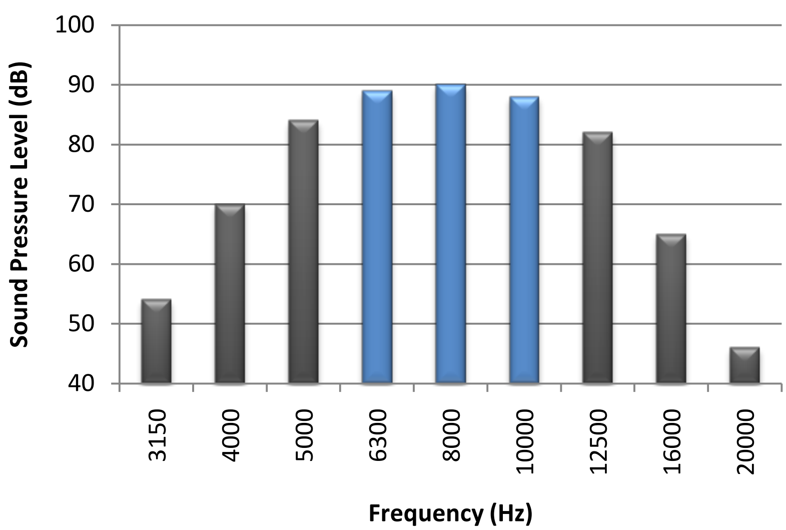


Figure 3. 8 kHz octave band noise with neighboring bands (as adapted from Pouyatos, Gearhart, & Fechter, 2004)

Research into the ototoxic effects of JP-8 in animals has been pioneered out of the Loma Linda VA Medical Center in Loma Linda, California. Researchers sought to examine the effects of inhalation exposure to JP-8 with and without subsequent noise exposure on hearing impairment in rats. The first of three auditory experiments conducted included a single four hour, nose-only inhalation exposure to 1000 mg/m^3 JP-8. The exposure group was then split, with half immediately receiving a four-hour noise exposure at 105 dB and the other half receiving no noise exposure. The second experiment group received five days of repeated nose-only inhalation exposures to 1000 mg/m^3 JP-8 for four hours per day. The group was then split each day with half receiving four hours of noise exposure at 97 dB and the other half receiving no noise exposure. The third experiment design followed the same repeated exposure and group splitting parameters outlined in the second experiment, but the noise exposure was increased to 102 dB and noise exposure duration was reduced to one hour. Noise exposures were conducted in a reverberant 40-liter chamber. Rats were placed in small wire-cloth enclosures within the chamber. Noise was generated using the same equipment and parameters outlined in the acrylonitrile study previously discussed, resulting in the same frequency sound level distribution and roll off values mentioned. Background sound levels in the fuel exposure chamber were below 60 dB at all sound frequencies. All sound pressure measurements were made using a sound level meter with a 1/3-octave filter set. Noise levels were within +/- 2 dB within the exposure chamber. In the first experiment, single exposures to JP-8 without subsequent noise exposure JP-8 exposure did not result in hearing impairment.

However, single JP-8 exposure with subsequent noise exposure produced additive disruption in outer hair cell function. In the repeated exposure experiments with five-day JP-8 exposure alone, impairment of outer hair cell function was observed, but partial recovery was observed over a four-week post-exposure period. Repeated exposures with JP-8 followed by noise caused greater hearing impairment and hair cell loss than noise alone. Examination also suggested an increase in outer hair cell death among rats treated with repeated exposure to JP-8 and noise when compared to noise alone. (Fechter L. , et al., 2007)

Results from both animal studies and human epidemiology studies discussed here indicate that chemical ototoxicity is of concern to hearing loss. The mechanism of action by which chemicals induce or potentiate hearing loss differs from the mechanism by which NIHL occurs. The primary target of JP-8 and its chemical constituents appears to be outer hair cells within the cochlear region, with a significant degradation in outer hair cell function that leads to either temporary or permanent hearing loss. Noise alone was shown to induce damage primarily to the stereocilia within the cochlea. Hearing loss induced by the combined insult of ototoxic chemicals and noise was shown to be greater and more permanent than either insult alone.

Results and methods of noise delivery presented by Fechter et al. in the JP-8 study discussed above were a kick-off point for the current NHRC protocol and are an impetus for this thesis effort. However, the NHRC facility will offer a distinct difference in the ability to deliver a whole-body JP-8 aerosol exposure and noise exposure simultaneously. No other literature was found relating to capabilities for simultaneous whole-body aerosol and noise exposures within the DoD or elsewhere in the United States. Additionally, no literature was found relating to novel methods of noise delivery, including the use of a shaker to induce sound waves within a chamber.

3. Methods

Facilities

All experiments conducted in this project were carried out at the NHRC inhalation laboratory at Wright-Patterson AFB, Ohio. The inhalation laboratory is located in a room with dimensions of approximately 8.53 m X 12.8 m (28' X 42') with a 3.05 m (10') ceiling and contains six whole-body aerosol exposure chambers. The chambers were custom built to in-house specifications and are designed to produce a laminar flow of an aerosol agent at constant concentration and equal distribution throughout the chamber. The exposure chambers are constructed of stainless steel with glass front and side windows. Angled plenums constructed of stainless steel are at the top and bottom of each chamber and are designed to provide airflow in from the top and exhaust out the bottom. A representative chamber is depicted in Figure 4.

Figure 4. Whole body aerosol exposure chamber

Each chamber has four sets of rails onto which cage assemblies slide. Cage assemblies are constructed of wire mesh with 1.27 cm (0.5 inch) spacing and each assembly is divided into eight individual compartments that hold one animal each (Figure 5).

Figure 5. Cage assembly with eight individual compartments

Each chamber can hold four cage assemblies for a maximum of 32 animals in any test. A chamber interior with four cage assemblies installed can be seen in Figure 6.

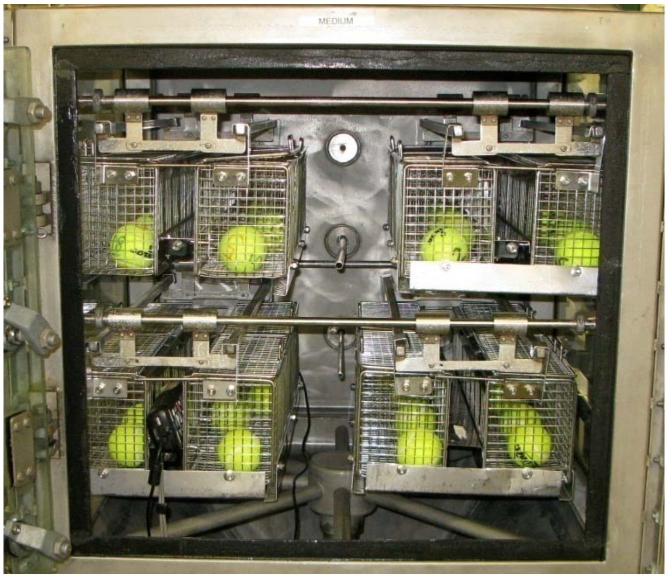


Figure 6. Chamber interior with four cage assemblies installed

Two large air compressor units are located in the southeast corner of the room and contribute greatly to overall background noise levels when running. Special treatment of the air compressors is discussed later in this section. A diagram of the overall room layout can be found in Appendix A.

Pilot Study

Initial Phase

During 29 April – 7 May 2009, students in the 10M Graduate Industrial Hygiene program at the Air Force Institute of Technology conducted a noise laboratory at the NHRC as part of the Industrial Hygiene II course (ENVR 543). This was the starting point of a pilot study to test the feasibility of various sound generation devices in delivering the noise requirements in the NHRC protocol and it was conducted in parallel with (and considered a portion of) this thesis effort. Numerous noise generation sources and configurations were tested, including placing various speakers against both the plenum and windows of a chamber, placing a trumpet horn against a port opening in the rear of the chamber, and utilizing a shaker to vibrate the plenum and create noise output. When comparing the outcomes of all configurations tested in the pilot study, using a shaker was the only option that achieved the protocol requirements of +/- 2 dB equal sound level distribution and sound levels greater than 95 dB inside the chamber. High frequency sound waves produced by speakers on the outside were easily attenuated by the chamber construction materials. Using a shaker or similar technology that vibrates the plenum and essentially turns it into a large speaker surface was determined to be an optimal solution. However, it must be noted that the necessary equipment to filter and generate a precise octave band of noise at 8 kHz was not available during the initial phase of the pilot study. (Stubbs, Ferreri, Graessle, & Horenziak, 2009)

Demonstration Shakers

Following through with the shaker concept that was determined to be a potential solution in the first phase of the pilot study, a search was conducted to select shaker candidates from vendors with specifications that could deliver the protocol noise requirements. Ideal candidates would have optimal performance in the 8 kHz octave band. Through discussions with shaker manufacturers, it was determined that larger and heavier shaker models designed to deliver heavy force loads begin to show performance decline at higher frequencies. A decision was made to consider shaker candidates rated at 111 N (25 lb force) or less. Two models were selected for the next phase of the pilot study and demonstration models were accepted on loan from vendors for further testing. The first candidate was a Modal Shop Model 2025E shaker (The Modal Shop, a PCB Company, Cincinnati, Ohio) paired with a Modal Shop SmartAmp power amplifier (The Modal Shop, a PCB Company, Cincinnati, Ohio). The 2025E was also supplied with a trunnion mounting base, stinger kit and attachment discs. The second candidate was a B&K Type 4809 shaker (Bruel & Kjaer, Naerum, Denmark) paired with a B&K Type 2719 power amplifier (Bruel & Kjaer, Naerum, Denmark). No additional accessories were supplied with the B&K shaker, making setup and testing difficult. A stinger kit was eventually expedited from Georgia. However, no trunnion bases were immediately available in the United States. A custom wood mounting stand was eventually built in-house to serve as a makeshift trunnion for the 4809. An additional professional audio power amplifier, QSC Audio Model RMX 1450 (QSC Audio, Costa Mesa, California) power amplifier, was also tested in conjunction with each of the shakers to see if it would perform satisfactorily. Using a mainstream amplifier such as the QSC model instead of a custom amplifier is beneficial in terms of both cost and ease of replacement if failure occurs.

Tapping versus Stinger Trials

Trials with the shaker during the initial pilot study were conducted by allowing the shaker head to vibrate against the plenum of the chamber. A similar attempt was made with the 2025E shaker by attaching a 10-32 threaded bolt into the shaker armature and allowing it vibrate against the plenum. For this trial, a white noise file was crudely filtered on a laptop computer to transmit an 8 kHz octave band of noise to the Modal Shop amplifier and to the 2025E shaker. Sound level measurements inside the chamber were taken with a Larson Davis Model 831 (Larson Davis, Depew, New York) integrating sound level meter.   At the point where the amplifier began to clip and eventually faulted, levels inside the chamber did not reach above 88 dB in the 8 kHz octave band. However, shakers are typically used in conjunction with a stinger versus being used as a tapping mechanism. A stinger is a rod that is affixed to the armature of a shaker and the opposite end is mounted to the test surface that is to be vibrated. Utilizing a stinger creates a solid connection between the shaker armature and the test surface and allows them to move in unison. This is beneficial to both the quality of the vibration spectrum generated and insuring a solid connection and position is maintained throughout a test.

A brief bench-top experiment was conducted to compare the results of tapping versus stinger attachment on a piece of metal bracket material. The metal bracket was mounted between two jack stands and raised to a level that allowed the shaker to rest beneath the bracket in a vertical position. Two experiments were conducted utilizing the same input white noise source noted above, one with the 10-32 bolt tapping against the bracket and the second with the 10-32 bolt screwed through the bracket into the shaker armature, forming an attachment similar to a stinger. Sound level measurements were taken approximately 2.54 cm (1 inch) above the bracket under each condition. Forming a solid, stinger-like, attachment produced a 12 dB greater sound pressure level at the 8 kHz octave band.

A stinger kit with test surface attachment discs was acquired from the Modal Shop for further testing. The attachment discs are small hexagonal-shaped units constructed of a composite material with concentric grooves machined in the back. The grooves allow for better application of glue or epoxy for attachment to a test surface. The front side of the discs has a 10-32 threaded hole in the center for stinger attachment. An attachment disc was affixed to the surface of the lower left (with respect to viewing straight on to the door) plenum wall with an epoxy putty and allowed to set for 24 hours. The shaker was then positioned beside the plenum wall and a stinger was connected between the shaker and the attachment disc on the plenum. The same noise file was then played again and sound pressure levels inside the chamber easily reached above the 95 dB requirement.

White Noise Generation

A search was conducted for robust computer software that could generate a more pure and precisely filtered white noise file. Audacity version 1.3 freeware (Audacity Development Team, audacity.sourceforge.net) was found to be well developed and fully capable of generating the required file. The software was installed on a laptop computer and a white noise file of one-hour duration was generated. A high pass filter with a 48 dB per octave roll-off was applied within the software to attenuate frequencies below 5.6 kHz, followed by a low pass filter with the same roll-off value to attenuate frequencies above 11.3 kHz. The processing produced a finished file filtered to one octave band wide, centered at 8 kHz as required by the protocol. The filtered file was then played through the 2025E shaker and measurements were taken inside the chamber. Levels above 95 dB were easily achieved. However, distribution between the 1/3 octaves comprising the 8 kHz octave band (6.3, 8, and 10 kHz) were observed and a stepwise increase favoring the 10 kHz 1/3-octave was noted. A graphic equalizer with 1/3-octave filter sliders was available within the Audacity software and was used to balance the sound file such that the shaker output was flat across the 8 kHz octave band.

Shaker Endurance Trials

With a well-defined white noise file created, extensive chamber testing was started to assess the shaker capability to perform consistently over a six hour run. Though the test protocol requires three chambers running simultaneously at 75, 85, and 95 dB, only the 95 dB level was tested during endurance runs. An assumption was made that running the system at 95 dB would tax the shaker maximally and that 75 and 85 dB could be easily achieved if there were no concerns at 95 dB. Additionally, at the time of the endurance runs there was confusion over whether the 95 dB level was to be a summation of the 8 kHz octave band or if each of the 1/3 octaves were to be 95 dB, producing a level closer to 100 dB for the full octave band. The latter assumption was chosen while waiting for an answer as it represented a worst case scenario. At the time of the endurance runs no microphone extension cable or preamplifier assembly was available for the sound level meter to allow for remote monitoring. Instead, the sound level meter was placed on one of the cage assemblies in the lower left quadrant of the chamber. A USB cable was then connected to the sound level meter and passed through a port on the back wall of the chamber. The other end of the USB cable was then connected to a laptop computer with Larson Davis SLM Utility software installed. The utility software allows for full control of the sound level meter through a virtual interface on the computer. Six-hour trials were started and sound level measurements were taken at the beginning and each hour thereafter. Over the course of a run, it was noted that there was a minimal increase in sound pressure levels and a frequency drift with the 8 kHz, 1/3 octave increasing slightly and the 10 kHz, 1/3 octave decreasing slightly. It was also noted that the external surface of the shaker was getting hot to the touch. During the next six hour trial a thermocouple was applied to the external surface of the shaker body between the trunnion and the shaker to measure temperature over the course of the run. Simultaneous temperature and sound level values were recorded throughout the run. All steps above were also repeated with the QSC professional audio amplifier substituted for the SmartAmp power amplifier. The QSC amplifier performed as well as the SmartAmp with no issues encountered.

The same procedures outlined above were repeated for the Type 4809 shaker. However, it was found that setting up and mounting the 4809 shaker was extremely difficult. Design limitations of the 4809 and other available shakers do not lend themselves to ease of use in this particular application. These limitations compared to inherent benefits of a unique design in the 2025E shaker are discussed later in this section.

Crossover Insertion

Though filtering provided by the Audacity software provided decent roll-off on the ends of the 8 kHz band, a Behringer Ultradrive Pro DCX2496 (Behringer, Willich, Germany) crossover was obtained to assess any benefits gained by adding a hardware filter inline versus the software filtering alone. Similar to filtering implemented by the Audacity software, the crossover was set to filter between 5.6 and 11.3 kHz with a 48 dB roll-off on each end using a Linkwitz-Riley filter type. Measurements were taken with and without the crossover inline for comparison. The DCX2496 crossover model is capable of simultaneously running three input channels and six output channels for consideration in running all three exposure chambers simultaneously.

Closed Loop Control

A closed loop control system was tested for potential use in the final system design. A VR-8500 (Vibration Research Corporation, Jenison, Michigan) vibration controller demonstration unit and associated software was accepted on loan. The closed loop control system software was used to generate a white noise output file filtered to the 8 kHz octave band and was passed from the VR-8500 to the amplifier and then to the shaker. A microphone with preamplifier was also provided on loan and was connected to the input channel of the VR-8500. The concept of a closed loop controller is to pass a known output signal, monitor that signal and pass results back to the controller, and then continuously modify the output signal automatically to adjust for inconsistencies at the monitoring point. Results with the VR-8500 produced a noticeable tonal ringing and it appeared that the system was attempting to control the total spectral power density in the 8 kHz band rather than maintain a flat profile across the band. The manufacturer was consulted and it was determined that a more capable controller designed specifically for audio applications would be required. Initial quotes on an audio controller were cost prohibitive and far beyond the budget of this effort. A decision was made to pursue a spectral data acquisition system with alarm and tolerance band settings, but without closed loop control. Control would be maintained by manual operator intervention using 1/3-octave graphic equalizers and manually adjusting frequency bands in response to alarm conditions on the data acquisition system.

Shaker Design Considerations

As noted above, the Bruel & Kjaer Type 4809 shaker was extremely difficult to setup and mount due to the design of the stinger attachment method and the lack of a trunnion. Though a wooden box was built in place of the missing trunnion to hold the shaker in place, precise angular positioning of the shaker to achieve a 90 degree approach to the plenum wall was difficult. Additionally, the stinger had to be cut to a specific length, screwed into place on the shaker end, and locked in place with an extremely small hexagonal set screw. The entire shaker then had to be picked up such that the other end of the stinger could be screwed into the plenum attachment point by turning the entire shaker. In addition to the difficulty of holding and turning the shaker for stinger attachment, there was a great margin of error in properly setting up and insuring the stinger was positioned at 90 degrees and that too much tension was not applied to the shaker armature if the stinger were offset. Insuring repeatability of results would be difficult due to the complication and imprecision of mounting a new stinger connection.

The Modal Shop Model 2025E overcame these limitations using a design that is unique to the industry. It was supplied with a trunnion base that allowed for full rotation of the shaker and positioning for perpendicular approach to the plenum. The stinger is attached through a unique through-hole armature design that allows the stinger to pass all the way through the center of the shaker. The stinger end has 10-32 threading that screws into the mounting discs on the plenum as previously noted and the stinger is locked tight to the shaker end using a chuck and collet attachment. The stinger connection design simplifies the setup between the shaker and the plenum, allows for the shortest stinger length to be achieved easily, and prevents unintentional binding of the armature during setup. Short stinger length is desirable as it reduces the amount of mechanical filtering the stinger may apply to the input signal. The design also allows for ease of repeatability from chamber-to-chamber and in the event of a failure and necessary stinger replacement. The 2025E also provides an inline fuse between the amplifier and the shaker to prevent damage to the shaker in the event of a power surge from the amplifier. These considerations and results of performance trials were taken forward to the acquisitions process.

Acquisitions Process

Results from the pilot study were used to recommend procurement of components for a final system design. Based on the proven performance, specifications, and unique design characteristics of the 2025E shaker, a recommendation was made to pursue a sole source contract for that shaker model. Additional equipment was procured through open solicitation contracts and local purchase. Detailed specification requirements for all open solicitations were provided to the contracting office. Table 2 provides a list of all equipment procured for the final system design.

Table 2. Equipment list for final system design

Equipment	Quantity	Manufacturer
Model 2025E Modal Shaker	4	The Modal Shop, Cincinnati, Ohio
Puma Data Acquisition System w/ PC and Monitor	1	Spectral Dynamics, San Jose, California
378B20 ½” Random Incidence Microphone w/ Preamplifier and 20’ Coaxial Cable	4	PCB Piezotronics, Depew, New York
Model 831 Integrating Sound Level Meter	1	Larson Davis, Depew, New York
CAL200 Acoustic Calibrator	1	Larson Davis, Depew, New York
Sony Vaio Laptop Computer w/ Windows Vista	4	Sony Corporation of America, New York, New York
Ultragraph Pro FBQ6200 31-Band EQ, 2 Channels	2	Behringer, Willich, Germany
Ultradrive Pro DCX2496 Crossover, 6 Channels	1	Behringer, Willich, Germany
RMX 1450 Power Amplifier	4	QSC Audio, Costa Mesa, California

Final Solution

System Installation

Upon receipt of all equipment, the system was inventoried and all components were installed. Locations of the Control, 75 dB, 85 dB, and 95 dB chambers were chosen and are noted on the floor plan diagram in Appendix A. An audio rack was assembled and equipment was mounted in the rack as depicted in Figure 7. The rack is located in a central area between all of the exposure chambers.

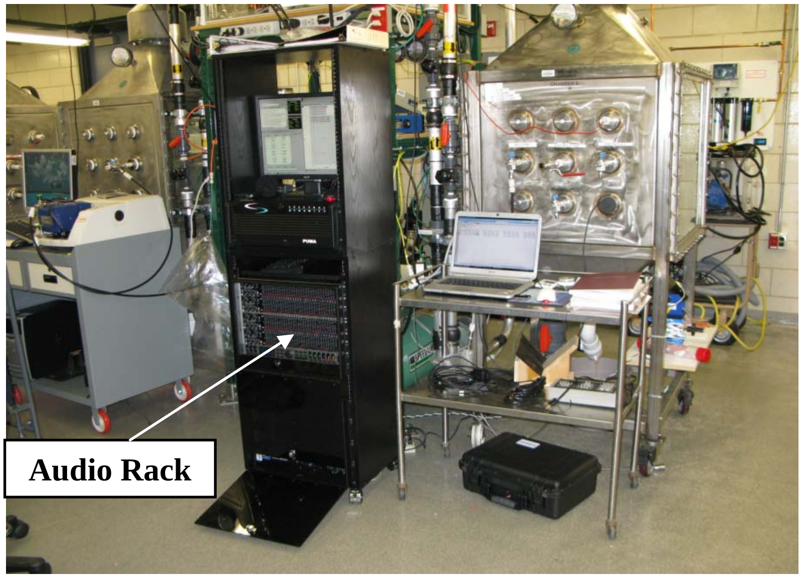


Figure 7. Audio rack housing system equipment

Shelves were designed and attached between the legs nearest the lower left plenum wall of the three exposure chambers where shakers were to be affixed. Jack stands were placed on top of each shelf and shakers were placed on each jack stand. The chambers are on wheels and having the shelves attached to the legs minimizes the risk of moving the shaker out of alignment in the event that the chamber is unintentionally bumped or moved. The stinger attachment disc utilized in the pilot study was left in place on the 95 dB chamber and two additional attachment discs were affixed to the 75 dB and 85 dB chamber plenums. A representative shaker mounting assembly can be seen in Figure 8.

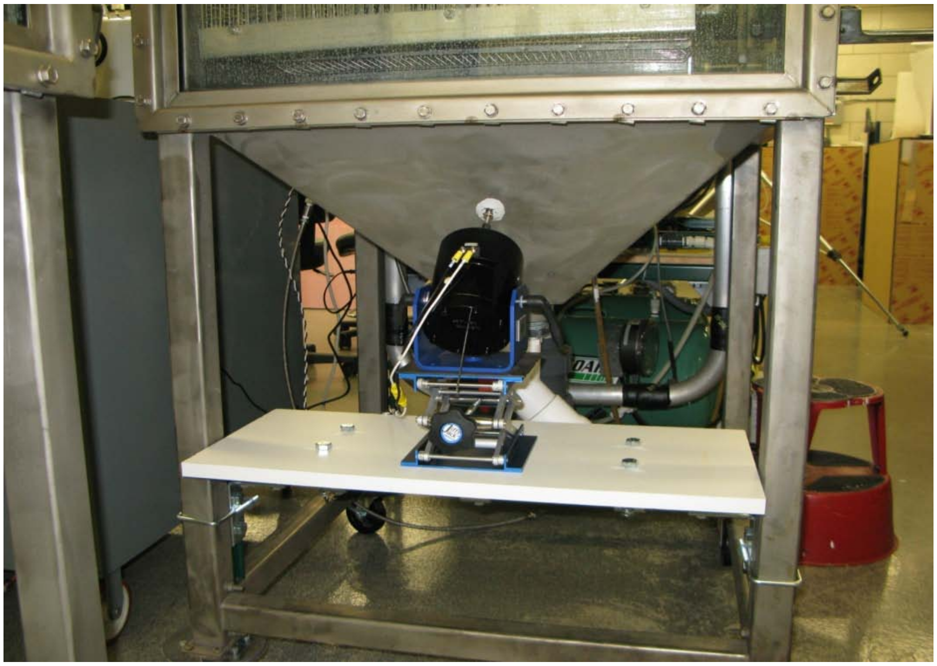


Figure 8. Shaker mounted on shelf between chamber legs

The Audacity audio editing software utilized in the pilot study was installed on one of the new laptop computers. A seven hour white noise file was created using the same high pass, low pass, and equalization procedures outlined in the pilot study. Utilizing a seven-hour file allows time for startup, six-hour run, and shutdown during live testing. Initial plans were to install audio software on three separate laptops and output an audio file to each chamber independently. However, with the insertion of three separate graphic equalizer channels inline, one audio file could be used and any necessary adjustments could be made on the equalizers. The Audacity software with the filtered white noise file was installed on one additional laptop as backup in case of failure.

An audio cable with a stereo mini-plug on one end and an RCA adaptor on the other end was connected to the audio output jack on the laptop computer on the mini-plug end of the cable. The RCA end of the cable was then connected to an RCA-to-XLR adaptor plugged into input channel A on the rear of the Behringer DCX2496 crossover. XLR-to-XLR cables were connected to output channels 1, 2, and 3 on the rear of the crossover. The other ends of the XLR-to-XLR cables were then connected to input channels 1 and 2 on the first equalizer and input channel 1 on the second equalizer. XLR-to-XLR cables were connected to output channels 1 and 2 of the first equalizer and output channel 1 of the second equalizer. Channel 2 on the second equalizer is unused. Output channel 1 of the first equalizer was then connected to XLR input channel 1 of the first QSC amplifier. The additional two equalizer output channels were then connected to the XLR input channel 1 on each of the two additional QSC amplifiers. Banana plugs were connected to the end of the input speaker wires connected to each shaker and the banana plugs were connected to output channel 1 on the rear of each amplifier. A wiring diagram of the system setup is provided in Appendix B.

The Spectral Dynamics Puma data acquisition system (Spectral Dynamics, San Jose, California) was installed and a company representative provided one day of familiarization training. The system as delivered has four active input channels for monitoring and recording real-time sound levels in the Control, 75 dB, 85 dB, and 95 dB chambers simultaneously. A 20-foot coaxial cable was connected to each of the output channels and a PCB Model 378B20 1.27 cm (0.5 inch) random incidence microphone assembly was connected to the other end of each cable. A 1.27 cm (0.5 inch) inside diameter PVC pipe was installed through the center port on the rear of each chamber. The microphone could then be passed through the PVC pipe and sit at a central point that serves as a reference measurement point in each chamber. Rubber o-rings were placed around the front and rear of each microphone preamplifier to isolate the microphone from the PVC pipe and provide tighter seating within the pipe. The channel 1 microphone was installed through the port on the control chamber and channels 2, 3, and 4 were installed in the 75, 85, and 95 dB chambers respectively. Figure 9 shows a microphone passed through a PVC pipe in the center of the 95 dB chamber.

Figure 9. Microphone passed through PVC pipe to central reference point

The Puma user interface screen was customized for use in the JP-8 study. Each channel was defined to the chamber being monitored and a built-in calibration function was used to calibrate each channel to the specific microphone attached. A Larson Davis CAL200 acoustic calibrator was used to produce the calibration tone. A real-time graphical interface was set to display a bar graph of the 8 kHz octave band decibel level inside each of the exposure chambers. Additionally, a function within Puma called

“Features” was used to both set alarm conditions and define data variables that are continuously recorded to a comma separated value file in the background. Alarms and recording functions were established for the full 8 kHz octave band and each of the 1/3 octaves within the 8 kHz octave band for each of the exposure chambers. Alarm conditions are displayed during a live run and values that are running below limits will display as yellow, values within the normal range as black, and values that are above limits as red. When yellow or red values stay consistently displayed, it is an indication that operator intervention may be required to adjust system gain or equalization levels. Additional values were set within the Features setup window to record neighboring octave bands (without alarm conditions) in order to collect data for future frequency spectrum mapping. A screen shot of the main Puma operating screen is provided in Appendix C. An SOP was developed to aid NHRC technicians in running the Puma system and is attached in Appendix D.

Chamber Characterization

The first phase of the NHRC protocol utilizes 10 rats in each exposure chamber. As discussed previously, each chamber holds four cage assemblies and each cage assembly can hold eight rats in individual compartments. For ease of discussion, the chamber and cage assembly locations can be described in terms of quadrants with quadrant 1 being the top left cage assembly and moving clockwise with quadrant 4 being the lower left cage assembly as shown in Figure 10. The rats will start with a 3-2-3-2 distribution pattern in the four quadrants, meaning three rats will be placed in the quadrant 1 cage assembly, two rats in the quadrant 2 cage assembly, etc. The position of the rats within the eight individual slots of each cage assembly is determined by a random assignment scheme developed by the NHRC. Rats will always be assigned to the same cage assembly, but will be randomized among the eight individual slots within the assembly daily. Additionally, each cage assembly will rotate clockwise to the next quadrant daily. Rotating the cage assemblies and randomizing positions of rats within assemblies daily insures each rat has an equal opportunity to be exposed to any point within the chamber for purposes of equal aerosol and noise dose exposure. The NHRC has historically used tennis balls to simulate live rats when conducting aerosol distribution characterization studies prior to live animal exposures. Tennis balls were also used to simulate rats in characterizing the chamber for noise distribution in this study. Two separate characterization trials were conducted.

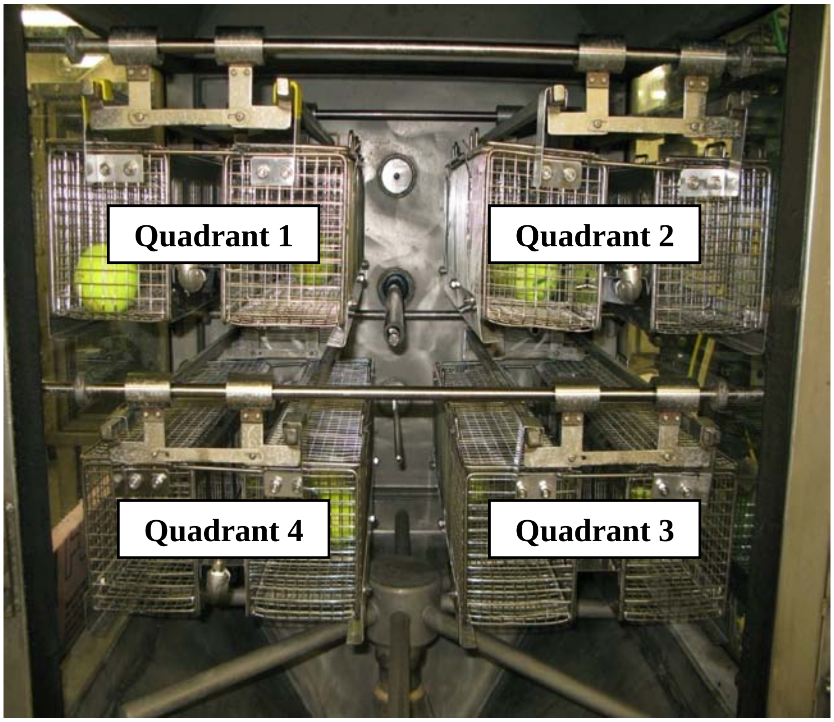


Figure 10. Exposure chamber quadrant identification

The first characterization trial was conducted by placing 10 tennis balls in each exposure chamber. The tennis balls were distributed throughout the four cage assemblies in each chamber using the 3-2-3-2 distribution pattern as discussed above. The NHRC randomization scheme was used to select the assignment points. The Puma data acquisition system was not used for this portion of the study. A Larson Davis Model 831 sound level meter with a 6.1 meter (20 foot) extension cable and microphone preamplifier was purchased by the NHRC and was used for chamber characterization. The sound level meter was calibrated using a Larson Davis CAL200 acoustic calibrator before and after each day of measurements. The microphone was placed in the central reference measurement position within the 95 dB chamber, the filtered white noise file was started, and the amplifier gain was adjusted to bring the reference level to 95 dB. The microphone was then moved to each of the 10 randomized positions containing tennis balls. Measurements were then taken over a 20-second interval at each point. A windscreen was also used on the microphone at all measurement points in order to prevent direct microphone contact with the metal cage assemblies and to insure approximately equal measurement position within each assembly slot. Placement of the microphone in a cage assembly is shown in Figure 11.

Figure 11. Microphone positioned with windscreen within a cage assembly

Two more sets of measurements were taken at each point with a span of 30 minutes between each measurement. Two additional cycles were conducted where the cage assemblies were rotated clockwise one position and the procedure was repeated each cycle. The identical procedure was repeated simultaneously for the 75 and 85 dB chambers with the exception of generating new random tennis ball assignment points. The chamber doors were closed and latched to insure realistic measurement conditions and maximal reverberation during each measurement.

The second characterization trial consisted of tennis balls placed in all 32 slots in each exposure chamber. Measurements were taken at the central reference point and each of the 32 slots within the cage assemblies using the same method as above. Due to time constraints and the labor intensive nature of microphone movement and placement within the assemblies, only one set of measurements was collected at each of the 32 points within each chamber. Sound level meter measurements were stored to the meter in both of the characterization trials and exported to a computer using the SLM Utility software. The data files provide spectral sound level distribution in dB with no conversion required.

Shaker Endurance Tests

Endurance tests were conducted to assess shaker performance over continuous six-hour runs. All three shakers were run simultaneously on each test day. Ten tennis balls were randomly assigned within each chamber, representing real world test conditions. All audio equipment was powered on and the filtered white noise file was started. The Puma system was started up with microphones placed at the center reference point of each chamber. Amplifier gain to each shaker was adjusted to bring the starting reference values to approximately 75, 85, and 95 dB within each of the three chambers respectively. Data acquisition was started and the system was allowed to run for a minimum of six hours. The process was repeated over five successive days. The comma separated value files were imported into Microsoft Excel (Microsoft, Redmond, Washington) for analysis. Though the Puma system displays real time values in dB, the recorded data is stored in pascals. Excel was used to calculate averages and standard deviations of the pascal data for each 1/3 octave and full octave band recorded over the full run period. The average pascal value was then converted to dB using Equation 1 below.

$$L_p(dB) = 20 * \log_{10}\left(\frac{p}{p0}\right) \quad (1)$$

L_p is the sound pressure level in dB, p is the pressure level in pascals, and p_0 is a reference value equal to 20 micropascals. The mean and standard deviation in pascals were then added together and the sum of the two was converted to dB using Equation 1. This served as an upper tolerance to calculate a standard deviation in dB. The mean in dB was subtracted from the upper tolerance in dB and the resultant value was reported as the standard deviation in dB.

Tennis Ball versus Rat Comparison

One week prior to the start of live animal testing an opportunity arose to conduct a brief characterization trial using ten live Fisher rats that were disqualified from the study. All settings on the audio equipment from the final day of endurance runs remained the same. The ten live rats were randomized within four cage assemblies using the normal 3-2-3-2 pattern and were placed in the 95 dB chamber. The noise generation system was started, a three-minute measurement was taken at the center reference point, and the system was shut down. The rats were then moved to the 75 and 85 dB chambers in succession and the procedure was repeated. Measurement duration was limited due to time constraints with animal handlers.

Engineering Control for Air Compressors

As noted previously, two large air compressors are located in the southeast corner of the NHRC inhalation lab. The compressors are the dominant noise source in the room when running. The corner of the room has a smooth concrete floor beneath the compressors and a glazed cinderblock wall behind them, both providing highly reflective surfaces for incident sound waves. The compressors are located in close proximity to the control chamber and the control animals must be moved past the compressors and remain exposed to the compressor noise during the chamber loading process and again during the unloading process. Two acoustical enclosures had been constructed by NHRC personnel to position over each compressor. The enclosures were constructed from 1.27 cm (0.5 inch) thick polycarbonate on the front, two side walls, and the top. They were designed to slide over the top of each air compressor and seat up against the wall behind each compressor. The enclosures were also lined with a layer of 2.54 cm (1 inch) thick pink polystyrene with a smooth reflective surface. The current design of the enclosures did not appear to reduce overall sound pressure levels from the air compressors significantly, if at all. Based on the design of the enclosures and the reflective surfaces surrounding the compressors, it was hypothesized that sound wave buildup may be occurring inside the enclosures and that an absorptive acoustic material may be useful in reducing overall sound pressure levels. The sound level meter was placed on a tripod at a position approximating the center of the control chamber door. Measurements were taken with and without the existing enclosures in place.

Sheets of Echo Eliminator Bonded Acoustical Cotton (Acoustical Surfaces, Chaska, Minnesota) were procured. The sheets are 5.08 cm (2 inches) thick with 3 pounds per cubic foot density and are class A/1 fire rated for industrial use. The fire rating is important to this application as it is well suited for potential temperature increases within the enclosures. The pink polystyrene liner material was removed from the polycarbonate enclosures and replaced with the Echo Eliminator material. Echo Eliminator sheets were also placed on the rear wall behind each compressor and on the floor beneath. Additionally, the compressor on the rear wall has an air intake that protrudes above the top of the enclosure and is surrounded by several pipes that make an enclosure difficult. A sheet of Echo Eliminator was cut into smaller sections and woven around the pipes to surround the air intake area. A sufficient gap was left to allow adequate airflow. Measurements were taken with the new control design in place at the same position in front of the control chamber door. The compressors with acoustical enclosures are in Figure 12. The control chamber door can be seen in the left foreground.

Figure 12. View of acoustical enclosures surrounding air compressors

4. Results and Discussion

Pilot Study

Tapping versus Stinger Trials

Trials were conducted to compare the 2025E shaker performance in tapping against a surface versus being mounted to the surface with a rigid attachment point. Results showed that a fixed attachment created a substantially greater overall SPL in both bench-top and chamber trials. In bench-top trials, forming a stinger-like attachment with the metal bracket resulted in a 12 dB increase in overall SPL at 8 kHz as compared to tapping against the bracket. Mounting the shaker to the plenum wall via a stinger versus allowing the armature to tap the plenum with the stinger resulted in a 14 dB increase in overall SPL at 8 kHz. Results of the tapping versus fixed stinger trials are summarized in Table 3.

Table 3. Tapping versus attached stinger trials with 2025E shaker

Test Performed	Overall SPL at 8kHz Octave Band (dB)
Shaker with 10-32 bolt tapping on metal bracket in bench-top trial	91.2
Shaker with 10-32 bolt attached to metal bracket in bench-top trial	103.1
Shaker with stinger tapping on chamber plenum	88.3
Shaker attached to plenum wall via stinger and mounting disk	102.2

Utilizing a stinger creates a solid connection between the shaker armature and the test surface and allows them to move in unison through both the push and pull phase of the armature stroke. The plenum wall is essentially turned into a large speaker surface which more accurately transmits the input energy from the shaker. This is beneficial to both the quality of the vibration spectrum generated and insuring a solid connection and position is maintained throughout a test. In contrast, tapping the surface does not result in complete transmission of the intended input signal, as much of the energy is likely filtered or altered by the test surface material upon impact.

White Noise Generation

The resultant frequency spectrum measured inside the chamber after initial filtering with the Audacity software showed a stepwise increase toward 10 kHz within the 1/3 octave bands comprising the 8 kHz octave band. A 31-band equalization function built into the Audacity software was successfully used to balance the 1/3 octaves, resulting in a flat profile across the 8 kHz octave band. Figure 13 shows a comparison of the filtered white noise with and without equalization performed in the Audacity software.

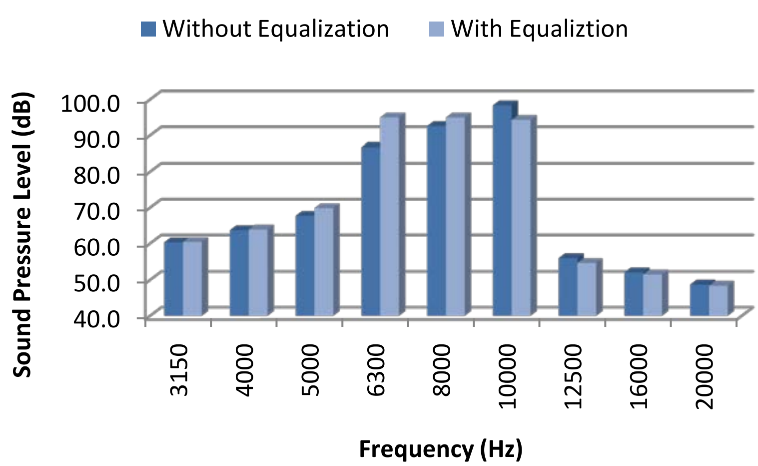


Figure 13. White noise spectrum before and after equalization in Audacity

Steps were taken to determine why the 2025E shaker with a flat white noise input file favored higher output toward 10 kHz when a flat sound profile was applied. An applications engineer at The Modal Shop indicated that the 2025E shaker has maximal performance with a resonant frequency in the range of 9 – 10 kHz. In that range, the shaker needs a lower voltage input applied to achieve the same level of output as neighboring frequency bands. With a flat white noise file applied, the 9 – 10 kHz range was getting more voltage than needed. Equalization compensated by dropping the input amplitude in that range, resulting in flat output. (Peres, Performance Curve of the 2025E Shaker, 2009)

Shaker Endurance Trials

Initial endurance trials conducted over six-hour periods indicated that there was a minimal increase in the overall SPL for the full 8 kHz octave band over the course of a run. Additionally, a slight frequency drift was observed with the 8 kHz, 1/3 octave SPL increasing slightly and the 10 kHz, 1/3 octave SPL decreasing slightly. This was concurrent with an increase in external shaker temperature. Simultaneous frequency spectral data and shaker temperature measurements were collected during a subsequent run and confirmed the finding as displayed in Table 4.

Table 4. Comparison of shaker performance versus temperature over time

Time	Shaker Temperature (°C)	8 kHz Full Octave SPL (dB)	6.3 kHz 1/3 Octave SPL (dB)	8 kHz 1/3 Octave SPL (dB)	10 kHz 1/3 Octave SPL (dB)
9:10:04	25.0	99.0	94.3	95.0	94.2
9:46:32	33.0	99.0	94.5	95.1	94.0
10:30:20	44.8	99.0	94.5	95.4	93.6
11:00:31	49.3	99.1	94.6	95.6	93.5
12:58:33	54.9	99.3	94.7	95.7	93.6
13:20:09	54.9	99.2	94.6	95.8	93.5
15:33:03	55.0	99.2	94.5	95.8	93.3

The shaker uses an electromagnet technology with a constant flow of electricity. The temperature increase over time is a direct result of the input current applied to the shaker and continues to rise until an equilibrium point is established. The characteristics of shaker performance shift with respect to temperature increase do not appear to be drastic and overall SPL values remained well within protocol requirements. A conversation with the shaker manufacturer also revealed that the measured surface temperatures were well within tolerance and of no concern for long-term shaker performance (Peres, 2009). Shaker temperature may become a more significant issue if future studies push beyond the current 95 dB upper limit and induce greater stress on the shaker.

Crossover Insertion

The crossover was inserted inline and set to provide an additional filter to the noise input file as described in the methods section. Octave band measurements were taken with and without the crossover inline. Results show that the crossover was able to better filter the input signal and produce a sharper roll-off from the 8 kHz octave band peak. A frequency spectrum comparison of the ambient chamber noise, noise file with no crossover, and noise file with crossover inline is shown in Figure 14. As compared to Lataye and Campo, the system is capable of a resultant 8 kHz octave band that is flatter across the 1/3 octaves. As compared to Pouyatos, Gearhart and Fechter, the overall spectrum is similar, though slightly flatter with steeper roll-off on the left and right side of 8 kHz. The resultant 8 kHz octave band noise with neighboring octave bands is shown in Figure 15.

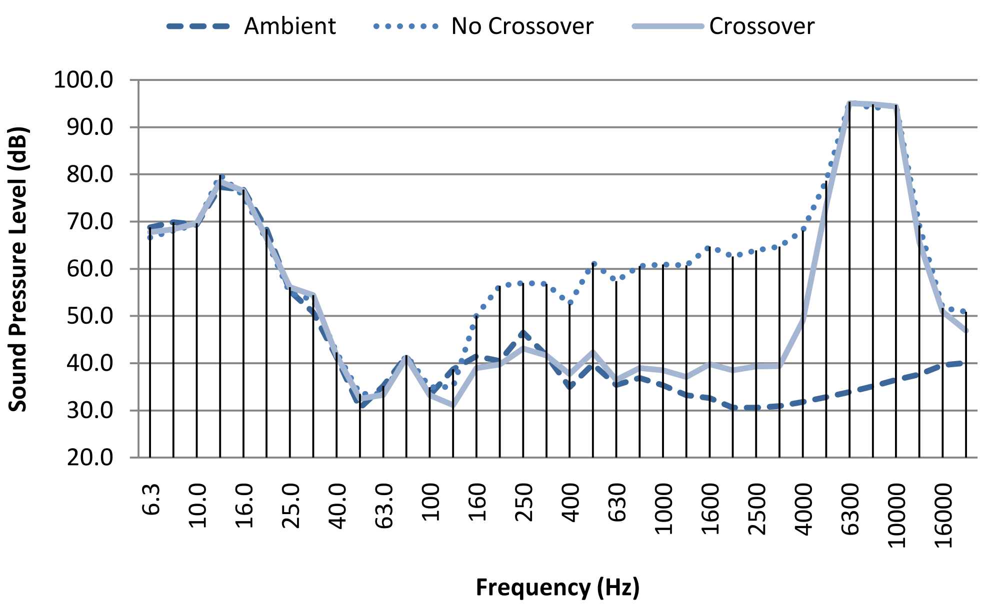


Figure 14. Comparison of frequency spectrum with crossover inline

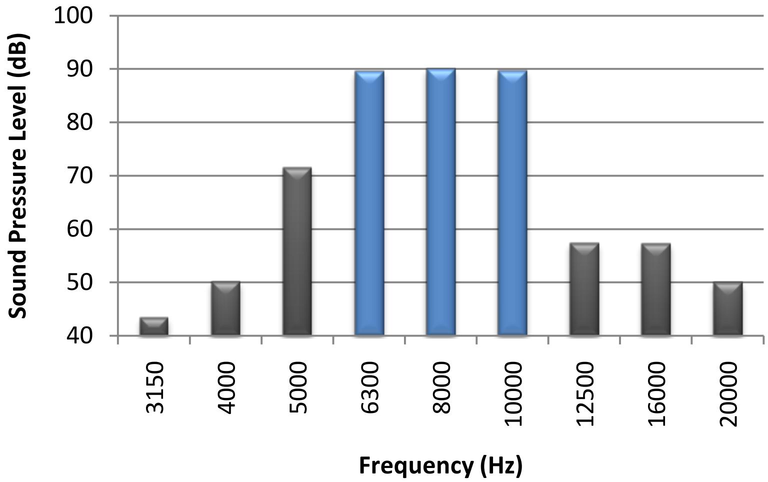


Figure 15. Resultant 8 kHz octave band noise with neighboring octave bands

Insertion of the crossover resulted in a much better roll-off on the left side, producing a better defined 8 kHz peak. The improved roll-off on the left side may be particularly useful in lessening the likelihood of a phenomenon known as upward spread of masking. Masking describes how the sensitivity for one sound can be affected by the presence of another sound. Research has shown that increases in masker intensity result in a considerable spread of masking effect upward in frequency (Gelfand, 2004). In this case, the concern would be that rat sensitivity at 8 kHz may be affected by masking from the neighboring 4 kHz octave band to the left. Separation in the SPL intensity between 4 and 8 kHz produced by the software filtering alone would likely prevent effects of masking. However, the increased separation provided by the crossover provides further assurance to that end. Additionally, the well defined output signal provided by the crossover likely requires less output from the amplifiers to drive the signal.

Final Solution

Chamber Characterization

Chamber characterization experiments were designed to measure the overall 8 kHz octave band SPL at various points throughout each chamber to test the +/- 2 dB distribution requirement of the NHRC protocol. During the first phase of characterization, 10 tennis balls were placed in each exposure chamber. Initial assignment points were chosen using the NHRC randomization scheme previously discussed. Numbering is from front to back of each cage assembly with one through four on the left side of the cage and five through eight on the right side of the cage. A series of three sound level measurements was taken at each point. Cage assemblies were then rotated one position clockwise within the chambers and measurements were repeated. The process was then repeated a third time. Positions of tennis balls for the three measurement rotations are identified in Appendix E. Summary results from the three chambers are in Table 5. Detailed results of individual sample points within each chamber are also located in Appendix E.

Table 5. Summary of three 10-point chamber characterization trials

		75 dB Chamber	85 dB Chamber	95 dB Chamber
Trial 1	Mean	75.5	84.9	94.6
	St Dev	0.7	0.6	0.6
	Range	74.0 – 76.9	83.9 – 86.1	93.9 – 95.9
Trial 2	Mean	74.9	85.0	94.9
	St Dev	1.0	0.9	0.8
	Range	73.4 – 76.3	83.4 – 86.5	93.9 – 96.5
Trial 3	Mean	74.7	84.9	95.2
	St Dev	0.5	0.6	0.7
	Range	73.8 – 75.6	83.6 – 85.6	94.1 – 96.1

Additionally, JMP 7 (SAS Institute Inc, Cary, NC) statistical analysis software was used to further analyze and describe the distribution of the data. All three measurement sets were combined into a single large data set for each chamber (N = 90 for each chamber). JMP was used to produce histograms describing each chamber and to compute both confidence intervals (CI) and prediction intervals (PI) at an alpha value of 0.05. All data sets passed the Shapiro-Wilk goodness-of-fit test for normality at alpha of 0.05 and showed good visual tracking on normal quantile plots. Results from JMP analysis are in Figure 16 and Table 6 and additional details are in Appendix F.

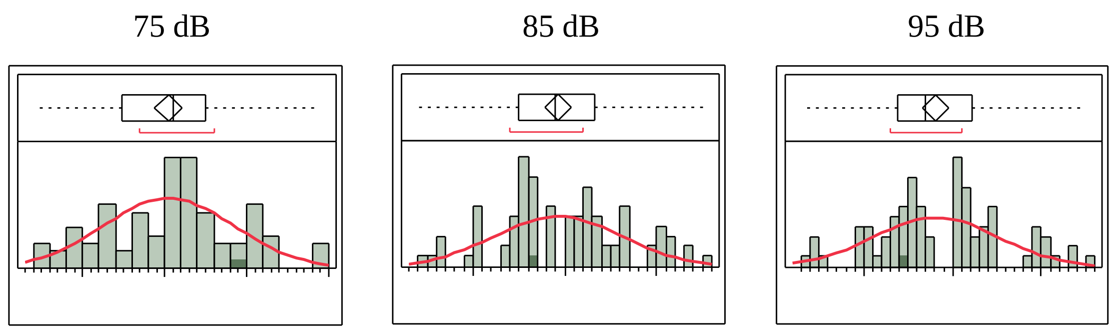


Figure 16. JMP histograms for ten-point randomization consolidated data set

Table 6. JMP statistics for consolidated ten-point randomization data set

	75 dB Chamber	85 dB Chamber	95 dB Chamber
N	90	90	90
Mean (dB)	75.05	84.92	94.81
St Dev (dB)	0.80	0.70	0.71
CI (α = .05)	74.88 – 75.21	84.77 – 85.06	94.66 – 94.96
PI (α = .05)	73.45 – 76.64	83.53 – 86.31	93.40 – 96.22

Finally, z-scores were calculated at the +/- 2 dB tolerance endpoints for each chamber to assess the probability of a future sample point falling out of limits. Equation 2 was used to calculate z-scores (McClave, Benson, & Sincich, 2008). The calculated z-scores and associated probabilities of exceeding the +/- 2 dB tolerance limits are in Table 7.

$$z = \frac{|X - \mu|}{\sigma} \qquad (2)$$

Table 7. Probability of exceeding +/- 2 dB distribution with 10 points

	75 dB Chamber	85 dB Chamber	95 dB Chamber
Z	2.51	2.87	2.83
Table Value	0.494	0.4979	0.4977
Probability of exceedance	0.012	0.0042	0.0046

All trials with 10 tennis balls resulted in distributions that were well within the NHRC protocol requirement of +/- 2 dB average distribution across 10 randomized exposure points. Repeated measurements within individual cage assembly compartments also showed good control from measurement-to-measurement over time. It is expected that any randomized assignment of rats to 10 exposure points utilizing the NHRC 3-2-3-2 assignment scheme should result in a similar outcome. This is supported by results of both the prediction intervals and z-score evaluation.

In the second phase of characterization, tennis balls were placed in all 32 cage positions in each exposure chamber, representing maximum capacity. As before, numbering is from front to back of each cage assembly with one through four on the left side of the cage and five through eight on the right side of the cage. One sound level measurement was taken at each point and only one set of measurements was taken in each chamber. Summary results from the three chambers are in Table 8. Detailed results for all points in each chamber are in Appendix E.

Table 8. Summary of 32-point chamber characterization

	75 dB Chamber	85 dB Chamber	95 dB Chamber
Mean (dB)	74.9	86.0	95.1
St Dev (dB)	0.92	0.97	1.30
Range (dB)	73.5 – 77.2	84.2 – 88.7	93.3 – 98.2

Results of the 32-point chamber characterization trials show that the 75 dB chamber is the only one of the three chambers that meets the +/- 2 dB distribution requirement. Both the 85 and 95 dB chambers fall outside of that limit. Filling the 85 and 95 dB chambers with 32 rats would require acceptance of a broader distribution tolerance. Localized “hot spots” can be observed in the lower left and lower right quadrants of all three chambers where sound pressure levels are higher than other points. Likewise, points with lower sound pressure levels can be observed in other areas.

Comparisons between 10-point distributions meeting the +/- 2 dB requirement, while 32-point distributions fail the requirement, can possibly be explained using Equation 3 for calculating sound pressure levels in reverberant fields (Bruce, Bommer, & Moritz, 2003).

$$L_p = L_w + 10 * \log_{10}\left(\frac{Q}{4\pi r^2} + \frac{4}{TA}\right) \quad (3)$$

L_p is the sound pressure level in dB, L_w is the sound power level, Q is a directivity factor, r is the distance from the sound source to the receiver, and TA is an absorption factor. Though the equation is intended for calculation in large rooms, it was used in the absence of other equations to describe small spaces. Within the logarithmic portion of the equation, the left side of the summation is related to a direct sound field while the right side of the summation is related to a reflected sound field. In the case of moving from trials with 10 tennis balls to trials with 32 tennis balls, all factors on the right side of the equation would remain constant with the exception of TA. Each additional tennis ball added to the chamber increases the absorptive surface area in the chamber and drives the TA value higher. The reflected sound field portion of the equation becomes less significant as the denominator increases and the direct sound field becomes more of a driving factor in the overall sound pressure level. However, in order for this to be significant, the reflected sound field portion of the equation would have to be dominant over the direct sound field portion prior to the insertion of additional tennis balls. Given the small volume of the exposure chamber and the short distance between the noise source and exposure points, it is more likely that the direct sound field is already dominant. A rough calculation can be made to test the likelihood. From Equation 3, the TA value can be calculated using Equation 4 below.

$$TA = \frac{S * \alpha bar}{1 - \alpha bar} \quad (4)$$

S is the total surface area of the chamber and 10 tennis balls and αbar is the average absorption coefficient of the chamber. The average absorption coefficient is calculated by summing the product of each surface area by its individual absorption coefficient and then dividing the sum by the total surface area. For a rough calculation, the chamber was assumed to be a rectangular box with the area of each side being 0.697 m^2 and the top and bottom each being 0.581 m^2 for a total surface area of 3.94 m^2. A standard tennis ball has a radius of 3.3 cm, making the surface area 0.0137 m^2 for a total surface area of 0.137 m^2 for 10 tennis balls. Using a generous estimate of absorption coefficients for each surface area, an α value of 0.05 was assumed for the glass and stainless steel walls of the chamber and 0.9 for the tennis balls. The αbar value in Equation 4 would calculate to approximately 0.079 and TA would be 0.35. To compare this to the direct sound field portion of Equation 3, the distance of the nearest tennis ball "hot spot" to the sound source was estimated at 0.152 m. Q in Equation 3 was assumed to be 4, meaning the noise source is located at the junction of a floor and wall and more directive (a value of 8 may be more realistic since the chamber is small and may be thought of as a corner). Calculating the direct and reflected portions of the logarithm in Equation 3 separately yields results of 13.7 for the direct field and 11.4 for the reflected. The results are too close to call the contribution of one field significantly greater than the other. When substituting 32 tennis balls into the calculation, the reflected portion drops to 5.85. The hot spots in the lower left and lower right quadrants of the chamber in the 32-point characterization trials may be related to the additional absorption provided by the tennis balls making the direct path more dominant. However, they may also be a result of disrupted transmission paths created by the additional tennis balls that interfere with equal path transmission to other points in the chamber.

Shaker Endurance Tests

Data from 6-hour endurance runs over five days was recorded by the Puma system using a single central reference microphone in each chamber. Data was exported to Microsoft Excel, converted from pascals to decibels, and analyzed as described in the methods section. Results are in Table 9.

Table 9. Results of 6-hour endurance runs over five days for the three exposure chambers

	Sample Size (N)	Parameter	75 dB Chamber	85 dB Chamber	95 dB Chamber
Day 1	13967	Mean	75.2	85.3	94.8
		St Dev	0.45	0.34	0.38
Day 2	14188	Mean	75.0	85.3	94.8
		St Dev	0.42	0.38	0.39
Day 3	13936	Mean	74.9	84.9	94.7
		St Dev	0.52	0.35	0.40
Day 4	13773	Mean	74.9	84.8	94.8
		St Dev	0.52	0.36	0.41
Day 5	12772	Mean	75.1	84.9	94.8
		St Dev	0.46	0.35	.37

JMP software was then used to further analyze and describe the distribution of the data. All five run days were combined into a single large data set (N = 68,636). All data points within each data set were shifted equally prior to consolidation to set each mean to the respective 75, 85, or 95 dB target value. This was done solely for ease in describing the data set. This adjustment is of no consequence to the accurate representation of the data set as the same treatment was performed on all data points. JMP was used to produce histograms describing each chamber as well as confidence and prediction intervals at an alpha value of 0.05. Results from JMP analysis are in Figure 17 and Table 10 and additional details are in Appendix F. As with the 10-point trials, z-scores were also calculated to determine the probability of data points exceeding the +/- 2 dB tolerance limits (Table 11).

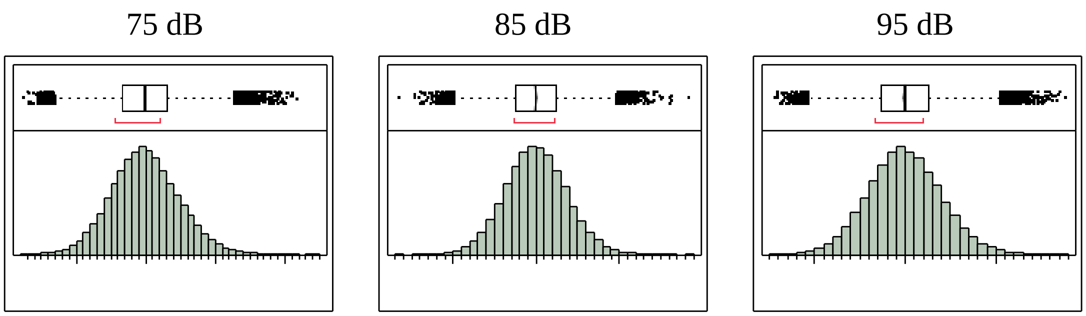


Figure 17. Distribution of five-day consolidated data set for each chamber

Table 10. JMP statistics for consolidated five-day endurance run data

	75 dB Chamber	85 dB Chamber	95 dB Chamber
N	68636	68636	68636
Mean (dB)	74.99	84.99	94.99
St Dev (dB)	0.48	0.36	0.39
CI (α = .05)	74.98 – 74.99	84.99 – 84.99	94.99 – 95.00
PI (α = .05)	74.04 – 75.93	84.28 – 85.70	94.22 – 95.77

Table 11. Probability of exceeding +/- 2 dB tolerance over a 6-hour run

	75 dB Chamber	85 dB Chamber	95 dB Chamber
Z	4.16	5.51	5.06
Table Value	Off table	Off table	Off table
Probability of exceedance	0.000	0.000	0.000

The shakers driving all three chambers performed exceptionally well over repeated 6-hour endurance runs, resulting in mean values well within protocol distribution requirements over time. The statistical analysis also indicates that the distribution of sample points is tightly controlled with 95% or more of the data points falling within +/- 1 dB of the mean. Additionally, as compared to external temperatures noted during the pilot study, shaker temperatures under final system operating parameters did not get noticeably hot to the touch.

Tennis Ball versus Rat Comparison

Results of measurements with ten live rats in each chamber were exported and analyzed using the same method described previously in the endurance run section. The results from the rat exposures were compared to the day five values detailed above for each exposure chamber. The objective was to compare measurements with tennis balls to those with live rats and gauge the accuracy of tennis ball use as rat surrogates in the study. Comparison of rat to tennis ball data for the three chambers is in Table 12.

Table 12. Rat versus tennis ball comparisons for each chamber

Subject		75 dB Chamber	85 dB Chamber	95 dB Chamber
Rat	Mean	75.5	84.8	95.1
	St Dev	0.41	0.38	0.47
	N	115	143	134
Tennis Ball	Mean	75.2	85.2	95.2
	St Dev	0.42	0.35	0.43
	N	13798	13798	13798

In all cases, tennis balls appear to be a suitable match to rats for purposes of characterizing the chambers prior to live exposures. The approximate size and density of the rats and tennis balls are likely equivalent enough to result in similar absorption coefficients, leading to the comparison points above.

Engineering Control for Air Compressors

A series of measurements was taken with a sound level meter in order to discern why the existing acoustical enclosures were not reducing the overall sound level contribution from the air compressors in the NHRL facility. A measurement of ambient noise in the room was taken for baseline comparison. A measurement was then taken with the two compressors running with enclosures removed in order to determine driving frequencies. Octave band analysis of the ambient noise compared to the two compressors without enclosures is shown in Figure 18 and Figure 19.

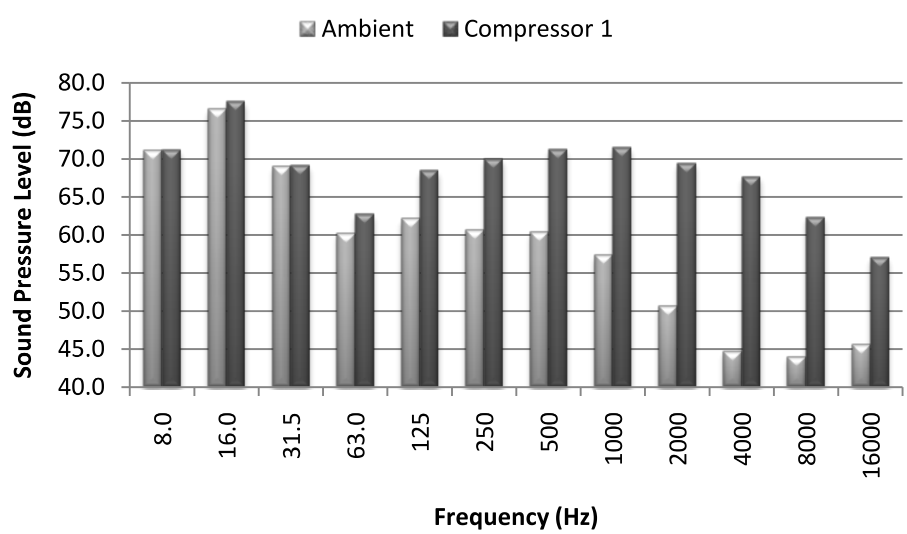


Figure 18. Compressor 1 running with no enclosure compared to ambient noise

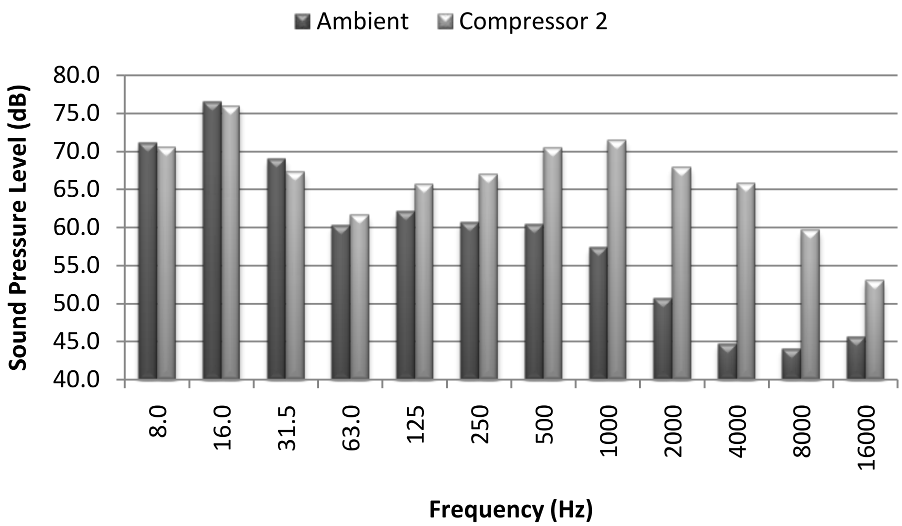


Figure 19. Compressor 2 running with no enclosure compared to ambient noise

Review of the spectra in Figure 18 and Figure 19 indicates that the compressors begin to contribute to room noise around 125 Hz and contribute significantly between 500 Hz and 16 kHz. The driving (or maximal) frequency of the compressors is 1 kHz. Also of interest is the significant contribution of the compressors to sound levels at 8 kHz, the frequency of greatest concern to rats in the NHRC study.

The existing acoustical enclosures were constructed of 1.27 cm (0.5 inch) thick polycarbonate. In the absence of sound transmission loss values for polycarbonate, comparable transmission loss values for 1.27 cm (0.5 inch) thick Plexiglas are given in Table 13 (Driscoll & Royster, 2003).

Table 13. Transmission loss values for 1.27 cm (0.5 inch) thick Plexiglas

Frequency (Hz)	125	250	500	1000	2000	4000	8000
Transmission Loss (dB)	21	23	26	32	32	37	37

The transmission loss values in Table 13 indicate that the polycarbonate material could offer significant reduction in overall room noise based on the transmission loss values afforded at the primary frequencies driven by the air compressors. In order to compare actual sound level reductions achieved by the enclosures to the textbook transmission loss values, octave band analysis of air compressors running with and without enclosures in place are shown in Figure 20 and Figure 21. It can be observed that the enclosures have minimal impact on reducing the sound transmission and do not approach the transmission loss values in Table 13.

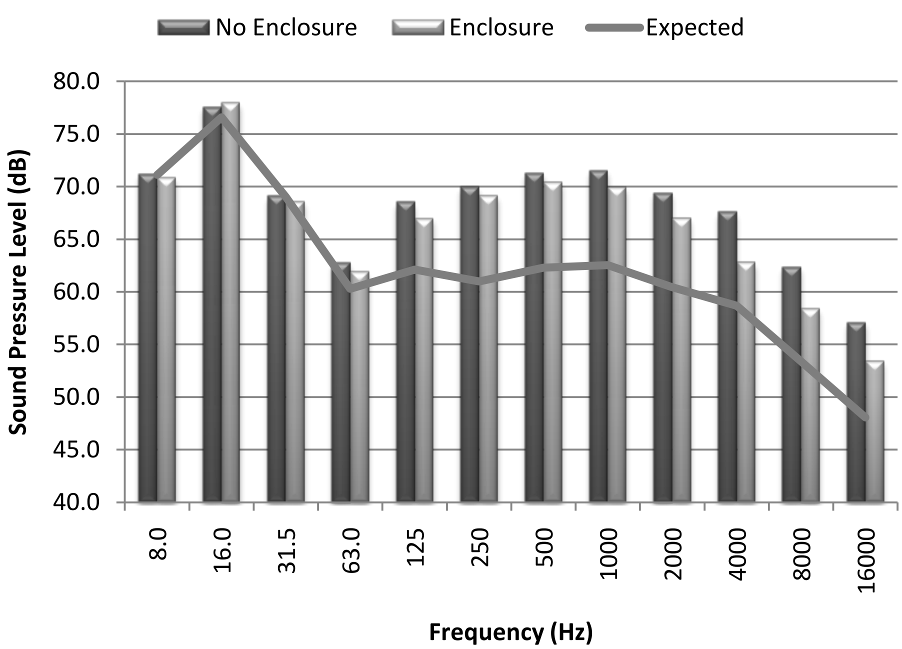


Figure 20. Compressor 1 running with and without acoustical enclosure

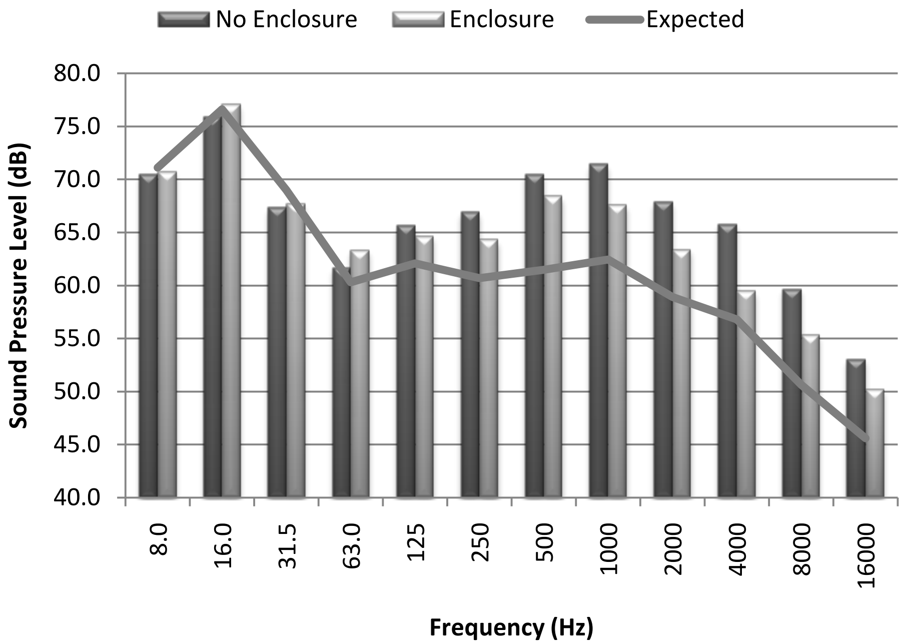


Figure 21. Compressor 2 running with and without acoustical enclosure

The transmission loss values in Table 13 represent a perfect scenario in which an enclosure would be constructed with no gaps and would completely encapsulate the noise source. The enclosures in the NHRC facility have openings that allow airflow to the compressors, particularly in the case of compressor 1 with approximately 10% openings. Openings are a potential reason that the enclosures offer poor performance in reducing overall sound levels in the facility. With an ideal transmission loss of between 21 and 32 dB across the frequency bands and an estimated 10% openings in the enclosure for compressor one, the actual attenuation potential would be reduced to approximately 9 dB at all frequencies (Driscoll & Royster, 2003). The expected reduction of the enclosures accounting for the openings is overlaid as an “expected” line in Figure 20 and Figure 21. The current enclosures do not attain even that reduced value. Though openings reduce the effectiveness of the enclosures, they are required for adequate airflow for compressor operation.

Though gaps in the enclosures are unavoidable, a second treatment option in this case was the addition of acoustical cotton liner to the enclosures as discussed in the methods section. The significant contribution of high frequency noise beyond 1 kHz to the overall sound level as seen in Figure 18 and Figure 19 make the enclosures ideal candidates for treatment with an absorptive material. High frequencies that build up from reflection off of the inside surfaces of the enclosure and surrounding walls and floors can be easily reduced by the addition of absorptive materials such as the bonded cotton used in this study. Sound absorption coefficients from manufacturer specifications for the Echo Eliminator Bonded Acoustical Cotton used for enclosure treatment are shown in Table 14 (Acoustical Surfaces, Inc, 2009). Sound absorption coefficients indicate the proportion of a sound wave that is absorbed by an incident surface at a particular frequency. Values of 1 or greater indicate the material is rated to absorb 100% of the incident sound at a given frequency under perfect laboratory conditions. The Echo Eliminator material offers outstanding sound absorption properties at frequencies of 250 Hz and higher.

Table 14. Sound absorption coefficients of Echo Eliminator acoustical material

Frequency (Hz)	**125**	**250**	**500**	**1000**	**2000**	**4000**
Sound absorption coefficient	0.35	0.94	1.32	1.22	1.06	1.03

Figure 22 and Figure 23 show a comparison of the octave band analysis with the addition of the cotton lining to the enclosures. Acoustic liners absorb reflected sound waves and prevent buildup of sound energy in the enclosure. However, the best case additional reduction that can be seen by adding acoustical lining is 3 dB. The modified expected reduction line is overlaid in Figure 22 and Figure 23 as well. The addition of absorptive material had a significant impact on sound level reduction between 250 Hz and 16 kHz, especially in the case of compressor one which is located nearest the control chamber. At some frequencies, the acoustical liner treatment exceeded expected reduction values. This is likely due to either the additional reduction from the acoustical cotton that was intertwined around the air intake point on compressor one or the density of the acoustical liner contributing as a transmission loss factor.

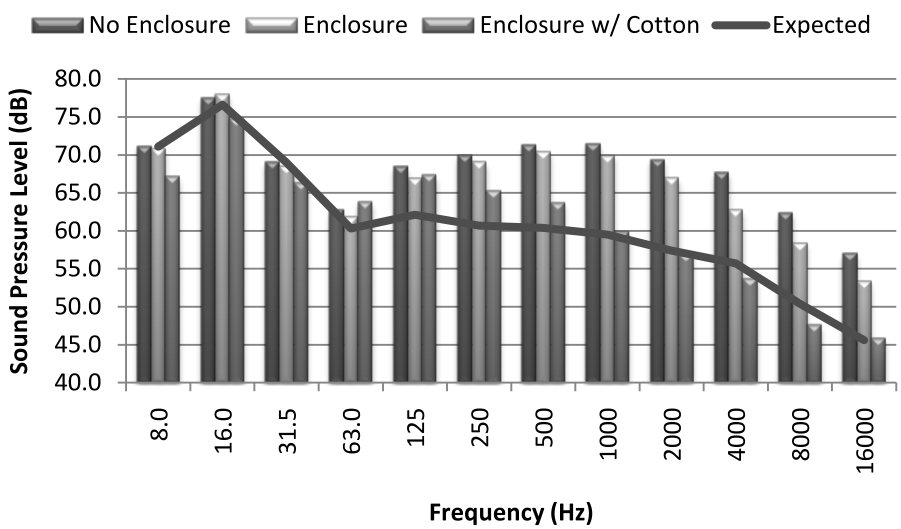


Figure 22. Comparison of compressor 1 with acoustical cotton liner added to enclosure

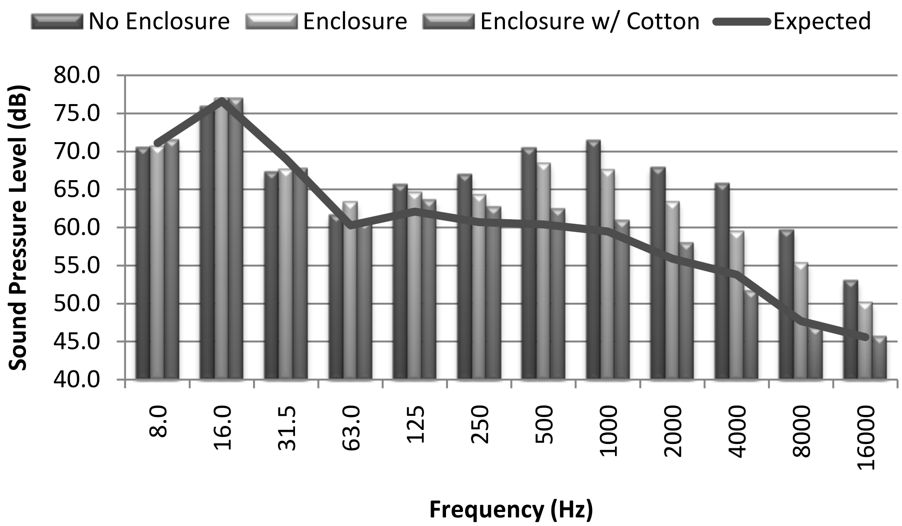


Figure 23. Comparison of compressor 2 with acoustical cotton liner added to enclosure

The net impact of adding absorptive material to the compressor enclosures was an overall sound pressure level reduction of 10.9 dB, from 76.3 dB to 65.4 dB. The overall ambient sound pressure level in the room was measured at 61.6 dB. Adding absorptive material to the compressor enclosures reduced the air compressor addition to overall room noise to 3.8 dB. The impact of the air compressors on room noise was essentially negated.

The addition of absorptive material also had a significant impact at the 8 kHz octave band that is of importance to rat exposures in the study. Prior to treatment with the cotton liner, compressor one was generating sound levels of 62.4 dB at the 8 kHz octave band. After treatment with the absorptive liner, the contribution was reduced to 47.7 dB at the 8 kHz octave band as compared to 44.0 dB in the ambient room noise at 8 kHz. Figure 24 compares the final acoustical enclosure treatment for compressor 1 to the overall ambient room noise, showing that the enclosure reduced the overall spectral contribution of the compressor down to near ambient levels.

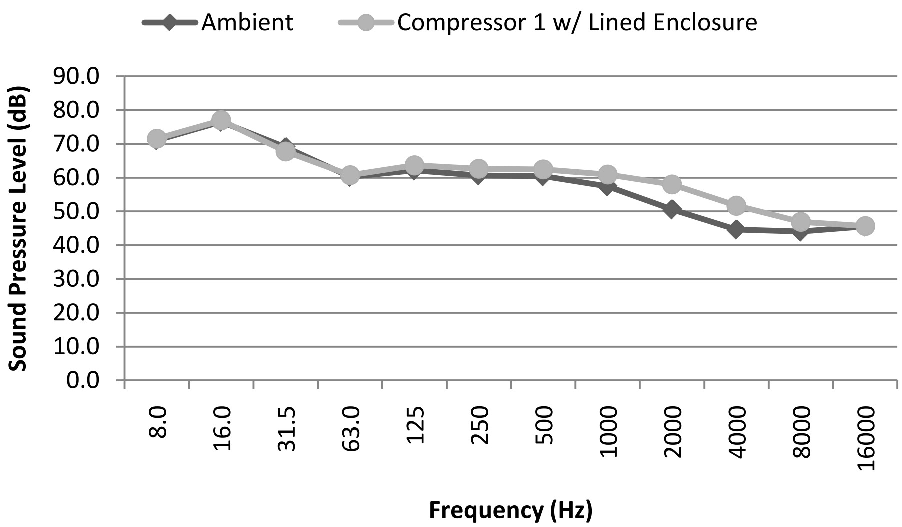


Figure 24. Comparison of compressor with final enclosure treatment to ambient room noise

Through this research effort, all requirements of the NHRC study protocol were met and the measures of effectiveness were evaluated (Table 15).

1. A system was designed to deliver protocol noise requirements and was installed on existing NHRC exposure chambers without major modification requirements.

2. The system is capable of generating an average 8 kHz SPL distribution within +/- 2 dB at 10 randomly assigned exposure points in each chamber.

3. The system can maintain a +/- 2 dB distribution at a central reference point over 6-hour runs.

4. The system can withstand repeated 6-hour runs without performance degradation.

5. The system provides a real-time view of operating status, including alarms for conditions that go out of limits. The system also continuously logs run data in the background and stores over 13,000 lines of data over a 6-hour run.

Table 15. Results of hypothesis tests

Test Performed	Null Hypothesis (H_O)	Alternative Hypothesis (H_A)	Result
Stinger versus tapping	A stinger affixed to the plenum surface will not produce a higher SPL at 8kHz than a stinger tapping against the plenum	A stinger affixed to the plenum surface will produce a higher SPL at 8kHz than a stinger tapping against the plenum	Reject H_O, accept H_A: stinger affixed to plenum surface resulted in a 12 dB increase in 8 kHz SPL
Temperature effect on shaker performance	Shaker temperature increase will not affect overall SPL and frequency distribution	Shaker temperature increase will affect overall SPL and cause frequency distribution	Reject H_O, accept H_A: shaker temperature increase showed mild correlation with frequency drift
Crossover insertion	Use of a crossover will not produce a better defined 8 kHz peak than use of audio filtering software alone	Use of a crossover will produce a better defined 8 kHz peak than use of audio filtering software alone	Reject H_O, accept H_A: crossover insertion resulted in much better roll-off on left side of 8 kHz
10-point chamber characterization	Distribution of average 8 kHz SPL across 10 randomly selected measurement points will not be within +/- 2 dB in each chamber	Distribution of average 8 kHz SPL across 10 randomly selected measurement points will be within +/- 2 dB in each chamber	Reject H_O, accept H_A: 95% PI and z-test show distribution within +/- 2 dB
32-point chamber characterization	Distribution of average 8 kHz SPL across all 32 chamber measurement points will not be within +/- 2 dB in each chamber	Distribution of average 8 kHz SPL across all 32 chamber measurement points will be within +/- 2 dB in each chamber	Fail to reject H_O: 85 and 95 dB exposure chambers had distributions beyond +/- 2 dB limits
Endurance test	Distribution of 8 kHz SPL measured at a central reference point will not be within +/- 2 dB over a 6-hour run	Distribution of 8 kHz SPL measured at a central reference point will be within +/- 2 dB over a 6-hour run	Reject H_O, accept H_A: 95% PI and z-test show distribution within +/- 1 dB
Compressor engineering controls	Modifying existing acoustical enclosures over air compressors will not reduce the SPL near the control chamber	Modifying existing acoustical enclosures over air compressors will reduce the SPL near the control chamber	Reject H_O, accept H_A: overall SPL at control chamber reduced by 10.9 dB

5. Conclusions

A novel noise delivery system was developed to produce a very specific sound exposure profile for use in JP-8 ototoxicity studies. Three electrodynamic shakers were successfully used to produce an octave band of noise, centered at 8 kHz, with sound pressure levels of 75, 85, and 95 dB in three separate exposure chambers simultaneously. The system proved to be stable over 6-hour runs with tight control over exposure amplitude and an essentially flat profile across the 6.3, 8, and 10 kHz 1/3 octave bands that comprise the full 8 kHz octave band. Additionally, characterization of the chambers showed that distribution of sound levels across 10 randomized exposure points was well within a +/- 2 dB range.

Shakers are typically used in industry for applications such modal failure testing or controlling vibration tables. This research effort represents the first known use of a shaker to induce a frequency profile into the plenum of an animal exposure chamber to produce an equivalent spectral sound distribution within the chamber. The final system design also gives the Naval Health Research Center a unique capability to deliver noise and whole body aerosol exposures to many animals and differing concentrations simultaneously. As of this writing, NHRC personnel have successfully utilized the system to complete the first phase of noise-only exposure with rats over a twenty day period.

Recent data from the Veterans' Administration underscores the growing problem of increased annual hearing loss claims across the Department of Defense. Within the United States Air Force, emphasis has been placed on identifying hazardous noise sources and enrolling hazardous noise exposed employees in hearing conservation programs. Unlike the United States Army, little emphasis is currently placed on identifying ototoxic exposures in the workplace. Studies by Kaufman et al., Fechter et al. and others led to our current state of knowledge and suggest that the USAF may have a cause for concern with simultaneous personnel exposures to noise and JP-8 jet fuel. The system designed in this thesis effort will enable NHRC research to add to that knowledge base. Results of the current NHRC study and future studies may one day lead to changes in the criteria by which hazardous noise exposure limits are set and account for the potential additive, potentiating, or synergistic effects that ototoxins may have on irreversible hearing loss. Bioenvironmental engineers in the USAF and industrial hygienists elsewhere may one day see the results of this effort through revised exposure standards and exposure assessment guidelines.

Appendix A

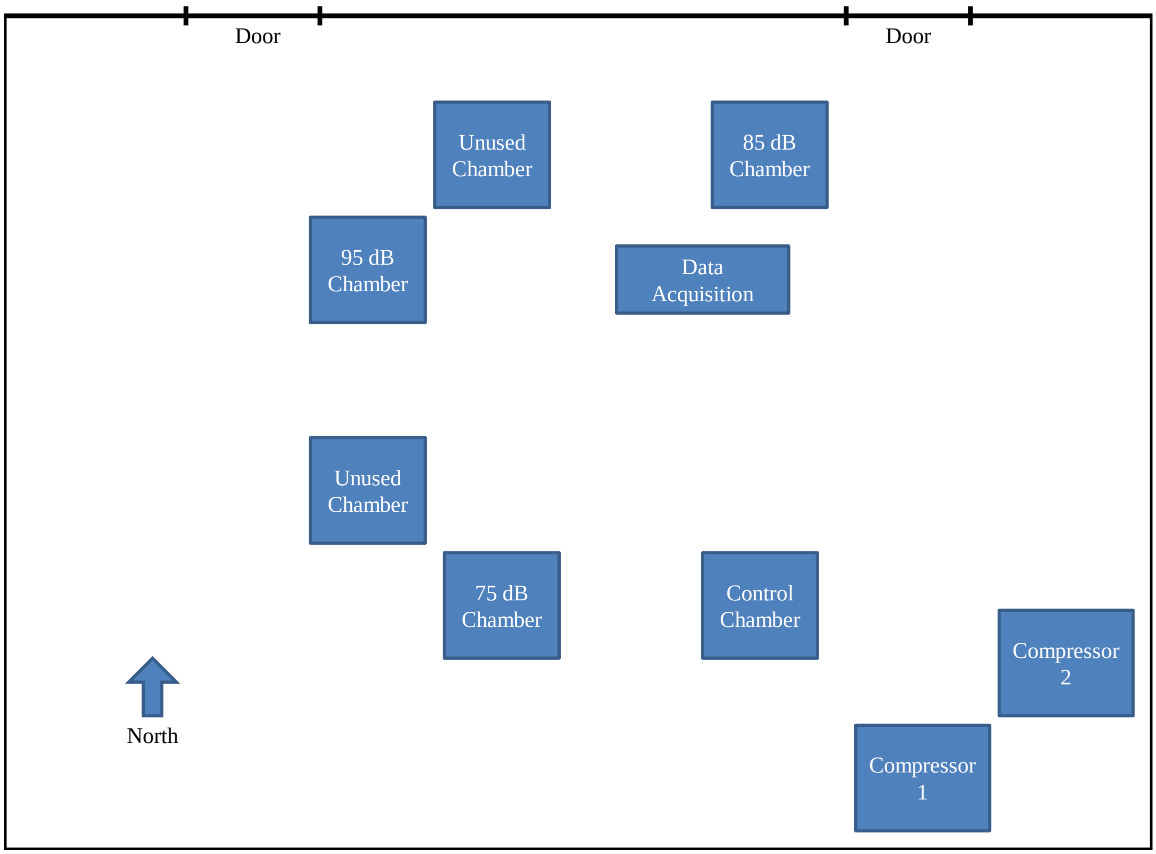


Figure 25. Layout of NHRC inhalation exposure laboratory

Appendix B

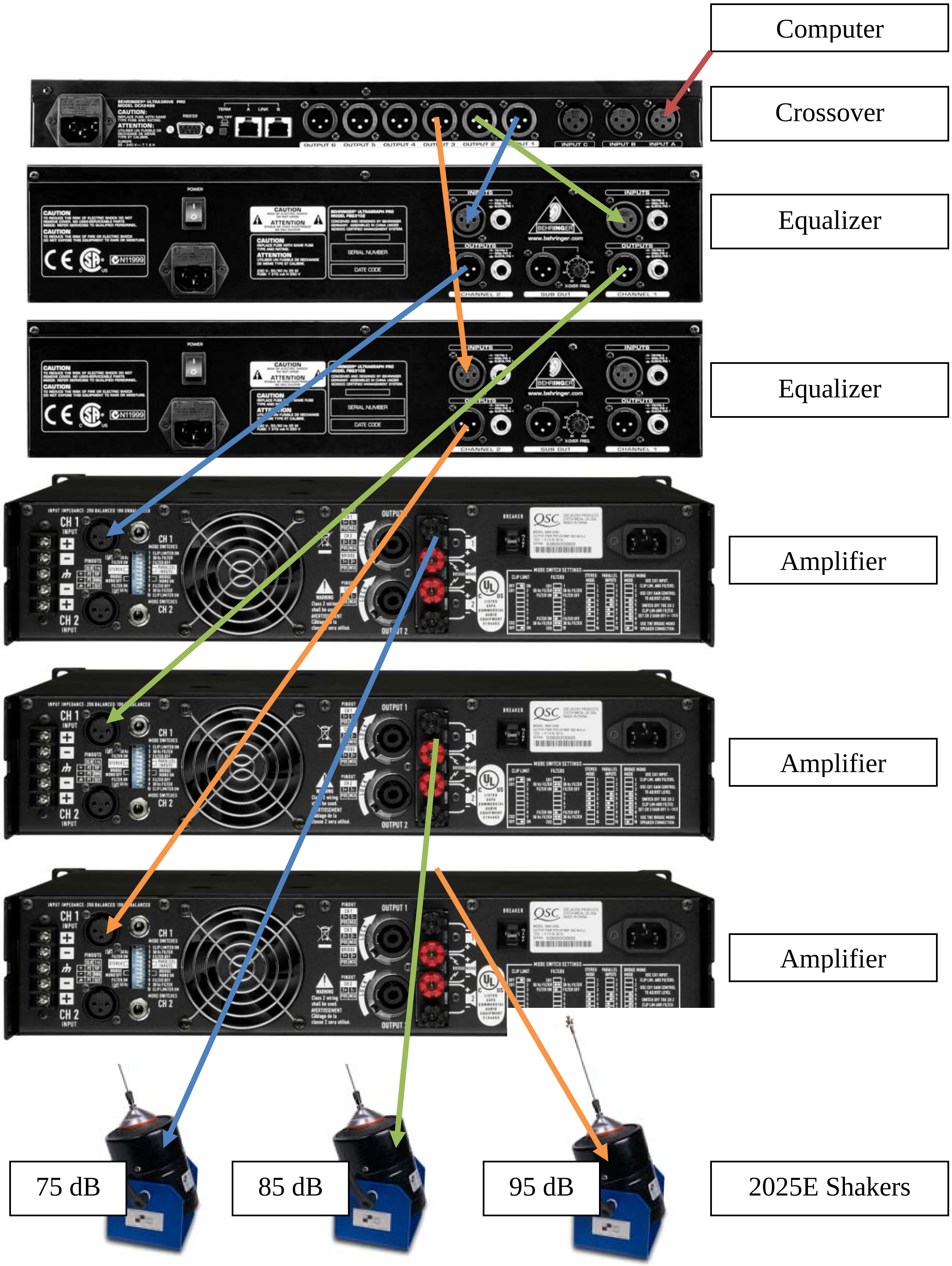


Figure 26. Wiring diagram for noise delivery system

Appendix C

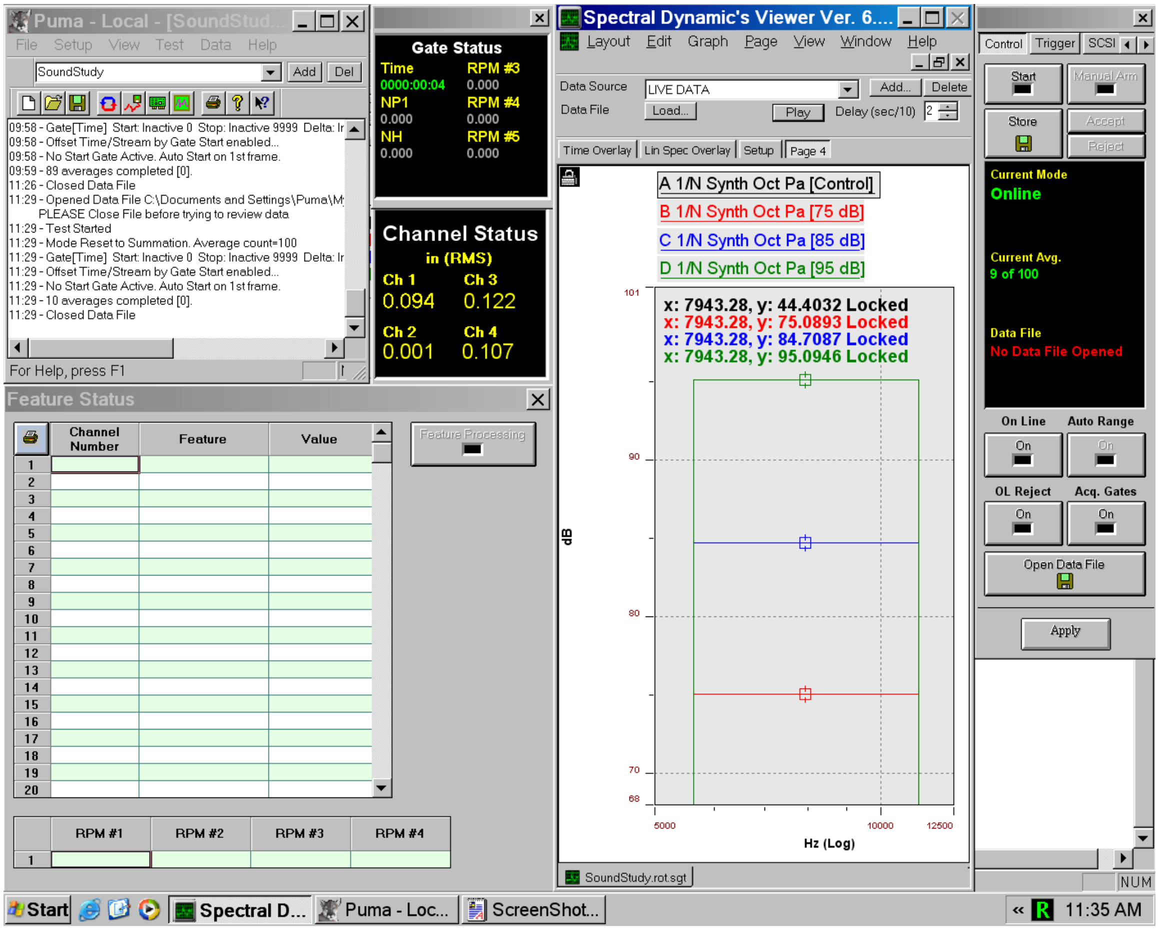


Figure 27. Screen capture of Puma data acquisition system

Appendix D

Sound Experiment: System Operation

New/Replaces: New
Effective Date: December 21, 2009
Approvals: Management:______________________________ ____________
Date
Technical Director:_________________________ ____________
Date
Quality Assurance:__________________________ ____________
Date

1. **PURPOSE.** To facilitate the proper startup of the sound generation system in building 837 room 264 – known as the Inhalation Exposure Lab

2. **EQUIPMENT**

2.1 Equipment rack: Multi tiered roll around cart which holds 4 amplifiers, 4 equalizers, and the data acquisition computer system.

3. **STARTUP ELECTRONICS AND DATA ACQUISITION**

NOTE: THESE DIRECTIONS SHOULD BE FOLLOWED IN THE ORDER THEY ARE WRITTEN. FAILURE TO DO SO CAN CAUSE DAMAGE TO THE EQUIPMENT.

3.1 Behind the equipment rack is a power strip. Turn on this power strip.

3.2 Just below the monitor on the equipment rack is the Computer for the sound system. Open the door on the front of the computer and turn on the computer system.

3.3 On the front of the rack are two plastic doors. The top door covers the equalizers. The bottom door covers the amplifiers.

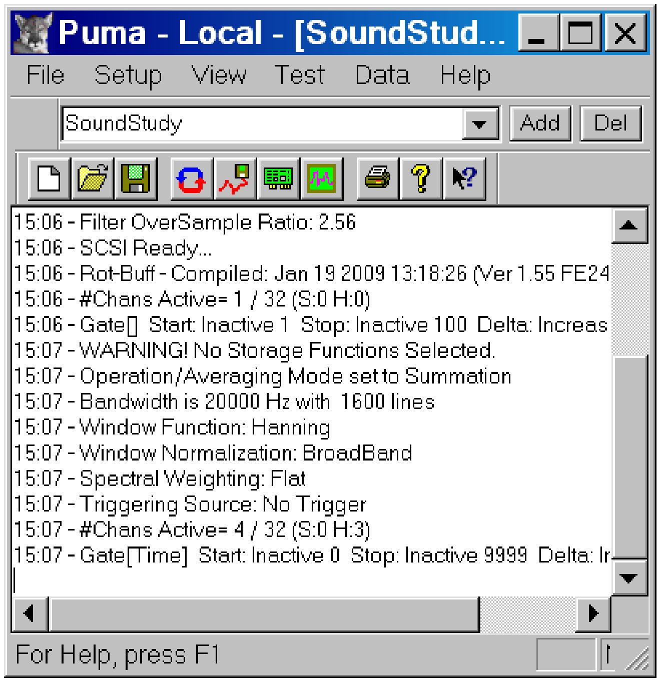
3.4 Open the bottom door and turn on the first three amplifiers starting with the topmost one.

3.4 Sitting to the right of the equipment cart is a metal roll around cart. Turn on the laptop computer sitting on this cart.

3.5 On the monitor in the equipment rack, use the mouse and click on the puma icon.

3.6 Look at the Puma-Local screen in Figure 1.

Figure 1

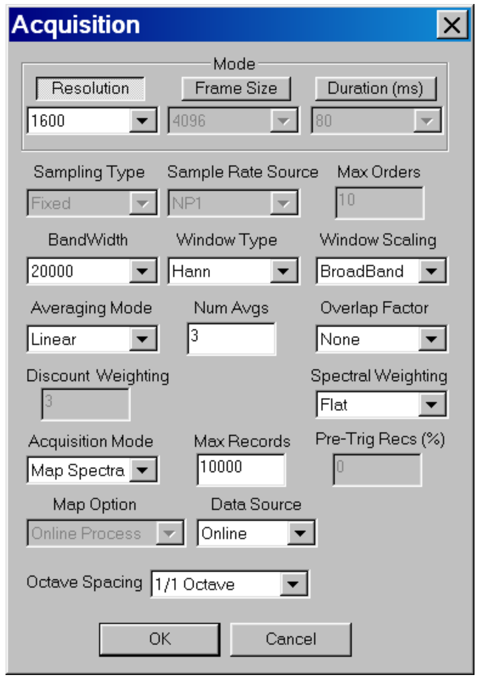


3.7 Click on the Setup tab.

3.8 Click on the Acquisition tab.

3. 9 Verify the settings are the same as shown in figure 2.

Figure 2

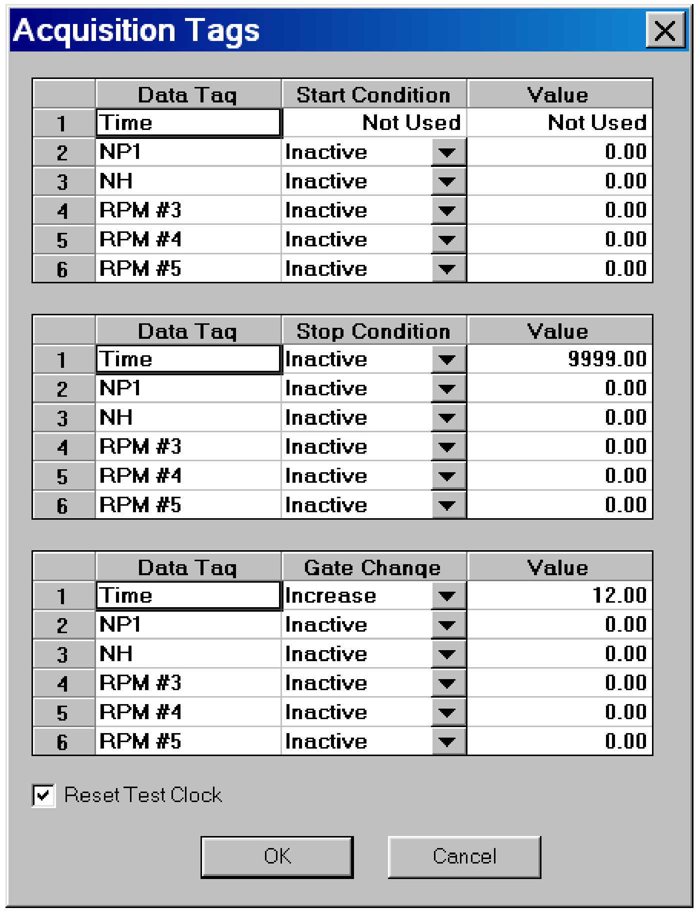


3.10 After viewing, and or correcting any values click OK.

3.11 Click on acquisition Gating located under the setup tab.

3.12 Verify the settings are the same as shown in figure 3.

Figure 3



3.12 After viewing and or correcting any values click OK.

3.13 From the local window click on the view tab.

3.14. Under the view tab click on the feature status.

3.15 The feature status menu as shown in figure 4 will appear.

3.16 Click on this window and move it to the lower left quadrant of the screen as shown in figure 5.

Figure 4

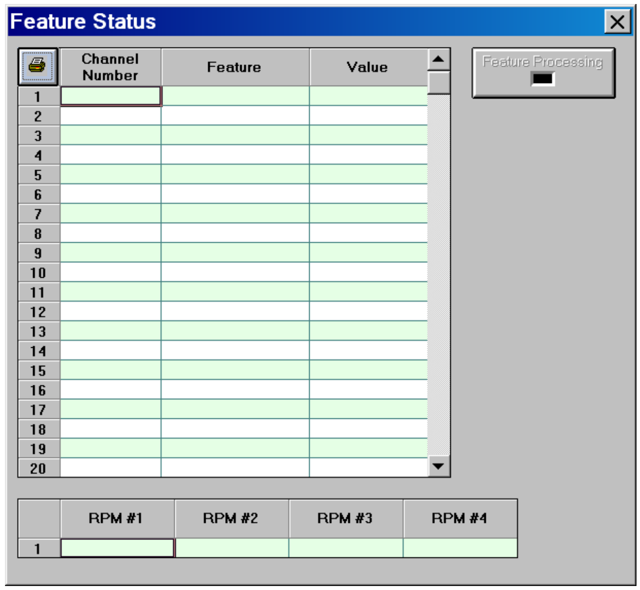
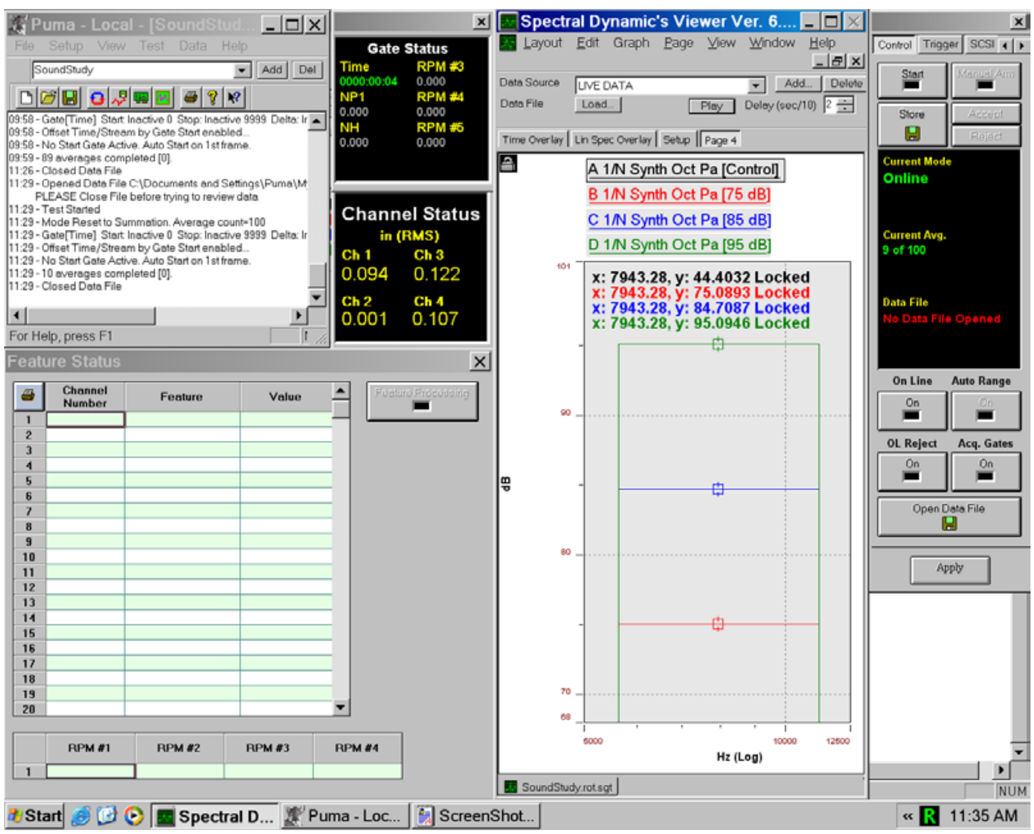


Figure 5



3.17 In the upper right hand corner of the screen click on the SCSI tab.

3.18 Make sure the “Store to SCSI “check box is UNCHECKED.

3.19 Click on the left arrow at the top right of the screen and then click on the control tab.

3.20 At this point the PUMA system is set up and ready for data acquisition.

4.0 STARTUP LAPTOP AND SOUND FILE

4.1 On the stainless steel cart just to the right of the electronics rack is the VIAO laptop.

4.2 Start up the laptop, ignore and close any pop ups that may show.

4.3 On the desktop click on the icon labeled “Sound Study.”

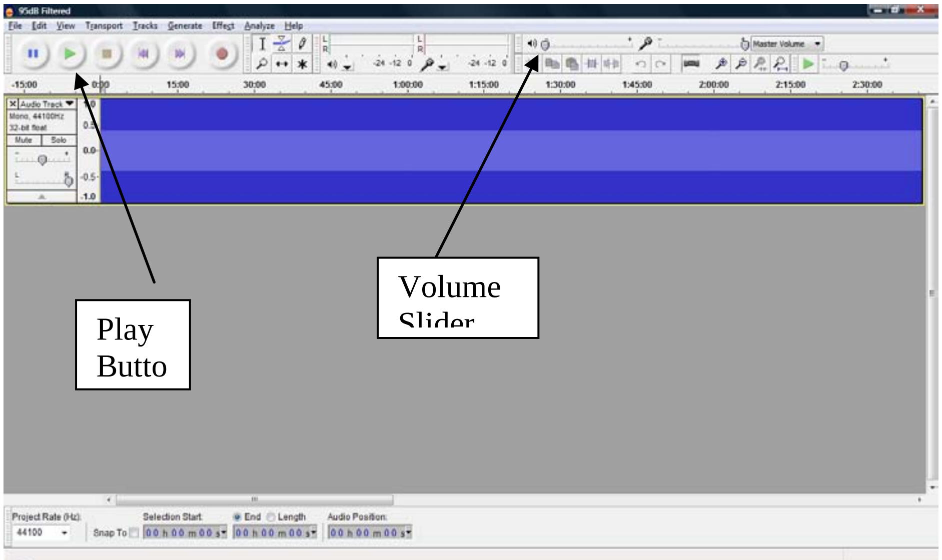
4.4 The audiology sound program will be loaded which allows creation of the correct sound levels.

5.0 CREATING SOUND AND COLLECTING THE DATA

5.1 After the animals are loaded press the play button on the menu at the top of the sound program (Figure 6).

5.2 Slowly over a period of 2 minutes slide the volume control from the minimum setting to the maximum setting (Figure 6).

Figure 6



5.3 On the puma main menu just under the control tab click on the button labeled "Start."

5.4 On the features menu click on the button labeled "Feature Processing."

6.0 SHUTTING DOWN THE ACQUISTION SYSTEM

6.1 On the laptop sound program move the volume control from maximum to minimum (Figure 6).

6.2 On the PUMA program click on the "features status" button to stop the program collection.

6.3 On the PUMA program click on the "Start" button located under the control tab to stop data acquisition.

6.4 On the PUMA local window click the File tab then click exit to close the program.

7.0 DATA STORAGE

7.1 On desktop of the computer on the electronics rack, click on the icon which is labeled Shortcut to RCT'S.

7.2 Locate the CSV file with the exposure day date.

7.3 Save a copy of this CSV file to a portable hard drive.

7.0 SYSTEM SHUTDOWN

8.1 Shut down the laptop.

8.2 Shut down the computer in the electronics rack.

8.3 Open the lower door in the electronics rack and turn off the first 3 amplifiers. Don't touch the equalizers which behind the upper door.

8.4 Behind the electronics rack turn off the power strip.

8.5 The system is now off and all electronics should be off.

Appendix E

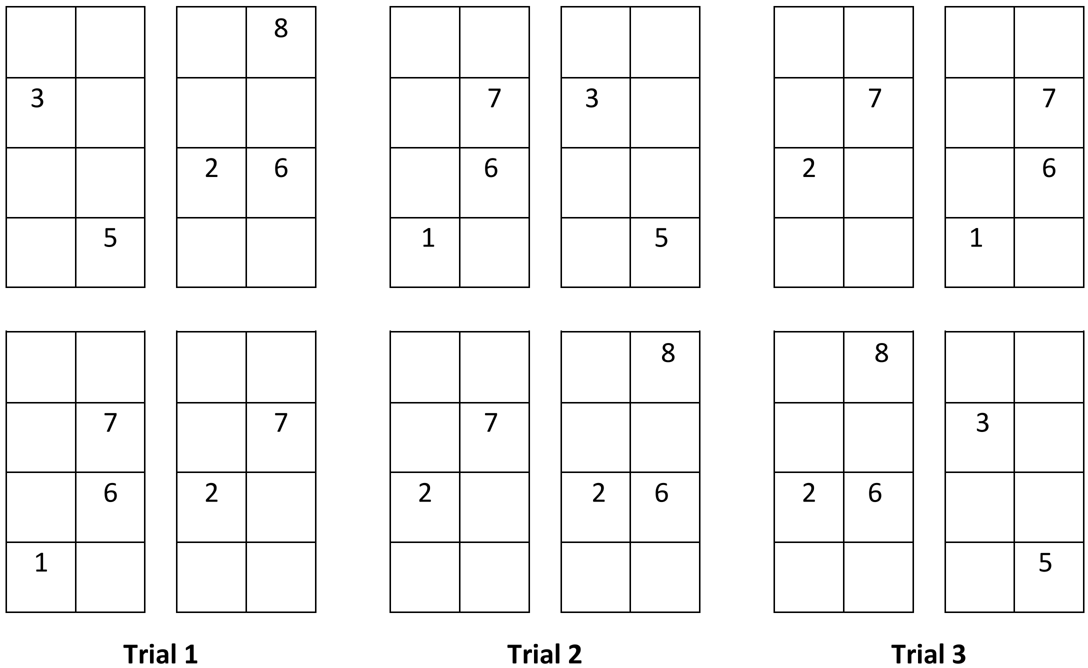


Figure 28. Measurement locations for 75 dB 10-point characterization trials

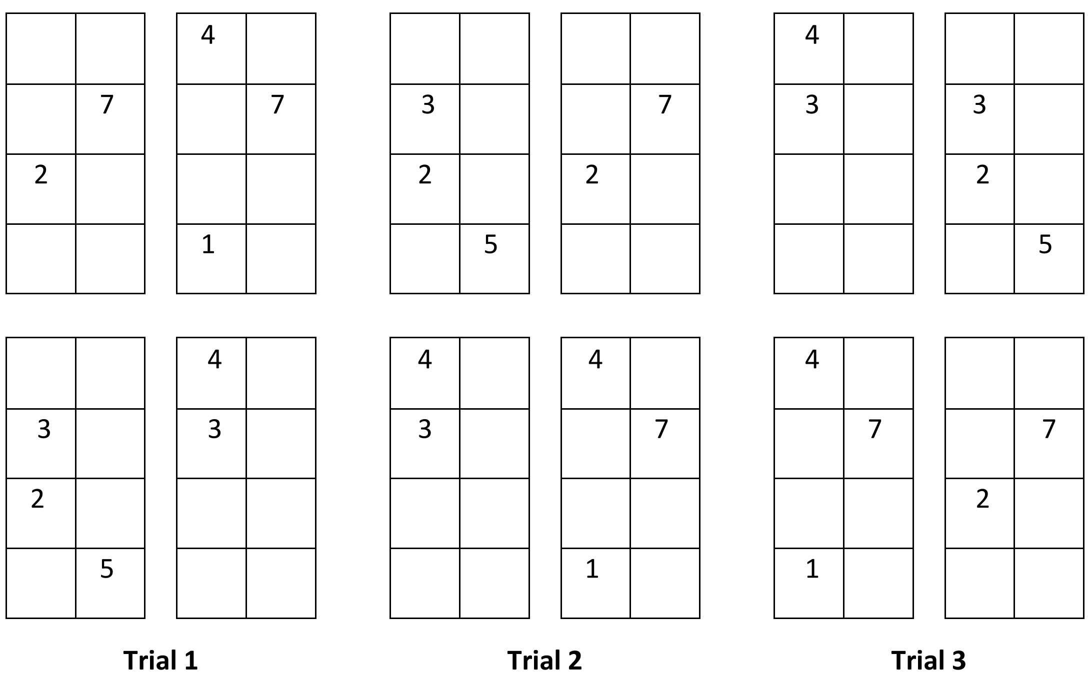


Figure 29. Measurement locations for 85 dB 10-point characterization trials

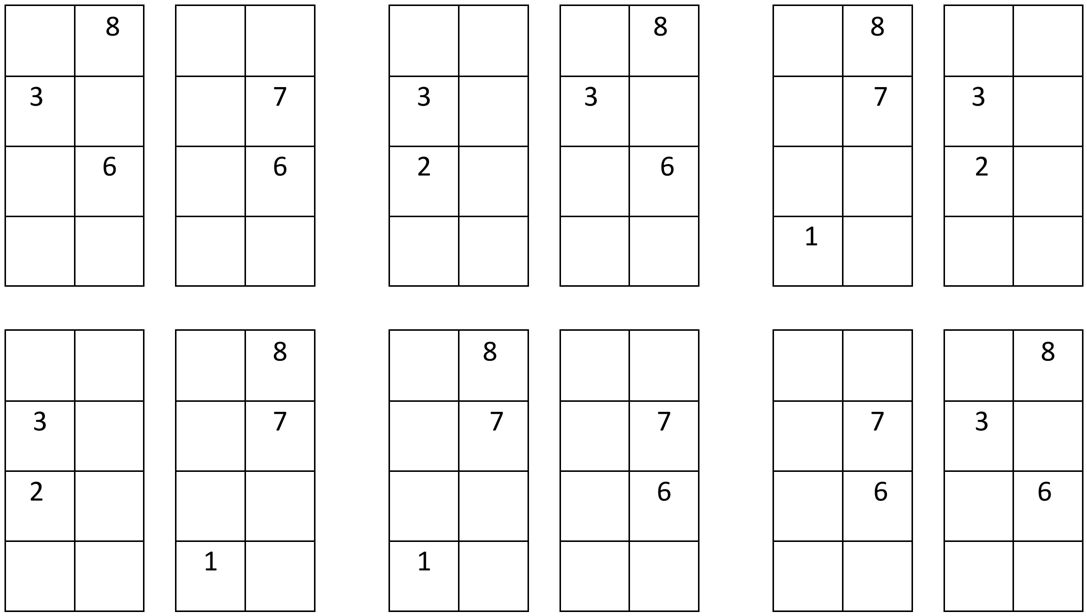


Figure 30. Measurement locations for 95 dB 10-point characterization trials

75.2 +/- 0.11	
	75.1 +/- .15

	75.2 +/- .15
74.1 +/- .18	76.0 +/- .12

	75.9 +/- .15
	75.3 +/- .11
76.8 +/- .15	

	76.1 +/- .13
75.3 +/- .11	

Overall Min: 74.0
Overall Max: 76.9
Overall Mean: 75.5 +/- 0.7

75 dB Chamber – Trial 1

Figure 31. Results of measurements at first 10 randomized points in 75 dB chamber. All values in dB.

	73.9 +/- .15	73.7+/- .19	
	73.5 +/- .16		
74.9 +/- .18			74.4 +/- .16
			75.6 +/- .17
	74.9 +/- .16		
76.1 +/- .17		75.5 +/- .15	76.2 +/- .14

Overall Min: 73.4
Overall Max: 76.3
Overall Mean: 74.9 +/- 1.0

75 dB Chamber – Trial 2

Figure 32. Results of measurements at second set of 10 points in 75 dB chamber. All values in dB.

	74.2 +/- .14		74.8 +/- .18
74.7+/- .12			75.0 +/- .10
		73.9 +/- .18	
	74.7+/- .12		
		74.2 +/- .17	
75.5 +/- .17	75.0 +/- .15		
			75.1 +/- .10

Overall Min: 73.8
Overall Max: 75.6
Overall Mean: 74.7 +/- 0.5

75 dB Chamber – Trial 3

Figure 33. Results of measurements at third set of 10 points in 75 dB chamber. All values in dB.

		85.1 +/- .12	
	84.4+/- .14		85.4+/- .16
84.5 +/- .12			
		84.6 +/- .14	

		86.0 +/- .12	
84.9 +/- .13		84.4 +/- .14	
85.4 +/- .13			
	84.6 +/- .12		

Overall Min: 83.9
Overall Max: 86.1
Overall Mean: 84.9 +/- 0.6

85 dB Chamber – Trial 1

Figure 34. Results of measurements at first 10 randomized points in 85 dB chamber. All values in dB.

83.5 +/- .17			84.4 +/- .15
84.0 +/- .14	84.8 +/- .14	84.4 +/- .17	

85.1 +/- .15		86.0 +/- .16	
86.4 +/- .1			86.0 +/- .15
		85.1 +/- .19	

Overall Min: 83.4
Overall Max: 86.5
Overall Mean: 85.0 +/- 0.9

85 dB Chamber – Trial 2

Figure 35. Results of measurements at second set of 10 points in 85 dB chamber. All values in dB.

84.5 +/- .11			
85.0 +/- .12		84.3 +/- .12	
		83.7 +/- .1	
			84.6 +/- .17

85.5 +/- .19			
	85.5 +/- .12		85.2 +/- .11
		85.2 +/- .13	
85.6 +/- .12			

Overall Min: 83.6
Overall Max: 85.6
Overall Mean: 84.9 +/- 0.6

85 dB Chamber – Trial 3

Figure 36. Results of measurements at third set of 10 points in 85 dB chamber. All values in dB.

	94.5 +/- .14		
94.6 +/- .16			94.2 +/- .13
	94.0 +/- .15		93.9 +/- .11

			95.1 +/- .12
95.9 +/- .12			95.1 +/- .13
94.4 +/- .13			
		94.5 +/- .12	

Overall Min: 93.9
Overall Max: 95.9
Overall Mean: 94.6 +/- 0.6

95 dB Chamber – Trial 1

Figure 37. Results of measurements at first 10 randomized points in 95 dB chamber. All values in dB.

			94.7 + .12
94.3 +/- .14		94.8 +/- .13	
94.0 +/- .11			94.3 +/- .15

	94.4 +/- .1		
	95.0+/- .14		96.4 +/- .13
			95.3 +/- .14
96.0 +/- .16			

Overall Min: 93.9
Overall Max: 96.5
Overall Mean: 94.9 +/- 0.8

95 dB Chamber – Trial 2

Figure 38. Results of measurements at second set of 10 points in 95 dB chamber. All values in dB.

	95.0 +/- .15		
	94.2 +/- .19	95.1 +/- .17	
		95.1 +/- .17	
95.4 +/- .18			

			95.3 +/- .16
	95.3 +/- .13	94.6 +/- .15	
	96.0 +/- .19		96.0 +/- .13

Overall Min: 94.1
Overall Max: 96.1
Overall Mean: 95.2 +/- 0.7

95 dB Chamber – Trial 3

Figure 39. Results of measurements at third set of 10 points in 95 dB chamber. All values in dB.

74.6	73.8		75.4	73.5
75.6	73.9		74.0	74.3
74.9	74.7		74.0	73.6
75.0	75.0		74.7	74.0

75.2	75.4		76.0	74.6
76.7	75.1		73.8	76.2
77.2	74.9		74.3	76.6
75.6	74.0		75.6	75.5

Overall Min: 73.5
Overall Max: 77.2
Overall Mean: 74.9 +/- 0.92

75 dB Chamber

Figure 40. Results of measurements at all 32 points in 75 dB chamber. All values in dB.

85.1	84.9		84.2	85.4
86.1	85.6		85.3	85.3
85.2	84.9		85.4	85.3
85.0	85.3		85.5	85.5

86.8	86.6		86.4	87.3
87.5	86.1		86.4	87.2
87.0	86.2		85.9	87.2
88.7	86.5		86.0	86.9

Overall Min: 84.2
Overall Max: 88.7
Overall Mean: 86.0 +/- 0.97

85 dB Chamber

Figure 41. Results of measurements at all 32 points in 85 dB chamber. All values in dB.

94.1	93.3		94.1	93.5
94.1	94.1		94.3	93.9
95.1	94.0		94.3	93.8
94.5	94.7		94.1	94.1

96.5	95.9		96.0	96.2
97.1	96.4		95.1	95.3
98.2	95.3		95.0	97.6
96.8	94.5		95.2	97.4

Overall Min: 93.3
Overall Max: 98.2
Overall Mean: 95.1 +/- 1.30

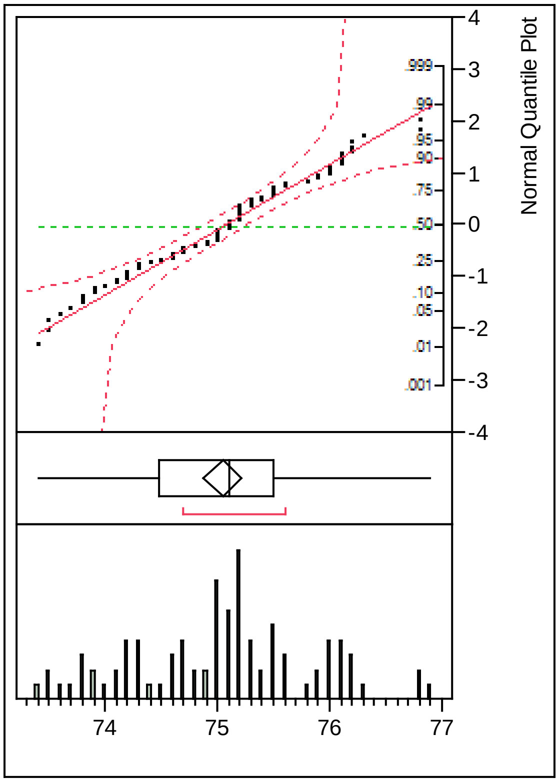
95 dB Chamber

Figure 42. Results of measurements at all 32 points in 95 dB chamber. All values in dB.

Appendix F

Supporting output data from JMP statistical analyses for 10-point randomization trials.

Distributions
75 dB 10pt



Quantiles

100.0%	maximum	76.900
99.5%		76.900
97.5%		76.800
90.0%		76.100
75.0%	quartile	75.500
50.0%	median	75.100
25.0%	quartile	74.475
10.0%		73.900
2.5%		73.500
0.5%		73.400
0.0%	minimum	73.400

Moments

Mean	75.047778
Std Dev	0.7975
Std Err Mean	0.0840639
upper 95% Mean	75.214811
lower 95% Mean	74.880745
N	90

Confidence Intervals

Parameter	Estimate	Lower CI	Upper CI	1-Alpha
Mean	75.04778	74.88074	75.21481	0.950
Std Dev	0.7975	0.69559	0.934675	

Prediction Interval

Parameter	Estimate	Lower PI	Upper PI	1-Alpha	Future N
Mean	75.04778	74.51957	75.57598	0.950	10
Std Dev	0.7975	0.431745	1.199023		

Prediction Interval

Parameter	Estimate	Lower PI	Upper PI	1-Alpha	Future N
Mean	75.04778	74.34805	75.74751	0.990	10
Std Dev	0.7975	0.345151	1.352566		

Fitted Normal
Parameter Estimates

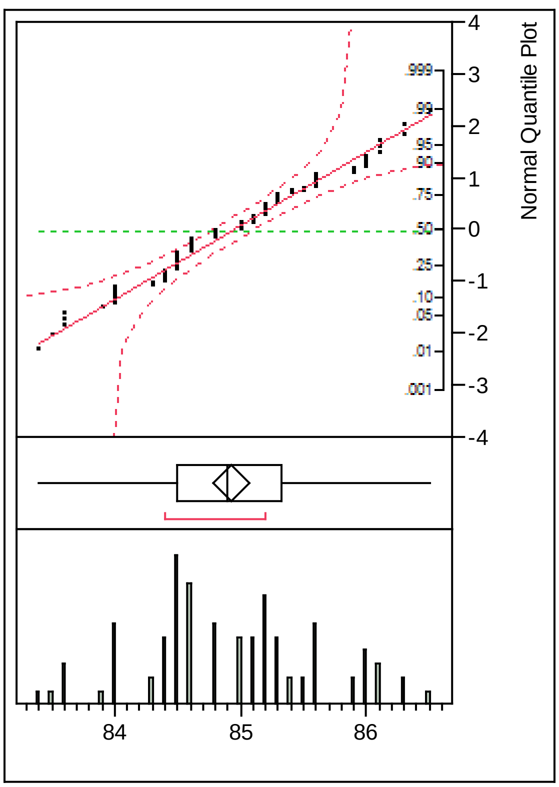
Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	75.047778	74.880745	75.214811
Dispersion	σ	0.7975	0.6955895	0.9346746

Goodness-of-Fit Test
Shapiro-Wilk W Test

W	Prob<W
0.981121	0.2160

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

85 dB 10pt



Quantiles

100.0%	maximum	86.500
99.5%		86.500
97.5%		86.300
90.0%		86.000
75.0%	quartile	85.325
50.0%	median	84.900
25.0%	quartile	84.500
10.0%		84.000
2.5%		83.528
0.5%		83.400
0.0%	minimum	83.400

Moments

Mean	84.923333
Std Dev	0.6971934
Std Err Mean	0.0734906
upper 95% Mean	85.069358
lower 95% Mean	84.777309
N	90

Confidence Intervals

Parameter	Estimate	Lower CI	Upper CI	1-Alpha
Mean	84.92333	84.77731	85.06936	0.950
Std Dev	0.697193	0.608101	0.817115	

Prediction Interval

Parameter	Estimate	Lower PI	Upper PI	1-Alpha	Future N
Mean	84.92333	84.46156	85.3851	0.950	10
Std Dev	0.697193	0.377442	1.048214		

Prediction Interval

Parameter	Estimate	Lower PI	Upper PI	1-Alpha	Future N
Mean	84.92333	84.31161	85.53505	0.990	10
Std Dev	0.697193	0.30174	1.182445		

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	84.923333	84.777309	85.069358
Dispersion	σ	0.6971934	0.6081009	0.8171147

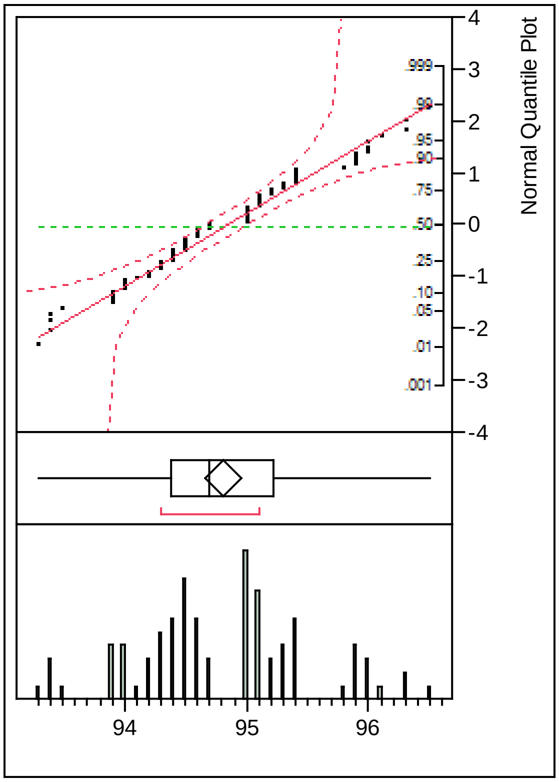
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.980003	0.1807

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

95 dB 10pt



Quantiles

100.0%	maximum	96.500
99.5%		96.500
97.5%		96.300
90.0%		95.900
75.0%	quartile	95.225
50.0%	median	94.700
25.0%	quartile	94.375
10.0%		93.910
2.5%		93.400
0.5%		93.300
0.0%	minimum	93.300

Moments

Mean	94.811111
Std Dev	0.7071774
Std Err Mean	0.074543
upper 95% Mean	94.959227
lower 95% Mean	94.662996
N	90

Confidence Intervals

Parameter	Estimate	Lower CI	Upper CI	1-Alpha
Mean	94.81111	94.663	94.95923	0.950
Std Dev	0.707177	0.616809	0.828816	

Prediction Interval

Parameter	Estimate	Lower PI	Upper PI	1-Alpha	Future N
Mean	94.81111	94.34273	95.27949	0.950	10
Std Dev	0.707177	0.382847	1.063225		

Prediction Interval

Parameter	Estimate	Lower PI	Upper PI	1-Alpha	Future N
Mean	94.81111	94.19063	95.43159	0.990	10
Std Dev	0.707177	0.306061	1.199378		

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	94.811111	94.662996	94.959227
Dispersion	σ	0.7071774	0.616809	0.828816

Goodness-of-Fit Test

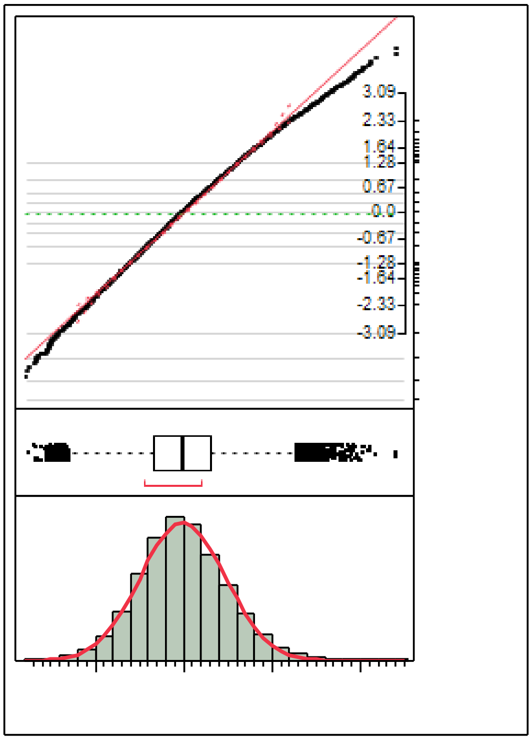
Shapiro-Wilk W Test

W	Prob<W
0.978055	0.1319

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Supporting output data from JMP statistical analyses for 5-day endurance runs.

Distributions
75 dB



Normal(74.989,0.48188)

Quantiles

100.0%	maximum	77.400
99.5%		76.350
97.5%		75.980
90.0%		75.610
75.0%	quartile	75.300
50.0%	median	74.970
25.0%	quartile	74.660
10.0%		74.390
2.5%		74.080
0.5%		73.800
0.0%	minimum	73.210

Moments

Mean	74.989045
Std Dev	0.481884
Std Err Mean	0.0018394
Upper 95% Mean	74.99265
Lower 95% Mean	74.98544

N	68636

Confidence Intervals

Parameter	Estimate	Lower CI	Upper CI	1-Alpha
Mean	74.98904	74.98544	74.99265	0.950
Std Dev	0.481884	0.479348	0.484447	0.950

Prediction Interval

Parameter	Future N	Lower PI	Upper PI	1-Alpha
Individual	1	74.04455	75.93354	0.950
Mean	1	74.04455	75.93354	0.950
Std Dev	1	.	.	0.950

Fitted Normal
Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	74.989045	74.98544	74.99265
Dispersion	σ	0.481884	0.4793483	0.4844468

-2log(Likelihood) = 94563.8416895508

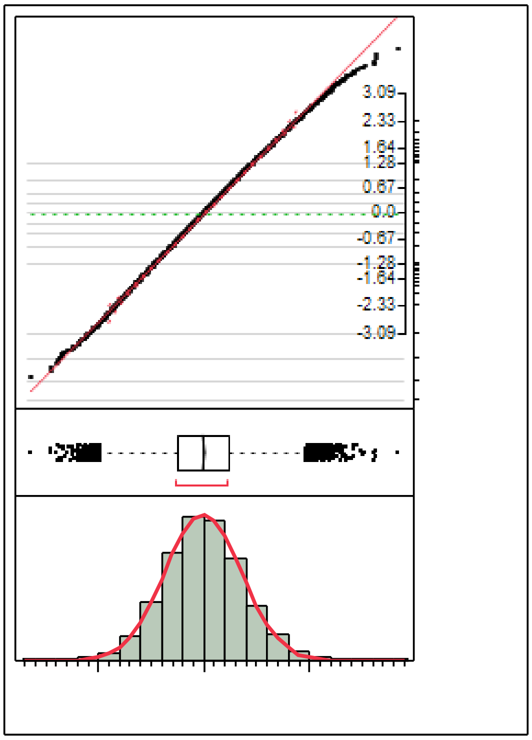
Goodness-of-Fit Test

KSL Test

D		Prob>D
0.020421	<	0.0100*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

85 dB



Normal(84.9939,0.36272)

Quantiles

100.0%	maximum	86.830
99.5%		85.968
97.5%		85.720
90.0%		85.460
75.0%	quartile	85.230
50.0%	median	84.990
25.0%	quartile	84.750
10.0%		84.530
2.5%		84.290
0.5%		84.080
0.0%	minimum	83.360

Moments

Mean	84.99387
Std Dev	0.3627247
Std Err Mean	0.0013845
Upper 95% Mean	84.996584
Lower 95% Mean	84.991156
N	68636

Confidence Intervals

Parameter	Estimate	Lower CI	Upper CI	1-Alpha
Mean	84.99387	84.99116	84.99658	0.950
Std Dev	0.362725	0.360816	0.364654	0.950

Prediction Interval

Parameter	Future N	Lower PI	Upper PI	1-Alpha
Individual	1	84.28292	85.70482	0.950
Mean	1	84.28292	85.70482	0.950
Std Dev	1	.	.	0.950

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	84.99387	84.991156	84.996584
Dispersion	σ	0.3627247	0.360816	0.3646538

-2log(Likelihood) = 55570.4580294835

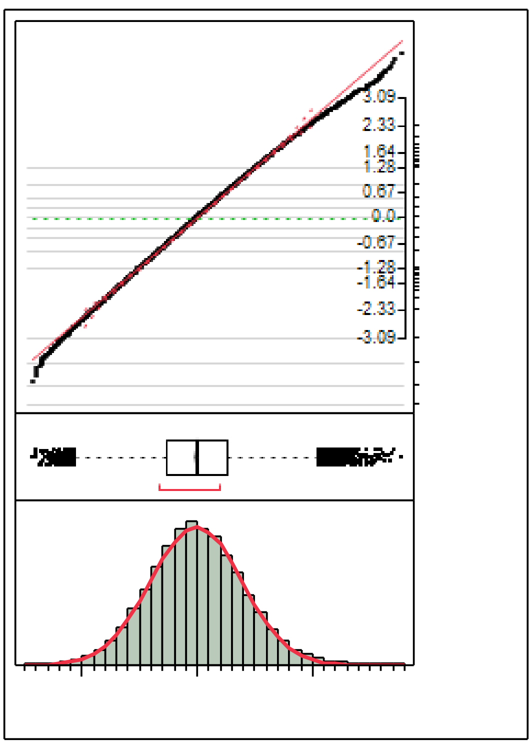
Goodness-of-Fit Test

KSL Test

D		Prob>D
0.011045	<	0.0100*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

95 dB



Normal(94.9927,0.39518)

Quantiles

100.0%	maximum	96.770
99.5%		96.078
97.5%		95.790
90.0%		95.500
75.0%	quartile	95.250
50.0%	median	94.980
25.0%	quartile	94.723
10.0%		94.490
2.5%		94.240
0.5%		94.010
0.0%	minimum	93.570

Moments

Mean	94.992713
Std Dev	0.3951762
Std Err Mean	0.0015084
Upper 95% Mean	94.99567
Lower 95% Mean	94.989757
N	68636

Confidence Intervals

Parameter	Estimate	Lower CI	Upper CI	1-Alpha
Mean	94.99271	94.98976	94.99567	0.950
Std Dev	0.395176	0.393097	0.397278	0.950

Prediction Interval

Parameter	Future N	Lower PI	Upper PI	1-Alpha
Individual	1	94.21816	95.76726	0.950
Mean	1	94.21816	95.76726	0.950
Std Dev	1	.	.	0.950

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	94.992713	94.989757	94.99567
Dispersion	σ	0.3951762	0.3930968	0.3972779

-2log(Likelihood) = 67332.9699690957

Goodness-of-Fit Test

KSL Test

D		Prob>D
0.014819	<	0.0100*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Bibliography

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Vita

Captain John E. Stubbs graduated from St. Stephens High School in Hickory, North Carolina in 1993. He then attended North Carolina State University in Raleigh, North Carolina where he graduated Suma Cum Laude with a Bachelor of Science degree in Industrial Engineering. During his undergraduate pursuits, Captain Stubbs also completed two engineering cooperative education rotations with DuPont in Wilmington, North Carolina and served as a resident assistant and resident director of a private dormitory with over 900 residents. After graduation, Captain Stubbs worked as a process engineer for Procter and Gamble in Greensboro, North Carolina where he led raw material supply chain management.

Following a strong desire to serve, Captain Stubbs received a direct commission into the Air Force in December 2001 as a bioenvironmental engineer. His first duty assignment was at Wright-Patterson AFB, Ohio where he was the principle investigator on a newly launched aircrew comfort and fatigue study. His next duty assignment was as Deputy Bioenvironmental Engineering Element Chief at McChord AFB, Washington where he managed the radiation and readiness programs. During his time at McChord AFB, Captain Stubbs was deployed for six months to Combined Joint Task Force-Horn of Africa at Camp Lemonier Naval Expeditionary Base in Djibouti. There he was assigned as an Air Force augmentee to the Army's 350th Civil Affairs Command, charged with carrying out medical and veterinary civic action projects throughout the Horn of Africa.

REPORT DOCUMENTATION PAGE	*Form Approved* *OMB No. 074-0188*

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From – To)*
26-03-2010	Master's Thesis	August 2008 – Mar 2010

4. TITLE AND SUBTITLE	
Development of a Novel Noise Delivery System for JP-8 Ototoxicity Studies	**5a. CONTRACT NUMBER**
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	**5d. PROJECT NUMBER**
John E. Stubbs, Captain, USAF	**5e. TASK NUMBER**
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(S)	**8. PERFORMING ORGANIZATION REPORT NUMBER**
Air Force Institute of Technology Graduate School of Engineering and Management (AFIT/EN) 2950 Hobson Way WPAFB OH 45433-7765	AFIT/GIH/ENV/10-M04
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) LT Vishwesh Mokashi, PhD, MSC, USN Comm: (937)904-9461 DSN: 674-9461 Naval Health Research Center Detachment Environmental Health Effects Laboratory 2729 R Street, Area B, Bldg 837 Wright-Patterson AFB, Ohio 45433-5707	**10. SPONSOR/MONITOR'S ACRONYM(S)** NHRC/EHEL **11. SPONSOR/MONITOR'S REPORT NUMBER(S)** F-WA-2008-0108-A

12. DISTRIBUTION/AVAILABILITY STATEMENT
APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

13. SUPPLEMENTARY NOTES
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14. ABSTRACT
Numerous chemicals with ototoxic properties may cause hearing loss directly, potentiate noise-induced hearing loss, or produce additive effects. Of interest to the US Air Force are studies showing ototoxic effects of JP-8 jet fuel and its hydrocarbon constituents. The Naval Health Research Center (NHRC) at Wright-Patterson AFB, Ohio, in conjunction with the USAF, is studying the ototoxic effects of JP-8 in rats. The study requires a white noise source that is one octave band wide, centered at 8 kHz frequency, delivered from outside of exposure chambers. Sound pressure levels must be within +/- 2 dB at all exposure points within each chamber and within +/- 2 dB over a 6-hour run. Electrodynamic shakers were successfully used to produce the required input noise in three exposure chambers by inducing vibration in chamber plenums. Distribution of sound pressure levels across chamber exposure points were well controlled within a +/- 1.5 dB prediction interval (= 0.05) or better. Stability at a central reference point was well controlled over 6-hour runs within a +/- 1 dB prediction interval (= 0.05) or better. The final system solution gives the NHRC a unique capability to deliver noise and whole-body JP-8 aerosol exposures simultaneously.

15. SUBJECT TERMS
Industrial Hygiene, Noise, Ototoxicity, JP-8 Jet Fuel, Shaker, Exciter

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** Jeremy M. Slagley, Maj, USAF (ENV)
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(Include area code)*
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39-18

	Form Approved *OMB No. 074-0188*