A noise delivery system for multi-animal multi-level whole body ototoxicity studies

John E. Stubbs[a] and Jeremy M. Slagley[b]
Air Force Institute of Technology, Department of Systems Engineering and Management, Wright-Patterson Air Force Base, Ohio 45433, USA

James E. Reboulet
Naval Medical Research Unit Dayton, Wright-Patterson Air Force Base, Ohio 45433, USA

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The Naval Medical Research Unit Dayton (NAMRU-D) at Wright-Patterson Air Force Base, Ohio, in conjunction with the U.S. Air Force, studied ototoxic effects of JP-8 in rats. NAMRU-D used a multi-chamber whole body exposure facility for up to 96 test animals and 32 control animals at different exposure levels. The objective was to design a noise delivery system that could provide a white noise source one octave band wide, centered at 8 kHz frequency, delivered from outside the exposure chambers. Sound pressure levels were required to be within ±2 dB at all exposure points within each chamber and within ±2 dB over a 6-h run. Electrodynamic shakers were used to produce input noise in exposure chambers by inducing vibration in chamber plenums. Distribution of sound pressure levels across exposure points was controlled within a ±1.5dB prediction interval ($\alpha = 0.05$) or better. Stability at a central reference point was controlled over 6-h runs within a ±1 dB prediction interval ($\alpha = 0.05$) or better. The final system allowed NAMRU-D to deliver noise and whole-body aerosol exposures to multiple animals at different levels simultaneously and study the effects that ototoxins may have on hearing loss. [http://dx.doi.org/10.1121/1.4935392]

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I. INTRODUCTION

Noise-induced hearing loss (NIHL) is a significant concern for both the U.S. Department of Defense (DoD) and private industry. Data from the U.S. Department of Veterans' Affairs indicate that over $8 billion were paid to veterans for hearing loss disabilities over the three decades spanning 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 (U.S. Army, 2008).

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 9 million are exposed to substances that are potentially ototoxic. However, that data are extrapolated from small sample populations due to the lack of a national occupational hearing loss and noise exposure surveillance system in the United States (Murphy and Tak, 2009). Actual figures could be much higher.

Though NIHL is easily identified and preventable 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 U.S. Army Public Health Command [formerly known as the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM)] has recognized the significance of ototoxins and noted 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 recommended 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 were 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 U.S. Army has recognized the significance and adopted CHPPM guidance in U.S. Army Pamphlet 40-501, *Hearing Conservation Program* (HCP). It states that personnel will be enrolled in a comprehensive HCP when they are exposed to known or suspected ototoxins (U.S. Army, 1998).

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 U.S. Air Force. Additionally, a retrospective epidemiology study with a relatively small sample size compared Air Force personnel who

[a]Electronic mail: john.stubbs@afit.edu
[b]Present address: Department of Safety Sciences, Indiana University of Pennsylvania, Indiana, PA 15705, USA.

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 *et al.*, 2005). The subsequent Navy study on ototoxicity of JP-8 in rats in conjunction with noise exposure at different levels using the noise delivery system explained in this article was published in 2012 (Fechter *et al.*, 2012).

A. Problem statement

The Naval Medical Research Unit Dayton (NAMRU-D) Environmental Health Effects Laboratory located at Wright-Patterson Air Force Base (AFB), Ohio, in conjunction with the Department of Veterans' Affairs and the Air Force, conducted a study on the ototoxic effects of JP-8 in rats. The study required a very specific white noise source that is one octave band wide, centered at 8 kHz (kHz) frequency. The average sound pressure level (SPL) was required to be within ±2 dB at all exposure points within each chamber and within ±2 dB over the course of a 6-h run. The noise system was needed to deliver the noise source to each of the three exposure chambers at the respective dB amplitude. Additionally, the system was required to provide real-time monitoring of noise levels inside of all four chambers and continuously log the data over each 6-h exposure day. The system design was complicated by the potential aggressive nature of JP-8 aerosol inside the chamber so that noise sources would ideally be transmitted from the exterior of the chamber.

The focus of this paper is the design and validation of the noise delivery and real-time analysis system for use in the NAMRU-D JP-8 ototoxicity study. The final solution represented the first known facility in the United States with the capability to deliver whole-body multi-animal, multi-level JP-8 aerosol and noise exposures simultaneously.

B. Literature review

Several studies have demonstrated the ototoxic effects of chemicals in humans and animals. Human studies included solvents in printing and paint manufacturing (Morata *et al.*, 1993), solvents in reinforced fabric manufacturing facility (Fuente *et al.*, 2009), styrene and toluene in yacht yard and plastic factory workers (Śliwinska-Kowalska *et al.*, 2003), and jet fuels (JP-4 and JP-8) in Air Force workers (Kaufman *et al.*, 2005). The human epidemiological studies show strong evidence of ototoxic effects of solvents with and without noise exposure. Animal studies offer a method to study the singular and combined effects of solvents and noise in a controlled environment.

Animal studies include toluene and noise simultaneously (Lataye and Campo, 1997) and in sequence (Lund and Kristiansen, 2008; Johnson *et al.*, 1990). The studies did not report chamber characterization across the entire cage volume, but Johnson *et al.* noted in their study that the cages were systematically repositioned within the chamber to minimize variation in individual noise exposure levels. Subcutaneous acrylonitrile injection followed by noise exposure was studied in rats as well, but the exposure route did not mimic human workplace exposures (Pouyatos *et al.*, 2005).

Research into the ototoxic effects of JP-8 in animals is of particular interest for the Air Force and has been pioneered out of the Loma Linda Veterans' Affairs Medical Center in Loma Linda, CA. 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 4-h, nose-only inhalation exposure to 1000 mg/m^3 JP-8. The exposure group was then split with half immediately receiving a 4-h noise exposure at 105 dB and the other half receiving no noise exposure. The second experiment group received 5 days of repeated nose-only inhalation exposures to 1000 mg/m^3 JP-8 for 4 h per day. The group was then split each day with half receiving 4 h 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 levels were within ±2 dB within the exposure chamber. In the first experiment, single exposures to JP-8 without subsequent noise 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 5-day JP-8 exposure alone, impairment of outer hair cell function was observed, but partial recovery was observed over a 4-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 *et al.*, 2007).

Researchers at the Montreal-based Institut de recherché Robert-Sauve en santé et en securite du travail (IRSST) published a review of their database of 224 human and animal ototoxicity experiments in 2012 (Vyskocil *et al.*, 2012). Fifty-one of the experiments combined the effects of a substance with noise. They reported that of 11 chemicals that may have interaction with noise, only two were supported by the research; toluene and carbon monoxide. Two chemicals were concluded as having no evidence of interaction by the studies. Finally, the investigators concluded seven of the 11 chemicals studied for ototoxic interaction were simply inconclusive. Particular criticisms included noting that several toluene otoxicity animal studies did not have simultaneous noise and toluene exposure. A multi-animal, multi-level, whole body exposure facility would allow sufficient test animals to more readily find the potential ototoxic effects of suspected substances.

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 2012 NAMRU-D protocol and were the impetus for this study. The NAMRU-D facility offered a distinct difference in the ability to deliver a whole-body JP-8 aerosol exposure and noise exposure at multiple levels to

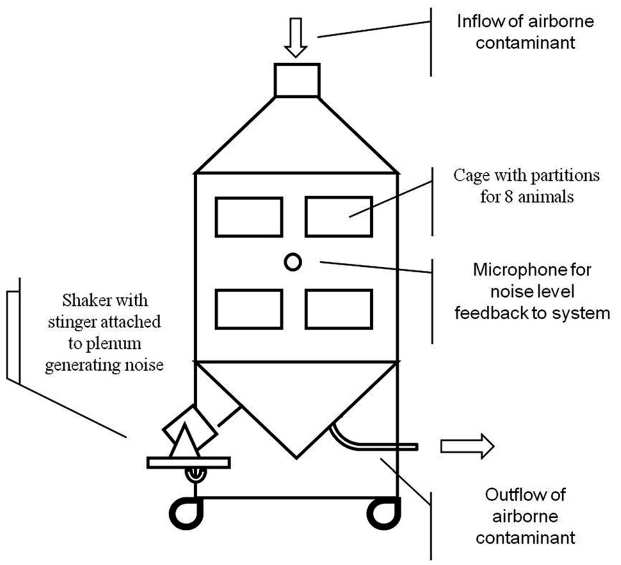

FIG. 1. Whole body aerosol exposure chamber. Representative locations of shaker, microphone, cage assemblies, and airborne contaminant inflow/outflow are shown. The shaker induces vibration into the chamber plenum in order to generate noise within the chamber.

multiple animals 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, particularly the use of a shaker to induce sound waves within a chamber.

II. METHODS

A. Facilities

All experiments conducted in this project were carried out at the NAMRU-D inhalation laboratory at Wright-Patterson AFB, OH. The inhalation laboratory is located in a room with dimensions of approximately 8.53 m × 12.8 m with a 3.05 m ceiling and contained 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 Fig. 1.

Each chamber has four sets of rails onto which cage assemblies slide. Cage assemblies are constructed of wire mesh with 1.27 cm spacing and each assembly is divided into eight individual compartments that hold one animal each. Each chamber can hold four cage assemblies for a maximum of 32 animals in any test.

B. Noise delivery system

A Modal Shop Model 2025E shaker (The Modal Shop, a PCB Company, Cincinnati, OH) paired with a pro audio, QSC Audio Model RMX 1450 (QSC Audio, Costa Mesa, CA) power amplifier and Ultragraph Pro FBQ6200 31-Band Two-Channel Equalizer (Behringer, Willich, Germany), used a stinger attached directly to the underside stainless steel plenum of the exposure chamber. The shaker was mounted in a trunion base on a shelf attached to the legs of the exposure chamber to prevent movement during the test period (see Fig. 1). The Model 2025E shaker used a unique design well-facilitated to the noise requirements of the study. The trunion base allowed for full rotation of the shaker and positioning for perpendicular approach to the plenum. The stinger was 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 had 10-32 threading that screwed into the mounting discs adhered to the plenum and the stinger was locked tight to the shaker end using a chuck and collet attachment. The stinger connection design simplified the setup between the shaker and the plenum, allowed for the shortest stinger length to be achieved easily, and prevented unintentional binding of the armature during setup. Short stinger length was desirable as it reduced the amount of mechanical filtering the stinger may apply to the input signal. The design also allowed for ease of repeatability from chamber-to-chamber and easy stinger replacement in case of failure. The 2025E also provides an in-line fuse between the amplifier and the shaker to prevent damage to the shaker in the event of a power surge from the amplifier.

Audacity version 1.3 freeware (Audacity Development Team, audacity.sourceforge.net) was used to generate the required noise signal 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 11.2 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.

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 in-line versus the software filtering alone. Similar to filtering implemented by the Audacity software, the crossover was set to filter between 5.6 and 11.2 kHz with a 48 dB roll-off on each end using a Linkwitz–Riley filter type. Measurements were taken with and without the crossover in-line for comparison. The DCX2496 crossover model is capable of simultaneously running three input channels and six output channels for future consideration in running all three exposure chambers

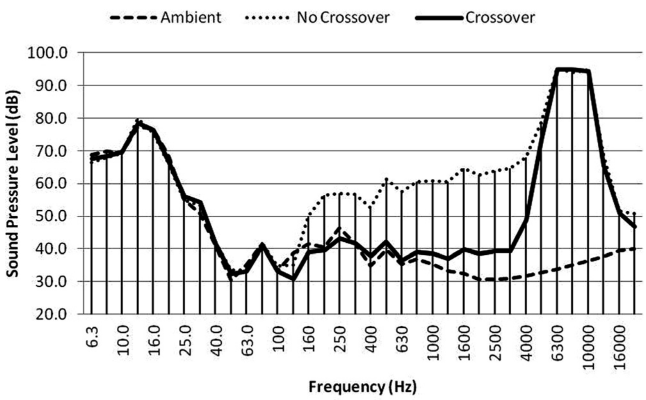

FIG. 2. Comparison of frequency spectrum with crossover in-line. Crossover hardware filtering improved conditioning, with steeper roll-off on the left side of the 8 kHz octave band as compared to software filtering alone.

TABLE I. JMP statistics for consolidated 10-point randomization data set. Mean sound pressure levels are well controlled around the target center points of 75, 85, and 95 dB. Confidence intervals indicate minimal spread around the center of the sample population. Prediction intervals indicate that the range for future expected values falls within the ±2 dB tolerance requirement for each of the three chambers.

	75 dB Chamber	85 dB Chamber	95 dB Chamber
Number	90	90	90
Mean ± std dev (dB)	75.05 ± 0.80	84.92 ± 0.70	94.81 ± 0.71
CI ($\alpha = 0.05$)	74.88 – 75.21	84.77 – 85.06	94.66 – 94.96
PI ($\alpha = 0.05$)	73.45 – 76.64	83.53 – 86.31	93.40 – 96.22

simultaneously. Figure 2 shows the frequency spectrum of the noise with and without the crossover inserted.

Sound measurement was conducted with a model 831 integrating sound level meter (Larson-Davis, Depew, NY) and 378B20 1.27 cm diameter random incidence microphone (PCB Piezotronics, Depew, NY). The sound measurement system was calibrated from the factory within a year and pre and post checked with a CAL200 acoustic calibrator (Larson-Davis, Depew, NY) daily.

C. Final system

1. System installation

A Spectral Dynamics Puma data acquisition system (Spectral Dynamics, San Jose, CA) was installed to collect and record noise measurements. The system as delivered had four active input channels for monitoring and recording real-time sound levels in the Control, 75, 85, and 95 dB chambers simultaneously. A 6.1 m coaxial cable was connected to each of the output channels and a PCB Model 378B20 1.27 cm random incidence microphone assembly was connected to the other end of each cable. A 1.27 cm 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 served 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 vibrations in the PVC pipe and provide tighter seating within the pipe.

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 display was set to display a bar graph of the 8 kHz octave band decibel level inside each of the exposure chambers.

2. Chamber characterization

The first phase of the NAMRU-D protocol utilized 10 rats in each exposure chamber. As discussed previously, each chamber held 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 cage assembly 4 being the lower left cage assembly (see Fig. 1.). The rats started with a 3-2-3-2 distribution pattern in the four quadrants, meaning three rats were 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 was determined by a random assignment scheme developed by the NAMRU-D. Tennis balls have been used to characterize contaminant flows through the chamber for inhalational studies, so tennis balls were also used in place of rats for the noise characterization study.

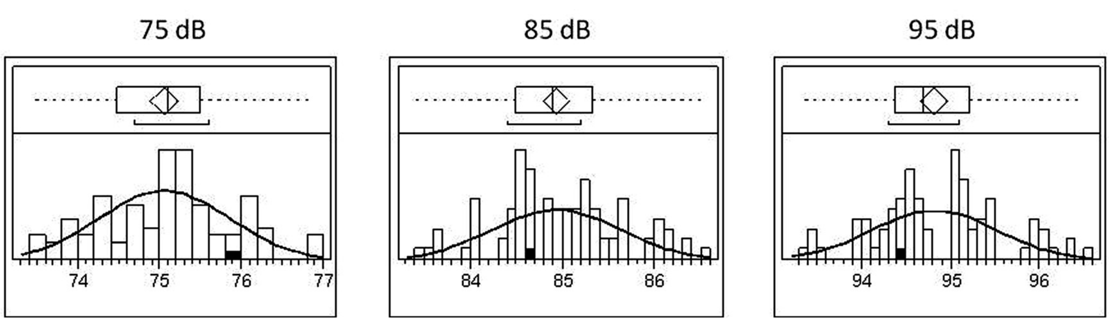

FIG. 3. JMP histograms for 10-point randomization consolidated data set. Plots show desired grouping within the required +/−2 dB range around 75, 85, and 95 dB center points, respectively, with no outliers.

TABLE II. Probability of exceeding ±2 dB distribution with 10 points. Results show that there is negligible probability that an individual sample point will fall outside the protocol sound pressure level tolerance range at any chosen exposure point within any of the three exposure chambers.

	75 dB Chamber	85 dB Chamber	95 dB Chamber
z-value	2.51	2.87	2.83
Probability of exceedance	0.012	0.0042	0.0046

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 NAMRU-D randomization scheme was used to select the assignment points. A Larson Davis model 831 sound level meter with a 6.1 m extension cable and microphone preamplifier 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 s 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.

Two more sets of measurements were taken at each point with a span of 30 min 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.

3. Shaker endurance tests

Endurance tests were conducted to assess shaker performance over continuous 6-h runs. All three shakers, one at each of three exposure chambers, were run simultaneously on each test day. Ten tennis balls were randomly assigned within each chamber, representing 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 were started and the system was allowed to run for a minimum of 6 h. The process was repeated over 5 successive days. The comma separated value files were imported into Microsoft Excel (Microsoft, Redmond, WA) for analysis.

III. RESULTS

A. 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 NAMRU-D protocol. During the first phase of characterization, 10 tennis balls were placed in each exposure chamber. Initial assignment points were chosen using the NAMRU-D randomization scheme previously discussed. 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.

JMP 8 (SAS Institute Inc, Cary, NC) statistical analysis software was used to 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 Fig. 3 and Table I.

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. The calculated z-scores and associated probabilities of exceeding the ±2 dB tolerance limits are in Table II.

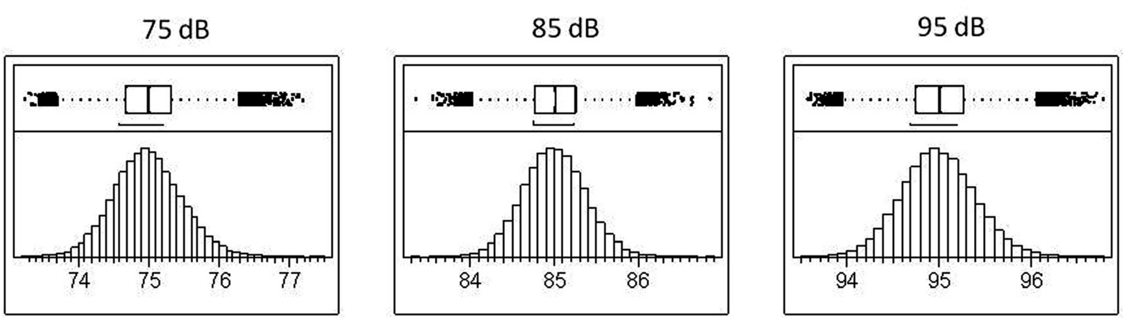

FIG. 4. Distribution of 5-day consolidated data set for each chamber. Plots show well-defined, normal distributions grouped within approximately +/−1 dB around 75, 85, and 95 dB center points, respectively. Results indicate that shakers performed within the required dB tolerance range over the course of 6-h endurance trials.

TABLE III. JMP statistics for consolidated 5-day endurance run data. Mean sound pressure levels are tightly controlled around the target center points of 75, 85, and 95 dB. Confidence intervals indicate negligible spread around the center of the sample population. Prediction intervals indicate that the range for future expected values falls within the ±2 dB tolerance requirement for each of the three chambers.

	75 dB Chamber	85 dB Chamber	95 dB Chamber
Number	68 636	68 636	68 636
Mean ± std dev (dB)	74.99 ± 0.48	84.99 ± 0.36	94.99 ± 0.39
CI ($\alpha = 0.05$)	74.98–74.99	84.99–84.99	94.99–95.00
PI ($\alpha = 0.05$)	74.04–75.93	84.28–85.70	94.22–95.77

All trials with 10 tennis balls resulted in distributions that were well within the NAMRU-D 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 NAMRU-D 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.

B. Shaker endurance tests

Data from 6-h endurance runs over 5 days were recorded by the Puma system using a single central reference microphone in each chamber. Data were exported to Microsoft Excel and converted from pascals to decibels. JMP software was then used to further analyze and describe the distribution of the data. All 5 run days were combined into a single large data set ($N = 68\ 636$). 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 Fig. 4 and Table III. 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 IV).

The shakers driving all three chambers performed exceptionally well over repeated 6-h endurance runs, resulting in mean values well within protocol distribution requirements over time. The statistical analysis also indicated 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.

TABLE IV. Probability of exceeding ±2 dB tolerance over a 6-h run. Results show that there is negligible probability that an individual measurement will fall outside the protocol sound pressure level tolerance range over the course of a 6-h run in any of the three exposure chambers.

	75 dB Chamber	85 dB Chamber	95 dB Chamber
z-value	4.16	5.51	5.06
Probability of exceedance	0.000	0.000	0.000

IV. DISCUSSION

Through this effort, all requirements of the NAMRU-D study protocol were met and the measures of effectiveness were evaluated. A system was designed to deliver protocol noise requirements and installed on existing NAMRU-D exposure chambers without major chamber modification. The system was capable of generating an average 8 kHz SPL distribution within ±2 dB at 10 randomly assigned exposure points in each chamber. The system maintained a ±2 dB distribution at a central reference point over 6 h runs for 5 days in a row. The system withstood repeated 6-h runs without performance degradation. The system provided a real-time view of operating status, including alarms for conditions that go out of limits. The system also continuously logged run data in the background and stored over 13 000 lines of data over a 6-h run.

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-h 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 as modal failure testing or controlling vibration tables. This research effort represented 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 gave the NAMRU-D a unique capability to deliver noise and whole body aerosol exposures to many animals at different concentrations simultaneously.

V. CONCLUSION

Recent data from the U.S. Department of Veterans' Affairs underscores the growing problem of increased annual hearing loss claims across the DoD. Studies by Kaufman *et al.* (2005), Fechter *et al.* (2007, 2012), and others led to the current state of knowledge and suggest that the Air Force may have a cause for concern with simultaneous personnel exposures to noise and JP-8 jet fuel. The system designed in this study enabled NAMRU-D research to add to that knowledge base. Results of the 2012 NAMRU-D 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.

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