

# Noise and Vibration in the Vivarium: Recommendations for Developing a Measurement Plan

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Noise and vibration are present in every room of laboratory animal vivaria, with great variability from room-to-room and facility-to-facility. Such stimuli are rarely measured. As a result, the many stakeholders involved in biomedical research, (for example, funding agencies, construction personnel, equipment manufacturers, animal facility administrators, veterinarians, technicians, and scientists) have little awareness of the effects such stimuli may have on their research animals. Noise and vibration present a potential source of unrecognized animal distress, and a significant, uncontrolled and confounding variable in scientific studies. Unmeasured and unrecognized noise and vibration can therefore undermine the fundamental goals of the 3R's to refine animal models and reduce the number of animals used in biomedical and behavioral research. This overview serves to highlight the scope of this problem and proposes a series of recommended practices to limit its negative effects on research animals and the scientific data derived from them. These practices consist of developing a written plan for managing noise and vibration concerns, assessment of noise and vibration both annually and whenever unexpected changes in the facility or animals are observed, and for maintaining levels of chronic noise below thresholds that might cause animal welfare concerns or disruptions in ongoing studies.

**Abbreviations:** db, decibel; g, gravitational acceleration; Hz, Hertz; JND, just noticeable difference; kHz, kilohertz; SPL, sound pressure level

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Noise, ultrasonic noise (sounds above 20 kHz which are not audible to humans, but are audible to research animals), and vibration are ubiquitous but seldom measured in our research animal vivarium and laboratory environments. As such, they represent largely uncontrolled, unmeasured, and unrecognized confounding variables that impact research and animal welfare.<sup>11,16,24,29,36,37,40,41,48,49</sup> The problem is perhaps even more significant when we consider that the vast majority of animals used in research are mice and rats, which are nocturnal, tunnel-dwelling species that have evolved to rely heavily on their senses of hearing and touch/vibration.

The Guide for the Care and Use of Laboratory Animals<sup>19</sup> mentions the problems of noise and vibration in the animal facility 39 and 28 times, respectively. The Guide effectively warns stakeholders (facility managers, technicians, veterinarians, and scientists) that noise and vibration in the research animal facility can be stressors for research animals, and can skew the outcome of the research. The Guide offers limited guidance on how to manage noise and vibration concerns, how/whether such variables should be measured, and provides no hard information about what levels or ranges of noise and vibration are normal or acceptable in the vivarium. Another resource sometimes used by research facilities, but which focuses more on concerns related to construction, is the US National Institutes of Health Design Requirements Manual (DRM).<sup>28</sup> The DRM also notes

the adverse effects of noise and vibration on lab animals, and helps inform all stakeholders regarding key issues during the design, construction, and commissioning of spaces. The DRM suggests that vivarium environments remain below NC45, in an empty room with no equipment or animals. However, the NC (noise criterion or noise rating curve) measure of room noise is designed for human hearing only, overemphasizing sounds in the human speech frequency range and only concerned with sounds between 63 to 8,000 Hz. As such, NC45 offers very little value for understanding how such noise levels are related to what research animals might hear. The DRM also notes that when animals are present or if ventilated caging or other equipment is used, the acoustical consultant and head veterinarian must decide how loud is too loud, on a per-project basis.<sup>28</sup> The DRM Manual lists no specific vibration level to avoid for animals (other than a standard for structural velocity of floors), instead noting that animals are very sensitive to vibration, that rooms housing animals should have low noise and vibration tolerances, and that researchers should be consulted regarding vibration levels acceptable to animals.

This dearth of information means that researchers responsible for animal husbandry have limited guidance on how to deal with noise and vibration concerns, what to measure, why measure it, how to measure it, and what levels to avoid. Others have reviewed the many problems associated with noise and vibration in the vivarium,<sup>11,16,24,29,36,37,40,41,48,49</sup> so the purpose of the current overview is to propose a series of noise and vibration practices that can provide conservative guidance for facility management and other stakeholders until such time that the research literature and/or other resources can provide more

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definitive guidelines. The recommended practices found here are based on both the research literature and direct experience with these measurements in many dozens of different research animal facility environments.

The problem of noise and vibration is compounded by the fact that every year we introduce more electronic and mechanical equipment into the vivarium and procedure rooms.<sup>25</sup> While new technology can help to solve some problems, such as ventilated caging systems helping to control odor and air particulates, such technology can sometimes simultaneously introduce potential new sources of audible and ultrasonic noise, and vibration. As a result, the problems associated with these factors in the vivarium and animal research environment are of increasing concern and constantly evolving as new equipment enters the vivarium and research laboratory.

The recommended practices described in this overview consist of the 4 items in Figure 1, and are further described in the following sections. These recommended practices are conservative, and facilities should generally have little problem achieving these standards with minimal resources and planning. Stakeholders should be aware that future guidelines might reveal that even lower levels of noise or vibration are desirable based on either new research, or the model/species-specific needs of the studies at a particular site (Figure 1).

## Plan

Facilities should maintain a written noise and vibration measurement, training, communication, and action plan. A written noise and vibration plan (NVP) need not be exhaustive, but it should briefly articulate the institution's position on staff training, regarding the recognition of aberrant noise and vibration levels and how to mitigate these problems. The plan should also describe the methods and frequency for the measurement of noise and vibration, and how and when those measurements are communicated to stakeholders. Having such a plan can promote open communication during normal operations of the facility, and particularly during the more stressful times associated with disease outbreak, construction, or equipment upgrades.

Developing a written plan for how to deal with noise and vibration helps create a climate of care and attention to these important variables. It also identifies noise and vibration as variables deserving attention from the institution, on par with others that are known to disrupt or harm animals, such as flood or viral outbreaks. In addition to defining what is measured, when, and by whom, the plan should recognize that much of the noise and vibration in the vivarium, based upon our measurements in animal facilities, is caused by personnel during normal vivarium operations such as cage changing and cleaning. Therefore, the NVP should include some annual training on the impacts of noise and vibration on research animals. In addition, the plan should recognize that noise and vibration concerns become most intense during construction or renovation projects. These typically produce high levels of noise and vibration that may impact animals, disrupt ongoing studies, and strain relationships between stakeholders and construction personnel. Construction projects are often blamed (appropriately or not) whenever any changes are observed in animal behavior or breeding, or when the researcher's studies simply do not work out as expected. To minimize the effects of construction-related noise and vibration on animals, ongoing studies and relationships with researchers, the construction process should be managed very carefully. Developing a training, communication, and action plan for facility administrators,

veterinarians, technicians, and scientists will minimize the effects of noise and vibration on animals and ongoing studies, set a standard for better communication between all stakeholders, and avoid misunderstanding and unwarranted concern or confusion.

Implementing a communication plan for normal day-to-day operations is advisable, but is absolutely critical during times of disease outbreak, new equipment installation, or during construction. Such a plan can help to maintain open lines of 2-way productive communication between all parties involved and help to prevent miscommunication.

Development of a master template plan for facilities has some appeal; however, such an undertaking is complicated in that every facility has different needs, different species, different kinds of research being conducted, different problems, different personalities, different administrative structures, and different histories. However, to aid facilities in their attempts to develop such a written plan, some key features of such a plan are provided in Figure 2.

## Annual Assessment

Facilities should conduct an annual noise and vibration assessment. The primary purpose for this annual assessment is to periodically review the written NVP and to consider whether noise and vibration concerns have emerged in the facility during the last year, and if so, what could be done to address them. This annual assessment could take many forms depending on the scope of the institution, its history of noise and vibration complaints/concerns, and its resources. Some facilities might opt to conduct a thorough annual noise and vibration measurement to provide an annual "check-up" of the facility. Others might elect to simply review their NVP and consider whether any changes need to be made to it, based upon the year's experiences. Annual assessments could include measurements from the macroenvironment and from the cage-level microenvironment, to determine which macroenvironmental noises and vibrations are reaching the animal's microenvironment. Ideally, such measurements could also be made in areas the animals experience during transit, and in laboratory spaces where animals are taken for procedures as these spaces often contain different sources of noise, ultrasonic noise and vibration (for example, computers, lab testing equipment, ultrasonic mixers, ultrasonic motion sensors).

Collecting annual noise and vibration measurements as part of the assessment provides a potential dual benefit in that, in addition to telling us what animals are hearing and feeling, this would allow tracking of the mechanical health of the aging equipment and components, which can cause the production of more noise, ultrasonic noise and vibration. Industrial settings routinely make use of noise, ultrasonic noise and vibration measurements from equipment to serve as predictors of mechanical faults. This process is called condition monitoring and is routinely used in industrial settings to measure the mechanical health of equipment and conduct data-driven, planned maintenance. This monitoring can identify if key components (pumps, blower motors, compressed gas leaks, etc.) need replacement to prevent the energy loss, downtime, and expense that comes with equipment failure.<sup>27</sup> For example, computers, test equipment, fluorescent lighting ballasts, and any equipment in the vivarium with a blower motor (ventilated caging, cage changing hoods) can generate greater noise, ultrasonic noise, and vibration as they age and components begin to fail.

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1. **Plan.** Facilities should maintain a written, comprehensive noise and vibration measurement, training, communication, and action plan.
  2. **Annual Assessment.** Facilities should conduct an annual comprehensive noise and vibration assessment.
  3. **Changes.** Facilities should conduct additional monitoring/measurements when changes in the animals are observed (for example, breeding problems) or when the vivarium itself changes (for example, construction or introduction of new equipment.)
  4. **Thresholds.** Thresholds of concern for animal welfare for chronic noise and vibration in the animal's cage/microenvironment should minimally be set to 70 dB for noise and 0.025g for vibration, recognizing that much lower levels of either noise or vibration can still disrupt more sensitive species, models, or assays.
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**Figure 1.** Recommended Practices (PACT) for minimizing negative effects on research animals, ongoing research studies, and relationships with scientists.

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1. Describe what individual(s) or group is in charge of developing and managing the plan, as well as the relevant stakeholders.
  2. Describe what information or training on noise and vibration is provided to staff and/or scientists.
  3. Describe the program's measurement plan. Describe who will be conducting the measurements, what will be measured, and how/whether those measures will be shared with PIs, administrators, construction team.
  4. Describe how the measurements will inform any relevant mitigation steps, and what findings will prompt what actions (placing vibration mats under racks, moving animals, etc.).
  5. Describe what animal behavioral and health observations will prompt measurements.
  6. Describe what construction/renovation actions will prompt measurements.
  7. Describe when key stakeholders will be alerted to noise and vibration levels of concern (for example, construction point person, veterinarian, the scientist's whose animals are impacted).
  8. For construction specifically, note what preventative measures will be taken to isolate the noise or vibration to the source, what construction practice modifications could be made IF required by the measurements to minimize spread to the animal spaces, and what practices in the vivarium can be employed if noise or vibration reaches unacceptable threshold levels. Clearly identify beforehand if and when construction will be stopped due to noise and vibration concerns in the animal facility, and what person or group is responsible for this decision.
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**Figure 2.** Recommended features of a noise and vibration measurement, training, communication, and action plan.

## Changes

Facilities should conduct additional monitoring/measurements when changes in the animals are observed (for example, breeding problems) or when the vivarium itself changes (for example, construction or introduction of new equipment.) Observed changes in animals themselves could include changes in breeding, behavior suggestive of the presence of a stressor, changes in the general health of lab animals, or changes reported by PIs in their study results that might be the product of an environmental stressor. Intentional changes introduced to the vivarium itself might include the introduction of new equipment to the vivarium or a renovation/ construction project. While it is important to monitor and mitigate noise and vibration levels experienced by animals during any significant renovation/construction process, consideration of noise and vibration should ideally be part of all phases of the construction process, from planning and design, to the choice of equipment and other materials, through the completed commissioning phases of the project to ensure that the new space is appropriate for habitation by research animals with respect to noise and vibration levels.

## Thresholds

Thresholds of concern for animal welfare for chronic noise and vibration in the animal's cage/microenvironment should minimally be set to 70 dB for noise and 0.025 g for vibration, recognizing that much lower levels of either noise or vibration can still be disruptive to more sensitive species, models, or assays (Figure 3).

### 70 dB Noise Threshold of Concern

Noise levels inside the cage should be maintained below 70 dB SPL. Summaries on the problem of noise in the vivarium have been published elsewhere.<sup>48,49</sup> Noise levels in the vivarium will vary dramatically depending upon many

factors, including the type of equipment used. Ventilated caging blower motors, as an example, can produce considerable noise, as can normal room ventilation, cage changing stations, and other equipment such as fluorescent lights and computers. Static caging rooms would typically expect to show much lower levels of background noise, due to the relative lack of equipment producing noise and vibration. Thus, we have observed that some of the most modern animal housing rooms have continuous background noise levels at or near 60 dB, due to ventilated caging blower motors. Moreover, intermittent sounds created by personnel in the room can be much louder than that.<sup>23</sup> For example, the act of snapping lids onto cages or connecting ventilated cages to a rack can produce intensities easily in the 85 to 100 dB SPL range; this level of noise is easily loud enough to produce an acoustic startle reflex in the animals inside or near the cage, as startle thresholds for mice and rats occur around 75 to 80 dB SPL.<sup>21,34</sup>

Independent of potentially stress-inducing short duration noise or vibration occurrences in the vivarium, continuous noise levels of 70 dB or greater could be expected to affect animals in a range of ways. For example, this level of background noise might mask vocalizations or other communications among animals. Although the Guide notes that noise levels of 85 dB can have wide ranging effects on hearing and nonauditory stress pathways, the 85 dB level is based on research designed to determine acceptable noise exposure for people in a typical 8-h human workday; applying that standard to a 24-h exposure period of a research animal is not appropriate. Indeed, the U.S. Environmental Protection Agency (EPA) recommends that humans maintain a 24-h noise exposure average of less than 70 dB to avoid hearing loss.<sup>52</sup> The World Health Organization (WHO) confirmed and adopted the EPA's 24-h noise exposure threshold of 70 dB in 1999,<sup>5</sup> and more recently, confirmed that limit by conducting a comprehensive review of the human and animal research data.<sup>53</sup> Both the EPA and WHO have also recognized

Noise	Vibration
<ul style="list-style-type: none"> <li>• <math>\geq 70</math> dB SPL Chronic Noise Level</li> </ul>	<ul style="list-style-type: none"> <li>• <math>\geq 0.025</math>g RMS Chronic Vibration Level</li> </ul>
<ul style="list-style-type: none"> <li>• *note that lower level signals <math>\geq 45</math> dB SPL might be disruptive to more sensitive models.</li> <li>• Set low and high-end frequency filters to the hearing range of the species being studied.</li> </ul>	<ul style="list-style-type: none"> <li>• *note that lower level signals might be disruptive to more sensitive models and breeding animals, so this level would be recommended for such situations.</li> </ul>
<ul style="list-style-type: none"> <li>• Use a measurement system capable of processing signals in the range of the filter settings for the animal being studied.</li> </ul>	<ul style="list-style-type: none"> <li>• Set low and high-end frequency filters to encompass the vibration range of the species being studied (2-500 Hz setting should normally be sufficient work).</li> </ul>
<ul style="list-style-type: none"> <li>• Report unweighted measurements in dB SPL (re 20 <math>\mu</math>Pa). Do not use human A-weighted measurements. Report whether sound is measured as instantaneous levels or time-weighted averages.</li> </ul>	<ul style="list-style-type: none"> <li>• Report vibration in acceleration (not displacement or velocity) using either g or m/s/s (<math>1g = 9.81 \text{ m/s}^2</math>).</li> <li>• Report whether vibration is measured in peak, peak-to-peak, or RMS and whether it is measured in the x, y, z or all 3 axes.</li> </ul>
<ul style="list-style-type: none"> <li>• Note that regardless of baseline background noise level, changes of 3 dB (JND) or more can likely be heard by the animal.</li> </ul>	<ul style="list-style-type: none"> <li>• Note that regardless of baseline background vibration level, changes of 10% (JND) or more can likely be felt by the animal.</li> </ul>

Figure 3. Key noise and vibration thresholds of concern and key features of measurement details.

that chronic exposure to levels of noise much lower than this 70 dB threshold, around 45 to 55 dB, does not cause hearing loss, but can have significant negative effects on a range of health metrics, largely impacting sleep patterns and cardiovascular function.<sup>5,52,53</sup>

Additional evidence from the laboratory animal hearing research field has demonstrated that chronic exposure to 70 dB SPL noise can affect auditory structures and functions ranging from the cochlea to the cortex, with changes in molecular and anatomic systems. This has implications for functional outcomes for any behavioral and electrophysiologic responses to sound.<sup>3,15,32,33,50</sup> Some evidence further indicates that such low-level, 70 dB noise effects can also be complicated by sex-specific effects.<sup>51</sup>

In addition to the direct effects on the auditory system, noise exposure (even sometimes at the low level of 70 dB) can activate a cascade of stress responses in animals, resulting in changes in many organ systems, including changes in reproductive efficiency.<sup>39,49</sup> The resulting widespread biologic and behavioral effects have the potential to influence virtually every area of biomedical research, ranging from immune system function and sleep/wake cycle disturbances, to cancer and cardiovascular disease.<sup>4,10,36,39,42,53</sup> These nonauditory effects of noise are often unrecognized by researchers, technicians and veterinarians and could represent a source of distress for animals and a potential design confound for many experiments.<sup>42</sup> Even at relatively low intensities, such noise can be damaging to research animals and humans alike. For example, a thorough review of animal and human data reported that environmental noise levels as low as 45 dB (especially while asleep) and in the range from 45 to 60 dB are associated with increased risk of a number of health concerns, including cardiovascular disease and hypertension.<sup>53</sup> Decades of human and animal research have demonstrated that subcritical noise levels can produce a variety of negative health effects due to the activation of stress pathways.<sup>4,43</sup> In addition, numerous additional negative consequences of noise, including sleep disturbances, cardiovascular stress, and learning and memory impairments can occur.<sup>6-8,10,13,36,46</sup>

Noise greater than 20 kHz is considered in the ultrasonic range and is not audible to humans. However, for many species of research animals, thresholds are near 0 dB for hearing ultrasonic noise in the 20 to 40 kHz range. Ultrasonic noise is therefore a potential source of animal stress and a serious experimental confound. Ultrasound noise above 20 kHz should be kept to at least below 45 dB SPL to minimize masking of vocalizations/communications and to limit its potential to disrupt

sleep. Certain ultrasound frequencies can have different effects on different species. For example, sound energy in the 18 to 37 kHz range provides an anxiety-related aversion call frequency range in a rat, whereas higher frequency ultrasonic calls in the 40+ kHz range serve appetitive, mating, and other prosocial interactions.<sup>44</sup> Although mouse vocalizations are less well understood and are perhaps more complex and context-dependent, lower-frequency ultrasonic vocalizations may signal aversive or threatening events and higher frequency vocalizations may aid social communication.<sup>12,35</sup> The laboratory environment generally contains many sources of ultrasonic noise, emanating from lighting, computers and other test equipment. This ultrasonic noise may potentially impact the animals or the tests being performed. As an example, consider a classic behavioral test in the learning and memory research field—the Morris Water Maze. In the Morris Water Maze, researchers work diligently to control all possible extraneous variables, such as light, orientation, and general visual cues. However, ultrasonic noise may serve as an invisible cue during training or testing. Because ultrasonic noise is highly directional in nature, it is reasonable to assume that rats and mice can localize this type of noise, which is often produced by laboratory test equipment, computers, lights, and cameras, to provide a directional cue aiding their navigation in the maze. Moreover, because ultrasonic noise levels can vary both within and among laboratories, its effects on animals are unpredictable, and can cause inconsistent and irreproducible effects on any data collected.

### 0.025g Vibration Threshold of Concern

Vibration levels inside the cage should be maintained below 0.025 g (RMS; see below). Note that vibration can occur in the x, y, or z axes and can be measured in all 3 axes or the greatest of the 3. Our experience is that most animal facility vibration reaching animals is in the z (vertical) axis. Recent work has identified the levels of vibration that are perceptible to rats and mice<sup>11,29,37</sup> and thereby potentially capable of causing significant biologic and behavioral impacts on research animals. Perhaps the most commonly reported finding in the vibration literature are elevated corticosterone levels.<sup>1,2,31,38</sup> At magnitudes as low as 0.1 to 0.3 g, fetal pigs showed a significant increase in plasma cortisol and adrenocorticotropin hormone levels.<sup>31</sup> Likewise, vibration levels of only approximately 0.025 g have been shown to increase fecal corticosterone metabolites in female (but not male) mice,<sup>2</sup> and to result in overt behavioral responses in female mice indicative of arousal.<sup>11</sup> In addition to stress systems, many secondary systems are in

turn affected by chronic exposure to vibration, as a result of the stress response. The effects of vibration can be observed in disturbances of sleep, changes in cardiovascular function, and even decreased pregnancy rates.<sup>2,24,47</sup>

In addition to the concern that chronic vibration presents a chronic stressor to research animals, vibration can also create an experimental confound by introducing unknown variability into research studies. Several studies have found significant biologic and behavioral changes in animals exposed to chronic vibration.<sup>30,45</sup> Furthermore, different species and strains may react differently to a given level of vibration, and levels of vibration may vary from cage to cage, rack to rack, and room to room, thus introducing variability within and across studies. Human standards for vibration preceded animal standards and research. International Organization for Standardization (ISO)<sup>20</sup> sets an action level for vibration for an 8-h work day at approximately 0.05 g (0.5 m/s<sup>2</sup>); the standard describes vibration in the approximately 0.05 to 0.1 g (0.5 to 1 m/s<sup>2</sup>) range as “fairly uncomfortable”, and levels over approximately 0.08 g (0.8 m/s<sup>2</sup>) as “uncomfortable”. While no current standards exist for vibration in animal facilities, the approximately 0.05 g (0.5 m/s<sup>2</sup>) action level is likely too high to be helpful. Furthermore, as noted earlier, vibration accelerations at half of that level, as low as 0.025 g, could be potential stressors for animals and confounding factors for research, especially for species, strains and models that are more sensitive.

Normal day-to-day personnel activity in the vivarium will typically generate more noise and vibration than is produced by typical construction activities. For example, the simple act of connecting a cage to a ventilated rack can easily generate startle-inducing 85 dB SPL bursts of noise, and vibration levels around 0.35 g, many times greater than the recommended lower limits. Similarly, animals being transported from a vendor or between locations on a cart can experience high levels of noise and vibration.<sup>18</sup> These handling-related noise and vibration exposures, together with other noise and vibration related to daily care, are likely to produce the greatest sources of noise and vibration experienced by animals. Furthermore, these levels are likely to be many times greater than any noise and vibration produced by construction activities at a facility, which are often viewed as a major concern. Also, the fact that a noise or vibration is felt or heard in the hallway by a human, does not mean that the signals are in the range of detection of the animals or are reaching the animal's microenvironment.

Finally, even if these signals do reach the home cage, research animals, like humans, demonstrate a perceptual phenomenon known as a just noticeable difference (JND), which is the lowest change in the stimulus that is reliably detectable. While these levels can be lower in highly controlled experimental situations, for more complex real-world purposes, the JND (sometimes measured as the intensity difference limen) for noise is approximately 3 dB,<sup>22,54</sup> and for vibration it is approximately a 10% increase from the background vibration.<sup>26</sup> Thus, any activities, such as construction, that do not generate an *increase* in the cage-level microenvironment of 3 dB for noise, or a 10% increase for vibration, might just be barely detectable by the animals, and can likely be considered benign. However, just because a stimulus is barely detectable does not mean it is meaningful, or that it causes problems. So individual facilities should use these values with interpretive caution, as it is likely that levels well beyond these minimum JND threshold levels would be needed to create a meaningful difference in the background that activates stress pathways or otherwise disrupts animals.

Additional research is needed before more definitive statements can be applied to this JND standard.

## Noise Measurement Details

A critical feature of noise measurement in both the macroenvironment (for example, hallways, center of vivarium room, outside cages) and microenvironment (inside cages) is that the microphone system must be capable of measuring the hearing frequency ranges of the species of interest. Mice, rats, and most other nonaquatic species used in biomedical research facilities can hear ultrasonic frequencies above the human upper limit of 20 kHz.<sup>14</sup> Therefore, the microphone and related processing equipment in a typical rodent facility should be capable of measuring sounds at least throughout the hearing range of a normal mouse, which often extends well into the 80 to 90 kHz range. Noise levels should be measured and reported as calibrated, unweighted dB sound pressure level (SPL) measurements. Reporting sound in dB SPL provides an absolute, calibrated sound level referenced to a standard pressure of 20 microPascal, which is generally considered the lowest intensity signal that can be heard (threshold) by a healthy young person (see<sup>17</sup> for a review of sound measurement). This can be accomplished with several methods; the most often used approach is to apply a calibration tone of known pressure of 1 Pascal, which is the pressure equivalent to 94 dB SPL, from an acoustic calibrator that has itself been calibrated within the last 12 mo. Most noise meters used by occupational or environmental health and safety offices (for example, for OSHA-based workplace noise exposure) are designed for measuring sounds audible for humans and are A-weighted, a process that adds gain to some sound frequencies and lowers gain to others, to fit the sound measurements to a range that is considered to be optimal for human speech. Although A-weighted measures are appropriate (and required) for determining human noise exposure, they are not appropriate for estimating noise exposures of animals. Noise measurements that are relevant for nonhuman animals remove the A-weighting and collect unweighted measurements (often referred to as Z-weighting, or unweighted).

The processing and analog-to-digital sampling rate of the microphone and meter system must be at least twice the frequency of the signal to be captured, to prevent signal loss or aliasing (Nyquist-Shannon Sampling Theorem;<sup>9</sup>). To measure a 96,000 Hz sound, one needs both a microphone with a flat response profile up to this frequency and a digitizer capable of digitally sampling the analog signal at a rate of at least 192,000 Hz (96,000 × 2). Additional information on comparative hearing across species can be found in<sup>14</sup> and a referenced listing of detailed hearing ranges of different species is maintained at [www.laboratoryforcomparativehearing.com](http://www.laboratoryforcomparativehearing.com).

Measurements of noise in the cage (microenvironment) should be taken to best simulate the experience of the animals, at the approximate head height of the animal and with bedding and any other elements typically present in the cage (enrichment, food, water bottle). The presence of bedding and other items better simulates the normal experience of the animal in the cage by providing similar sound absorption and reverberation features. In our experience, and consistent with the physics of sound, the presence of food and bedding serve to lower noise reverberation and the levels of noise in the animal cage, so measurement taken without bedding or food, as an example, can provide intensity readings that can be louder than they really are for animals housed in bedded cages.

## Vibration Measurement Details

Vibration, as with noise, should be measured in both the macroenvironment (for example, floor, wall, rack) and the microenvironment (inside cages). Microenvironmental vibration measurements should be collected with normal bedding, enrichment items, and food in place to better simulate the real experience of the animal, but also because such items add more mass to the cage, which depending on the bedding type and thickness, can also help to limit/absorb some of the vibration (and noise). Vibration should be measured from the bottom middle of the cage surface itself, as species like mice and rats often burrow down into the bedding material such that their bodies directly contact the cage. In addition, vibration can occur in the x, y, or z axes. Some prefer to measure all 3 axes while others prefer to measure just the vertical (z) axis, or the greatest of the 3. Our experience is that the greatest vibration in animal facilities tends to occur in the vertical z axis under normal circumstances.

Many building/architectural engineers report vibration in terms of length of displacement of a structure, as in meters (m; how far the object moves), or in speed of movement of a structure, as in m/s (how fast the object moves). Vibration acceleration is change in velocity, represented in m/s/s ( $m/s^2$ ) or in the equivalent g (gravitational acceleration). The m/s/s metric is more commonly used in countries using the metric system and g is more commonly used in the United States. However, 1 g of gravitational acceleration is = 9.81 m/s<sup>2</sup>, making conversion estimates easy to accomplish by using a multiplier of 10 (within 2% accuracy). As a result, the vibration health literature generally reports findings in m/s<sup>2</sup> or g, whether the results are from crash tests, roller coasters or space flight, or studies on the effects of concussion in football, the effects of vibration on workers using heavy equipment, or the effects of vibration on research animals. We recommend use of RMS (root mean square) as it is a commonly used standard in vibration measurement. Vibration accelerations can also be measured as peak level or peak-to-peak levels, but which type is used should be noted, as conversions between the 3 can be easily estimated. RMS is the most commonly used standard in vibration acceleration measurement, because it accurately measures a time-varying phenomenon with positively and negatively moving waves, as is found in vibration. RMS is also used in sound measurement but it is typically not designated in the label as the dB SPL computation implies/requires use of RMS data for its calculation.

As with sound, measurements of vibration must capture the relevant frequency content that is perceptible by that particular species. Fortunately, commonly used research species generally have a vibration perception range that is quite similar to that of humans, and most off-the-shelf accelerometers will easily accommodate this range. Vibration should minimally include the range of frequencies detectable by research animals, measured as Hz = number of cycles or oscillations per second. Just as a violin will vibrate at a different frequency than a cello, species with different body sizes will vibrate maximally within different frequency ranges. This is known as the resonance frequency range. For mice, the body cavity vibrates optimally between approximately 30–100 Hz<sup>37</sup> and mice appear to be most impacted by frequencies in the 70 to 100 Hz range.<sup>41</sup> As the species' body gets progressively larger (for example, from rat to cat to human), the resonant frequency range adjusts down accordingly. However, different species appear to show substantial overlap in touch perception sensitivity, as skin touch mechanoreceptors, whether in the foot pads of mice or on the skin surface of a human, show similar features. There-

fore, vibration measurement devices that include frequencies down to approximately 2Hz and up to at least 500 Hz should be more than adequate for most animal species used in research. Nevertheless, just as different frequencies of sound might have different behavioral or ecological significance to a species, different frequencies of vibration might also have differential effects.<sup>11</sup> Therefore, vibration frequencies that overlap most with a species resonance frequency range would likely be most harmful to them. For a particularly thorough, recent review of these and related vibration effects on research animals, see.<sup>41</sup>

The recommended overall levels of noise and vibration proposed here focus on maintaining levels below certain key intensity thresholds, within the perceptible frequency range of the species being studied. While different frequencies of sound and different frequencies of vibration likely have differential impacts on animals, adding such frequency-dependent qualifiers or some form of complicated frequency weighting system would unnecessarily complicate the recommendations and their implementation. Indeed, the field of human noise exposure, where much more research is available, follows a similar principle. While different frequencies of sound have different auditory and nonauditory impacts on humans, standards set for human occupational settings still limit overall average noise levels for an 8-h workday to 85 dBA, without regard to frequency content. The WHO<sup>53</sup> still argues for 45 dBA being a threshold of concern for sleep disruption and increased risk of health concerns. While the current recommended levels of noise and vibration focus on intensity within the perceptible frequency range of that particular species, all sound or vibration frequencies may not have the same impact on lab animals. However, building such qualifiers or complicated weighting systems into recommended levels would only serve to obscure the goals of measuring and limiting unnecessary noise and vibration exposure, and would severely hamper implementation of reasonable measurement practices. However, these recommended levels should also be considered a minimum, conservative standard. Much lower levels, or a frequency-dependent version of such levels, could best serve a particular site/program. Furthermore, with more widespread measurement practices, additional research will necessarily follow that will further refine the conservative levels proposed here. As with temperature, humidity, and light levels, the standards published in the Guide<sup>19</sup> are merely starting points and require additional information for their optimal use. We expect a similar path will be taken for noise and vibration and future work will further refine these conservative starting points.

## Conclusion

The noise we hear, ultrasonic noise we do not hear, and vibration we feel can all serve as potential stressors to research animals and can introduce confounds into our research studies. Noise and vibration are ubiquitous and vary greatly across our facilities, within facilities from room-to-room, and even within a room from rack-to-rack and cage-to-cage. This can introduce unrecognized variability to our research models, which plays havoc with our ethical goals of reduction and refinement. Stakeholders in the laboratory animal science field should engage in a concerted effort to measure and manage noise and vibration in the vivarium to help better understand their effects on our model systems, and to help bring these important variables under control.

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