

# Evaluation of Vibration as an Extrinsic Variable in In Vivo Research

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Vibration is inherent in research animal facilities due to the mechanical systems and practices required for animal care and use. Ample evidence indicates that vibration can change behavior and physiology in multiple species, potentially altering the results of research studies. Although one cannot eliminate environmental vibration, its control is important in research animal environments to decrease the possibility of introducing a research variable due to vibration effects. To assess the potential for a vibration source to alter experimental results and variability, one must understand the principles of vibration, its likely sources, and control methods. The literature regarding the effects of vibration, as it applies in a practical sense, can be challenging to interpret because the vibration frequencies tested to date have often not been within or near the most sensitive ranges of the species being tested. Some previous studies have used unrealistic vibration magnitudes and provided insufficient detail to duplicate or build upon conclusions. Standardization is essential for research examining the effects of vibration on animals to validate knowledge of this extrinsic variable in animal research and identify ways to mitigate the variable in research facilities.

**Abbreviations and Acronyms:** Hz, hertz; HVAC, heating, ventilation, and air condition

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## Introduction

Vibration can produce positive effects in animals, such as increased bone density<sup>11,12,15,18,55</sup> and bone healing,<sup>8,12,27</sup> attenuation of insulin resistance,<sup>33</sup> and decreased skin healing time in mice with diabetes.<sup>53</sup> Animals may serve as models for the study of these factors in humans.<sup>44</sup> However, for the most part, vibration in the animal facility is considered a potential general stressor to the animal population.<sup>10,43</sup> Studies have shown that even routine husbandry practices and equipment commonly used in the animal facility can expose animals to vibration,<sup>4,13,35</sup> leading to concerns that vibration could affect animals being used in research studies. For example, increased hair corticosterone levels in mice on the top row of individually ventilated racks, relative to mice on lower levels, were attributed to the higher level of vibration caused by proximity to blower motors on top of the rack.<sup>13</sup> The number of cages on the rack has also been shown to affect each cage's vibration level.<sup>4</sup> However, these subtle differences in animal housing may or may not cause a variable in research studies because any effects on animals would depend on the frequency and magnitude of the vibration, the sensitivity of the species, and the nature of the study.

We still have a lot to learn about vibration characteristics that could introduce research variability and the nature of these effects on animal anatomy, physiology, and behavior. As a stressor, vibration exposure has been shown to trigger a startle response and other fear-related behaviors in mice.<sup>16</sup> Increased vibration has been associated with elevated stress hormones in mice, rats, and pigs<sup>2,3,6,20,40,42</sup> and affects the cardiovascular system of mice, indicating stress.<sup>31</sup> Vibration may also have effects at

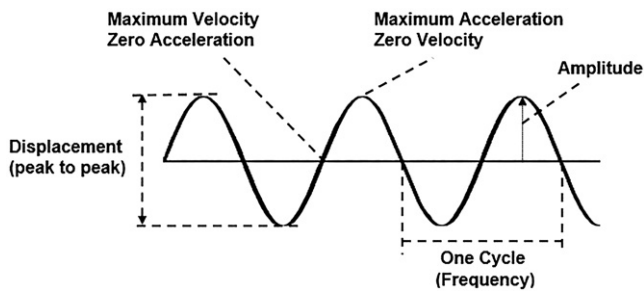
the cellular level.<sup>43</sup> To assess the potential impact of vibration on animals and understand how to mitigate its effect, the basic principles of vibration must be understood. These principles include vibration waveform, directionality, the concept of resonance frequency, and the potential for animals to adapt to vibration. The following overview summarizes the physical principles of vibration, the most common sources in the research animal facility, and general ways to mitigate vibration. Further, this review includes a summary of factors to consider when conducting vibration studies on animals and the subsequent publication of these studies.

**Principles of vibration.** Two primary factors associated with the adverse effects of vibration on physical structures are the amount of inherent energy contained within the vibration and how fast the vibration movement occurs. One can visualize the oscillating nature of a vibration waveform by envisioning slow-motion vibration in a metal bar as it pushes upward to a point where it stops and then moves back past its neutral position until it stops in the downward position, only to move back up. One cycle of the repetitive motion of vibration is the movement from a point on the wave to the same point on the next wave, including both upward and downward motion. (Figure 1). The number of cycles that occur per second is called the frequency, which is measured in hertz (Hz) units: one Hz is one cycle per second. The amplitude is a measure of vibration intensity and is the distance that the bar moves in one direction from its resting position.<sup>19</sup>

As illustrated in Figure 1, the three measures of vibration magnitude are displacement (peak to peak of the waves), velocity, and acceleration.<sup>19</sup> Displacement, as measured from the peak where the vibrating bar moves in one direction to the movement's peak in the other direction (peak to peak), can help standardize the total movement for reporting. Displacement is measured in distance (e.g., millimeters) and can occur in three

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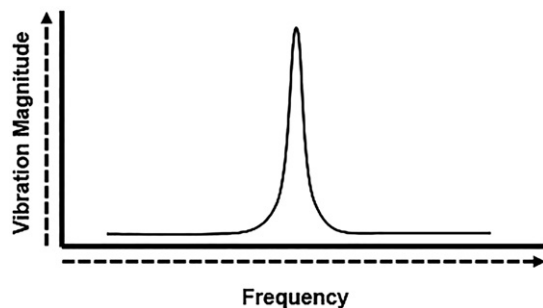
**Figure 1.** Fundamental properties of vibration waves, including amplitude, frequency, displacement, velocity, and acceleration.

axes: the X-axis (horizontal), the Y-axis (front to back, back to front), and the Z-axis (vertical). Different vibration sources can cause more pronounced vibration in one of these axes relative to others. Vibration in the vertical (Z-axis) is thought to be the predominant direction of the vibration that reaches animals.<sup>49</sup>

Velocity is a measure of the speed of movement and is expressed in units of distance of displacement over time (e.g., m/s). Because of the wavelike pattern, the movement's speed slows as the bar reaches either extreme, which is where velocity reaches zero before it starts to move in the other direction. Velocity is greatest when the bar passes through the neutral position.<sup>19</sup>

In contrast to velocity, acceleration is greatest at the peaks of the waves due to the force to move in the other direction; acceleration is at zero when it is in the neutral position. Acceleration is the rate at which the velocity of the bar changes as the bar speeds up to go toward the other extreme and is expressed in units of  $m/s^2$  or gravitational force. Measurements of displacement, velocity, and acceleration emphasize the magnitude at low, medium, and high frequencies, respectively.<sup>36,48</sup>

One of the more interesting aspects of vibration is that an object will not vibrate equally at all frequencies, such that some frequencies cause a large amount of vibration of the object and some have less or no effect.<sup>16,31,37,41</sup> To illustrate, Figure 2 plots increasing vibration frequency against increasing vibration magnitude to show how an object will vibrate at some frequencies but not others. Even though the same force strikes the object at all frequencies, each object (including humans and animals) has a narrow range of frequencies, called resonance frequencies, in which the measured magnitude of the resulting vibration is greater than at other frequencies, and the object can amplify the vibration it receives. Here, the curve represents an abrupt increase in the vibration magnitude that subsequently decreases as the frequency increases. An example would be a cage washer that produces vibration



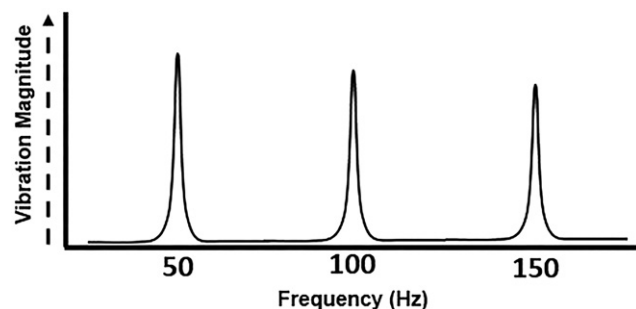
**Figure 2.** Even though the exposure magnitude of a striking or vibrating force remains unchanged, an object will tend to vibrate as a result at a relatively narrow range of frequencies at or near the resonance frequency.

on the floor at various frequencies. Vibration frequencies produced by the cage washer that are further away from the resonance frequency of the floor would have less or no effect on causing floor vibration relative to frequencies near the floor's resonance frequency.<sup>34</sup> Many floors that are problematic regarding vibration-induced discomfort for humans have a resonance frequency of 5 to 8 Hz, which matches the resonance frequency of human internal organs.<sup>9</sup> Therefore, vibration is most problematic when the resonance frequency of the object that is vibrating is close to the resonance frequency of the object exposed to this vibration. Because resonance frequency depends on the object's stiffness divided by its mass, inanimate objects, people, animals, and different species of animals can have different resonance frequencies that cause more adverse effects than other frequencies.<sup>16,31,36,37,41</sup>

Another interesting phenomenon is that vibration can have more significant effects on an object, not only at or near the object's fundamental frequency but also at specific higher frequencies. These secondary frequency ranges are called harmonic frequencies. As shown in Figure 3, these frequencies occur at whole-number multiples of the first number. In this example, the fundamental frequency of an object is 50 Hz, but greater vibration also occurs at frequencies of 100 and 150 Hz (2 and 3 times 50 Hz). Because of this, one must consider not only the fundamental frequencies of vibration exposure but also other secondary frequencies that may cause an object to vibrate at an exaggerated level.<sup>16</sup>

Similar to human sensory adaption to a continuous odor or sound, prolonged exposure to vibration can render the stimulus less noticeable over time, and animals may habituate to vibration. Vibration may become less of an extrinsic variable in these circumstances. Studies in humans have shown that adaptation of mechanoreceptors to vibration reduces the vibration sensation after a period of continuous or repeated exposure.<sup>6,7,21,30</sup> These studies have demonstrated that continuous vibratory stimulation is associated with an exponential decrease in the perceived intensity as time increases. The studies have also shown that this adaption lasts for a defined time, measured in minutes after the vibration stops and then complete sensitivity is regained.<sup>6,7,30</sup> Some work suggests that mice habituate to successive short periods of whole-body vibration, as indicated by a decrease in startle response as the number of exposures increased.<sup>16</sup> A reasonable assumption is that animals have some ability to habituate to vibration; however, the practical relevance of this ability has yet to be determined.

Likely related to habituation, vibration that starts and stops frequently may have more of an effect on animal behavior than continuous vibration. One study showed that it was the initial increase in environmental vibration that was more likely to elicit



**Figure 3.** Harmonic frequencies occur at whole number intervals from the fundamental frequency. In this example, the fundamental frequency is 50 Hz; increases in vibration magnitude occur at 100 and 150 Hz.

a behavioral response than vibration that does not change.<sup>16</sup> When studying the vibration acceleration and frequency needed to produce a startle response, mice that exhibited a startle response resumed their normal activity within 10 s of the start of the vibration, even though vibration continued.<sup>16</sup> The authors speculated that after the mice had assessed the disturbance, they did not consider it a threat and did not demonstrate further concern. However, this possibility does not discount the possible effects of continuous vibration on distress or other physiologic processes.

**Sources of vibration.** Vibration cannot be eliminated in an animal facility. Like sound, it can exist at different levels and change rapidly, depending on the conditions in or outside of the facility. Vibration is inherent to animal facilities and is caused by such factors as physical plant systems (e.g., heating, ventilation, and air conditioning [HVAC]), lighting, husbandry activities (e.g., cage changing), research activities (e.g., moving of carts), equipment (e.g., sterilizers and change hoods), and construction/renovation activities.<sup>45</sup> Sources outside of the facility (e.g., construction of nearby buildings, municipal traffic, and industrial sites) and transportation of animals are other areas of concern.<sup>17,26,36,43,46</sup> Due to the different resonance frequencies of structures, some objects may vibrate when exposed to a vibration source while others may not.<sup>19,37,47</sup> A less obvious source of vibration is that caused by sound.<sup>43</sup> For example, in our experience, the sound produced by an HVAC unit at the resonance frequency of a housing rack can cause cages to vibrate without affecting the floor underneath the cage rack. Due to the relationship of vibration to sound, one must also be cognizant that one can cause the other.

**Vibration control.** The concept of fundamental and harmonic resonance frequencies is also important because avoiding or damping these frequencies may be necessary to protect an object from vibration. Damping is the use of material that has been placed between the vibration source and object to absorb the energy of vibration at the object's resonance frequency, thus damping the object's exposure to vibration.

Vibration isolation introduces a material or apparatus with a different resonance frequency from that of the object being protected, shifting the frequency of vibration the object receives away from its resonance frequency.<sup>14,26</sup> Although large-scale changes to a building to control vibration should be implemented with the help of a professional vibration mitigation expert, some practical ways are available to minimize the potential for vibration to affect research animals.<sup>23,46,57</sup> Minimizing inherent vibration in the animal facility includes addressing issues with equipment, animal housing location, husbandry procedures, and transportation (Table 1). Examples of measures used for maintenance/construction projects are listed in Table 2. However, the suggestions are not appropriate if they increase the potential risk to human safety or if there are structural limitations.

**Necessary standards for vibration research.** Numerous variables involved in studying the effects of vibration on animals, if not addressed, will lead to difficulty in interpreting results or making comparisons to other studies. In the literature, the various frequencies that are used may or may not be in the more sensitive frequency range of an animal and so may miss the full potential of the effects. Many studies do not control for the effects of sound produced by vibration, so any changes

**Table 1.** Considerations for minimizing vibration caused by routine procedures in the animal facility

<b>Equipment</b>
Employ low-vibration-producing equipment (e.g., individually ventilated cage racks and cage changing stations)
Keep equipment well maintained (e.g., ensure preventative maintenance is performed appropriately for equipment)
Use noise abatement tiles in high sound areas <sup>56</sup>
Use anti-vibration mounts or flexible couplings on equipment that produces excessive vibration
<b>Housing Location</b>
House larger species that generate more noise away from more sensitive species (e.g., avoid putting breeding mice next to a dog room or cage washing area)
Encourage randomization of study groups between the rows of a rack to account for higher potential cage vibration near the top of the rack (for racks with top-mounted motor blowers) and consider avoiding the top row of these racks for more sensitive studies. In addition, researchers should be aware that the number of cages on the rack may cause a variable in cage vibration exposure.
Avoid establishing animal facilities near to high sound or vibration generation (e.g., near railroad tracks or a place of business with high sound and vibration generation)
Keep physical plant well maintained (e.g., no slamming doors in facility)
<b>Husbandry Procedures</b>
Address high-impact activities in the animal facility that can cause vibration (e.g., moving carts or racks into walls or dropping heavy equipment on the floor)
Educate employees that cage changing, or placing the cage top on the cage for any reason, can expose animals to high levels of vibration
Handle cages containing animals slowly and gently
Avoid shouting or using loud voices in animal rooms
<b>Transportation</b>
A study found that a double-folded bath towel on a metal cart was the method of hand transportation that caused the least amount of vibration <sup>24</sup>
Use rubber wheels on transport carts or other mobile equipment
Maintain vehicles and their suspension systems in good working order
Push carts over routes that are as free as possible of bumps and cracks
Drive slowly over rough surfaces
Secure transport enclosures so that vibration is minimized
Allow appropriate animal acclimation time after transport

**Table 2.** Considerations for mitigating maintenance/construction vibration (items that are not referenced are included based on authors' experience)

<b>Administrative controls</b>
Consider relocation of animals when an option <sup>46</sup>
Perform high-impact physical plant work before or after business hours so that experiments conducted during business hours will not be affected <sup>46</sup>
Communicate and coordinate with research investigators during planning stages of any potentially disruptive facility work to allow them to adjust their experimental schedule <sup>46,56</sup>
Educate facility maintenance/construction personnel on the effects of vibration and ask them to minimize this as much as possible <sup>46</sup>
Before construction, discuss the following with the construction unit's administration <sup>46</sup> :
<ul style="list-style-type: none"><li>• Specific animal-related concerns</li><li>• Training of maintenance/construction staff on sound and vibration mitigation and</li></ul>
How can deficiencies be addressed
<ul style="list-style-type: none"><li>• Steps that will be taken to minimize sound and vibration concerns</li><li>• The nature of the monitoring that will occur</li><li>• What will occur if sound and vibration are exceeding appropriate levels and/or causing changes in the animals</li></ul>
<b>Procedural controls</b>
Premanufacture ducting, pipes, and other materials in dimensions as large as possible off the jobsite <sup>46</sup>
Prefabricate piping for sprinkler lines off site <sup>46</sup>
Thread piping for gas lines outside of the building <sup>46</sup>
Bring building materials into the work area on carts with rubber tires <sup>46</sup>
Bring materials into buildings by a route that will be least disruptive to the animals
Position noise sources as far away from animals as possible
<b>Equipment used</b>
Perform proper and regular maintenance of equipment <sup>23,29</sup>
Choose a saw blade with the greatest number of teeth and the smallest width and the gullets (space between the teeth) as possible <sup>29</sup>
Use equipment with built-in vibration dampening <sup>29</sup>
Use compressors and generators designed to reduce sound and vibration <sup>29</sup>
Isolate vibrating machinery or components from their surroundings (e.g., with antivibration mounts or flexible couplings) <sup>23</sup>
Fit silencers to air exhausts and blowing nozzles <sup>23</sup>
Use a muffler on a nail gun <sup>29</sup>
Remove cinder block walls with power tools instead of a sledgehammer <sup>46</sup>
Remove vinyl tiles with power machines instead of scrapers and chisel bits <sup>46</sup>
<b>Engineering controls</b>
Add material to reduce vibration of machine panels <sup>23</sup>
Use electric scissor lifts with rubber tires <sup>46</sup>
Use metal-clad electrical cabling, with the wiring preinstalled <sup>46</sup>
Use "bolt-up" connections for ducting rather than slip-and-drive connections <sup>46</sup>
Use hanger brackets that do not require drilled-in anchors <sup>46</sup>
Remove ducts with a beam clamp and a block, and tackle <sup>46</sup>
Place enclosures around machinery to reduce the amount of sound to the environment <sup>23</sup>
Use barriers and screens to block the direct path of sound <sup>23,29</sup>
Use absorptive materials within the building to reduce reflected sound (e.g., open cell foam or mineral wool) <sup>56</sup>
Use rubber mats on the floor during demolition <sup>46</sup>
Place heavy material and equipment on softeners or pallets <sup>46</sup>

in an animal could also be attributed to sound. Lastly, some studies report magnitudes of vibration that are more excessive than any that an animal would reasonably experience under realistic conditions. For these reasons, the body of knowledge concerning the effects of vibration on animals remains limited.

Information in the spirit of the ARRIVE guidelines should be incorporated into publications on vibration.<sup>39</sup> A recent publication by an international group of experts has provided a consensus statement on reporting guidelines for whole-body vibration studies.<sup>50</sup> Use of this information on factors to consider when planning and reporting results of animal vibration studies will help control for the many relevant variables and facilitate valid comparison with other studies. Because this consensus

statement focuses on reporting guidelines for experimentally induced vibration, Tables 3, 4, and 5 below summarize this information, and, in addition, contain information relevant to studies involving environmentally-induced (e.g., from the physical plant or infrastructure), as well as requirements that would be germane to both experimentally-induced and adventitious vibration originating from the environment. Another source of information on the standardization of conducting and reporting vibration studies, particularly structural vibration, is the Institute of Environmental Sciences and Technology's (IEST) guidance published in *Measuring and Reporting Vibration in Microelectronics Facilities*.<sup>25</sup> This document and any subsequent updates provide maximum vibration velocity standards in the

**Table 3.** Information on vibration source and characteristics to include in publications on whole-body vibration in animals: items without reference numbers are included based on the authors' experience

Information about the Vibration Source	
<b>Induced experimentally</b>	<ul style="list-style-type: none"> <li>• For commercial shaker devices, the manufacturer and model number<sup>50</sup></li> <li>• For fabricated shaker devices, a description of the device<sup>50</sup></li> <li>• A description of the cage or other container where the animals were maintained during vibration exposure (e.g., the height, width, and depth in centimeters) and whether the enclosure included a lid<sup>50</sup></li> </ul>
<b>Induced in the environment (e.g., cage washers or HVAC units)</b>	<ul style="list-style-type: none"> <li>• Description of source, including construction of the area's floors and walls</li> <li>• Description of any measures taken to attenuate sound or vibration</li> <li>• Distance from measured source</li> <li>• The equipment, manufacturer, and model number of the vibration source</li> <li>• If known, the specific mechanical component of the equipment responsible for the vibration</li> <li>• Sound and vibration frequencies and magnitudes of interest</li> </ul>
<b>Induced both experimentally and environmentally</b>	<ul style="list-style-type: none"> <li>• Where the accelerometer was attached and how was it affixed to the surface<sup>50</sup></li> </ul>
Information about the Characteristics of the Vibration	
<b>Induced experimentally</b>	<ul style="list-style-type: none"> <li>• The parameter settings used to induce vibration<sup>50</sup></li> <li>• Whether the vibration parameters were verified<sup>50</sup></li> </ul>
<b>Induced in the environment</b>	<ul style="list-style-type: none"> <li>• Whether an increase in vibration of the measured object occurred with or without a simultaneous increase in floor vibration in the area</li> <li>• Whether the increase in vibration occurred with a simultaneous increase in sound</li> </ul>
<b>Induced both experimentally and environmentally</b>	<ul style="list-style-type: none"> <li>• The direction of the vibration (or the relative percentage) in the X, Y, and Z axes<sup>50</sup></li> <li>• The magnitudes of interest reported per specific frequency for acceleration, velocity or displacement in root mean square values<sup>50</sup></li> <li>• Definition of each reported parameter (e.g., displacement, acceleration, or velocity)<sup>50</sup></li> <li>• Whether the frequency and magnitude were constant or changed during the measurements<sup>50</sup></li> <li>• Specifics of how data analysis was performed<sup>50</sup></li> </ul>

form of vibration criterion curves for people and equipment at various frequencies and under different settings (e.g., office, operating room, or a room with an electron microscope).

One factor that can confound studies on animal exposure to vibration on ventilated racks is any inherent vibration originating within the caging system. Due to motors forcing airflow through individually ventilated cages, vibration can exist at a constant baseline level. Therefore, studies should be performed with motors running to obtain accurate total animal vibration exposure levels. In addition, any habituation of the animals to the vibration of the caging system might increase the threshold of animal sensitivity to additional vibration. Animals in conventional housing will also be exposed to vibration caused by a facility's infrastructure. As a result, ambient vibration levels in animal housing rooms should be considered and reported when studying the effects of additional vibration sources.

As noted in Table 3, an important detail in obtaining accurate vibration measurements is how and where the accelerometer is attached to the object of interest. If the animal exposure level of vibration is being studied, the accelerometer should be attached to the surface that contacts the animal. Otherwise, resonance frequencies of structural components between the accelerometer and the animal's contact surface may alter the exposure level of the animal. To measure maximal levels of vibration exposure, the accelerometer should also be placed close to the vibration source of concern. For example, measurements should include the top row of cages on racks with blower motors attached to the top to determine maximal vibration that is present in the rack. If floor vibration is of concern, the bottom row of cages would be included in the testing. In addition, the accelerometer must be oriented in a direction, as indicated on the accelerometer, that will accurately measure the vibration in the direction of interest. Lastly, the method of accelerometer attachment to

the object should be carefully considered and reported. Screws can be used for more permanent attachment, and various options for temporary attachment include glues (e.g., epoxies or cyanoacrylate) and waxes (e.g., beeswax or paraffin).<sup>5</sup> Accelerometer mounting clips can be used to avoid applying adhesive substances directly to the accelerometer. The manufacturer of the accelerometer should be consulted to ensure that the vibration measurement is accurate and the accelerometer is not damaged. A detailed description of how and where the accelerometer is attached should be included in publications because the attachment could be a source of a high degree of variation.

An issue that has complicated comparisons among vibration studies is the use of different published units for vibration magnitude (i.e., velocity, acceleration, and displacement) without providing enough information to convert one to the other. For example, if the frequency is not given, the vibration magnitude reported as velocity in one study would be difficult to compare with the acceleration values reported in another study. Although modern monitoring equipment can provide all 3 measurements simultaneously, they can easily be inter-converted if the value is given for a specific frequency. Reporting vibration magnitude as velocity may be advantageous because the IEST uses this unit to develop vibration criterion curves,<sup>25</sup> allowing data to be compared directly with these standards.

Two remaining factors that affect the consistency of reported vibration magnitude involve the data analysis. The first factor is to specify the bandwidth of frequencies used to report the magnitude. The IEST uses the resolution provided by one-third octave analysis commonly used in other applications.<sup>25</sup> The second factor is that the magnitude of vibration at various frequencies should be expressed as root mean square values, which give a more accurate indication of vibration energy than do peak vibration values at different frequencies. Additional detailed information on presenting data as root mean square

**Table 4.** Information on animals and exposure that should be included in publications on whole-body vibration in animal (items without reference numbers are based on the authors' experience)

<b>Information about Animal Exposure to Vibration</b>	
<b>Induced experimentally</b>	<ul style="list-style-type: none"> <li>• Whether animals were housed individually or in groups<sup>50</sup></li> <li>• Whether the animals have prior habituation to the environment and equipment, and, if so, how this was done and the time involved</li> <li>• Whether and, if so, how the animals were restrained during vibration exposure<sup>50</sup></li> <li>• Whether and, if so, how the animals were anesthetized during vibration exposure and the rationale for anesthesia<sup>50</sup></li> <li>• Any prior exposure to experimentally induced or known increased environmental vibration and how long it has been since the exposure</li> <li>• Whether vibration was delivered continuously in short periods followed by rest and the time each period of exposure persisted in relation to rest</li> <li>• The length of time that the animals were exposed<sup>50</sup></li> <li>• How controls were used to consider the effects of vibration (include sound)<sup>50</sup></li> </ul>
<b>Induced environmentally (for example by, cage washers or HVAC units)</b>	<ul style="list-style-type: none"> <li>• Whether the vibration was constant or changed with time</li> <li>• The approximate length of time the animals had been exposed to the vibrations before they were assessed</li> <li>• What animals were used for control observations to compare with animals exposed to vibration</li> </ul>
<b>Induced both experimentally and environmentally</b>	<ul style="list-style-type: none"> <li>• The changes in observed behavior /parameters and whether they changed after the initial response as the vibration continued</li> <li>• Whether the animals had a change in posture (e.g., lying, standing, or awake from sleep) during the vibration<sup>50</sup></li> <li>• The ambient sound and vibration</li> </ul>
<b>Information about the Animal Subjects</b>	
<b>Induced experimentally</b>	<ul style="list-style-type: none"> <li>• Age and body weight (before and after vibration exposure if chronic exposure)<sup>50</sup></li> <li>• Any alteration in whiskers (e.g., by barbering)<sup>50</sup></li> </ul>
<b>Induced in the environment (for example, by cage washers or HVAC units)</b>	<ul style="list-style-type: none"> <li>• Type of cages (e.g., conventional, microisolation, or individually ventilated racks)</li> <li>• Manufacturer and model number of racks</li> </ul>
<b>Induced both experimentally and environmentally</b>	<ul style="list-style-type: none"> <li>• Species, breed, and strain<sup>50</sup></li> <li>• Effects relative to vibration exposure: acute (directly after or during the vibration exposure and up to 15 min), short term (after 15 min and up to 3h), and long term (3h or longer)<sup>50</sup></li> </ul>

values and octave band analysis is available elsewhere.<sup>1,36</sup> All vibration units referenced in this manuscript are presented as root mean square values unless otherwise specified.

The vibration magnitude used in studies should be within the range of what would be reasonably expected to occur in a research animal facility. As a guide to what might be considered reasonable, established threshold magnitudes can help. With regard to construction criteria for buildings, the level cannot exceed 1.26 m/s<sup>2</sup> at 20Hz without jeopardizing structural integrity.<sup>32</sup> Therefore, magnitudes above this level would not likely occur in an animal facility. If the purpose of the study is to mimic vibration that humans can perceive, other vibration magnitudes may be considered. The median perception threshold for people sitting or standing was 0.01 m/s<sup>2</sup> to vertical vibration ranging from 1 to 100 Hz.<sup>37</sup> The maximal vibration level cannot exceed 0.1 m/s<sup>2</sup> at 20 Hz for human comfort.<sup>32</sup> Generally, vibration over 0.315 m/s<sup>2</sup> at 0.5 to 80 Hz, weighted to consider the human resonance frequency, also can be considered uncomfortable to a human.<sup>38</sup> As discussed below, the frequencies tested in rodents might differ from those presented above. However, structural integrity limits and human limits for perception and discomfort due to vibration may serve as a guide when choosing a magnitude of practical relevance to animals housed in animal facilities.

Testing at higher magnitudes may be reasonable in studies on transport vibration. To provide a reference for the vibration magnitude that can occur during transportation, the highest vertical vibration that dairy cows experienced was 2.27 m/s<sup>2</sup> at 70 km/h (about 45 miles/h). The resonance frequencies noted

were 1.3, 5.1, and 12.6 at about 23 Hz.<sup>17</sup> Movement of a cage of mice within a facility exposed the mice to a mean of 1.98 m/s<sup>2</sup> (6.22 m/s<sup>2</sup> maximum) in one study when carried by hand and to 8.6 m/s<sup>2</sup> (17.31 m/s<sup>2</sup> maximum) when transported on a plastic cart.<sup>24</sup> Therefore, higher magnitudes can be used to assess vibration-induced transport stress.

Another factor to consider in studies involving vibration is the frequency of applied vibration relative to the most sensitive frequencies of the animal. This is especially important when studying the effects of a particular vibration magnitude on an animal. For example, studies suggesting that exposure to a specific vibration magnitude did not affect mice might be invalid if the vibration frequency was not in a range close to the resonance frequency for mice. As discussed above, the fundamental resonance frequency of mice appears to range between 70 and 100 Hz.<sup>20,31</sup> Because resonance frequencies become lower as the mass of the animal increases, these frequencies in other common research species would be lower, assuming that the stiffness of biological tissue is similar for each species. The reason for this relationship between mass and resonance frequency can be understood by noting that in the resonance frequency formula ( $1/2\pi \times \sqrt{k/mass}$ ), mass is a denominator, and with k being constant, the resonance frequency will decrease as the mass increases. For example, for vibration in the vertical direction, a 25-g mouse has a resonance frequency range starting at approximately 70 Hz,<sup>16,31</sup> whereas the range appears to begin at 30 Hz for rats<sup>41</sup> and 1.3 Hz for cattle.<sup>17</sup> These are approximate minimal values. Much work is needed to elucidate these ranges

**Table 5.** Information on general protocol or general environmental conditions that should be included in publications on whole-body vibration in animals (items without reference numbers are based on the authors' experience)

<b>Induced experimentally</b>	<ul style="list-style-type: none"> <li>• Where vibration exposure occurred relative to the animals' housing room</li> <li>• The parameters of the temperature, humidity, and lights where the vibration exposure occurred and in the housing room</li> <li>• Whether animals were in bedded cages and, if so, how much bedding and type and size of bedding</li> <li>• Whether the animal was in its home cage or a different cage</li> </ul>
<b>Induced in the environment (for example, cage washers, HVAC units)</b>	<ul style="list-style-type: none"> <li>• The distance between the vibration source and animals and between the construction source and animals</li> <li>• The parameters of the temperature, humidity, and lights where the animals were located</li> <li>• The manufacturer, model number, or other identifying characteristics of animal housing racks and cages</li> <li>• The size of the animal room and the number of racks in the room, if applicable</li> </ul>
<b>Induced both experimentally and environmentally</b>	<ul style="list-style-type: none"> <li>• The manufacturer, model number, and description of the accelerometer and associated data collection and analysis equipment/software<sup>50</sup></li> <li>• The time of day that the animals were observed for the effects of vibration relative to the light/dark cycle<sup>50</sup></li> <li>• When measurements or observations occurred relative to the initiation of vibration</li> <li>• What was included in the cage such as enrichment materials and type of bedding (including manufacturer and identifying information)</li> </ul>

so that studies can be performed in the most sensitive range for a species. In addition, environmental exposure to vibration, such as higher fundamental or harmonic frequencies of the physical plant surrounding the animal, could result in a vibration magnitude that affects animals at higher frequencies.<sup>16</sup>

A previous publication<sup>49</sup> proposed that a maximum frequency of 500 Hz would likely include the highest frequencies of concern for producing effects on the whole animal. This limit is likely to capture animal resonance frequencies, including harmonics of concern. Therefore, for studies using vertical whole-body vibration in animals smaller than cattle, frequencies should range between 2 and 500 Hz in the vertical direction, with more weight given to frequencies from 2 (or as low as can be reliably administered) to 150 Hz, are reasonable for experimental application and concerns about environmentally induced vibration.

Not only do objects have resonance frequencies, but parts of an object, in isolation from the whole, likely have resonance frequencies higher than that of the entire object due to their lower mass. For example, the whole body of a rat has a resonance frequency of approximately 31 to 50 Hz.<sup>41</sup> In contrast, the rat tail has a resonance frequency of 125 to 300 Hz.<sup>28,54</sup> Therefore, higher frequencies may need to be considered if the measured vibration is for only part of the body. Another variable that can lead to an increase in resonance frequency is the object's stiffness. The resonance frequency is higher for an active muscle than for a muscle at rest.<sup>52</sup> This change in resonance frequency underscores the importance of allowing adequate animal acclimation to testing apparatus before vibration exposure for whole- or partial-body studies so the animal is more likely to be relaxed.

## Discussion

Vibration exposure causes a change in an animal's environment that the animal may perceive as a threat, causing the animal to take protective measures, including changes in behavior or physiologic parameters that allow it to avoid detection or to escape. Unfortunately, these changes may introduce an unwanted variable that could alter study results. Because some vibration is inherent in an animal facility, a necessary precaution is to determine when vibration control measures are necessary. Unfortunately, standards are lacking for maximum acceptable vibration thresholds for individual species of research animals.

The matter is complicated because the most sensitive frequencies likely differ for each species due to resonance frequencies. One study found that vibration produced by various construction/demolition equipment resulted in intracage vibration magnitudes at the theoretical mouse and rat resonance frequencies, as extrapolated from the human abdomen, thorax, and head data, and tended to be greater in rats and mice than in humans.<sup>35</sup> Therefore, animal perception depends on the animal's most susceptible vibration frequencies and the spectrum of frequencies produced by the vibration source.

Another consideration when determining vibration exposure limits in animals may be the distribution and type of mechanoreceptors in body regions. In the mouse<sup>51</sup> and rat,<sup>21</sup> predominant types and distribution of mechanoreceptors vary between the foot pads and digits of the same foot. Furthermore, the distribution of these receptors appears to be different in mice and rats. Because different vibration frequencies preferentially stimulate mechanoreceptors in the skin,<sup>22</sup> their type and distribution may also influence whether an animal might be adversely affected by exposure. Another factor that might determine whether vibration could cause behavioral or physiologic changes is the animal's body position in relation to the surface exposed to vibration.

Fortunately, animals appear to habituate to low vibration and no longer deem it a threat. Research into an animal's ability to habituate to continuous and intermittent vibration is needed, as is research on the magnitudes and frequencies of vibration that will cause adverse effects and the nature of these effects. Other necessary areas of vibration research include the differential effects of vibration in the *x*-, *y*-, and *z*-directions, design criteria that prevent fundamental and harmonic frequencies from affecting animals, additional studies on the magnitude and frequencies of vibration produced during construction relative to the associated effects on animals, and transportation methods that mitigate vibration. Research into these areas should be conducted and reported in a manner that ensures the resulting findings apply to animal research facilities and provide a body of knowledge that will address the many remaining questions.

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