TRANSPORT AND WORKS ACT 1992

TRANSPORT AND WORKS ACT (INQUIRIES PROCEDURE) RULES 2004

THE NETWORK RAIL (CAMBRIDGE SOUTH INFRASTRUCTURE ENHANCEMENTS) ORDER

STATEMENT OF CASE

SUBMITTED PURSUANT TO RULE 7(3) OF THE

TRANSPORT AND WORKS ACT (INQUIRIES PROCEDURE) RULES 2004

ON BEHALF OF OBJECTORS

UNIVERSITY OF CAMBRIDGE ESTATES DIVISION

MILLS & REEVE

1 INTRODUCTION

1.1 This Statement of Case ("**Statement**") is submitted on behalf of the University of Cambridge Estates Division (the "**University**"), the owner of land and interests in land in parts of the Cambridge Biomedical Campus ("**CBC**") included within the Order scheme ("**Scheme**"). As such the University is a registered statutory objector to the proposed Network Rail (Cambridge South Infrastructure Enhancements) Order (the "**Order**") which has been applied for by Network Rail.

1.2 The University submitted its objection to the Order by letter dated 30 July 2021 ("**Objection**").

1.3 The CBC is the largest centre of medical research and health science in Europe. As outlined in the Objection, whilst the University supports the broad objectives of the Scheme, it has serious concerns that the Scheme will cause significant harm to the CBC throughout all phases, including construction and operation of the Scheme. It therefore opposes the Scheme unless and until these concerns can be resolved.

1.4 Network Rail has failed to sufficiently consider the impacts on the University arising from the Scheme and the measures that may mitigate such impacts. The suite of Transport and Works Act Order 1992 application documents (the "**Application**") does not contain sufficient information to ensure that significant harm to the interests of the University can be avoided or mitigated by the Scheme.

1.5 In preparing this Statement, the University seeks further information from Network Rail regarding the proposed Scheme which it is hoped will enable the University to better assess the impacts of the Scheme on the University's land and interests in land. The level of detail provided by Network Rail in the Application on matters, including mitigation, is inadequate. As such, the University cannot undertake a full assessment of the impacts of the Scheme.

1.6 If the impacts of the Scheme cannot be sufficiently mitigated, the University would need to relocate its research facilities (assuming that such relocation is possible, as no alternative location exists within the CBC), in particular the world-leading research facility on the CBC, known as the Anne McLaren Building ("**AMB**"). The financial cost of such relocation would be extremely substantial and, notwithstanding the consequential detrimental impacts on the University, there is no evidence that the implications of any potential relocation have been considered as part of the cost estimations or funding for the Scheme (see Section 13 of this Statement below).

2 BACKGROUND TO THE UNIVERSITY

2.1 As set out in the Objection, the University has been at the heart of Cambridge for generations. Having been founded in 1209, it is the second oldest university in the English-speaking world and one of the most prestigious academic institutions in the world. It is a top ranked Russell Group University and was globally ranked 3rd in the recently published 2022 QS World University Rankings. The 2020 Research Excellence Framework results place 99% of the University's research activity to be "world leading", "internationally excellent" or "internationally recognised", with the University being recognised as excellent in disciplines which span the full range of academic research.

2.2 The University's core activities are world class academic teaching and research and its ability to provide an environment in which these activities can successfully flourish is therefore critical. The AMB is a vital component of this in that it supports the wider research activities of the CBC. As explained in Section 1 above, the University does not (nor do wider campus occupiers who use the AMB) have access to an equivalent facility within the CBC or the wider University estate should the AMB be unable to operate within its core design parameters because of the construction and operation of the proposed Scheme. This would lead to the loss of both direct research grant income to the University (see Sections 3 and 4 below) but also have a devastating impact upon the vital research activities that rely upon the AMB, including preventing projects from proceeding for a number of years (see Section 4).

2.3 The mitigation set out in the Environmental Statement for the Application ("**ES**") does not adequately protect this world leading and sensitive site. The University has a number of significant issues of concern which must be addressed to its satisfaction to ensure that the vital research within the AMB can continue, with particular regard to the significant impacts upon the AMB (and the University's future development of "Plot 9" within the CBC – see section 5 below).

2.4 As a globally recognised centre for research excellence and, as the University will show in its evidence, given the breadth of research activities and the specialist nature of the work undertaken, the AMB has specialist working requirements in respect of environmental laboratory conditions, with a narrow tolerance range beyond which research outcomes would be rendered unreliable. The University will show how much of the equipment in the AMB is noise and vibration sensitive and will be materially adversely affected by the Scheme without specific bespoke mitigation (if such mitigation is possible).

2.5 As set out in the Objection, the AMB (and Plot 9) are the closest of the University's affected land interests to the Scheme, alongside tenanted space within the Laboratory of Molecular Biology (marked as "LMB" in Figure 1 below), but the University also has other interests in buildings within the CBC. The University is the principal leaseholder of the soon to be completed Heart and Lung Research Institute Building (marked "HLRIB" on Figure 1 below), which sits between the Royal Papworth Hospital building and Francis Crick Avenue, under which it has access and servicing rights along Francis Crick Avenue and Robinson Way. It also has freehold and leasehold interests in other property to the north of the CBC, which are accessed from Robinson Way. These are known as "CRUK", "JCBM" (and are shown marked as such of Figure 1 below) and two car parks.

2.6 Each of the interests noted above comprise the University's estate ("**Estate**") for the purposes of this Statement. Separate objections have been raised in relation to impacts on other buildings within the Estate (other than the AMB and Plot 9) and the content of those separate objections are not repeated here.

3 THE ANNE MCLAREN BUILDING (AMB)

Location and the University's land interest

3.1 The University has a long leasehold interest in the AMB. Located immediately next to the AMB is Plot 9 (see Section 5 below). The location of the AMB and Plot 9 are shown outlined in red in Figure 1 below, together with the locations of other buildings within the CBC forming part of the Estate.

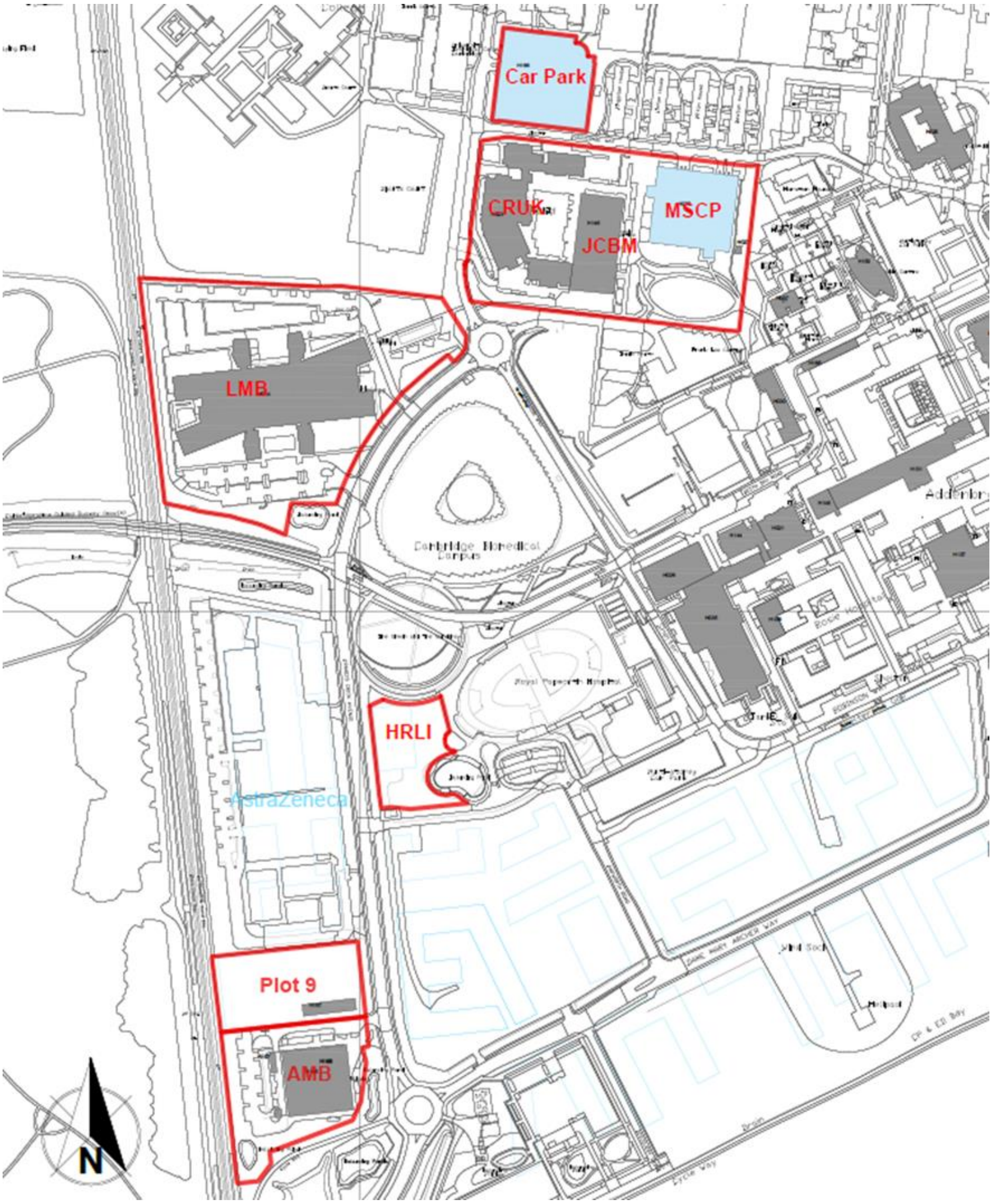


Figure 1 - Estate Plan

3.2 The extent of the University's interest in the AMB (both the building itself and the wider plot on which the AMB sits) is also shown outlined in red in Figure 2 below, with the AMB (i.e. the building itself) shown separately outlined in blue.

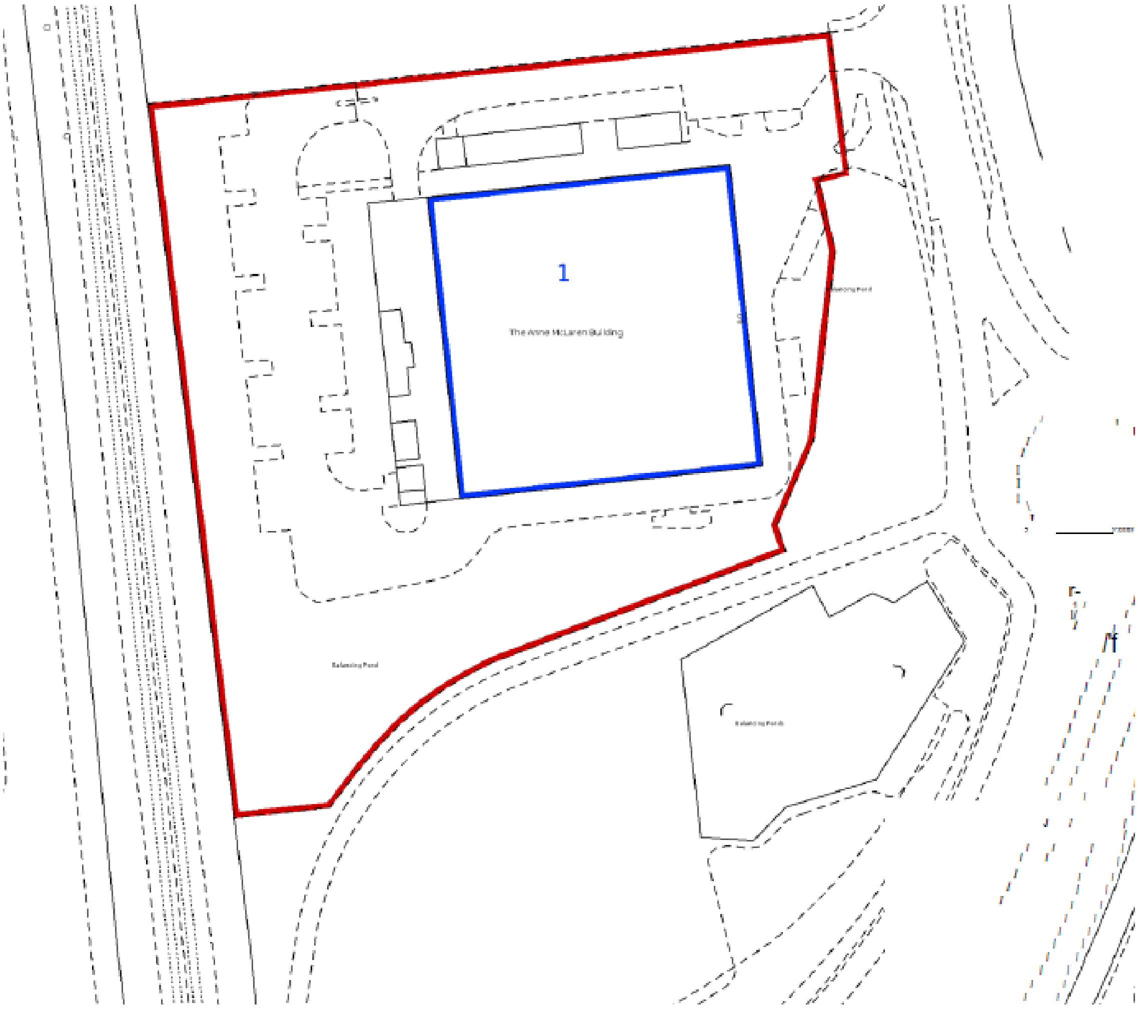


Figure 2 - AMB

The importance of the AMB

3.3 The biomedical research facility at the AMB represents a major investment in UK scientific research and is a one of a kind facility within the CBC. The AMB was opened in October 2019 as a new biomedical research facility to help further our understanding of how diseases occur and in the development of new treatments for conditions including cancer, dementia and diabetes. It includes a high-throughput centre providing material that supports globally important research activities across the CBC, including pioneering research into Covid-19.

3.4 As is common in biomedicine, research at the AMB involves the use of rodents (mice and, to a lesser extent, rats) ("**rodents**") and some fish. This necessitates a Home Office licence ("**Licence**") to be held[1], to which Establishment Licence Conditions attach.

3.5 The Licence conditions dictate, but in any event the University and other occupiers of the AMB aim to meet, the highest standards of animal welfare, underpinned by the principles of the '3Rs[2]' of animal research. This being to ensure that the AMB facility

---

[1] The University is granted such Licence under the Animals (Scientific Procedures) Act 1986

[2] The principles of the '3Rs' (**R**eplacement, **R**eduction and **R**efinement) provide a framework for performing more humane animal research. Since the introduction of the 3Rs 50 years ago, they have been embedded in national and international legislation and regulations on the use of animals in scientific procedures, as well as in the policies of organisations that fund or conduct animal research.

consistently produces the highest possible quality and consistency of scientific research. The design of the AMB is bespoke and was designed specifically to house rodent colonies. This includes, for example, a range of air handling units (Skyflow and WiFlow) that will continually monitor and control the air within the housing system. The AMB provides the very best environment for housing rodents, maintaining their welfare and standardising the research environment.

3.6 The AMB also houses (across five experimental zones) many high tech and industry leading pieces of specialised equipment (see below) which require specialist working environments. It operates 24 hours a day, 365 days a year, and is highly sensitive to both external noise and vibration factors.

3.7 Whilst delays due to COVID-19 mean that the AMB is still currently expanding its operational capacity, the AMB is nonetheless currently hosting 119 Individual Research Grants (it has hosted others to date). These grants overall accounted for £28.1m (12%) of research activity in the School of Clinical Medicine in 20/21 and £6.7m (5.5%) in the School of Biological Sciences.

3.8 The AMB itself provides unique service and support space not found anywhere else within the CBC. This means that the AMB researchers use it as a specialist hub, whilst maintaining their own labs elsewhere on the CBC, often taking samples and working back and forth.

The design of the AMB

3.9 The AMB was designed as a Home Office secure facility, with fixed vibration criteria as part of the embedded design built to withstand vibration impact from the existing operational railway.

3.10 VC-C vibration criteria is required for Magnetic Resonance Imaging ("**MRI**") and other imaging equipment housed within the AMB (see Section 4 below), alongside providing for stable laboratory conditions and the AMB has been designed to accommodate normal use of the building (footfall) and the railway in its current operation (i.e. trains passing by between Cambridge Central Station and the signals before Shelford Branch points where the line splits to either Kings Cross or Liverpool Street).

3.11 The design of the AMB does not consider the effects of any major construction activity or trains / passengers at a station in close proximity to the AMB beyond the existing operational railway line.

3.12 The AMB has specialist working requirements in respect of environmental laboratory conditions, with a narrow tolerance range beyond which research outcomes would be rendered unreliable. As such, the equipment and the subjects of the research within the AMB are noise and vibration sensitive and will be materially adversely impacted by the Scheme.

4 THE IMPACTS OF THE SCHEME ON THE AMB AND RESEARCH

Overview

4.1 As set out above in Section 3, the research undertaken within the AMB requires specialist working requirements and laboratory conditions in respect of both the sensitivity of equipment being used and the sensitivity of animal receptors within the AMB (comprising rodents and fish).

4.2 The AMB facility is rare. Neither the University, nor wider operators within the University's Estate have access to an equivalent facility should the AMB be unable to operate within its core design parameters. This would lead to the loss of both direct research grant income to the University in the region of £35m per annum but also have a devastating impact upon the vital research activities that rely upon the AMB, possibly preventing these from proceeding for a number of years. This will in turn have a detrimental impact on a number of careers, including those of PhD students.

4.3 The University's concerns raised by the noise and vibration effects of the proposed Scheme on the AMB break down into two main categories:

4.3.1 effects of noise and vibration on highly sensitive research equipment; and

4.3.2 effects of noise and vibration on research outputs where rodents and fish are used.

Effects of Vibration on Highly Sensitive Research Equipment

4.4 As explained above, the AMB contains state-of-the-art equipment for high resolution imaging, which is acutely sensitive to vibration and noise. This includes several pieces of extremely valuable and specialist equipment. Notable among these are the AMB's imaging equipment, including a circa £1m "3T MRI" machine and two Two-Photon Microscopes. The imaging suite is set inside a purpose built area of the AMB designed to the VC-C threshold.

4.5 University researchers at the AMB use MRI equipment for testing disease treatments, and to acquire sub-millimetre images in lab specimen and animals. The University will show that increased ground vibrations will cause image artefacts[3] (i.e. abnormalities in the images) which in turn would render certain vital pieces of research useless. It is critical that this is avoided in order for on-going research and experiments to be continued.

4.6 The University's research studies include imaging abdominal and brain cancers, brain aneurysms, thrombectomies, human cadaveric specimen, and cardiac and respiratory diseases. Work is performed with the University and Addenbrooke's Hospital researchers and with pharmaceutical companies, including AstraZeneca. High resolution imaging with limited noise and vibration interruptions are vital to ensure that these studies run efficiently and with limited disruptions to ensure the highest clinical impact.

4.7 MRI equipment in the AMB is in high demand and is used by researchers at the University throughout the week (and is expected shortly to extend into weekend use) with scanning sessions taking several hours at a time.

Effects of Noise and Vibration on Research Outputs where Animals are used[4]

4.8 As stated above, the AMB must meet the highest standards of animal welfare, which is one of the key reasons why it was so important for the University to establish a set standard for vibration (VC-A) wherever the rodents or fish might be located throughout the AMB (which accounts for approximately 75% of the operational floorspace within the AMB - see paragraphs 6.3 and 6.4 below). The University will

[3] An anomaly seen during visual representation in MRI i.e. a feature appearing in an image that is not present in the original object. This can affect diagnostic quality and/or be confused with pathology.

[4] The University will produce evidence in relation to effects of noise and vibration on Rodents and fish as part of its case, including the scientific articles listed at the back of this Statement of Case

show that it is vital to establish a consistency in the environment for the rodents and fish and minimise vibration stressors.

4.9 Elevated levels of noise are associated with a number of adverse physiological and behavioural changes in rodents and fish. In relation to fish, exposure to noise and vibration causes stress response behaviour, increased susceptibility to disease and reduced survival. Intermittent or persistent noise levels also have the potential to affect studies and the breeding potential of the rodents within the AMB. The most commonly recorded consequence of elevated vibration levels is an increase in stress hormones with breeding animals being most sensitive.

4.10 The University will show that, as well as affecting the welfare of the rodents and fish, the stress and anxiety caused by noise and vibration from the proposed Scheme could cause significant impact to research with these species, by substantially disrupting and invalidating research results. By way of illustration, the Institute of Metabolic Science ("**IMS**") performs a considerable number of studies involving mice at the AMB which may be impacted by low intermittent noise and vibrations, which result in stress-related effects and behaviours in the mice.

4.11 **Short term concerns:** The results of many procedures performed are sensitive to the animals' stress levels. As noted above, IMS performs behavioural studies on mice (especially those that have previously undergone surgical interventions) and acute noise and vibration interference would have potentially very serious impacts on the outcome of these sensitive experiments. The University also performs continuous monitoring of rodent vital signs through telemetry and intermittent noise and vibrations may introduce errors and variability in the data. Stress-sensitive parameters would need larger numbers of experimental animals to derive significance, since the variability of the data would increase (in conflict with the principles of the 3Rs). In the worst case of interruptions, experiments may need to be delayed or cancelled. In addition to increased costs, this would lead to delays to the completion of research, publication of results and students and researchers failing to complete work by the end of grant deadlines. The overall implication would be reduced competitivity for funding for research teams and the University overall, which accounts for circa £35m per annum (see paragraph 4.2 above).

4.12 **Longer term concerns:** Many of the readouts that IMS researchers examine are the result of long-term or ageing experiments. Chronic release of stress hormones affect these parameters and whole body metabolic status more generally. The net result of this is two-fold:

4.12.1 Data generated during these conditions could lead to the same variability issues as mentioned above, requiring larger group sizes as well as increased costs in species and caging. The same effect on the abandonment of work (as described in paragraph 4.11 above) will occur but with higher risk of occurance, since these experiments are long-term and the frequency of major interruptions will be higher.

4.12.2 Secondly, the data becomes less comparable to that in other facilities, and with previously generated datasets in the AMB.

4.13 A lack of reproducibility is a significant problem in biomedical science and with significant levels of interference caused by noise and vibration impacts are likely to cause issues with result validation. Moreover, some strains of rodents used at the AMB are also sensitive to stress which would result in poor breeding performance. Not only could there be increased litter losses, but larger numbers of rodents would need to be used in breeding (in conflict with the principles of the 3Rs), with the additional consequence of increased costs for the research.

4.14 Each research grant is for a period of five years on average. Even assuming that the University were only to repeat the grants of the last two years to maintain consistency of results, that could amount to circa £60m of total grant funding.

4.15 The University will show that the volume of the work undertaken at the AMB, its interdependency with the rest of the CBC, as well as the elongated time period of the disruption at the AMB because of the proposed Scheme, means that relocation or other mitigation alternatives (as well as being prohibitively expensive), will pause or terminate vital research for years.

4.16 For example, any planned "short-term" disruption to allow for construction of the Scheme (because of the length of some studies or time to prepare them) will themselves turn into long term disruptions for many researchers at the AMB.

4.17 The University will show that the impact of the proposed Scheme on numerous research studies will cost millions of pounds each year. Network Rail have not fully investigated any and all mitigation measures to ensure that the original design criteria for the AMB are met.

5 PLOT 9

5.1 The University has a long leasehold interest over Plot 9. Plot 9 is shown outlined red in Figure 3 below (as well as in Figure 1 above) and currently has the benefit of outline planning permission (ref 16/1078/OUT) granted on 3rd July 2017 for a further research building to be constructed in this location ("**OPP**").

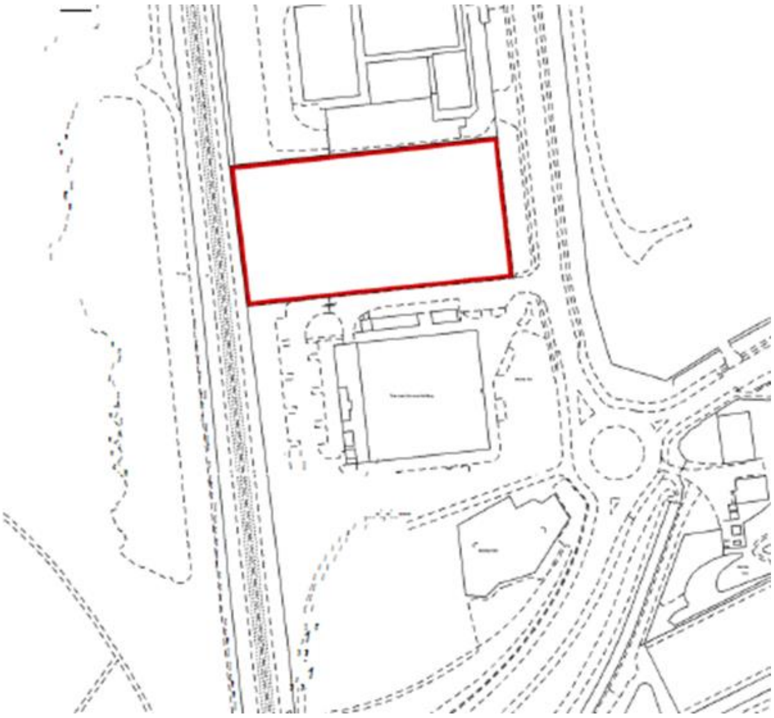


Figure 3 - Plot 9

5.2 The AMB and the future Plot 9 development are vital components of the CBC.

5.3 The University will demonstrate as part of its case the importance of Plot 9 to the strategic plan for the CBC. The grant of OPP for development of Plot 9, has established the principle of development on Plot 9, which is also confirmed through the allocation of the site in the Cambridge Local Plan 2018 (Policy 17) for research and development uses. The Plot 9 site therefore has significant development potential, which could include activity similar to that carried out within the AMB.

5.4 The Scheme risks significantly interfering with the University's future development options for Plot 9. Plot 9 is likely to be developed, in due course, with a facility involving biomedical and/or biotechnology uses. The University is concerned that the Scheme will have detrimental impacts on its plans in this regard, including in relation to drainage (see section 8 below) and also the potential loss of developable area (particularly should any part of Plot 9 be permanently acquired).

6 IMPACTS OF THE SCHEME - NOISE AND VIBRATION

6.1 As set out in the Objection and in the sections above, there are a number of important buildings within the CBC which are directly adjacent to or within the vicinity of the proposed works for the Scheme.

6.2 The fundamental issue is that there will be very significant noise and vibration effects on the AMB but the University finds itself without any measures being secured by Network Rail to mitigate these effects. The AMB was designed to function in the context of the existing rail infrastructure near the CBC but not the proposed Scheme.

Vibration

6.3 The table below identifies the uses within the operational areas of the AMB. All of the rooms in these operational areas are sensitive to noise and, in particular, to vibration. The vibration criteria for each operational area is quoted, together with the approximate proportion of the overall floorspace within the operational area of the AMB taken up by the three quoted areas:

Area	Proposed vibration criteria	Approx. % of operational floorspace
Laboratory areas (holding and procedure rooms)	VC-A	70
Supporting Laboratory Areas (surgeries, staff area, corridors)	R-4	25
Imaging Laboratory Areas	VC-C	5

6.4 As can be seen from the table above, approximately 75% of the operational researcg floorspace within the AMB is acutely sensitive to vibration.

6.5 In order for the University to properly understand the potential impacts and scope for mitigation, urgent further work needs to be undertaken by Network Rail to provide adequate information on noise and vibration issues. This is broken down into construction and operation as follows.

6.5.1 Vibration: Construction - Assessment of Impacts

(i) Detailed predictions of vibration from construction of the proposed Scheme, including durations, must be carried out by Network Rail. It is unclear what works are proposed by the Scheme that would affect the AMB and, in places, the ES is contradictory. For example, the assessment summary on page 17 of Chapter 6 of the ES (Acoustics Assessment Part 2 – Vibration) states *"no use of vibratory piling techniques, except for at Shepreth Branch Junction where they may be required due to limited time periods for track possessions."* Yet, Table 6-2-5 of Appendix 6.2 of the ES

(Construction Phase Impact Assessment) presents a prediction of 2.43 mm/s for the AMB due to vibratory piling in the Track Construction Zone and 0.65 mm/s due to vibratory piling in the Station Area Construction Zone.

(ii) Sources of vibration are referred to which have not been assessed: "*Compaction for the ballasted track will be carried out by means of a Main Line Tamper. This has a negligible impact on induced vibration when compared with other vibratory methods of compaction*." (Page 21 of Appendix 6.2). It is necessary for Network Rail to provide a prediction of vibration from a main line tamper. Tamping will be required not only during construction of the proposed Scheme, but periodically after completion of the works, including tamping on the new loop closer to the AMB.

(iii) A prediction of vibration from each activity and item of plant likely to be used in constructing the Scheme on each site is also required from Network Rail. Worst case predictions are insufficient when a significant effect is likely because durations and locations become critical.

(iv) It must also be clarified by Network Rail when the predictions presented are for the external ground surface or the internal building structure.

(v) Page 22 of Appendix 6.2 of the ES states "*where a potential significant impact is predicted for a receptor the likely effect of the building has been taken into account to establish if this would materially affect the assessment*." It is unclear whether or how this has been done by Network Rail. Page 28 of Appendix 6.2 makes the general statement that "*heavyweight building will reduce vibration levels at ground floor but potential for amplification at upper levels to offset benefit. Probable reduction from Moderate to Minor impact but does not alter significance*." This is an insufficient level of prediction.

(vi) Measurements should be carried out on site by Network Rail to establish the local properties of the soil layers and establish a site-specific loss factor for the purpose of predicting the effect of distance on vibration.

(vii) Measurements should also be carried out on site to establish transfer functions between external source locations and internal building structures where sensitive receptors are located.

6.5.2 Vibration: Construction - Mitigation

(i) Full and proper consideration of potential mitigation options for the AMB has not been carried out by Network Rail and is required. Consideration of mitigation measures is partial and incomplete.

(ii) Alternatives to vibratory piling are not assessed, such as hydraulic press-in piling. Whilst "low vibration construction techniques" are reference on page 25 of Appendix 6.2 of the ES, these are not explained or assessed.

(iii) Vibration from all construction activities referred to should be predicted by Network Rail.

(iv) A noise management, monitoring and control protocol is also required. Page 25 of Appendix 6.2 of the ES mentions "vibration monitoring with real-time feedback". However, a system of continuous monitoring of vibration is required in which warning levels are automatically monitored, so that the approach of critical levels of vibration is detected in advance. It is necessary to establish a site management structure capable of ensuring that work can be stopped immediately when critical levels are approached.

6.5.3 Operation: Assessment of Impacts and Mitigation

(i) Freight trains should be assessed by Network Rail in the ES. Although not all timetable train paths are used, there is a significant number of freight train movements in the Network Rail working timetable.

(ii) The baseline vibration currently attributable to freight trains should be measured. Additionally, the effect of freight trains passing over switches and crossings should also be measured at Shepreth Junction (in addition to the measurements which were made relating to passenger services). Moreover, future intentions regarding use of the proposed loops through the Scheme by freight trains should be made clear by Network Rail.

(iii) Simultaneous train movements should also be taken into account by Network Rail in its assessment. Table 6-11 of Chapter 6 of the ES ("*Assumptions made in the impact assessment*") states as follows: *"the assessment was based on single train pass-by events. During the baseline surveys, there were periods when trains travelling in opposite directions passed each other in the CBC region, but the frequency of this occurrence was less than 5-10% of all train pass-bys observed. The vibration levels in the as-developed case will be dominated by the S&C locations and hence the chance of trains passing this location at the same time and at the high speeds associated with greatest impact will be lower. However, sometimes trains will pass over S&C simultaneously and may cause higher levels, but this is expected to be sufficiently infrequent to not warrant forming the basis of assessment".* In the case of the vibration sensitive equipment, and operations in continual use in the AMB, exceedance of vibration criteria by any combination of train movements is a potential significant adverse effect. Predictions of maximum vibration due to all combinations of train operations should be provided and assessed by Network Rail in the ES.

(iv) Effects of speeds and distance should also be measured on site by Network Rail. It is pointed out in the ES that a line speed of 60 mph will apply to the loop lines (compared with 90 mph on the current track). Measurements of the relationship between vibration and speed must be carried out by Network Rail on site and at Shepreth Junction. The actual proposed speed profiles that will apply (a) on the loops; and (b) on the through lines should be simulated. Whilst 90 mph will not be reached in the vicinity of the station by stopping trains, the proportion of stopping and non-stopping trains (if any) should be reported by Network Rail, and the likely speeds of trains passing over the crossings should be considered and compared with the speeds of trains measured while passing over the crossing at Shepreth Junction.

(v) The relationship between vibration and distance from the track should be measured on site so that the combined effects of speed changes and distance changes can be accurately predicted.

(vi) The use of speed limits as a mitigation measure should also be fully considered, including further measurements at Shepreth Junction to establish the relationship between speed and crossing vibration.

(vii) It is unclear which vibration predictions in the ES are of ground surface vibration outside buildings and which take account of the transfer function between ground surface and building structure at the location of sensitive equipment. Where it has been previously measured, the transfer function should be presented. In other cases site measurements of simultaneous ground surface and internal structural vibration should be made.

(viii) The effect of mitigation measures mentioned but not assessed in the ES should be predicted or clarified by Network Rail. VC curves from VC-C downwards have a flat frequency response in velocity terms. Of the operational mitigation measures listed in the ES, all but one have the effect of shifting the peak in the loaded track natural frequency towards a lower point in the spectrum, with no change in amplitude. Thus no improvement in VC levels results. The one measure which does reduce amplitude is the use of swing-nose points or movable frogs. This is dismissed in the ES with the comment "*this option has been assessed and found not to be feasible given the site constraints*" (Table 6-13, "*Options for track vibration mitigation measures*").This approach is inadequate.

(ix) The prediction of vibration in Figures 6-38 and 6-39 of Appendix 6.3 of the ES may be exceeded when freight trains have been taken into account and the conclusions of "*not significant*" on page 6-21 of Chapter 6 may not apply to freight trains. Paragraph 6.2.40 of Chapter 6 states that "the south western area of the building at ground floor level requires VC-C to be achieved." Whereas, Figure 6-38 shows VC-C reached on the ground floor and Figure 6-39 shows VC-C exceeded for the second floor of the AMB.

(x) A full assessment of the option of installing swing-nose points and movable frogs should be carried out by Network Rail. Where there are site constraints the removal of those constraints should be fully considered as well.

(xi) In the ES, "*it is assumed there are no other track discontinuities such as joints in rails"* (Table 6-11, *"Assumptions made in the impact assessment".* However, in addition to joints, rail welds made using the aluminothermic process also have the effect of discontinuities because the metal used in the weld is softer than rail steel, and impulses occur when axles pass over them. There has been no assessment of mitigation by way of ensuring that only flash-butt or arc welds are used.

Noise

6.6 The ES predicts a major impact on the AMB due to construction noise, yet there is no detailed consideration of mitigation beyond "embedded mitigation" described as site hoarding and the use of "Best Practicable Means". It is stated that "*Site hoarding of 2.4m would be installed around the site perimeter, where mitigation is required and practicable. Guidance provided in BS 5228:2009+A1:2014 Annex B, states that a screen can provide 5 dB attenuation for partial line of sight from source to receiver, and up to 10 dB attenuation where there is no line of sight between source and receiver*." (Paragraph 5.5.4 of Chapter 5 of the ES)

6.7 Notably, however, where there is a clear line of site above the hoarding there is no noise barrier effect. Added to this, given the height of the AMB, this will be the case for many parts of the building. It thus appears that the predicted noise level of 72 dBA may in truth be 77 dBA where there is line of sight from the noise source over the top of the hoarding to parts of the AMB facade. The University is therefore concerned that predicted noise levels may exceed those that have been assessed i.e. reach up to 77 dBA. Accordingly, it is necessary for Network Rail to carry out a proper assessment of the potential for mitigation, including more effective noise barriers than standard site hoarding and also the possibility of alternative plant selection.

6.8 The University considers that Network Rail should employ an example programme for the Scheme construction works, based on the construction stages identified in Volume 3: Appendix 5.3 of the ES to indicate the likely periods when the "worst case" prediction will arise (and their durations) in order that the magnitude of the disturbance to activities in the AMB can be properly assessed.

6.9 The sound insulation of the external facade of the AMB was selected assuming the continuance of the current ambient noise climate. The University is concerned that the predicted exterior construction noise levels are substantially in excess of the pre-existing ambient noise climate, meaning that the noise criteria for internal spaces within the AMB will be exceeded.

7 IMPACTS OF THE SCHEME - EMI

Introduction

7.1 Significant concerns with the Scheme have caused the University, following its Objection, to consider further potential effects on Electromagnetic Compatibility ("**EMC**"). EMC is the ability of electronic and electrical equipment, systems and installations to operate satisfactorily in their electromagnetic environment without generating excessive levels of Electromagnetic Interference ("**EMI**") that could degrade the performance and functionality of other systems operating in the same environment.

7.2 The University is concerned that the Scheme could prejudice the functionality of systems within the AMB due to EMI, including systems degradations and loss of functions. The implications of this for research within the AMB could include data errors and interruption to research timescales. Further, potential EMI effects are not limited to laboratory and research equipment – there are also risks of adverse effects on AMB electrical services (for example emergency power supply and lighting).

7.3 The AMB has been specially designed to accommodate the existing electromagnetic environment. Accordingly, all equipment within the AMB currently operates satisfactorily and is compatible with the external environment. The University is concerned that the implementation of the Scheme could alter the electromagnetic characteristics of the AMB environment and cause existing electromagnetic levels to be exceeded (both during construction and operation of the Scheme).

Lack of information: assessment of effects in the ES

7.4 The only mention of EMI/EMC in the ES appears in Chapter 2 (EIA Methodology) in a limited section spanning paragraphs 2.2.18 – 2.2.32 entitled "*Electromagnetic Compatibility*". In this EMC section, Network Rail confirms that several stakeholders during informal scoping stage of the Application referenced the possibility of EMI having an effect on sensitive equipment as a result of the overhead line equipment and/or new track associated with the proposed Scheme. Such stakeholders included the Medical Research Council Laboratory and the University.

7.5 Network Rail acknowledges in the ES that there remains a risk that the proposed Scheme would have EMC effects on various receptors, including the AMB. The University understands that an immunisation study is proposed by Network Rail at GRIP Stage 4 (between April to December 2021 – see paragraph 2.2.28 of Chapter 2 of the ES) when the outline designs for the proposed Scheme are produced. The University does not know anything further about this study (including whether it has been carried out or not) and Network Rail must share the results with the University as soon as possible.

7.6 The purpose of the immunisation study is stated (at paragraph 2.2.28 of Chapter 2 of the ES) as being to provide an assessment of the proposed Scheme design to fulfil the following requirements:

7.6.1 *"verify the earthing and bonding proposed design and demonstrate that the design will mitigate the risks of touch voltages…;*

7.6.2 *assess the impacts of the proposed design on signalling and telecom cables…;*

7.6.3 *determine the expected magnetic fields along the line…"*

7.7 As is clear from the above, the requirements set out for the proposed immunisation study are focused only on increasing the protection of the railway assets, including safety aspects. The proposed immunisation study does not take into consideration the EMI impacts on the external neighbouring environment. The immunisation study, as proposed, will have no effect on the EMC interaction between the railway and the outside world, which is of major concern to the University.

7.8 Network Rail has failed to assess or, in the alternative, to provide any assurance in the ES that the EMC/EMI effects associated with the electromagnetic emissions of the railway environment onto the AMB and other neighbouring sensitive receptors during the construction and operation of the proposed Scheme will be mitigated.

Lack of information: mitigation

7.9 The potential for measures to reduce likely significant EMC effects on the AMB must be considered as early as possible. This is due to the railway environment having different types of electromagnetic sources operating at different frequencies. Railway environments are considered "Harsh" with respect to EMC.

7.10 Mitigation cannot simply be left to a Code of Construction Practice ("**CoCP**"), as is suggested by Network Rail in Chapter 2 of the ES (referred to in paragraphs 2.2.32

and 2.2.49). Notably, there is no mention of the approach to the immunisation study in the CoCP. This omission suggests that the immunisation study will only provide protection to the railway systems (in that the study is limited to addressing EMC / safety interactions within the railway environment only) and has no influence on environmental impact outside of the immediate railway environment.

7.11 Preliminary EMC assessments must be undertaken based on worst case assumptions and typical emissions data from railway studies and EMC standards[5]. Electromagnetic site survey measurements must also be undertaken by Network Rail at the boundary of the AMB during the construction and operational stages of the proposed Scheme to quantify the existing electromagnetic environment.

7.12 Mitigation measures will be needed for the sensitive equipment within the AMB, should the levels measured exceed the susceptibility limits of such systems. Such mitigation might need to include electromagnetic shielding of rooms within the AMB or architectural shielding of exterior sides of the building.

7.13 As matters stand, Network Rail has not carried out sufficient assessment work on EMC/ EMI effects and has failed to identify any adequate mitigation to address the potential effects of the Scheme.

8 IMPACTS OF THE SCHEME - DRAINAGE

Overview

8.1 The impacts of the proposed Scheme on the drainage of the existing AMB and proposed buildings at Plot 9 at the CBC are of concern to the University, as highlighted in the Objection.

8.2 The University notes from Table 3 of Network Rail's Planning Statement (which contains a summary of the key features of the proposed Scheme) that (for drainage and culverts), reference is made to the "r*econstruction of Tibbets Culvert to minimise flood risk and provision of additional sustainable drainage for the railway infrastructure and modification of several existing culverts to receive new track layout"* but not to the surface drainage that serves AMB and Plot 9, which is part of the overall drainage network serving the CBC.

8.3 As set out in the Objection, any alterations to the drainage arrangements proposed by the Scheme would need to be undertaken in a manner that preserves the normal operation of the AMB, both temporarily and permanently. It is currently unclear what impacts there are upon the swale and attenuation pond that exist within the University's Estate, particularly given that the limits of deviation shown on the Application drawings appear to straddle on site drainage infrastructure. The ES does not obviously assess this, nor does Network Rail commit to any mitigation measures which take into consideration the implications of the Scheme on the drainage arrangements for the AMB and Plot 9. The proposed mitigation is inadequate and the ES is deficient in this regard.

8.4 As also explained in the Objection, the University must also understand the intended implications for the management and maintenance of drainage and landscape features going forward to protect future maintenance and building operations. At present, whilst the submitted Flood Risk Assessment ("**FRA**") suggests that it is Network Rail's intention to manage features within the Order Limits, there appears to

[5] For example BS EN 50121-2: Railway Applications – Electromagnetic Compatibility, Part 2, Emission of the Whole Railway System to the Outside World

be no further information provided in this regard to clarify which elements are temporary and which are permanent management issues, despite the deposited Application drawings suggesting that some of the University's existing surface water drainage features fall within land that Network Rail is looking to compulsorily acquire.

8.5 As confirmed in the Objection, the surface water drainage outlet from the AMB and Plot 9 discharges into the balancing ponds to the south of the AMB (within the control of Cambridge Medipark Limited). The University understands that the water from the balancing ponds subsequently feeds into the Hobson's Conduit via the south ditch further to the south of the AMB and Plot 9 (outside of the University's demise for these interests). The University has given covenants to the Hobson's Conduit Trust to protect the Hobson's Conduit from damage and contamination ("**Conduit Covenants**"). These include (inter alia) obligations on the University to control the flow rate into the Conduit; to liaise with the Trustees prior to any development of the University's land and ensure such development does not result in pollution of the Conduit; and to remediate any damage or contamination caused during such development. The Conduit Covenants are referred to in the FRA, as set out in paragraph 8.10 below.

8.6 Given the interdependency between the AMB and Plot 9 drainage design and the potential impact upon the Hobson's Conduit, suitable mitigation measures must be put in place by Network Rail to ensure the outfall drainage from the AMB and Plot 9 remains unaffected by the Scheme. Whilst there are protective provisions included (within Part 4 of Schedule 12) in the draft Order in relation to the Hobson's Conduit itself, Network Rail has not committed to any specific mitigation measures to protect the outfall drainage from the AMB and Plot 9.

8.7 From the information provided in the ES, it is not possible to determine how the efficacy of the existing drainage system will be maintained or whether any mitigation is proposed to offset any impacts.

Existing Drainage Arrangements

8.8 The AMB and Plot 9 both drain to the south via a series of pipes, swales, ditches and ponds and ultimately discharge to the Hobson's Conduit as shown illustrated below in Figure 4:

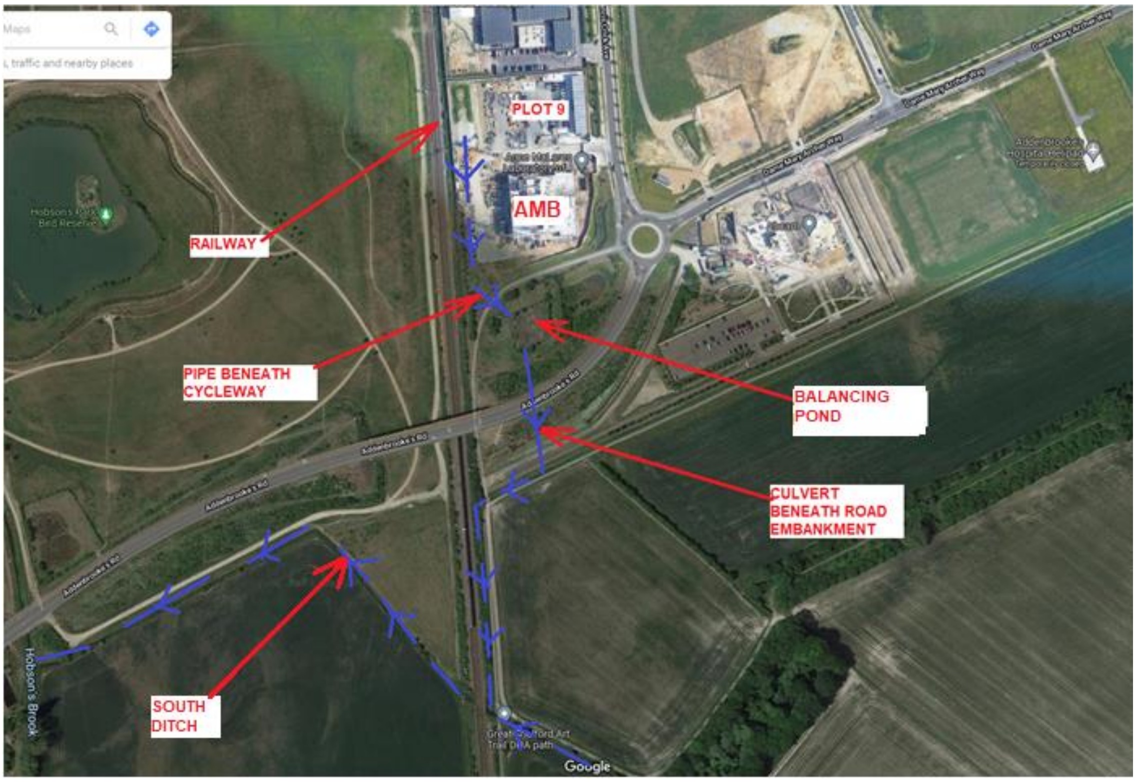


Figure 4

8.9 The drainage system is designed to maintain an agreed peak discharge rate, as well as maintaining the quality of runoff into the receiving watercourse. The proposed Scheme could reduce the effectiveness of this system by:

8.9.1 altering or interfering with the alignment, maintenance of ditches;

8.9.2 adding more flow into an existing network; and

8.9.3 altering the effective capacity or operation of the ponds or ditches.

8.10 The FRA makes reference to this in section 6.2 as below:

"The proposed Development is partially located in the Cambridge Biomedical Campus and information on the drainage regime of the Campus has been gathered from the Surface Water Strategy report for the extension of the Campus. The existing drainage network for the Cambridge Biomedical Campus is served by a series of SuDS, ditches, gullies and attenuation features which have been designed to receive and attenuate flows from the wider surface water drainage system of the Campus. The Cambridge Biomedical Campus is covered by covenants with the Hobson's Conduit Trust regarding drainage and special arrangements are in place to safeguard and monitor the quality of surface water entering Hobsons Brook and Hobsons Conduit. These covenants govern the right to access, for the purpose of carrying out works, the Hobsons Conduit. Discharge of surface water into Hobsons Conduit, through the North Ditch and/or the South Ditch and/or other ditches constructed through the green corridor between the Cambridge Biomedical Campus and Hobson's Conduit, must also be controlled under the covenant".

Proposed Drainage for the Scheme

8.11 The proposals for surface water drainage for the proposed Scheme are set out in the FRA, in Appendix C. In essence the runoff from the trackside components and station is collected in an interceptor drain which runs southwards to a new balancing pond and then on to the Hobson's Conduit via the South Ditch. The drainage layout in Appendix C shows the new pipe crossing the existing ditch to enter the balancing pond but there is no indication that this can be achieved based on the levels of the ditch or the pipe.

8.12 Furthermore, there is no indication whether any activities either during construction or operation of the proposed Scheme would impact on the existing drainage system for either AMB or Plot 9 and, in particular, the swale to the west. Chapter 18 of the ES sets out proposed principles for surface water drainage but the University is unable to find the evidence that Network Rail has considered the detail of what exists and how it works (as explained above).

Hobson's Brook/Conduit

8.13 The same basic observations apply to the impacts on the Hobson's Conduit watercourse, but the University does not comment in detail as the Hobson's Conduit Trust is separately represented.

Lack of information: assessment

8.14 There are a number of pieces of information which are lacking from Network Rail's assessment. This means that the University cannot be confident as matters stand that the existing drainage arrangements have been considered and assessed in full.

8.15 The ES and FRA do not include the following detail:

8.15.1 a plan showing the detailed existing drainage scheme as exists currently.

8.15.2 a plan showing the detail of the proposed drainage scheme with line and level of pipes and ponds and the intended controls to preserve water quality, minimum flows and maximum flows in accordance with the Conduit Covenants;

8.15.3 a plan showing the extent of temporary or permanent works proposed within the curtilage of the University properties and in particular the impacts these might have on existing drainage infrastructure.

8.16 The above information should have been included in the FRA. Without it, it is unclear how Network Rail's assertions on minimal impacts (as set out in the ES) can be evidenced. The University has provided information relating to paragraph 8.15.1 above (a plan showing the existing drainage scheme) to Network Rail but there is no evidence that this has been considered in the ES or FRA.

Lack of information: mitigation

8.17 Without the above details, it is not possible to conclude that there would be minimal impacts on the efficacy of the existing drainage system within the ES and FRA. Network Rail has not provided any mitigation measures in the ES to justify the conclusion of "not significant" impacts, whether this might be relocating the proposed drainage infrastructure for the Scheme; the relocation of existing drainage infrastructure, or the relocation of temporary or permanent works - which could themselves affect the existing drainage system for the AMB and Plot 9.

8.18 Further information is required from Network Rail to demonstrate that there are no likely significant effects on the drainage of the AMB and Plot 9. Specifically, the University is concerned with impacts of the Scheme on the swale to the west of the

AMB (to the extent existing drainage from the AMB or proposed drainage from Plot 9 would be impacted) and whether Network Rail's proposed drainage infrastructure can be delivered without obstructing existing drainage. If there are such impacts and/or obstruction, the University needs to understand what mitigation is proposed by Network Rail to offset these.

9 IMPACTS OF THE SCHEME - HIGHWAYS

Overview

9.1 The impact of construction of the Scheme on the AMB, given the proximity of the proposed Scheme to the AMB, is of major concern to the University. This includes impacts on car parking, access to and from the AMB, as well as servicing for the AMB.

9.2 The proposed works for the Scheme will include a series of major earth moving and major construction works during the construction period. Chapter 17 of the ES contains details of the proposed construction access points and quantifies the number of vehicles proposed to access the Scheme site, with an estimated peak in numbers occurring in 2023.

9.3 Figure 5 below (an extract taken from Figure 17-1 in the ES) shows the location of construction site compounds in the vicinity of the AMB. The proposed main site compound "CC1" is located immediately west of the AMB, separated by the road bridge over the tracks. Figure 6 below (an extract taken from Figure 17-2 in the ES) shows the location of access roads in the vicinity of the AMB. Site access roads "AR1" and "AR2" are the closest to the AMB.

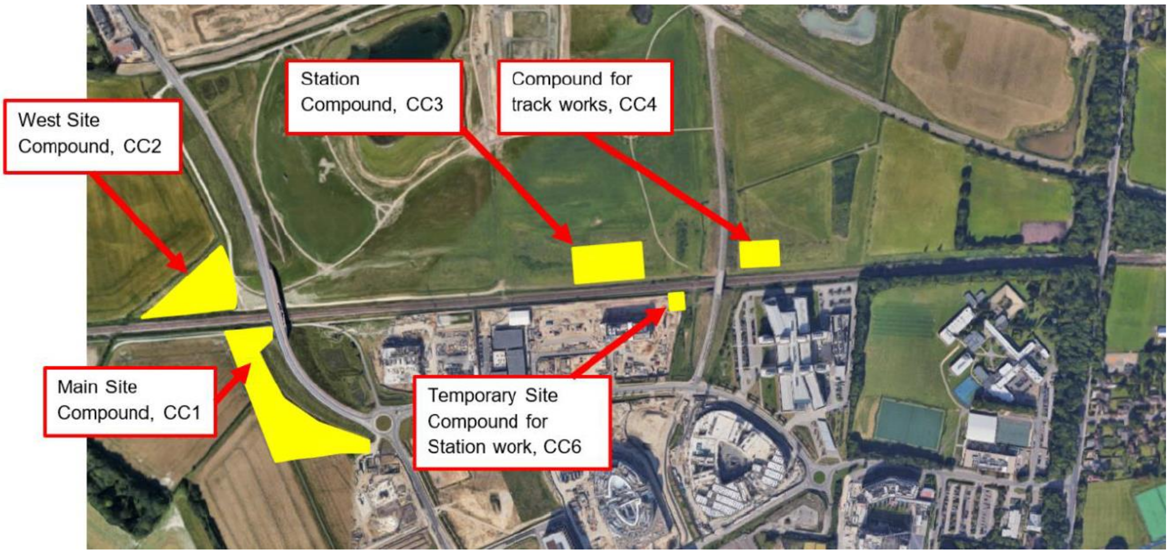


Figure 5 - Location of construction compounds (extract taken from Figure 17-1 in the ES)

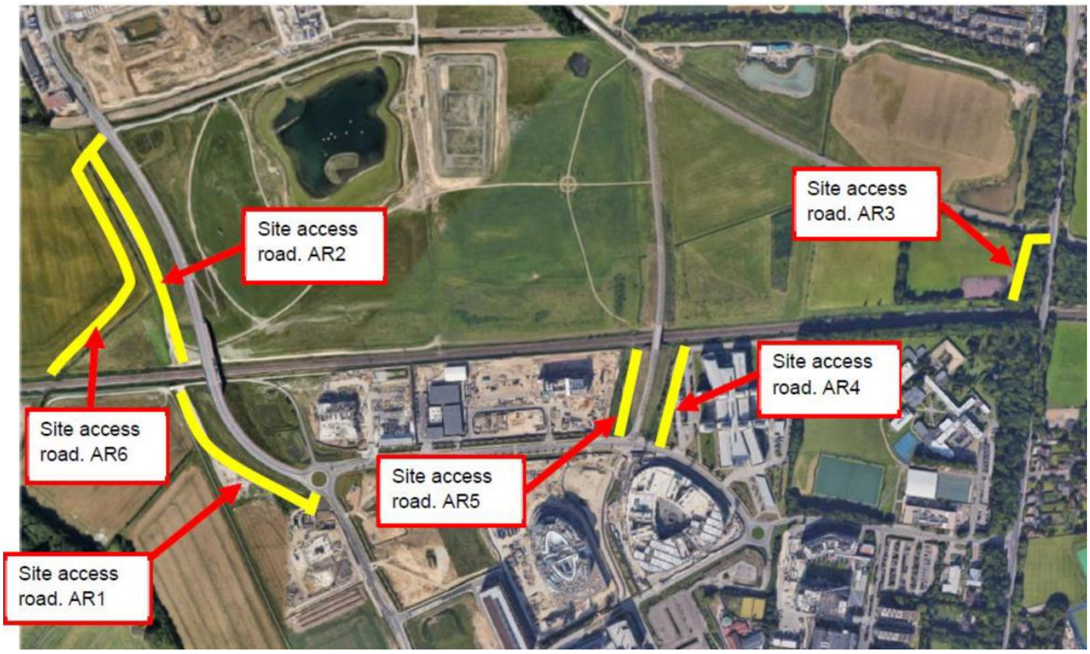


Figure 6 - Location of haul/access roads
(extract taken from Figure 17-1 in the ES)

9.4 Figure 17.1 in the ES (partially shown above in Figure 5) shows the proposed temporary construction compounds to build the station. As previously identified in the Objection, the information provided within the Application does not include the as built development at the AMB. For example, the Figures above are out of date as much of what is shown has since been substantially further developed and/ or completed. Not only does this make it difficult to interpret the extent of proposed temporary and permanent acquisition along the University's property boundary, it also makes the highways impacts difficult to assess.

9.5 Figure 17.2 in the ES (partially shown above in Figure 6) shows the proposed haul roads from the highway network to access the Scheme's site compounds. Given that the track works and the proposed station buildings will be on both sides of the existing railway line, compounds and haul roads are proposed on both sides of the existing railway track. It is not clear from the ES whether possible alternative site compounds and haul roads, which don't impact on the operation of the AMB (or at least have a lesser impact), have been considered by Network Rail. The University notes that the ES does not include a plan showing possible alternative site compounds and haul roads.

9.6 Paragraphs 17.4.3 – 17.4.24 of Chapter 17 of the ES outline the proposed approach to assessing construction impacts and mitigation. In reality, this amounts to what is generally standard good practice and does not seem to acknowledge the special circumstances of the AMB. Table 17.12 in Chapter 17 of the ES contains details of the projected number of construction vehicles accessing the Scheme site. It is notable that 90% of the total predicted movements for the Scheme site as a whole (464 / 516) are on access roads AR1 and AR2, which are the closest in proximity of all the proposed access roads to the AMB.

9.7 Proposed site compounds, haul roads and major road network connections are detailed in the ES but there is no real discussion of alternatives or alternative forms of mitigation. As noted above, the only mitigation that is proposed in the ES is good operational practice. Given the special characteristics of the AMB and the nature of the research activities undertaken, additional mitigation measures must be assessed and secured.

9.8 Whilst operational impacts are assessed in the ES, it is unclear when in the operational phase temporary site compound land will be returned to the land owners.

Effects and mitigation

9.9 Given the nature of the construction works for the proposed Scheme and their proximity to the AMB, it is likely that the ability to mitigate the construction impacts of the proposed works for the Scheme is limited. These impacts include noise and vibration from vehicles accessing the site. Mitigation could include restrictions on times of deliveries and size of vehicles used and works to be agreed in conjunction with the University in advance. However, the location of the site compounds and the haul roads mean that the likely significant effects on the AMB may not be capable of being fully mitigated. Accordingly, the University seeks further details of alternatives that have been considered for the locations of site compounds and haul roads.

9.10 As set out in the ES, the AMB is a sensitive receptor site because equipment and operations within the building will be significantly impacted by vibration and noise including vibration and noise arising from construction vehicle movements and activities. The assessment carried out in the ES does not take into account the special nature of the AMB and the assessment is therefore deficient, not least because alternative locations for site compounds and haul roads do not appear to have been identified or assessed. The potential impacts on the AMB are such that alternative locations should have been assessed by Network Rail.

10 COMPULSORY ACQUISITION POWERS

10.1 The University will rely on the points made in its Objection, in relation to which further information and clarity is still awaited from Network Rail on what compulsory powers are sought and/or intended for the following plots: (084a, 084, 005a, 006, 006a, 006b, 006c, 007, 008, 010, 011, 065, 064, 062, 061, 057, 056, 055, 054, 053, 052, 020, 051, 050, 049, 048, 021, 047, 046, 045, 044, 043, 042, 041, 040, 039, 038, 022, 037, 024, 025, 036, 035, 028, 030, 029, 008a).

10.2 It is not clear in all cases which plots will be subject to permanent acquisition powers and it is important for the University to understand the full extent of land (or rights) being proposed to be used or taken (whether permanently or temporarily and for what purpose).

10.3 Sheets 2 and 3 (refs 158454-ARC-00-ZZ-DRG-EMF-200002 Rev P02.1 and 158454-ARC-00-ZZ-DRG-EMF-200003 Rev P02.1) of the deposited plans and sections include the plots of land owned by the University within the limits of deviation for the Works in the Draft Order. However, the University is concerned that neither drawing includes a sufficiently updated base map to enable a detailed comparison between the Scheme as proposed and the University's Estate. Neither plan illustrates buildings that have been built and operational in the last 3-4 years, including the AMB. The University has provided additional information to Network Rail to enable a clearer comparison but, despite requests for this, Network Rail has not produced an accurate as built base plan within their Application documents that the University can rely on to assess the impacts on the University's Estate.

10.4 Whilst a number of Deemed Planning Drawings, including Sheet 1 of 5 (ref 158454-ARCZZ-ZZ-DRG-LEP-000051) illustrate the committed AstraZeneca developments, the only plans that provide more detail in respect of the University's Estate, such as drawing. 158454-ARC-ZZ-ZZ-DRG-LEP-000101, are at an unhelpful scale to allow the University to assess the implications of the Scheme.

10.5 The University has insufficient information to determine the impacts on the operation of its Estate, including upon features such as the car park to the AMB (and access to this), surface water drainage and landscape features which run parallel to the western boundary of the CBC, or the impact upon the size and functional area of Plot 9. Similarly, the impacts on other buildings within the Estate cannot be assessed. The lack of information in the Application means that the University is also concerned as to the impacts of the Scheme on the operation of other buildings within the Estate (not only the AMB). These concerns with the clarity and precision of the drawings, and extent of proposed compulsory acquisition, have already been raised with Network Rail, but to date this information is still awaited from Network Rail. It is critical that this information is supplied as soon as possible to enable the University to understand and respond to the case being presented by Network Rail. The University will supplement this Statement as necessary in response to any further details that are received

11 PLANNING CONTEXT

11.1 The following headings and appraisals directly follow those in Network Rail's Planning Statement (NR14).

Background to the Proposed Development

11.2 Network Rail's Planning Statement submitted as part of the Application correctly identifies the CBC as the largest centre of medical research and health science in Europe (paragraph 2.2.2). Consultation and engagement in relation to the proposed Scheme is set out in Section 2.8 of the Planning Statement. This summarises consultation and engagement with the Greater Cambridge Shared Planning Service, Cambridgeshire County Council, Public Consultation, and Astra Zeneca. Schedule 5 and 6 consultees (excluding land owners, tenants and lease holders) are also identified. There is no reference, however, to consultation and engagement with the University. The University had in fact raised specific concerns relating to the likely potential for noise and vibration impacts of the Scheme on sensitive research in the AMB, car parking as well as access and servicing for AMB, and drainage for AMB and Plot 9.

11.3 Whilst the AMB is referred to (paragraph 3.1.4 of the Planning Statement), it is not identified as a noise and vibration sensitive receptor. Nor is there any obvious reference to Plot 9 (or for that matter certain Medical Research Council and Astra Zeneca development).

Planning Context

11.4 The Planning Statement appears to acknowledge that there is no specific planning support for the proposed Scheme. The University notes that a number of planning policies will be breached by the Scheme as proposed in the Application. The analysis that follows outlines the specific national and local policies of concern.

NPPF

11.4.1 Reference is made within the bullet points at 5.2.4 of the Planning Statement to a number of sections of the National Planning Policy Framework ("**NPPF**"), including "Paragraph 181 (2019)"[6]. Paragraph 181

[6] This has since been updated in July 2021, post submission of the Application and the corresponding paragraph is now paragraph 186 (2021)

provides details in relation to ground conditions and pollution, explaining that:

"Planning policies and decisions should sustain and contribute towards compliance with relevant limit values or national objectives for pollutants, taking into account the presence of Air Quality Management Areas and Clean Air Zones, and the cumulative impacts from individual sites in local areas. Opportunities to improve air quality or mitigate impacts should be identified, such as through traffic and travel management, and green infrastructure provision and enhancement"

11.4.2 However, there is no reference to paragraph 185 of the NPF, which is relevant for noise impact and has not been demonstrated to be satisfied by the Scheme:

"Planning policies and decisions should also ensure that new development is appropriate for its location taking into account the likely effects (including cumulative effects) of pollution on health, living conditions and the natural environment, as well as the potential sensitivity of the site or the wider area to impacts that could arise from the development. In doing so they should: a) mitigate and reduce to a minimum potential adverse impacts resulting from noise from new development – and avoid noise giving rise to significant adverse impacts on health and the quality of life (emphasis added)*"*

11.4.3 Similarly the Planning Statement does not make any reference to paragraphs 167 and 169 of the NPPF relative to sustainable drainage:

"167. When determining any planning applications, local planning authorities should ensure that flood risk is not increased elsewhere. Where appropriate, applications should be supported by a site-specific flood-risk assessment. Development should only be allowed in areas at risk of flooding where, in the light of this assessment (and the sequential and exception tests, as applicable) it can be demonstrated that: a) within the site, the most vulnerable development is located in areas of lowest flood risk, unless there are overriding reasons to prefer a different location; b) the development is appropriately flood resistant and resilient such that, in the event of a flood, it could be quickly brought back into use without significant refurbishment; c) it incorporates sustainable drainage systems, unless there is clear evidence that this would be inappropriate; d) any residual risk can be safely managed; and e) safe access and escape routes are included where appropriate, as part of an agreed emergency plan.

169. Major developments should incorporate sustainable drainage systems unless there is clear evidence that this would be inappropriate. The systems used should: a) take account of advice from the lead local flood authority; b) have appropriate proposed minimum operational standards; c) have maintenance arrangements in place to ensure an acceptable standard of operation for the lifetime of the development; and d) where possible, provide multifunctional benefits. (emphasis added.)"

11.4.4 The information provided by Network Rail does not demonstrate that these policy objectives have been met.

PPG

11.4.5 Paragraphs 001 and 002 of the National Planning Practice Guidance ("**PPG**") (guidance for noise) are summarised in the Planning Statement, but not paragraph 003, which makes specific reference to significant adverse effects including those arising from construction activity. Network Rail has not demonstrated compliance with this aspect of policy:

"Plan-making and decision making need to take account of the acoustic environment and in doing so consider:

> *-whether or not a significant adverse effect is occurring or likely to occur;*
>
> *-whether or not an adverse effect is occurring or likely to occur; and*
>
> *-whether or not a good standard of amenity can be achieved.*
>
> *In line with the Explanatory note of the noise policy statement for England, this would include identifying whether the overall effect of the noise exposure (including the impact during the construction phase wherever applicable) is, or would be, above or below the significant observed adverse effect level and the lowest observed adverse effect level for the given situation. As noise is a complex technical issue, it may be appropriate to seek experienced specialist assistance when applying this policy.*
>
> *Paragraph: 003 Reference ID: 30-003-20190722*
>
> *Revision date: 22 07 2019"*

Guidance for the Natural Environment 2019

11.4.6 Similarly, brief mention is made of Guidance for the Natural Environment (2019) in the Planning Statement but without specific reference to sustainable drainage features (paragraph 004), or the management of flood risk (005, 006), or to paragraph 008 which contains guidance that:

"<u>Green infrastructure opportunities and requirements need to be considered at the earliest stages of development proposals, as an integral part of development and infrastructure provision</u>, and taking into account existing natural assets and the most suitable locations and types of new provision" (<u>our emphasis added</u>).

Cambridge Local Plan (2018)

11.4.7 It is noted in the Planning Statement that Cambridge Local Plan (2018) Policy 17 'Cambridge Biomedical Campus (including Addenbrooke's Hospital) Area of Major Change' sets out to support the continuing growth and development of the CBC. However, specific land uses for biomedical and biotechnology research and development, related higher education and sui generis medical research institutes, are not referenced in the Planning Statement. It is common for noise and vibration sensitive research and equipment to be present within that group of uses, and therefore to maintain consistency with policy objectives it is important to ensure that any proposals involving noise and vibration generating development – including those arising from construction activity – are managed and designed to mitigate negative effects on the CBC, with specific measures identified at an early stage. This has not been demonstrated by Network Rail.

11.4.8 The Planning Statement also confirms that Cambridge Local Plan (2018) Policy 17 requires any proposal for development to retain and incorporate the existing watercourses. This has not been demonstrated satisfactorily by Network Rail. The Application includes a proposed haul road built over the AMB and Plot 9. However, how drainage is provided and maintained (in compliance with Policy 17) has not been demonstrated by Network Rail contrary to policy (see section 8 above). And whilst reference is made in the Planning Statement to Policy 31 'Integrated water management and the water cycle', there has been no proper demonstration that specific requirements for sustainable drainage are satisfied, including that:

(i) surface water is managed close to its source and on the surface where reasonably practicable to do so;

(ii) the features that manage surface water are commensurate with the design of the development in terms of size, form and materials and make an active contribution to making places for people;

(iii) there is no discharge from the developed site for rainfall depths up to 5 mm of any rainfall event;

(iv) the run-off from all hard surfaces shall receive an appropriate level of treatment in accordance with Sustainable Drainage Systems guidelines, SUDS Manual (CIRIA C753), to minimise the risk of pollution;

(v) watercourses are not culverted and any opportunity to remove culverts is taken;

(vi) all hard surfaces are permeable surfaces where reasonably practicable, and having regard to groundwater protection.

Cambridgeshire Flood and Water SPD

11.4.9 Whilst general reference is made to this SPD in the Planning Statement there is no reference to specific requirements, or to the specific requirement to plan in SuDS from the start (page 57 of the SPD).

Greater Cambridge Sustainable Design and Construction SPD

11.4.10 General reference is made to this SPD in the Planning Statement but not to the specific requirement that "*in certain situations, for instance where there is a proposal for a substantial development or infrastructure project, a Noise and Vibration Demolition and Construction Environmental Management Plan, detailing the management and control of noise and vibration, will be required as part of planning consent*" (paragraph 3.6.126 of the SPD).

11.4.11 There has been no sufficient Noise and Vibration Demolition and Construction Environmental Management Plan provided by Network Rail which provides sufficient detail having regard to the specific nature and effects of this Scheme.

11.5 Planning Considerations

11.5.1 The relevant planning considerations for the proposed Development are examined in Section 6 of the Planning Statement and considered against the relevant planning and transport policies set out above.

Noise

11.5.2 In relation to noise impact, paragraphs 6.8.2 to 6.8.5 of the Planning Statement identify that it is predicted that the construction phase of the proposed Scheme will result in significant but temporary effects on a number of locations/receptors, including the AMB. It is concluded that "*in order to appropriately control construction plant noise, the CoCP Part B will set out construction methodologies and methods for noise control".*

11.5.3 This approach is not agreed. The University's case is that a significant adverse effect from noise arising from construction activity, however temporary, will have a harmful and potentially catastrophic impact on research quality and outputs, funding and animal welfare (see section 4 above). It is essential for Network Rail to demonstrate that the proposed Scheme can proceed without harming research operations within the AMB through noise impact. This requires certainty that the Scheme is capable of effective mitigation, through the early identification of measures secured in the Application in order to comply with NPPF paragraph 185, NPPG paragraph 003, the Greater Cambridge Sustainable Design & Construction SPD paragraph 3.6.126.

Vibration

11.5.4 In relation to vibration impact, it is stated at paragraphs 6.8.6 to 6.8.12 in the Planning Statement that *"significant effects are predicted in relation to the most sensitive imaging equipment located within the Laboratory of Molecular Biology, however this can be reduced to acceptable levels through the use of mitigation measures. The approach will be developed in the detailed design stage of the Development and as part of ongoing consultation with users of the Laboratory of Molecular Biology"* (paragraph 6.8.11). However, no reference is made to the vibration sensitive equipment and research in the AMB, despite the fact that, as confirmed by Network Rail "*respective building users have been consulted in order to agree their sensitivity as part of the assessment*" (paragraph 6.8.8).

11.5.5 It is stated in the Planning Statement that the *"approaches to mitigation of potential significant effects from construction activities are set out within ES Chapter 5 and 6, with more detail to be included within the CoCP Part B. The CoCP Part B will include guidance and measures to be implemented to reduce the vibration levels as far as practicable, and set out the proposed construction vibration monitoring and the consultation and liaison plan with neighbouring properties. These measures will ensure activities that have the potential to lead to significant effects are reduced to a minimum where achievable and communicated well in advance with those that could be affected".* The University fundamentally disagrees with that approach. Chapter 6 of the ES (Acoustics Assessment Part 2 Vibration Report) identifies moderate impacts for the AMB from works that are in close proximity, resulting in a significant adverse effect even with the mitigations proposed by Network Rail. A significant adverse effect from vibration will have a harmful and potentially catastrophic impact on research quality and outputs, funding and animal welfare (see section 4 above). It is essential for Network Rail to demonstrate that the Application proposals will not harm research operations within the AMB through vibration. This requires certainty that the Scheme is capable of effective mitigation, through the early identification of measures secured in the Application, in order to comply with NPPF paragraph 185, NPPG paragraph 003, the Greater Cambridge Sustainable Design & Construction SPD paragraph 3.6.126.

Water Resources and Flood Risk

11.5.6 Water is considered in section 6.20 of the Planning Statement. This simply states that the CoCP (Part B), sets out best practice protocols which will be applied to prevent an increase in flood risk to both the site and the surrounding area runoff. However, as detailed in section 8 above there is no assessment of whether any activities (either during construction or operation of the proposed Scheme) would impact on the existing drainage system for either AMB or Plot 9 and in particular the swale to the west. Moreover, there is no evidence in the Application that the proposals have considered the detail of what exists and how it works or, critically, how construction and operational impacts on drainage – including for the AMB and Plot 9 – can be mitigated satisfactorily.

11.5.7 Mitigation cannot simply be deferred to the CoCP, in order to comply with NPPF paragraphs 167 and 169, NPPG paragraphs 005, 006 and 008, Cambridge Local Plan Policy 31, and the Cambridgeshire Flood and Water SPD page 57.

11.6 Planning Conditions

11.6.1 Proposed planning conditions are set out in the Request for Deemed Planning Permission ("**Deemed Permission**") (Document NR12). This includes a pre-commencement condition requiring the submission and approval of a CoCP, to include a Noise and Vibration Management Plan (Condition 10).

11.6.2 The proposed planning conditions also include a proposed pre-commencement condition for the submission and approval of a surface water drainage scheme (Condition 13).

11.6.3 For the reasons set out above, it is the University's case that it is not appropriate for mitigation measures relating to noise, vibration or surface water drainage to be dealt with through pre-commencement conditions where significant adverse effects have been identified. Rather, details should be approved as part of the Application and be subject to conditions in the Deemed Permission that require implementation in accordance with the approved details.

11.6.4 The Planning Statement does not therefore properly acknowledge the extent to which the inadequacy of information identified above causes conflict with planning policy.

12 INADEQUATE CONSULTATION

12.1 Government guidance in the form of "A Guide to Transport and Works Act Processes" dated 2006 (and last updated in November 2013) stresses the need for thorough consultation before Transport and Works Act Orders are made. The

University will show that notwithstanding this clear guidance, Network Rail have not undertaken meaningful engagement with the University.

12.2 As such, despite meetings having taken place with Network Rail, no meaningful progress has been made and Network Rail has failed to take into account the University's detailed concerns. Moreover, the University has not been provided with sufficient clarity by Network Rail around the proposals for the Scheme.

12.3 Network Rail has neither understood nor fully assessed the impacts of the Scheme on the University. They have therefore not provided any detailed or tailored mitigation which is vital given the unique nature of and impacts on the University. Had Network Rail consulted meaningfully with the University, this may have been avoidable.

13 COSTS AND FUNDING

13.1 The overall estimated cost of the scheme is stated to be £183,661,399 as set out in Network Rail's Estimate of Costs document (reference NR06). The Estimate of Costs also states that within the overall cost, acquisition of land and rights over land compensation is estimated to be in the sum of £7,673,614.

13.2 As matters stand, the University has not been provided with sufficient information to demonstrate that impacts on the AMB have been properly addressed or could be satisfactorily mitigated. If the effects of the Scheme could not be demonstrated to be acceptable, and it became necessary to relocate the AMB, the costs of doing so (even assuming a suitable site could be identified) would be extremely substantial and likely to be significantly greater than the sum identified for compensation in the Estimate of Costs, even without taking into account the potential losses from research work, including loss of grants (as indicated in Section 4 above).

13.3 There is no evidence that the viability of meeting the potential costs of relocation, to the extent that these are capable of being compensated, have been considered through the funding of the Scheme.

14 CONCLUSION

14.1 The University maintains its objection to the Scheme unless and until Network Rail satisfactorily addresses the issues expressed above in this Statement.

14.2 Given the identified inadequacies in the information to support the Scheme, the University emphasises the importance of immediate engagement by Network Rail to address these serious concerns.

Annex A - List of Documents

1 Network Rail's TWAO Application Documents, including Draft Order (NR02), Funding Statement (NR05), Estimate of Costs (NR06) Book of Reference (NR08), Deposited Plans and Sections (NR08), Request for Deemed Permission (NR12), Planning Statement (NR14), Design and Access Statement (NR15), Environmental Statement (including Appendices and Figures) (NR16).

2 Network Rail's Cambridge South Outline Business Case.

3 National Planning Policy Framework (2012, as updated 20 July 2021) – extracts appended at Appendix 1

4 Planning Practice Guidance for Noise (2016, as updated 24 June 2021) – extracts appended at Appendix 2

5 Planning Practice Guidance for the Natural Environment (2019) – extracts appended at Appendix 3

6 Cambridge Local Plan (2018) – extracts appended at Appendix 4

7 Cambridgeshire Flood and Water SPD, originally adopted in November 2016 and readopted by South Cambridgeshire District Council in November 2018 and Cambridge City Council in December 2018 – extracts appended at Appendix 5

8 Greater Cambridge Sustainable Design and Construction SPD, adopted by South Cambridgeshire District Council on 8 January 2020 and Cambridge City Council on 14 January 2020 – extracts appended at Appendix 6

9 A Guide to Transport and Works Act Processes, 2006 (and last updated in November 2013) – extracts appended at Appendix 7

10 Article entitled "Comparison of Mice' Sperm parameters exposed to some hazardous physical agents" - Mohammad-Bagher Abdollahi, Somayeh Farhang Dehghan, Faezeh Abasi Balochkhaneh3, Manouchehr Ahmadi Moghadam, Hamzeh Mohammadi – included at Appendix 8

11 Article entitled "Vibration in mice: A review of comparative effects and use in translational research" Randall P. Reynolds, Yao Li2, Angela Garner, John N. Norton – included at Appendix 8

12 Article entitled "Noise and Vibration in the Vivarium: Recommendations for Developing a Measurement Plan" Jeremy G Turner, Journal of the American Association for Laboratory Animal Science – included at Appendix 8

Appendix 1 – NPPF extracts

NPPF (2019) paragraph 181

181. Planning policies and decisions should sustain and contribute towards compliance with relevant limit values or national objectives for pollutants, taking into account the presence of Air Quality Management Areas and Clean Air Zones, and the cumulative impacts from individual sites in local areas. Opportunities to improve air quality or mitigate impacts should be identified, such as through traffic and travel management, and green infrastructure provision and enhancement. So far as possible these opportunities should be considered at the plan-making stage, to ensure a strategic approach and limit the need for issues to be reconsidered when determining individual applications. Planning decisions should ensure that any new development in Air Quality Management Areas and Clean Air Zones is consistent with the local air quality action plan.

NPPF (2021) paragraph 185

185. Planning policies and decisions should also ensure that new development is appropriate for its location taking into account the likely effects (including cumulative effects) of pollution on health, living conditions and the natural environment, as well as the potential sensitivity of the site or the wider area to impacts that could arise from the development. In doing so they should:

a) mitigate and reduce to a minimum potential adverse impacts resulting from noise from new development – and avoid noise giving rise to significant adverse impacts on health and the quality of life[65];

b) identify and protect tranquil areas which have remained relatively undisturbed by noise and are prized for their recreational and amenity value for this reason; and

c) limit the impact of light pollution from artificial light on local amenity, intrinsically dark landscapes and nature conservation.

[65] See Explanatory Note to the *Noise Policy Statement for England* (Department for Environment, Food & Rural Affairs, 2010).

NPPF (2021) Paragraph 167

167. When determining any planning applications, local planning authorities should ensure that flood risk is not increased elsewhere. Where appropriate, applications should be supported by a site-specific flood-risk assessment[55]. Development should only be allowed in areas at risk of flooding where, in the light of this assessment (and the sequential and exception tests, as applicable) it can be demonstrated that:

a) within the site, the most vulnerable development is located in areas of lowest flood risk, unless there are overriding reasons to prefer a different location;

b) the development is appropriately flood resistant and resilient such that, in the event of a flood, it could be quickly brought back into use without significant refurbishment;

c) it incorporates sustainable drainage systems, unless there is clear evidence that this would be inappropriate;

d) any residual risk can be safely managed; and

e) safe access and escape routes are included where appropriate, as part of an agreed emergency plan.

[55] A site-specific flood risk assessment should be provided for all development in Flood Zones 2 and 3. In Flood Zone 1, an assessment should accompany all proposals involving: sites of 1 hectare or more; land which has been identified by the Environment Agency as having critical drainage problems; land identified in a strategic flood risk assessment as being at increased flood risk in future; or land that may be subject to other sources of flooding, where its development would introduce a more vulnerable use.

NPPF (2021) Paragraph 169

169. Major developments should incorporate sustainable drainage systems unless there is clear evidence that this would be inappropriate. The systems used should:

a) take account of advice from the lead local flood authority;

b) have appropriate proposed minimum operational standards;

c) have maintenance arrangements in place to ensure an acceptable standard of operation for the lifetime of the development; and

d) where possible, provide multifunctional benefits.

Appendix 2 – PPG for Noise (2019) extracts

How can noise impacts be determined?

Plan-making and decision making need to take account of the acoustic environment and in doing so consider:

- whether or not a significant adverse effect is occurring or likely to occur;
- whether or not an adverse effect is occurring or likely to occur; and
- whether or not a good standard of amenity can be achieved.

In line with the Explanatory note of the noise policy statement for England, this would include identifying whether the overall effect of the noise exposure (including the impact during the construction phase wherever applicable) is, or would be, above or below the significant observed adverse effect level and the lowest observed adverse effect level for the given situation. As noise is a complex technical issue, it may be appropriate to seek experienced specialist assistance when applying this policy.

Paragraph: 003 Reference ID: 30-003-20190722

Revision date: 22 07 2019

Appendix 3 – PPG for the Natural Environment (2019) extracts

What can green infrastructure include?

Green infrastructure can embrace a range of spaces and assets that provide environmental and wider benefits. It can, for example, include parks, playing fields, other areas of open space, woodland, allotments, private gardens, sustainable drainage features, green roofs and walls, street trees and 'blue infrastructure' such as streams, ponds, canals and other water bodies. References to green infrastructure in this guidance also apply to different types of blue infrastructure where appropriate.

Paragraph: 004 Reference ID: 8-004-20190721

Revision date: 21 07 2019

Why is green infrastructure important?

Green infrastructure is a natural capital asset that provides multiple benefits, at a range of scales. For communities, these benefits can include enhanced wellbeing, outdoor recreation and access, enhanced biodiversity and landscapes, food and energy production, urban cooling, and the management of flood risk. These benefits are also known as ecosystem services.

Paragraph: 005 Reference ID: 8-005-20190721

Revision date: 21 07 2019

What planning goals can green infrastructure help to achieve ?

Green infrastructure can help in:

- **Building a strong, competitive economy**
  Green infrastructure can drive economic growth and regeneration, helping to create high quality environments which are attractive to businesses and investors.
- **Achieving well-designed places**
  The built environment can be enhanced by features such as green roofs, street trees, proximity to woodland, public gardens and recreational and open spaces. More broadly, green infrastructure exists within a wider landscape context and can reinforce and enhance local landscape character, contributing to a sense of place and natural beauty.
- **Promoting healthy and safe communities**
  Green infrastructure can improve the wellbeing of a neighbourhood with opportunities for recreation, exercise, social interaction, experiencing and caring for nature, community food-growing and gardening, all of which can bring mental and physical health benefits. Outdoor Recreation Value (ORVal) is a useful online tool that can be used to quantify the recreational values provided by greenspace. Green infrastructure can help to reduce health inequalities in areas of socio-economic deprivation and meet the needs of families and an ageing population. It can also help to reduce air pollution and noise.
- **Mitigating climate change, flooding and coastal change**
  Green infrastructure can contribute to carbon storage, cooling and shading, opportunities for species migration to more suitable habitats and the protection of water quality and other natural resources. It can also be an integral part of multifunctional sustainable drainage and natural flood risk management.
- **Conserving and enhancing the natural environment**
  High-quality networks of multifunctional green infrastructure contribute a range of benefits, including ecological connectivity, facilitating biodiversity net gain and nature recovery networks and opportunities for communities to undertake conservation work.

Paragraph: 006 Reference ID: 8-006-20190721

Revision date: 21 07 2019

How can green infrastructure be considered in planning decisions?

Green infrastructure opportunities and requirements need to be considered at the earliest stages of development proposals, as an integral part of development and infrastructure provision, and taking into account existing natural assets and the most suitable locations and types of new provision.

Depending on individual circumstances, planning conditions, obligations, or the Community Infrastructure Levy may all be potential mechanisms for securing and funding green infrastructure.

Green infrastructure will require sustainable management and maintenance if it is to provide benefits and services in the long term. Arrangements for funding need to be identified as early as possible, and factored into the design and implementation, balancing the costs with the benefits. Local community engagement can assist with management and tailoring provision to local needs.

Paragraph: 008 Reference ID: 8-008-20190721

Revision date: 21 07 2019

Appendix 4 – Cambridge Local Plan (2018) extracts

(Policy 17 & Policy 31)

Policy 17: Cambridge Biomedical Campus (including Addenbrooke's Hospital) Area of Major Change

Development proposals will be permitted at Cambridge Biomedical Campus (including Addenbrooke's Hospital) where it can be demonstrated that development is required to meet local, regional or national health care needs or for biomedical and biotechnology research and development activities within class B1(b), related higher education and sui generis medical research institutes.

Associated support activities for the site as a whole, including a hotel, seminar conference centre and small scale A1 (local shop), A3 (café), A4 (public house) and D1 (crèche) type uses, would be acceptable to meet the needs of employees and visitors and to add to the vibrancy of the area.

Section 106 agreements and planning conditions will be used to ensure occupation accords with this mix of uses and that sufficient land is available to meet the hospital's future development needs.

Any proposals for development should:

a. respect key views, especially of and from the chalk hills, create new vistas, and create an attractive landscape and building edge along the railway and landscape buffer areas of at least 20 metres along the southern boundary;
b. maximise opportunities to improve the 'legibility' of the Cambridge Biomedical Campus by providing a network of cycle and pedestrian routes, high quality new public realm and open space;
c. retain and incorporate the existing watercourses;
d. include measures to enhance access to the Cambridge Biomedical Campus including for cyclists, pedestrians, wheelchair users and other disabled people, and mitigate the impact on the existing road network and parking in the surrounding area;
e. include provision for the extension of existing conventional bus services, the Cambridgeshire Busway and Park and Ride services to meet the needs of the resident and working populations, including disabled people; and
f. connect to the Addenbrooke's Hospital energy network, where feasible and viable.

Supporting text:

3.42 The Cambridge Biomedical Campus is an international centre of excellence for patient care, biomedical research and healthcare education. It plays a local, regional and national role in providing medical facilities and medical research. The local plan will support its continuing development as such, and as a high quality, legible and sustainable campus. It also reinforces the existing biomedical and biotechnology cluster in the Cambridge area.

3.43 This policy covers the existing campus, and sufficient land to allow its expansion to meet the health needs of the expanding city and for public and private organisations with a biomedical focus to co-locate.

3.44 The total area of the site is approximately 68 hectares comprising:

3.45 **Existing NHS Trust area**: this is the existing built-up area bounded by Robinson Way to the west. At the north west of the site outline approval has been granted for:

- a learning/seminar/conference centre;
- a hotel; and
- ancillary and support activities.

3.46 **Area covered by outline approval**: this is the area between the existing buildings and the railway line to the west, and the southern access road (under construction) to the south. The current outline approval allows for:

- the relocation of Papworth Hospital;
- other NHS and private clinical development;
- clinical research and biomedical and biotechnology research and development activities within class B1(b);
- sui generis medical research institutions; and
- related support activities.

3.47 The Medical Research Council Laboratory of Molecular Biology is within the outline permission site.

3.48 **Expansion area**: this is the area south of the southern access road. Development here will be similar to that approved for the remainder of the site, with approximately a third of the land to be developed for NHS and private clinical development and two-thirds for biomedical and biotechnology research and development activities.

3.49 When approval is granted for this land, or other proposals within the Cambridge Biomedical Campus, conditions or legal agreements will be used to ensure future development and occupation will be in accordance with this mix of uses.

3.50 **Energy centre**: Addenbrooke's Hospital has identified the need for a new clinical waste facility (energy from waste) to replace the existing facility. In response, the Cambridgeshire and Peterborough Minerals and Waste Core Strategy (July 2011) made a strategic site specific allocation for the replacement clinical waste facility (Policy CS19, area of search site W2). It also provided a waste consultation area around this to protect the site allocation (Policies CS19 and CS30). The area of search and the waste consultation area are shown on the Policies Map. An application has been approved by Cambridgeshire County Council for an energy innovation centre (energy from waste facility) within the site allocation.

3.51 This will allow Addenbrooke's Hospital to benefit from an energy innovation centre and energy network serving the Cambridge Biomedical Campus as a whole. Developments within the site should, therefore, seek to connect to this energy network, subject to feasibility and viability. A benefit is that it can provide developers with a cost-effective way to meet the carbon reduction requirements sought by the local plan.

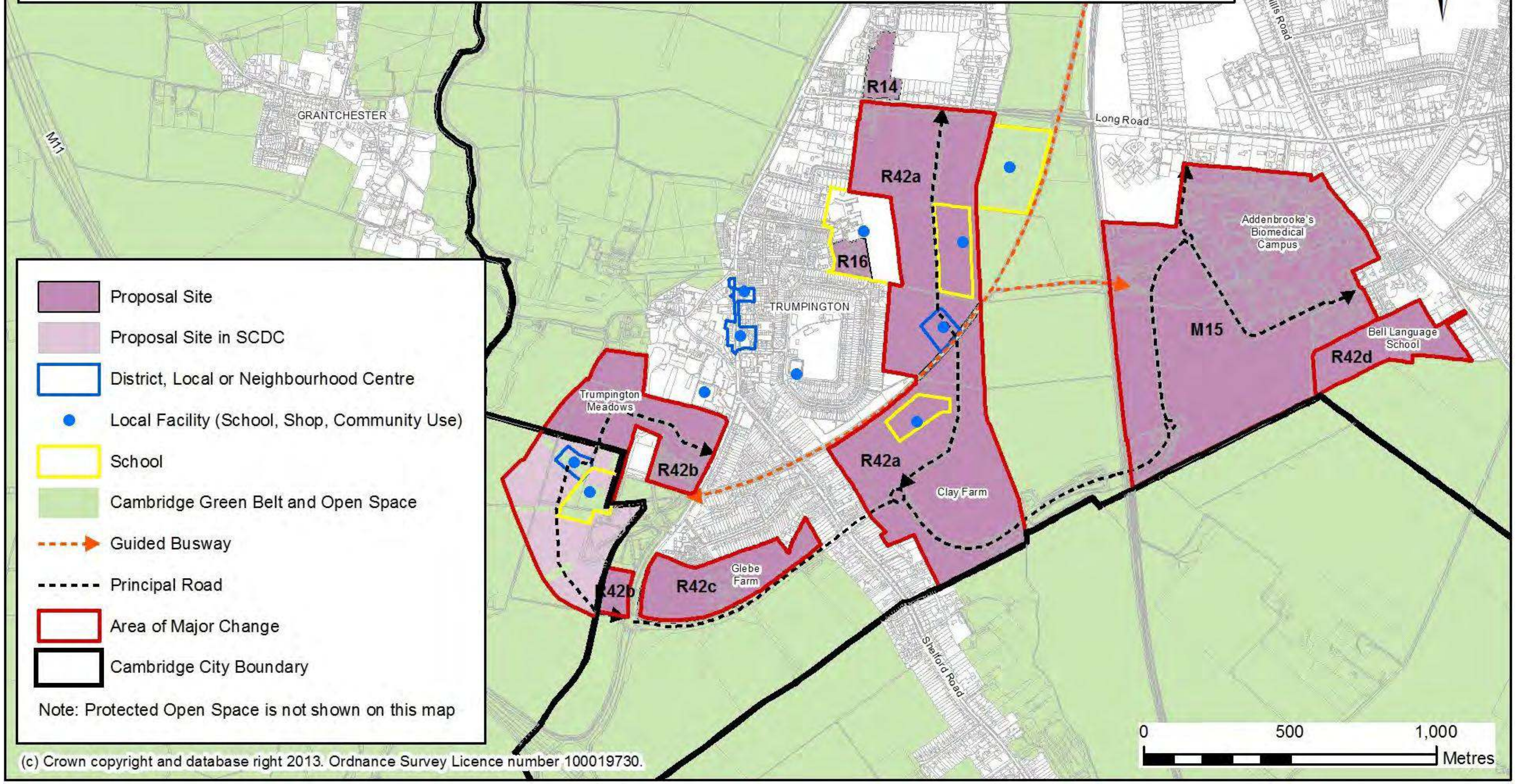
3.52 **Strategic masterplan**: the Cambridge University Hospitals NHS Trust (the Trust) has developed a 2020 Vision for the extended campus area. It completed a strategic masterplan in 2010 which includes the following:

- key routes and street hierarchy;
- public realm strategy and open space;
- building massing;
- potential uses;
- development phasing; and
- sustainability.

3.53 The public realm strategy aims to achieve an environment that is attractive, well-designed and distinctive, accessible and inclusive. The masterplan is being partially updated by the Trust as developments come forward, and does cover development beyond 2020, working with development partners and the city and county Councils. These updates will be reported to the Council as appropriate to inform the consideration of applications as they come forward.

3.54 Figure 3.5 provides a diagrammatic representation of the principal land uses, access and transport arrangements and landscape provision for the Cambridge Biomedical Campus and its relationship with the Southern Fringe, and the rest of the city.

Figure 3.5: Cambridge Biomedical Campus (including Addenbrooke's Hospital) and Southern Fringe Areas of Major Change



Supporting text:

4.18 In order for Cambridge to play a role in meeting national targets for carbon reduction, there is a need to reduce emissions from existing buildings as well as new ones. This policy seeks to utilise the opportunities that arise for making cost-effective energy efficiency improvements when works to extend existing homes are undertaken. Applicants will be asked to complete a simple online home energy questionnaire, which will help to identify suitable measures. Where an applicant has recently had a Green Deal assessment undertaken or the property has an Energy Performance Certificate (EPC), these could also be submitted as part of the planning application to demonstrate the need to comply with the policy.

4.19 The aim of the policy is to help homeowners implement measures that will enhance the energy efficiency of their homes, helping to reduce fuel costs at a time of rising energy prices. This might help reduce the risk of some homeowners finding themselves in fuel poverty, or in cases where residents are already in fuel poverty, help get them out of this situation. There is also some evidence to suggest that carrying out energy efficiency measures can also increase the value of properties. The focus is on cost-effective measures with a simple payback of seven years or less and that would be relatively simple to install with limited disruption.

4.20 Care will need to be taken in applying the policy to listed buildings and other heritage assets, to ensure that they are not damaged by inappropriate interventions. The implementation of the policy will be case by case, with officers recommending measures that would be suitable for that particular property, bearing in mind its age, type of construction and historic significance. There may be cases where improvements cannot be made to an existing dwelling without causing harm to the significance of the heritage asset, and in such circumstances the requirements of this policy will not be implemented.

Policy 31: Integrated water management and the water cycle

Development will be permitted provided that:

a. surface water is managed close to its source and on the surface where reasonably practicable to do so;
b. priority is given to the use of nature services[17];

[17] Nature services are defined by the National Planning Policy Framework (2012) as: 'The benefits people obtain from ecosystems such as, food, water, flood and disease control and recreation'. These are also known as ecosystem services.

c. water is seen as a resource and is re-used where practicable, offsetting potable water demand, and that a water sensitive approach is taken to the design of the development;
d. the features that manage surface water are commensurate with the design of the development in terms of size, form and materials and make an active contribution to making places for people;
e. surface water management features are multi-functional wherever possible in their land use;
f. any flat roof is a green or brown roof, providing that it is acceptable in terms of its context in the historic environment of Cambridge (see Policy 61: Conservation and Enhancement of Cambridge's Historic Environment) and the structural capacity of the roof if it is a refurbishment. Green or brown roofs should be widely used in large-scale new communities;
g. there is no discharge from the developed site for rainfall depths up to 5 mm of any rainfall event;
h. the run-off from all hard surfaces shall receive an appropriate level of treatment in accordance with Sustainable Drainage Systems guidelines, SUDS Manual (CIRIA C753), to minimise the risk of pollution;
i. development adjacent to a water body actively seeks to enhance the water body in terms of its hydromorphology, biodiversity potential and setting;
j. watercourses are not culverted and any opportunity to remove culverts is taken; and
k. all hard surfaces are permeable surfaces where reasonably practicable, and having regard to groundwater protection.

Supporting text:

4.21 The Surface Water Management Plan[18] and Strategic Flood Risk Assessment for Cambridge[19] have found there is little or no capacity in our rivers and watercourses that eventually receive surface water run-off from Cambridge and that it needs to be adequately managed so that flood risk is not increased elsewhere. The appropriate application of sustainable drainage systems to manage surface water within a development is the approach recommended within the planning practice guidance to the National Planning Policy Framework[20] (NPPF, 2012) as a way of managing this risk.

4.22 Current best practice guidance such as the SUDS Manual and Planning for SUDS (CIRIA C753 and C687) should be followed in the design of developments of all sizes, with design principles that are important to Cambridge set out in this policy. Smaller, more resilient features

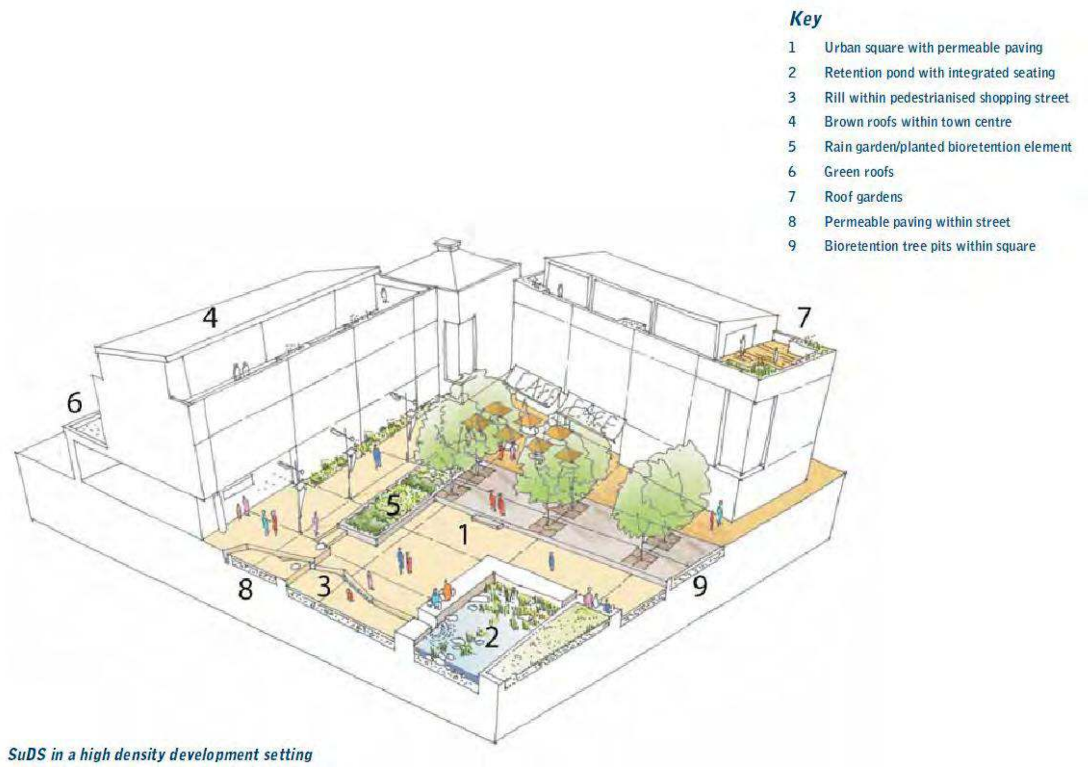
[18] Cambridge and Milton Surface Water Management Plan (2011), Cambridgeshire Flood Risk Partnership
[19] Cambridge and South Cambridgeshire Level 1 Strategic Flood Risk Assessment (2010)
[20] Planning practice guidance - Flood risk and coastal change (2014)

distributed throughout a development should be used, instead of one large management feature. Figure 4.3 provides examples of how to successfully integrate SuDS into a range of developments.

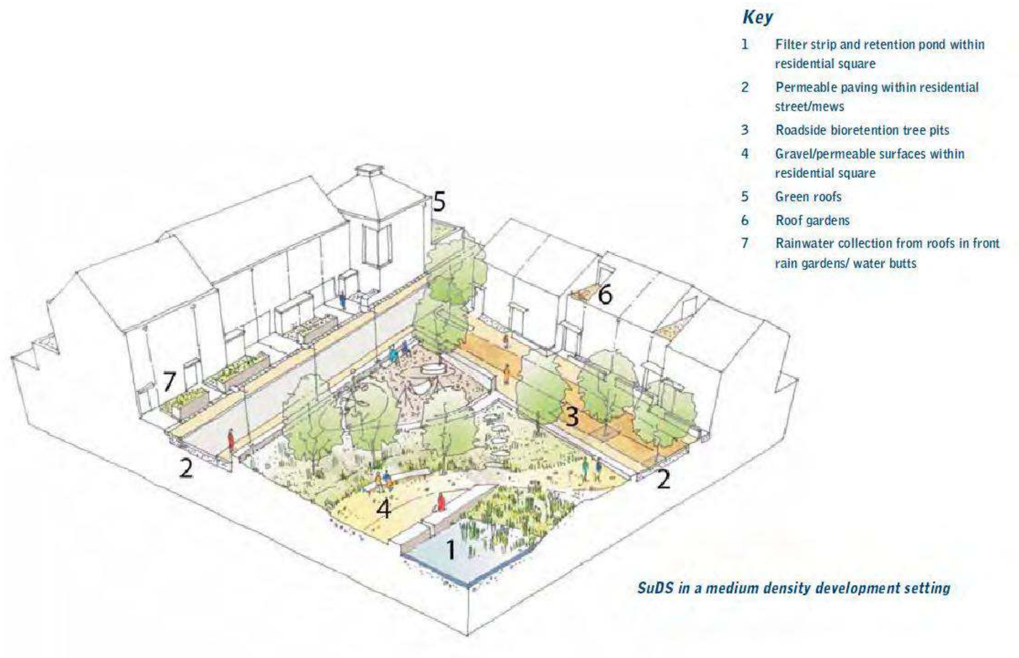
4.23 Managing water close to where it falls and on the surface is often the most cost-effective way to manage surface water. Early consideration in the design process helps achieve this. Managing water on the surface is an opportunity to celebrate water and create developments distinctive to Cambridge.

4.24 Climate change will in future see times of too much water and times of too little water more frequently than now. The design of new developments should reflect this change and value water as a resource that can be stored in times of plenty for re-use in times of deficit.

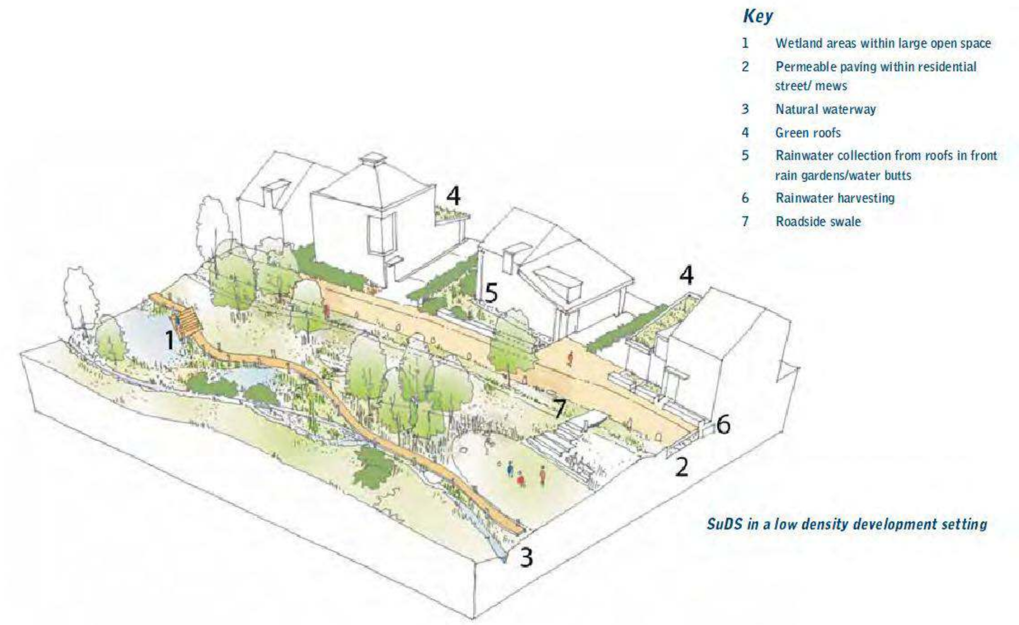
Figure 4.3: Examples of integrating SuDS into developments[21]



[21] Source: Dickie, S, McKay, G, Ions, L, Shaffer, P (2010) Planning for SUDS - Making it happen, CIRIA, C687, London (ISBN: 978-0-86017-687-9) Go to: www.ciria.org



SuDS in a medium density development setting



SuDS in a low density development setting

4.25 Green and brown roofs are a key measure in terms of Cambridge's climate change adaptation policy. They offer multiple benefits for a comparatively small additional construction cost, including forming part of an effective sustainable drainage solution, reducing the amounts of storm water run-off and attenuating peak flow rates. In the summer, a green roof can typically retain 70-80 per cent of rainfall run-off. Predicted climate change means that Cambridge will experience increasing risks of flooding, overheating and drought, manifested through hotter drier summers and warmer wetter winters. Living roofs can reduce the negative effects of climate change, for example by improving a building's energy balance and reducing carbon emissions. The use of vegetation on a roof surface ameliorates the negative thermal effects of conventional roof surfaces through the cooling effect of evapotranspiration, which can also help ameliorate the urban heat island effect (UHI). It can also provide benefit in the form of insulation, helping to reduce the internal cooling load of buildings, thereby reducing energy use and associated carbon emissions. The biodiversity benefits of green roofs are manifold, supporting rare and interesting types of plant, which in turn can host a variety of rare and interesting fauna. Accessible roof space can also provide outdoor living space, particularly in high-density developments. As such, accessible roof space should be viewed as an integral element of a well-designed, high-quality, high-density, more efficient, attractive and liveable city.

4.26 Green/brown roofs can be more cost effective than a traditional roof over the lifetime of a development. A flat roof is defined as a roof with a pitch of between 0° and 10°**.**

4.27 The EU Water Framework Directive and the associated River Basin Management Plan for the Anglian region[22] require public bodies to have a positive impact on the quality of lakes, rivers and groundwater, collectively called water bodies. The water bodies in Cambridge are currently failing to achieve the required status of 'good'. Quality refers to the quality of the water body in terms of the quality of the water itself, the quality of the shape and form of the water body, and the quality of its biodiversity.

4.28 This policy seeks to ensure all surface water that is discharged to ground or into rivers, watercourses and sewers has an appropriate level of treatment to reduce the risk of diffuse pollution.

4.29 The policy also recognises that development adjacent to a water body provides an opportunity for both the development and the water body, and that they should complement and enhance each other.

Policy 32: Flood risk

Potential flood risk from the development

Development will be permitted providing it is demonstrated that:

a. the peak rate of run-off over the lifetime of the development, allowing for climate change, is no greater for the developed site than it was for the undeveloped site;

[22] Environment Agency (2009). Water for life and livelihoods. River Basin Management Plan – Anglian River Basin District

Appendix 5 – Cambridge Flood and Water SPD extracts

Plan in SuDS from the start

6.3.2 Considering SuDS during the preliminary stages of site design provides the opportunity to incorporate features that are appropriate to the local context and character of an area. Integrated design to achieve multi-functional benefits is inherent to the site master planning and layout process; therefore it is most efficient and cost effective to design SuDS schemes into a site as early as possible. When drainage is accounted for from the beginning of the design process, it provides opportunity for the built up areas to be designed in-line with the topography, rather than to fit the drainage around the site at a later stage which is much less effective.

6.3.3 Land uses that have different pollution potential can also be clustered and phased so that management trains can be designed most effectively. The result of early inclusion of SuDS is a more effective and efficient layout which will avoid the need for abortive work and changes at a later stage which can escalate costs.

6.3.4 The better the SuDS design the more options for adoption that might be available to a development. The stages described in Figure 6.1 to Figure 6.5 show how a design can integrate SuDS spatially through the evolution of a masterplanning exercise.

Appendix 6 – Greater Cambridge Sustainable Design and Construction SPD extracts

Construction and Demolition Work

3.6.126 The Control of Pollution Act 1974 is primary legislation which can deal with the control of noise from construction sites. However in certain situations, for instance where there is a proposal for a substantial development or infrastructure project, a Noise and Vibration Demolition and Construction Environmental Management Plan, detailing the management and control of noise and vibration, will be required as part of planning consent.

Appendix 7 – A Guide to Transport and Works Act Procedures (2013) extracts

The importance of pre-application consultations

Undertaking thorough and effective consultations before an application is made will almost certainly reap dividends later. The extent of consultations required will depend upon the size and nature of the scheme. But having a constructive and meaningful dialogue with those likely to be interested in or affected by a project can provide helpful feedback into its design, can help to allay fears and suspicions that may be based on a lack of understanding of the scheme, and can help greatly to limit the number of objections once an application is made.

In particular, promoters are asked to consult key players in their area, such as local authorities, development agencies, public service providers, MP's etc. The importance of meaningful pre-application consultation is reinforced by the statutory procedure rules which require a report summarising the consultations that have been carried out to accompany the application.

Appendix 8 – Scientific Articles

- "*Comparison of Mice' Sperm parameters exposed to some hazardous physical agents*": Mohammad-Bagher Abdollahi, Somayeh Farhang Dehghan, Faezeh Abasi Balochkhaneh3, Manouchehr Ahmadi Moghadam, Hamzeh Mohammadi.

- "*Vibration in mice: A review of comparative effects and use in translational research*": Randall P. Reynolds, Yao Li2, Angela Garner, John N. Norton

- "*Noise and Vibration in the Vivarium: Recommendations for Developing a Measurement Plan*" Jeremy G Turner, Journal of the American Association for Laboratory Animal Science

EAHT
Environmental Analysis Health and Toxicology
eISSN: 2671-9525
OpenAccess
Vol: 36(3), Article ID: e2021013, 9 pages
https://doi.org/10.5620/eaht.2021013

Original Article

Comparison of mice' sperm parameters exposed to some hazardous physical agents

Mohammad-Bagher Abdollahi[1], Somayeh Farhang Dehghan[2], Faezeh Abasi Balochkhaneh[3], Manouchehr Ahmadi Moghadam[3], Hamzeh Mohammadi[4,*]

[1] Shoushtar Faculty of Medical Sciences, Shoushtar, Khuzestan, Iran
[2] Environmental and Occupational Hazards Control Research Center, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Department of Occupational Health, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
***Correspondence:** Hamzeh.Mohammadi5h@gmail.com

Received: December 13, 2020 **Accepted**: June 28, 2021

Abstract
The present study was aimed to compare the effects of exposure to noise, vibration, lighting, and microwave on male mice' sperm parameters. The mice were randomly assigned to five groups of eight, which comprised of the unexposed group and exposure groups including the lighting (1000 lux), noise (100 dB(A)), vibration (acceleration of 1.2 m/s^2) and microwave (power density of 5 watts). The exposure groups were subjected to the four agents for 8 hours a day, 5 days a week during a 2-week period. Semen analysis were done according to World Health Organization guidelines. The highest significant mean difference in sperm count (-1.35×10^6/mL) had being observed between the microwave group and the control one (P=0.001). The highest difference in immotile percent (25.88 %) had being observed between the noise group and the control one (P=0.001). The highest difference in normal morphology (-27.06 %) observed between the lighting exposure group and the control group (P=0.001). The four agents can cause changes in different sperm parameters, however for definite conclusion; more laboratory and field studies are required. In total, exposure to microwave has had the greatest effect on sperm count and exposure to light has had the greatest effect on normal morphology and non-progressive motility. Moreover, exposure to noise has had the greatest effect on progressive motility and immotile percent, respectively.

Keywords: Sperm parameters, Exposure, Physical agents, Mice

Introduction

The advancement of technology in all areas and the process of industrialization has led to the widespread use of different devices, tools and machinery in various industries [1]. This phenomenon has caused humans to be increasingly exposed to varying degrees of hazardous agents both in the workplace and in everyday life. On the other hand, in the preceding decades, studies on the effects of occupational exposure on the reproductive system have been expanded greatly [2], especially since any damage to the reproductive system can lead to permanent or temporary infertility, genetic mutations or hereditary cancer [3]. Research has shown that the quality of sperm has deteriorate over the past fifty years and this has raised questions regarding the negative effects of hazardous physical and chemical agents on it [4].

Common harmful physical agents at workplaces and environments include microwaves, lighting, noise and vibration. Noise pollution is defined as exposure to unwanted or unpleasant sound [5] and is dependent on various factors like noise exposure duration and frequency characteristics. Exposure to noise levels exceeding occupational exposure limits leads to reduced efficiency among workers and the enterprise as a whole [6]. Noise exposure can cause cardiovascular, gastrointestinal, behavioral, psychological and sleep related disorders. It can also cause reduced hearing, visual impairment, disruption of the vestibular system and can affect sperm parameters [7,8,9]. The results of a study conducted by Swami et al. regarding the effects of noise exposure on steroidogenic hormones in men showed that exposure to noise at 100 dB(A) can cause a meaningful reduction in serum testosterone levels [10].

Vibration is another hazardous occupational agent in industrialized and developing countries that can cause discomfort and dissatisfaction among workers [11]. Occupational exposure to Whole Body Vibration (WBV) can be found among workers engaged in vibrating platforms and stone cutting machines as well as drivers [12]. The body response to vibration depends on many variables such as vibration frequency, vibration amplitude, exposure duration and body posture

[13]. The effects of vibration on sperm parameters have been proven in previous studies. The results of a study by Penkov et al. regarding the effects of WBV exposure on sperm morphology showed an increase in Oligospermia and Azoospermia, a reduction of ejaculate volume, reduced motile spermatozoa and an increase in the rate of sperm deformation in the exposure group [14].

Lighting can also be a harmful physical occupational factor, though is itself an essential element of occupational safety as it helps in the detection of sizes, shapes or colors, and can increase the accuracy of the workers and help prevent visual errors and occupational accidents [15]. An adult uses his eyes for around 16 hours each day, so the level of lighting must be suited to the precision of work being performed [16]. Advancements in technology and the need for extended or 24-hour work shifts means that workers are subject to prolonged periods of exposure to high levels of lighting, especially in occupations requiring precision such as watch making, cartography and electronics [17]. Brandt et al. conducted a study on the effects of artificial lighting (300 to 350 lux) on the semen quality of adult boars. Their findings showed reduced semen volume and reduced number of motile sperm in the exposure group [18]. Alternative studies however, suggest that exposure to low intensities of visible spectrum and infra-red light can actually increase sperm motility [19].

Technological advancement and the ever-increasing usage of tools and devices has made exposure to electromagnetic fields at home, work, hospitals or industries, inevitable. This has made researchers and the public increasingly concerned regarding the potential biological effects of exposure to these fields [20]. The International Commission on Non-Ionizing Radiation Protection (ICNIRP) insists on the seriousness of the problem regarding electromagnetic radiation and its negative effects on human health and consider it to be a prevalent environmental risk [21]. Therefore, controlling the exposure to electromagnetic fields is as important as the controlling of other hazardous occupational and environmental agents from a health and safety perspective. The daily use of electronic devices has caused people to become increasingly exposed to electromagnetic waves [22]. The electromagnetic spectrum covers a wide frequency range among which, microwave radiation falls between 300 MHz to 300 GHz [23]. A study by Dasdag et al. showed that whole body exposure to microwave radiation had no meaningful effect on sperm count [24]. A study by Mailankot et al. however showed that exposure to microwave radiation can reduce the quality of the ejaculate [25].

Considering the fact that workers are exposed to various occupational risk factors such as noise, vibration, lighting or microwave, and since the results of other studies investigating the effects of these factors on semen indices are conflicting; the present study was designed with the aim of comparing the effects of exposure to these hazardous agents on male mice' sperm parameters.

Materials and Methods

This case-control study was conducted on 40 male adult NMRI (Naval Medical Research Institute) mice with a weight of 30±2 g and an age of 50 days old. The mice were provided from Neuroscience Research Center, Shahid Beheshti University of Medical Sciences. The mice were randomly assigned to five groups of eight, which comprised of the unexposed group (control group) and the lighting, noise, vibration and microwave exposure groups. The exposure groups were subjected to the four harmful occupational factors for 8 hours a day, 5 days a week for a total of 80 hours of exposure during a 2-week period. This exposure duration was chosen because the regeneration cycle for the Epithelium cells inside the seminiferous tubules of male mice is 8.6 days. This also better approximates the exposure conditions of workers in various industries.

Storage and test conditions

The test mice were initially kept at the animal house under standard conditions (22±2°C, 40-60% humidity, <35 dB(A) background noise, 12-hour light/dark cycle, 100 lux lighting intensity during light cycle, free access to plate and water) [26]. All protocols were in accordance to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by student research committee, Shahid Beheshti University of medical science. The storage space where the mice were kept was a box made of double-pane glass (for internal visibility) with a dimension of 100×90×60 cm and enough space for eight lab mice in each group. Holes were made in each box for observation, measurement (12 times per hour) and ventilation. The storage boxes were designed in a way as to be able to dynamically control the flow of air and to be able to maintain steady temperature and humidity (Figure 1). The mice were killed by the guillotine on the last day of exposure.

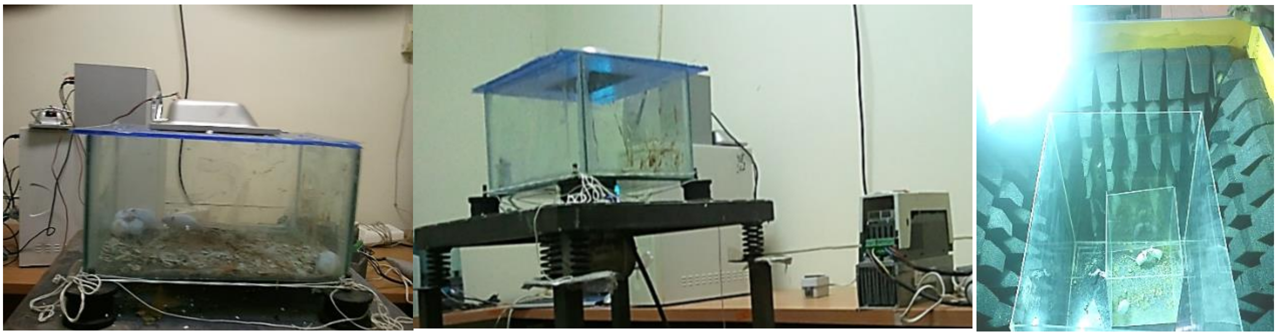


Figure 1. Images of the exposure chamber.

Noise exposure

The noise exposure group was subjected to compound noise with a frequency of 500 to 8000 Hz at a sound pressure level (SPL) of 100±3 dB(A) [27]. The noise was amplified using an AS 2000 amplifier (Taiwan) and played back using a pair of Microlab M563 speakers (Iran). The speakers were placed at equal distance from the four corners of the storage box and the sound pressure level was constantly controlled during the exposure period using a calibrated B&K 2245 sound level meter (Denmark).

Vibration exposure

The vibration exposure group was subjected to regular intervals of vibration for a duration of 60 minutes. A SVANTEK 583 whole body vibration meter (Poland/USA) equipped with a triple axis frequency analyzer was used to monitor the vibration acceleration. The vibrating mechanism was designed in a way as to ensure a dominant Z axis affective vibration acceleration of 1.2 m/s^2 while keeping vibration along the other axes at a minimum level (less than the maximum allowed threshold of RMS 0.315 m/s^2).

Lighting exposure

Light intensity during exposure was 1000 lux measured by Hanger EC1 lux meter (Sweden). The amount of light needed for testing was only provided through a projector equipped with 400watt metallic halide bulbs with white light. Remarkably, the lamp was installed upside the chamber and the mice were directly exposed to lighting at a distance of 50-cm.

Microwave exposure

The microwave exposure group was subjected to microwaves at a frequency of 850 to 960 MHz with a modulation frequency of 100 to 200 kHz and an output power density of 5 watts [28]. Holadays HI-1501 Microwave Survey Meter (USA) was used for this purpose. The radiation-emitting antenna of this device was placed above the center of the storage box as to ensure an equal degree of radiation dissipation.

Sperm analysis

Sperm parameters such as sperm count (10^6/mL) and sperm motility were analyzed manually. For this purpose, the epididymis tail was separated by making an incision and then placed in a phosphate buffer solution which was kept at 37 °C. In order to further facilitate the extraction of sperm from the epididymis tail, the samples were segmented using forceps. After this step, the samples are placed on a warm surface for 15 to 30 minutes as this increases sperm movement and eases the counting of sperm and morphology assessment. Sperm counting was done using a microscopic slide and neubauer haemocytometry slide. A drop of the sample solution is taken using a micropipette and placed between the microscopic slide and the Neubauer slide. Then the sample is placed under Olympus AH2 microscope (Japan) at 400x magnification and the sperm count is performed.

In order to manually measure sperm motility, a simple scaling system is used without the need for sophisticated tools. At least 5 microscopic fields must be studied systematically in order to categorize 200 sperm. The motility of the studied sperm is categorized as follows:

a) Percentage of progressive motile sperm.
b) Percentage of non-progressive motile sperm.
c) Percentage of immotile or static sperm.

The shape of the sperm is analyzed via colorization using Diff-Quik™ staining set. Normal sperm should have no abnormalities at the head, neck and tail while abnormal sperm will have deformed tails, no heads, two heads or microcephaly of the head [29].

Data analysis

Data was statistically analyzed using SPSS v20.0 (SPSS Inc., Chicago, Ill., USA) with descriptive statistics presented as mean, standard deviation and range. The Shapiro test was used to determine the normality of the data distribution. The one-way analysis of variance (ANOVA) test was used to compare mean sperm parameters in the studied groups. Dunnett's test was used to compare the mean sperm parameters of each exposure group with the control group. The effect size of each type of exposure on the sperm parameters was determined using univariate analysis of variance. A significance level of 0.05 was used in this study.

Results

(Table 1) presents mean and standard deviation for all sperm parameters in the control group and the various exposure ones. The results show that the mean difference for all studied sperm parameters among the various exposure groups is statistically significant (P=0.001). The lowest sperm count belonged to the microwave exposure group (3.16±0.55 10^6/mL) and the highest sperm count belonged to the lighting exposure group (4.12±0.79 10^6/mL). In case of percentage of progressive motile sperm, it was found that all exposure groups had a lower mean compared to the control group (64.76±0.89 %). The lowest percentage of progressive motile sperm belonged to the noise exposure group (31.41±1.28 %) and the highest belonged to the lighting exposure group (60.67±0.77 %). The highest percentage of non-progressive motile sperm belonged to the lighting exposure group (34.01±1.51 %) and the lowest belonged to the vibration exposure group (31.88±1.26 %). The highest percentage of immotile sperm belonged to the microwave exposure group (36.58±1.24 %) and the lowest belonged to the lighting exposure group (5.31±1.66 %). The highest percentage of normal morphology belong to the vibration exposure group (74.11±0.64 %) and the lowest belong to the lighting exposure group (45.50±2.15%).

The mean sperm count in all exposed groups was lower than the ones of the control group (4.51±0.22 10^6/mL). The mean percent of sperm progressive motility in all exposed groups was lower than the ones of the control group (64.76±0.89 %). The percentage of sperm non-progressive motility was higher among the exposure groups compared to the control group (26.12±1.72%). The percentage of immotile sperm was higher among the exposure groups compared to the control group, except for lighting one (9.11±2.22 %). The percentage of normal morphology was lower in the exposure groups compared to the control group (72.56±1.52%)

Table 1. Mean and standard deviation of sperm parameters in each exposure scenario.

Sperm parameters	Mean±SD					Control group
	Vibration	Noise	Microwave	Lighting	P-value*	
Total count (10^6/mL)	4.11±0.25	3.23±0.76	3.16±0.55	4.12±0.79	0.001	4.51±0.22
Progressive motility (%)	39.43±1.03	31.41±1.28	35.22±1.43	60.67±0.77	0.001	64.76±0.89
Non-progressive motility (%)	31.88±1.26	33.58±2.55	33.27±1.96	34.01±1.51	0.001	26.12±1.72
Immotile (%)	28.67±1.24	35.00±2.62	36.58±1.24	5.31±1.66	0.001	9.11±2.22
Normal morphology (%)	74.11±0.64	70.30±1.41	60.10±1.59	45.50±2.15	0.001	72.56±1.52

[1] * Comparison of the mean difference of a sperm variable between different exposure groups (One-way ANOVA)

According to (Table 2), the results of Dunnett's test show significant difference in terms of sperm count between the control group with noise and microwave exposure group (P=0.001). The sperm count of the noise and microwave exposure group was 1.28 and 1.35 units lower than the control group, respectively. The mean difference of motility parameters between the control group and all of the exposure ones was statistically significant (P<0.01). The significant difference in terms of normal morphology between the control group with noise, lighting and microwave exposure group (P<0.01).

Table 2. A two-by-two comparison of sperm parameters in the control group and the exposure groups.

Group	Mean Difference (P-value*) with Control Group				
	Total Count	**Progressive Motility**	**Non-Progressive Motility**	**Immotile**	**Normal Morphology**
Vibration	-0.40 (0.431)	-25.32 (0.001)	5.76 (0.001)	19.56 (0.001)	1.55 (0.159)
Noise	-1.28 (0.001)	-33.35 (0.001)	7.46 (0.001)	25.88 (0.001)	-2.26 (0.020)
Microwave	-1.35 (0.001)	-29.53 (0.001)	7.15 (0.001)	22.38 (0.001)	-12.46 (0.001)
Lighting	-0.40 (0.431)	-4.08 (0.001)	7.88 (0.001)	-3.80 (0.007)	-27.06 (0.001)

[1] * Dunnett's test

The results of the one-way variance analysis used to determine the effect size of each exposure type on sperm parameters are presented in (Table 3). The microwave exposure had the largest significant effect size for sperm count (B=-1.35; P= 0.001). In case of exposure to microwave, the chance that the sperm count will be decreased comparing to the control group is 1.35 times. The noise exposure had the largest significant effect size for progressive motility (B=-33.35; P= 0.001). In case of exposure to noise, the chance that the progressive motility percent will be decreased comparing to the control group is 33.35 times. The lighting exposure had the largest significant effect size for non-progressive motility (B=7.88; P= 0.001). In case of exposure to lighting, the chance that the non-progressive motility percent will be increased comparing to the control group is 7.88 times. The noise exposure had the largest significant effect size for immotile percent (B=22.38; P= 0.001). In case of exposure to noise, the chance that the immotile percent will be increased comparing to the control group is 22.38 times. The lighting exposure had the largest significant effect size for normal morphology (B=-27.06; P= 0.001). In case of exposure to lighting, the chance that the normal morphology percent will be decreased comparing to the control group is 27.06 times.

Table 3. Results of univariate analysis of variance on the sperm parameters

Group	B (P-value*)				
	Total count	**Progressive motility**	**Non-progressive motility**	**Immotile**	**Normal morphology**
Vibration	-0.40 (0.165)	-25.32 (0.001)	5.76 (0.001)	19.56 (0.001)	1.55 (0.052)
Noise	-1.28 (0.001)	-33.35 (0.001)	7.46 (0.001)	25.88 (0.001)	-2.26 (0.006)
Microwave	-1.35 (0.001)	-29.53 (0.001)	7.15 (0.001)	22.38 (0.001)	-12.46 (0.001)
Lighting	-0.40 (0.165)	-4.08 (0.001)	7.88 (0.001)	-3.80 (0.002)	-27.06 (0.001)

Discussion

The aim of this study was to assess sperm parameters including sperm count, sperm motility, and sperm morphology in mice after exposure to four hazardous occupational agents (noise, vibration, lighting and microwave).

Overall, the results show that sperm count, percent of progressive motility sperm and normal morphology were significantly reduced in all exposure group comparing to the control group. In addition, the percent of immotile sperm and non-progressive motility in the all exposure groups was significantly higher comparing to the control group.

One of the physical agents investigated in the present study was microwave radiation. This kind of radiation can disrupt the spermatogenesis process and reduce the fertility of the sperm. The results of the present study confirm it and also, indicate that microwave, as an inducing element, can affect the motility, morphology and total count of the sperm and therefore create changes in spermatogenesis and consequently endanger fertility. The results of the present study show that the mean difference of sperm parameters in microwave exposure group had a significant difference with the ones of control group, and also microwave exposure had the largest significant effect size on sperm count among the studied groups. The results of a study carried out by an infertility clinic regarding the effects of microwave exposure on sperm parameters showed that exposure to electromagnetic radiation can cause an increase in the number of abnormally shaped sperm and reduce sperm motility [30], which agrees with the findings of the present study. The results of Dasdag et al. regarding the effects of whole-body exposure to microwave radiation emitted by cellphones on sperm count in rats showed no significant difference in sperm count among the exposure group and the control group [24]. In another study, Kesari et al. (2010) assessed the effects of electromagnetic fields (EMFs) on testicular performance in Wistar rats. The results showed sperm morphology being more normal among the group exposed to 900MHz fields and concluded that exposure to EMFs caused an increase in testosterone levels, which led to a more normal sperm morphology [31]. The findings of Kesari et al. and Dasdag et al. did not agree with the present study, which may be due to differences in frequency, type of test animal and exposure duration. However, the results of a study conducted on the effects of electromagnetic fields on spermatogenesis showed that exposure to these fields causes reduced sperm mobility [32] which is in accordance with the present study.

Exposure to noise can induce stress and disrupt the synthesis and release of sex hormones such as testosterone, which can lower the production of sperm, and other sex hormones. This reduction can be the main cause of changes in the testicular tissue, which is due to the increase in the concentration of the luteinizing hormone (LH) (the reduction of testosterone in the noise exposure group is probably the reason for reduced serum LH concentration). The increase in cortisol levels due to noise exposure can induce negative effects on the synthesis or testosterone, spermatogenesis and steroidogenesis in the testes. Studies have shown that chronic cortisol elevation can cause reduced steroidogenesis in the testicular tissue [33]. The results of the present study confirm that noise exposure can adversely affect sperm parameters. The results of the present study indicate that all the sperm parameters in noise exposure group had a significant difference with the ones of control group, and also noise exposure had the largest significant effect size on percent of progressive motility and immotile sperm count among the studied groups. A study by Abbate et al. showed that exposure to noise caused a reduction in sperm count [34], which agrees with the findings of the present study. Moreover, the study by Jalali et al. (2012) regarding the effects of noise exposure on sperm motility revealed that exposure to noise during a full spermatogenesis cycle causes a significant reduction in sperm motility [33]. Another research concluded that exposure to noise had reduced the concentration of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) suggesting that noise can lead to reduced sperm motility, which agrees with the findings of the present study [35].

Vibration exposure can have negative effects on the secretions deriving from the epididymis wall tissue, preventing the sperm from maturing and reducing their motility [36]. Therefore, vibration exposure can affect sperm parameters. The results of the present study show that all the sperm parameters in noise exposure group had a significant difference with the ones of control group, except for total count and normal morphology. The study by Saeed et al, in 2018 however, showed no significant difference between the sperm count of the vibration exposure group and the control group [37]. The study by Penkov et al. regarding the effects of whole-body vibration on sperm morphology indices showed that vibration can lead to reduced sperm count [14], which agrees with the findings of the present study.

Exposure to lighting can change the redox state of the sperm cell, which is accompanied by induced production of Reactive oxygen species (ROS). Changes in ROS play a vital role in the controlling of sperm movement and sperm fertilization capacity in mammals. Therefore, lighting exposure can affect sperm parameters by causing these changes [38]. The results of the present study indicate that all the sperm parameters in lighting exposure group had a significant difference with the ones of control group, except for total count and also lighting exposure had the largest significant effect size on percent of normal morphology among the studied groups. A study by Brandt et al. regarding the effects of artificial lighting (300 to 350 lux) on the semen quality of adult boars showed that the volume of semen and the overall number of motile sperm was lower in the exposure group compared to the control group [18]. Sayed et al. (2018) conducted a study on the effects of red, yellow, green, blue and white LED lights on testosterone concentration and sperm quality among roosters [39]. Their results showed that green light prevents the growth of the testes and has negative effects on almost all sperm characteristics monitored in their study. They also found that the green and blue exposure groups have a considerably lower testosterone level compared to the control group, suggesting that light can affect sperm quality, which is in agreement with the findings of the present study.

Workers are at risk of many different hazardous physical agents at the workplaces and each of these factors can have detrimental physical and psychological health consequences depending on the nature and the conditions of exposure. On the other hand, the health of the sperm is a necessary factor for fertility and any endogenous or exogenous cause can lead to it being damaged and become infertile. This makes the study of influential factors on sperm parameters ever more important. Based on the results of the present study, it can be said that noise, vibration, lighting, and microwave can cause changes in different sperm parameters, however for definite conclusion; more laboratory and field studies are required in this regard.

Conclusions

The results of the present study clearly show that exposure to noise, vibration, lighting and microwave can have an adverse effect on sperm count, sperm motility and sperm morphology. Among the four exposure scenarios, exposure to microwave has had the greatest effect on sperm count. Exposure to noise has had the greatest effect on progressive motility and immotile percent and exposure to light has had the greatest effect on normal morphology and non-progressive motility. Investigating the exposure to different levels of noise, vibration, lighting and microwave radiation are suggested for future lab and field studies for a better understanding of this issue. The limitations of the present study include the short exposure duration, problems related to working with lab animals and maintaining environmental conditions inside the storage containers of mice during the exposure period. Manual assessment of sperm parameters is another limitation of the present study, so it is recommended for obtaining a better test reliability, the all-semen analysis have done by the computer-automated semen analyzer. Moreover, histopathology of testis is needed to assure the effect of spermatogenesis which is ignored at the present study.

Ethical statement

This study is approved by student research committee- Shahid Beheshti University of Medical Sciences (28-92/10/03).

Acknowledgement

The author thanks to the Shahid Beheshti University of Medical Sciences for providing financial support.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT author statement

MBA: Conceptualization, Methodology, Investigation, Software, Writing- Original draft preparation; HM: Conceptualization, Methodology, Investigation, Data curation, Software, Supervision, Writing- Reviewing and Editing; SFD: Conceptualization, Methodology, Investigation, Supervision, Writing- Reviewing and Editing; FAB: Methodology, Investigation, Data curation, Writing- Reviewing and Editing; MAM: Methodology, Investigation, Software, Writing-Reviewing and Editing.

ORCID

Hamzeh Mohammadi: 0000-0001-5566-4339

Informed consent

The study was approved by the ethical committee of school of public health & neuroscience research center- Shahid Beheshti University of Medical Sciences (Research ID. 28-92/10/03).

References

[1] Szirmai A. Industrialisation as an engine of growth in developing countries, 1950–2005. Structural change and economic dynamics 2012; 23(4):406-420. https://doi.org/10.1016/j.strueco.2011.01.005

[2] Rim KT. Reproductive Toxic chemicals at work and efforts to protect workers' health: a literature review. Safety and health at work 2017; 8(2):143-150. https://doi.org/10.1016/j.shaw.2017.04.003

[3] Sengupta P, Dutta S, Tusimin MB, İrez T, Krajewska-Kulak E. Sperm counts in Asian men: Reviewing the trend of past 50 years. 2018. https://doi.org/10.4103/2305-0500.228018

[4] Daoud S, Sellami A, Bouassida M, Kebaili S, KESKES LA, Rebai T, et al. Routine assessment of occupational exposure and its relation to semen quality in infertile men: A cross-sectional study. Turkish journal of medical sciences 2017; 47(3):902-907. https://doi.org/10.3906/sag-1605-47

[5] Dehghan SF, Nassiri P, Monazzam MR, Aghaei HA, Moradirad R, Kafash ZH, et al. Study on the noise assessment and control at a petrochemical company. Noise & Vibration Worldwide 2013; 44(1):10-18. https://doi.org/10.1260/0957-4565.44.1.10

[6] Nassiri P, Monazzam MR, Asghari M, Zakerian SA, Dehghan SF, Folladi B, et al. The interactive effect of industrial noise type, level and frequency characteristics on occupational skills. Performance Enhancement & Health 2014; 3(2):61-65. https://doi.org/10.1016/j.peh.2015.01.001

[7] Münzel T, Sørensen M, Schmidt F, Schmidt E, Steven S, Kröller-Schön S, et al. The adverse effects of environmental noise exposure on oxidative stress and cardiovascular risk. Antioxidants & redox signaling 2018; 28(9):873-908. https://doi.org/10.1089/ars.2017.7118

[8] Michaud DS, Feder K, Keith SE, Voicescu SA, Marro L, Than J, et al. Exposure to wind turbine noise: perceptual responses and reported health effects. The Journal of the Acoustical Society of America 2016; 139(3):1443-1454. https://doi.org/10.1121/1.4942391

[9] Guo L, Li P-h, Li H, Colicino E, Colicino S, Wen Y, et al. Effects of environmental noise exposure on DNA methylation in the brain and metabolic health. Environmental research 2017; 153:73-82. https://doi.org/10.1016/j.envres.2016.11.017

[10] Swami CG, Ramanathan J, Jeganath CC. Noise exposure effect on testicular histology, morphology and on male steroidogenic hormone. The Malaysian journal of medical sciences: MJMS 2007; 14(2):28.

[11] Vitharana V, Chinda T. Factors affecting health problems among construction workers due to whole body vibration (WBV): literature review. Proceedings of the TIMES-iCON 2016;28-30.

[12] Bayat R, Aliabadi M, Golmohamadi R, Shafiee Motlagh M. Assessment of exposure to hand-arm vibration and its related health effects in workers employed in stone cutting workshops of Hamadan city. Journal of Occupational Hygiene Engineering 2016; 3(1):25-32. https://doi.org/10.21859/johe-03014

[13] Du BB, Bigelow PL, Wells RP, Davies HW, Hall P, Johnson PW. The impact of different seats and whole-body vibration exposures on truck driver vigilance and discomfort. Ergonomics 2018; 61(4):528-537. https://doi.org/10.1080/00140139.2017.1372638

[14] Penkov A, Stanislavov R, Tzvetkov D. Male reproductive function in workers exposed to vibration. Central European journal of public health 1996; 4(3):185-188.

[15] Mohammadi H, Farhang Dehghan S, Abdollahi MB, Kalantar M, Kaydany M. Effect of High Light Level on Sperm parameters in Mice. Iran Occupational Health 2019; 16(4):11-21.

[16] Zamanian Z, Barzideh M, Ghanbari S, Daneshmandi H. The survey of noise and light effects on body posture during the study in male dormitory of Shiraz University of Medical Sciences. Tolooebehdasht 2014; 13(4):48-56 (Persian). https://www.sid.ir/en/journal/ViewPaper.aspx?id=419252

[17] Cember H, Johnson TE, Alaei P. Introduction to health physics. Medical Physics 2008; 35(12):5959. https://doi.org/10.1118/1.3021454

[18] Brandt KE, Diekman MA. Influence of supplemental lighting on serum LH, testosterone and semen quality in prepubertal and postpubertal boars. Animal Reproduction Science 1985; 8(3):287-294. https://doi.org/10.1016/0378-4320(85)90033-8

[19] Iaffaldano N, Meluzzi A, Manchisi A, Passarella S. Improvement of stored turkey semen quality as a result of He–Ne laser irradiation. Animal reproduction science 2005; 85(3-4):317-325. https://doi.org/10.1016/j.anireprosci.2004.04.043

[20] Barsam T, Monazzam MR, Haghdoost AA, Ghotbi MR, Dehghan SF. Effect of extremely low frequency electromagnetic field exposure on sleep quality in high voltage substations. Iranian journal of environmental health science & engineering 2012; 9(1):1-7. https://doi.org/10.1186/1735-2746-9-15

[21] Zilberti L, Bottauscio O, Chiampi M. Assessment of exposure to MRI motion-induced fields based on the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Magnetic resonance in medicine 2016; 76(4):1291-1300. https://doi.org/10.1002/mrm.26031

[22] Woldanska-Okonska M, Karasek M, Czernicki J. The influence of chronic exposure to low frequency pulsating magnetic fields on concentrations of FSH, LH, prolactin, testosterone and estradiol in men with back pain. Neuroendocrinology Letters 2004; 25(3):201-206.

[23] Banik S, Bandyopadhyay S, Ganguly S. Bioeffects of microwave—a brief review. Bioresource technology 2003; 87(2):155-159. https://doi.org/10.1016/S0960-8524(02)00169-4

[24] Dasdag S, Ketani M, Akdag Z, Ersay A, SarıI, Demirtas Ö, Celik M. Whole-body microwave exposure emitted by cellular phones and testicular function of rats. Urological research 1999; 27(3):219-223. https://doi.org/10.1007/s002400050113

[25] Mailankot M, Kunnath AP, Jayalekshmi H, Koduru B, Valsalan R. Radio frequency electromagnetic radiation (RF-EMR) from GSM (0.9/1.8 GHz) mobile phones induces oxidative stress and reduces sperm motility in rats. Clinics 2009; 64(6):561-565. https://doi.org/10.1590/S1807-59322009000600011

[26] Vosoughi S, Khavanin A, Salehnia M, Mahabadi HA, Soleimanian A. Effects of simultaneous exposure to formaldehyde vapor and noise on mouse testicular tissue and sperm parameters. J Health Scope 2012; 1(3):110-117. https://doi.org/10.17795/jhealthscope-7973

[27] Vosoughi S, Khavanin A, Shahverdi A, Esmaeili V, Ghasemkhan AH. Investigation of Simultaneous Exposure to Noise and Formaldehyde Vapor on Mouse Reproductive Function. International Journal of Fertility & Sterility 2013; 7:71.

[28] Ghanbari KM, Mortazavi SB, Khavanin A, Khazaei M, Safari VA. The effect of the cell phone waves and severity noise on sperm motility and sexual hormones in male rats. Journal of Clinical Research in Paramedical Sciences 2014; 3(1):7-15.

[29] Prakash S, Prithiviraj E, Suresh S, Lakshmi NV, Ganesh MK, Anuradha M, et al. Morphological diversity of sperm: A mini review. Iranian journal of reproductive medicine 2014; 12(4):239.

[30] Wdowiak A, Mazurek PA, Wdowiak A, Bojar I. Effect of electromagnetic waves on human reproduction. Ann Agric Environ Med 2017; 24(1):13-18. https://doi.org/10.5604/12321966.1228394

[31] Kesari KK, Kumar S, Behari J. Mobile phone usage and male infertility in Wistar rats. Indian Journal of Experimental Biology 2010; 47:987-992. http://hdl.handle.net/123456789/10343

[32] Shafik A. Effect of electromagnetic field exposure on spermatogenesis and sexual activity. Asian jiurnal of andrology 2005; 7(1):106. https://doi.org/10.1111/j.1745-7262.2005.00015.x

[33] Jalali M, Saki G, Sarkaki AR, Karami K, Nasri S. Effect of noise stress on count, progressive and non-progressive sperm motility, body and genital organ weights of adult male rats. Journal of human reproductive sciences 2012; 5(1):48. https://doi.org/10.4103/0974-1208.97801

[34] Abbate C, Concetto G, Fortunato M, Brecciaroli R, Tringali MA, Beninato G, et al. Influence of environmental factors on the evolution of industrial noise-induced hearing loss. Environmental monitoring and assessment 2005; 107(1):351-361. https://doi.org/10.1007/s10661-005-3107-1

[35] Crino OL, Johnson EE, Blickley JL, Patricelli GL, Breuner CW. Effects of experimentally elevated traffic noise on nestling white-crowned sparrow stress physiology, immune function and life history. Journal of Experimental Biology 2013; 216(11):2055-2062. https://doi.org/10.1242/jeb.081109

[36] Stansfeld SA, Matheson MP. Noise pollution: non-auditory effects on health. British medical bulletin 2003; 68(1):243-257. https://doi.org/10.1093/bmb/ldg033

[37] Saeed GT, Al-Azzawi KSA, Al-Wasti HSH. The Effect of Mechanical Vibration on Human Sperm Activity in Vitro. Biomedical and Pharmacology Journal 2018; 11(3):1617-1621. https://dx.doi.org/10.13005/bpj/1529

[38] Grzelak A, Rychlik B, Bartosz G. Light-dependent generation of reactive oxygen species in cell culture media. Free Radical Biology and Medicine 2001; 30(12):1418-1425. https://doi.org/10.1016/S0891-5849(01)00545-7

[39] Sayed M, Abdelfatah M. Effect of light-emitting diode (led) light color on testicular growth, circulating testosterone concentration and sperm quality in dandarawi roosters. Egyptian Poultry Science Journal 2018; 38(1):195-205. https://doi.org/10.21608/epsj.2018.5600

Received: 9 March 2018 | Accepted: 11 June 2018
DOI: 10.1002/ame2.12024

REVIEW ARTICLE

Vibration in mice: A review of comparative effects and use in translational research

Randall P. Reynolds[1] | **Yao Li**[2] | **Angela Garner**[1] | **John N. Norton**[1,3]

[1]Division of Laboratory Animal Resources, Duke University Medical Center, Durham, NC, USA
[2]Department of Laboratory Animal Science, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[3]Department of Pathology, Duke University Medical Center, Durham, NC, USA

Correspondence
Randall P. Reynolds, Division of Laboratory Animal Resources, Duke University Medical Center, Durham, NC, USA.
Email: randall.reynolds@duke.edu

Abstract
Sound pressure waves surround individuals in everyday life and are perceived by animals and humans primarily through sound or vibration. When sound pressure waves traverse through a solid medium, vibration will result. Vibration has long been considered an unwanted variable in animal research and may confound scientific endeavors using animals. Understanding the characteristics of vibration is required to determine whether effects in animals are likely to be therapeutic or result in adverse biological effects. The eighth edition of the "Guide for the Care and Use of Laboratory Animals" highlights the importance of considering vibration and its effects on animals in the research setting, but knowledge of the level of vibration for eliciting these effects was unknown. The literature provides information regarding therapeutic use of vibration in humans, but the range of conditions to be of therapeutic benefit is varied and without clarity. Understanding the characteristics of vibration (eg, frequency and magnitude) necessary to cause various effects will ultimately assist in the evaluation of this environmental factor and its role on a number of potential therapeutic regimens for use in humans. This paper will review the principles of vibration, sources within a research setting, comparative physiological effects in various species, and the relative potential use of vibration in the mouse as a translational research model.

KEYWORDS
animal models, mice, translational, vibration

1 | INTRODUCTION

Translational research is commonly referred to as the combining of various scientific disciplines and using the expertise of individuals working within those disciplines to accelerate basic scientific findings into advances for novel therapeutics, medical devices, and treatment regimens for human patients.[1] Basic scientific endeavors may use various in vitro methodologies, but prior to clinical use in humans, studies in animals are imperative to fully assess diagnostic or therapeutic modalities. Animals and humans share the same organ systems, and many therapeutics and procedural regimens are comparable as well. These similarities lead to the use of animals as translational models of human disease. The animal model is selected because it is predictive of the specific disease in humans and in whole or part, the animal model will respond to medical intervention similar to humans.

Novel therapeutics require assessment of efficacy in animals, but the lack of validation of the animal model can result in erroneous interpretation of data from the model and lead to lack of predictability during extrapolation to humans.[2] Success rates of novel therapeutics in humans during clinical development remain low due to the lack of relative levels of efficacy in preclinical testing, including animal models and in humans during clinical trials.[2,3] Careful attention to the assessment of a proposed animal model is critical to ensure species differences are identified and considered in the process. Similarly, reproducibility and transparency of published research using animals is imperative to ensure characterization of a model that will be predictive of human biology and disease.[4,5] Thus, it is critical to define the criteria being assessed within the animal model to ensure translational success in humans.

This paper reviews the current understanding of vibration in the research setting. The most recent revision of the "Guide for the Care and Use of Laboratory Animals" highlights the importance of considering vibration and its effects on animals in research.[6] Vibration likely elicits stress-mediated effects, as reported in the literature, but scant information is available on the level of vibration (threshold) that will cause effects or on the nature of the effects in animals. Understanding the threshold effects of vibration ultimately will assist in the evaluation of this environmental factor and its potential role in a number of therapeutic regimens in humans. This paper summarizes the basic principles of vibration, sources within a research setting, comparative physiological effects in various species and the potential use of vibration in the mouse, relative to other species, as a translational research model.

2 | PRINCIPLES OF VIBRATION

Sound and vibration are forms of energy that travel in waves with sound being perceived by what we hear and vibration by what we feel. In fact, sound is comprised of pressure waves caused by movement of air particles that can be detected by either a human or animal. These waves are oscillatory in nature and have both an amplitude and frequency. The amplitude contributes to the intensity of the sound or vibration and is represented by how far the peak of the wave moves past the position of equilibrium. Frequency is the amount of time that it takes to complete one cycle from a point on one wave to the same point on the next wave. The term "Hertz" is used as a unit of measure for frequency and is the number of cycles per second. One Hertz (Hz) is one cycle per second.[7] The magnitude or loudness of sound is measured in decibels, whereas the magnitude of vibration can be measured in relation to the amplitude by displacement from the point of equilibrium (often measured in millimeters), the velocity of wave movement (quantified in meters per second) or acceleration past the neutral point measured in meters per second squared (m/s^2).[8,9]

Both the magnitude and frequency of sound and vibration are important in the perception and potential adverse or therapeutic effects in humans and animals. For example, the human hearing range is from 20 Hz to 20 kHz and the mouse hearing range is from about 1 kHz to 100 kHz.[10] Likewise, an object will vibrate differentially based on its physical composition and will also tend to vibrate at some frequencies more than others. The frequency where vibration occurs most readily and can amplify the vibration is called the resonance frequency. The resonance frequency is located within the resonance frequency range (RFR), where the vibration would become greater at frequencies closer to the resonance frequency and somewhat less at the ends of the range. These frequency ranges are unique to an animal, a body region, or any other object and are dependent on that subject's physical composition with regard to "stiffness" and mass. Any object or part of an animal's body has a resonance frequency (Fn), which is calculated by the formula $Fn = 1/(2\pi) \times \sqrt{(k/m)}$, where k is the stiffness constant, and m is the mass.[11,12] Knowledge of resonance frequencies is important because vibration near these frequencies, compared with other frequencies, will be perceived more strongly and ultimately will induce more physiological effects, including those considered harmful.[13] Therefore, different species or size of animals may perceive vibration to a lesser or greater degree depending on the frequency of the vibration. In addition to the frequency of vibration, other factors that will determine the effects on animals include magnitude, duration, whether the vibration is directed at the whole body or is localized, and potentially individual variation in perception across species or within the same species.

Because both frequency and magnitude impact the exposure level of vibration, the interpretation of the literature with regard to both beneficial and adverse effects of vibration can be difficult. Until recently, only the resonance frequency of the liver had been determined in mice, which is 2-7 Hz.[14] The predicted resonance frequency for the mouse was calculated[11(p655)] and then studied by performing measurements in both rats and mice.[15] Importantly, the resonance frequency for a single organ is quite different than the vibrating frequency of the entire body in cumulative. Similarly, different body regions of a human or animal will have different RFRs. For example, the human abdomen has a resonance frequency of 4-8 Hz, the thorax of 5-10 Hz, and the head from 20 to 30 Hz.[16] In rats, the RFR was 27-29 Hz for the abdomen, 225-230 Hz for the thorax, and 75-80 Hz for the head.[17] Although resonance frequencies had not been reported for mouse anatomical regions, the predicted RFRs for mice were 85-92 Hz for the abdomen, 711-727 Hz for the thorax, and 237 to 253 Hz for the head when assuming equivalent inherent stiffness of tissue is similar in mice and humans.[11(p655)] Anesthetized mice that were exposed to vibration generally attenuated vibration that would have been detected by a mounted accelerometer on their back, except for vibration in the ranges of 30-100 Hz. Instead, the magnitude of the vibration in these ranges was either equal to or greater than the applied vibration, indicating that the RFR for these animals lie within these ranges.[15(p1963)] Mice that were exposed to vibration at 80 and 90 Hz showed increases in blood pressure and/or heart rate, whereas no increases were observed with frequencies 70 Hz or less or with 100 Hz or greater.[18] A recent study has demonstrated that mice show more behavioral alterations due to whole- body vibration (WBV) predominantly between the frequencies of 70-100 Hz.[19] Therefore, mice appear to be the most sensitive to vibration between frequencies of 70-100 Hz. Within this RFR, mice should be most susceptible to low level vibration, which would likely most affect an animal's normal physiological and behavioral functions.

3 | SOURCES OF VIBRATION IN RESEARCH SETTING

Because the care of animals requires the use of mechanical systems and equipment, vibration will be present in the animal facility to some degree. There are three general sources of vibration: vibration produced from mechanical systems or procedures within the animal facility; vibration produced outside, but near the animal facility; and vibration resulting from the transportation of animals from the vendor or to locations within an animal program. Sources of vibration that occur within the animal facility include ventilation systems, husbandry-associated cleaning and sterilizing equipment, ventilated racks, and cage change stations.[11(p656),20] There are several studies regarding the numerous effects of construction noise and vibration on rodents that have detailed effects such as increases in corticosterone levels and other alterations in biochemical parameters or reproductive efficiency.[21-28] Recently, studies have begun to separate the effects of construction noise vs vibration. In one study, dams of two strains of mice were exposed to vibration levels comparable to that produced in an animal facility from proximal construction.[23(p3)] While no changes in overall fertility were noted, nursing dams did show some alterations in normal maternal behavior. The study raised several important points to consider with regard to construction-induced vibration. Specifically, vibration from outside sources (ie, construction, trains) is often produced in sudden, intermittent bursts in contrast to vibration produced over long continuous time periods. Intermittent vibration is thought to produce more adverse effects than continuous vibration due to its unpredictability.[23(p3)] While no changes in fertility were detected in this study, other research has demonstrated increased rates of abortion, cannibalism, and resorptions following construction procedures in proximity to animal facilities.[29] High rates of cannibalism were also observed in a mouse housing room located near an active railroad.[22(p737)] Measurements were taken to adequately characterize the frequency and magnitude of both the sound and vibration produced by the passing train. While most sound that was produced was outside the range of mouse hearing, significant vibration of up to 0.25 m/s^2 was generated. In addition, the exposed female mice exhibited higher corticosterone levels relative to female mice that were not vibrated.[22(p737)] Lastly, the transportation of animals by vehicle, by a hand-pushed cart, or by hand has been shown to produce a relatively high degree of vibration exposure.[30,31] Using an accelerometer placed inside a standard polycarbonate mouse cage, vibration was measured during transportation by either hand-carrying or with several types of carts. With transport of the cage along a set pathway, vibration within the cage varied by as much as 35 m/s^2 between the transportation methods, suggesting that movement of animals even between rooms and buildings, which is common in many research environments, can subject animals to considerable vibration.[31(p544)] For this reason, animals should be provided with an opportunity to recover from vibration exposure before being used in scientific experiments. Mice that were transferred from their housing room to another room across the hall and placed on a shaker apparatus, with no vibration administered, took between 1.5 hours to approximately 24 hours for their active behaviors (eg, locomotion, rearing, sniffing) and inactive/maintenance behaviors (eg, sleeping, grooming, eating) to return to pre-transport levels.[19]

While it is not always possible to completely mitigate vibration from sources such as trains, subways or proximal construction, these factors should be taken into consideration during the design and location site planning for animal facilities. In addition, care should be taken to reduce vibration from cage movement and disturbances within the animal room or between locations within an institution. Even when rodents are exposed to movement from opening cages for routine experiments or normal husbandry activities, animals may be stressed. For example, rats have been shown to have higher corticoid metabolites in their feces following husbandry procedures.[32] Appropriate training of research personnel and staff can help mitigate some of these effects with proper handling. Even simple measures and policies, such as limiting cell phone use in animal facilities can have an effect. In a study with rats, exposure to intermittent noise and vibration from cell phones increased anxiety-like behavior during plus maze testing.[33] Vibration-induced effects should also be considered when obtaining materials and equipment for animal facilities. For instance, most modern individually ventilated racks have a heavy construction with clips to hold cages in place. Such racks may be better at dampening short bursts of vibration compared to other types of racks. In addition, in one study that looked at vibration produced by common transport carts used in a facility, metal carts with large wheels helped to decrease vibration at the cage level. Using padding on the carts also helped to further dampen vibration's accelerative forces.[31(p546)]

4 | ADVERSE VIBRATION EFFECTS AND POTENTIAL BENEFITS IN ANIMALS AND HUMANS

In humans excessive vibration can cause effects on bone, joints, nerves, muscles, and blood vessels that can be profound and debilitating.[34,35] Because of these effects, regulations and standards have been employed to limit vibration exposure in humans.[36,37] Similarly, animal studies have shown that vibration can have a myriad of adverse effects in many different species, including altering the normal physiology and even cell structure. Information regarding the adverse effects of vibration in animals and humans is summarized in Table 1.

Stress as a result of vibration, not unexpectedly, causes increases in heart rate in mice and humans. Conscious mice exposed to vibration can exhibit increases in heart rate (HR) and mean arterial blood pressure (MAP). When mice were anesthetized and unconscious,

TABLE 1 Adverse effects of vibration in various species

Species	Adverse effect	References
Mouse	Decreased the number of litters born relative the number bred	22(p737)
Mouse	Nursing dams exhibited noticeable agitation and disruption in nursing	23(p8-10)
Mouse	Increased both heart rate and mean arterial blood pressure	18(p374,375)
Mouse	Decreased the number of blood vessels per muscle fiber in the soleus muscle	66
Mouse	Startle response and fear-related behaviors	19
Mouse	Increased blood levels of corticosterone	22(p737)
Mouse, Pig	Changes in reproduction associated with hormonal changes with an increase in stress hormones	Mouse [22(p737)], Pig[67]
Rat	Disrupted myelin in axons, decreased the arterial lumen size, and an increased arterial smooth muscle vacuolization in the tail	68,69
Rat	Altered serotonin levels in the brain	70(p15)
Rat, Dog	Caused stress leukograms	Rat[71], Dog[72]
Dog	Increased aortic flow rate and pulse pressure during anesthesia	40
Rabbit	Alterated neuropeptides in the dorsal root ganglion associated with ultrastructural changes in cellular structure	73

neither HR nor MAP were elevated under the same vibratory conditions, suggesting that consciousness is a requisite for these cardiovascular effects in mice.[18(p374,375)] To assess the effect of noise and vibration on heart rate in humans, study participants were exposed to experimentally induced vibration, equivalent to that produced from a train, during sleep. In 79% of participants subjected to the high-vibration condition, an average increase of at least 3 beats per minute per train was observed and cardiac responses were generally higher in the high-vibration condition than in the low vibration condition.[38] The increased HR in humans was characterized by an initial and then a delayed response, indicating that a startle response was associated with awakening and a more conscious response ensued as the vibration continued. Similarly, the HR of participants receiving vibration during squat training had higher HR than individuals not receiving vibration. The HR of individuals that received vibration was increased on the initial training day and declined during subsequent training days, showing a rapid cardiovascular adaptation to the vibration stimulus.[39] Therefore, both humans and mice may perceive vibration as a psychological stressor and subsequently undergo increases in HR. However, vibration may have other cardiovascular effects that do not require consciousness since vibration at very high magnitudes (9.8-29.4 m/s^2) caused an increase in aortic blood flow and pressure during anesthesia in dogs and pigs.[40(p386)]

In larger species, vibration associated with transportation is considered one of the factors involved in transportation stress.[41] Exposure of swine to WBV, to mimic transportation stress, caused behavioral avoidance of the vibration produced.[42] Transportation-induced vibration in poultry causes stressed-induced behaviors and the stress-related effects of increased heart rate and blood circulation.[43] Vibration levels during transport can become high, which may contribute to observed behavioral alterations. The vibration levels produced from routine animal facility transport methods such as carts and hand carrying have been measured.[31(p544)] In some instances, vibration magnitudes reached as high as 17.31 m/s^2 for some of the carts tested.[31(p546)] These levels are much higher than ambient vibration levels of approximately 0.024 m/s^2 measured in animal rooms.[11(p655)]

Some studies have shown potential benefits of vibration on bone, muscle, fat accumulation, metabolism, and in wound healing (Table 2). The studies demonstrating the positive effects of vibration point to exciting potential for vibration to be used in the therapy for conditions that affect humans as well as areas for future translational studies using animal models. Because of the potential positive effects, vibration has been used to treat musculoskeletal diseases as well as to increase athletic performance in humans. Work still needs to be done, however, to determine the accelerations and frequencies that are most beneficial.[44,45] As discussed below, because the frequency, magnitude, and duration of exposure can determine if vibration will have negative, positive or no effects, animal models will be important in developing these therapeutic uses.

5 | CHALLENGES IN ANIMAL STUDY DESIGN

Because of the varied nature of experimental design applied to WBV studies reported in the literature, it is challenging to determine which vibration protocol is likely to have the greatest benefit, adverse effects, or no effects at all. For example, in studies to use vibration exposure for promoting bone growth or maintenance, there were acceleration ranges between 2.94 and 29.43 m/s^2, frequency ranges between 8 and 90 Hz, varied durations of exposure, as well as animal age and species.[46(p1059),47(p349),44-46,48,49] Higher magnitude WBV of 19.62 and 29.43 m/s^2 was only osteogenic in ovariectomized rats,[50(p316)] whereas low magnitude vibration applied to osteoporotic (ovariectomized) rats at approximately 2 m/s^2 reversed some of the negative effects of osteoporosis and accelerated early peri-implant osseointegration.[51] An evaluation of WBV effects on

TABLE 2 Potentially beneficial effects of vibration in various species

Species	Potentially beneficial effects	References
	Bone	
Mouse	Increased bone formation on the endocortical surface of the metapaphysis during skeletal growth	74
Mouse	Increased cortical bone area and cortical thickness in the femur and tibia diaphysis	75
Mouse	Increased trabecular metaphyseal bone formation and percentage of mineralizing surfaces	76
Mouse	Increased trabecular bone volume of the proximal tibial metaphysis	77
Rat	Mitigated negative effects of bone repair and bone callus formation due to ovariectomy	78
Rat	Improved fracture callus density, enlarged callus area and width, accelerated osteotomy bridging, upregulated osteocalcin expression and suppressed osteoclast activity after ovarectomy	79
Rat	Improved stiffness and increased endosteal and trabecular bone densities during fracture repair after pharmacological induction of osteoporosis and ovariectomy	80
Rat	Attenuated the loss of bone mass and trabecular bone microstructure after spinal cord injury	81
Rat	Promoted migration of mesenchymal stem cells and fracture healing, upregulation of several osteogenic proteins, up-regulation of the expression of chondrogenesis-, osteogenesis-, and remodeling-related genes	82-84
Sheep	Increased femoral trabecular bone formation	47,85
	Muscle	
Humans	Prevented a shift in myofiber type during extended bed rest	86
Humans	Increased isometric muscle strength, explosive muscle strength, and muscle mass in men older than 60 y of age	87
Human	Caused muscle relaxation in the neck and back	88
	Other effects	
Mouse (diabetic)	Attenuated hyperglycemia and insulin resistance, reduced body weight, normalized muscle fiber diameter, mitigated adipocyte hypertrophy in visceral adipose tissue, and reduced hepatic lipid content	89
Mouse (diabetic)	Decreased skin wound healing time, increased wound –associated angiogenesis and granulation tissue formation, accelerated wound closure and re-epithelialization, and increased expression of insulin-like growth factor-1, vascular endothelial growth factor and monocyte chemotactic protein-1 in the wounds	48
Humans	Increased the oxygen carrying capacity of the blood during exercise	49

bone formation in healthy rats using a constant acceleration and 45 or 90 Hz demonstrated that only a frequency of 90 Hz stimulated bone formation,[46] indicating that studies performed only at the low frequencies would have yielded a different conclusion regarding the effects of vibration. Although there have been varied experimental regimens used in vibration research, some consistency in findings is starting to emerge. For example, a second study has demonstrated that WBV at 90 Hz stimulates trabecular bone cellular activity, accelerates cortical bone growth, and increases bone mineral density in mice.[52] The WBV of 90 Hz is consistent with our established RFR for mice.[15(p1963)] Previous studies have been conducted without regard to the RFR of the animal and thus, the results may have been different if a frequency within the RFR had been used. Therefore, when designing vibration studies in animals careful consideration should be given to the frequency used as well as the magnitude.

There are also species considerations in animal study design. For example, techniques to study the effects of vibration at the molecular level are more available in mice than non-rodent species. Rats, however, may be a more appropriate rodent model for some studies, such as the study of vibration effects on the tail blood vessels and nerves, since they are larger in size. Rats share the same advantage as mice in that larger numbers can generally be used due to lower cost, reduced space requirements, rapid generation time, and increased availability.

6 | USE OF VIBRATION IN ANIMAL MODELS

The effects of vibration in animals is varied and can be either destructive or beneficial, likely depending on magnitude, duration, whole-body or localized, and presumably the sensitivity to the vibration for the species. The use of the mouse as a model to study human conditions has the advantage that transgenic, knock-out and knock-in strains are available to delineate the function of various genes in contributing to the harmful or beneficial effects of vibration in humans.

Vibration- induced effects in people include hand-arm vibration syndrome (Raynaud's phenomenon) consisting of vasospasm in hands and fingers,[53] lower back pain,[54] motion sickness, bone damage, varicose veins/heart conditions, stomach and digestive conditions, respiratory effects, endocrine and metabolic changes, impairment of vision/balance, and reproductive organ damage.[55] In mice, vibration-induced effects have been demonstrated in bone, muscle, hormones, metabolism, and reproduction as well as altering cardiovascular parameters, causing weight loss and increasing stress.[44,56] The mouse, therefore, is a valuable model to study many of the adverse conditions caused by vibration in humans.

In both humans and animals, diminishment of skeletal strength and muscle atrophy can lead to decreased mobility and function.

However, the musculoskeletal system responds to dynamic load in an anabolic manner and vibration therapy may serve to augment pharmacological therapy to strengthen bone and muscle.[56] The musculoskeletal system is able to tolerate a high level of vibration without damage due to its inherent elasticity and plasticity of the system, including the natural shock absorbers of the articulating joints. As previously noted, vibration has shown positive effects on both muscle and bone in mice, and therefore, the mouse model would be useful in the study of muscle and bone health.

Osteoporosis or bone fracture repair is another area where vibration may be beneficial and rodents may serve as a translational model. However, in humans, both osteoblastic and chondroblastic osseous repair occurs, while endochondral bone formation predominates in rodents. Fracture repair of the long bones in animal models has been well described, but vibration was not assessed as an adjunct to traditional intervention.[44] Considerable variation in bone morphology and healing processes exist among animal species; thus, characterization of each model is critical to appropriately correlate experimental outcomes to a skeletal condition in human. The bones in larger species (eg, canine, caprine, ovine swine, and nonhuman primates) do not undergo the continuous growth or modeling observed in rodents, while fracture fixation methods and biomechanics of fractures in these larger species mimic those used in humans.[56] Thus, preclinical research is commonly performed in these larger species instead of rodents. Despite this difference in bone healing, 53% of animals used in fracture studies over a 10-year period were either rats or mice and the large percentage of rodents used correlates to their applicability to molecular biology techniques, the ability to use a larger number of animals, and faster healing rates.[56]

Experimentally induced vibration has been used commonly in various behavioral, physiological, and psychological research models for decades as a source of stress.[57-59] In these studies, stress is defined as a physical, chemical, or emotional factor that causes physical or mental tension.[58] Often stress is a chronic condition and animal studies utilizing vibration are an important part of modeling the pathological effects of stress. Depending on the model, use of vibration or shaker stress often may prove advantageous over other models of induced stress such as physical restraint, foot shocks, or forced-swim testing in rodents. Use of shaker stress in animal studies provides a mild form of stress that has been used reliably to induce a form of stress that results in changes in blood pressure, heart rate, and stress hormones. Since shaker stress can be delivered remotely to an animal's home cage, it reduces the potential for artificial enhancement of the stress response from factors such as handling, restraint, noise, or pain.

Some of the most common models that utilize shaker stress are those used to study conditions such as depression and post-traumatic stress disorder (PTSD).[58,59(p320)] PTSD affects nearly 10% of Americans, but finding appropriate animal models is difficult due to the co-morbidities PTSD shares with other conditions such as anxiety and depressive disorders.[59] It is important for animal models to exhibit similar underlying characteristics or components of the corresponding disorder being studied. This allows for adequate study of the various factors that may contribute to disease processes, such as genetic or environmental factors. It also ensures that more reliable predictions are made about treatment effects. A study of rats exposed to intermittent shaker stress as part of a chronic stress schedule assessed the effects of the chronic unpredictable stress on anxiety-like behavior and cognitive deficits.[59(p320)] In conditions such as depression, human patients can also display cognitive changes.[60,61] Rats exposed to chronic unpredictable stress displayed cognitive deficits and increased anxiety similar to effects seen in the human condition. Rats also showed improvement in cognitive deficits when common treatments were tested, such as selective serotonin reuptake inhibitors and other drugs, indicating the appropriateness of the model.[59(p320)] Because shaker stress has also been shown to cause stress in mice and induce behavioral changes,[19] vibration in mice may also provide an appropriate stressor for the study of anxiety and depression.

The availability and current use of many genetically altered strains of mice offer a wide array of potential mouse models of human disease. For example, shaker stress has been used to study how early development factors affect the stress response in later life. In one study, progeny from NOS-3 knock-out mice were exposed to shaker stress to determine how the intrauterine environment affects the cardiovascular response to stress. NOS-3 is an enzyme responsible for the generation of nitric oxide in endothelial cells. Nitric oxide is a smooth muscle relaxant that plays a vital role in maintaining uteroplacental perfusion via vasodilation. NOS-3 deficient knock-out mice are susceptible to hypertension and reduced fetal growth during gestation. In the study, mature mice born to NOS-3 knock-out dams had greater changes in blood pressure in response to intermittent two-minute shaker sessions that were repeated over 24 hours relative to wild type mice.[62] Other studies have used shaker stress to study the interplay between circadian patterns and cardiovascular responses to stress.[63(p768)] All of these animal models are valuable tools in advancing the knowledge of the numerous factors that determine how stress affects various disease processes in humans.

Mice may also serve as a good model to study the potential of vibration as a therapy for wound treatment. Because local vibration has been shown increase blood flow in the skin of humans, it has been proposed as a treatment for pressure ulcers or other skin wounds.[64] Pressure wounds and other skin injuries may be more prevalent or of concern in diabetics. Because wound healing time in diabetic mice decreases when vibration exposure occurs,[48] the mouse model needs to be explored further with regard to wound healing.

There is evidence that vibration therapy may be beneficial in many age-related conditions.[65(p319)] WBV has been suggested to attenuate muscle atrophy resulting from bed rest, and may increase postural balance and gait. Similarly, exercise supplemented with WBV increases muscle strength and speed in older women following 24 weeks of treatment. Mice could play a very valuable role in studying the effects of vibration to prevent or treat conditions related to age.

7 | SUMMARY

Vibration experienced by animals can elicit stress-mediated effects and increased emphasis is being placed on vibration with regard to the welfare of animals and as a research variable. To understand the threshold for these effects, the sensitivity of a species to vibration is crucial to determine the utility of the animal as a translational model that is predictive in humans for a therapeutic effect. The mouse is a commonly used model in biomedical research, particularly when investigating molecular and cellular effects. This species, through genetic engineering and humanization, is appropriate for investigating the effects of vibration in a number of therapeutic modalities. There are numerous effects of vibration on the mouse, both those considered adverse as well as those with the potential to be used as a translational model for human therapeutics. Continued characterization of the effects of vibration in the mouse model will facilitate its use as a translational model for various therapeutic endeavors.

ORCID

Randall P. Reynolds http://orcid.org/0000-0002-1012-3851

REFERENCES

1. Woolf SH. The meaning of translational research and why it matters. *JAMA*. 2008;299:211-213.
2. Arrowsmith J. Trial watch: phase II failures: 2008–2010. *Nat Rev Drug Discov*. 2011;10:328-329.
3. Denayer T, Thomas Stöhr T, Van Roy M. Animal models in translational medicine: validation and prediction. *New Horiz Transl Med*. 2014;2:5-11.
4. Jilka RL. The road to reproducibility in animal research. *J Bone Miner Res*. 2016;31:1317-1319.
5. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*. 2010;8:e1000412.
6. National Research Council. *Guide for the Care and Use of Laboratory Animals*, 8th edn. Washington, D.C.: National Academy Press; 2011.
7. Crocker MJ. Fundamental of Acoustics, Noise and Vibration. In: Crocker MJ, ed. *Handbook of Noise and Vibration Control*. Hoboken, NJ: John Wiley and Sons, Inc.; 2007:1-16.
8. Vibration-Introduction. Canadian Centre for Occupational Health and Safety. https://www.ccohs.ca/oshanswers/phys_agents/vibration/vibration_intro.html. Updated October 21, 2008. Accessed December 11, 2017.
9. Frequency. Encyclopedia Britannica. https://www.britannica.com/science/frequency-physics. Published July 20, 1988. Accessed December 11, 2017.
10. Reynolds RP, Kinard WL, Degraff JJ, Leverage N, Norton JN. Noise in a laboratory animal facility from the human and mouse perspective. *J Am Assoc Lab Anim Sci*. 2010;49:592-597.
11. Norton JN, Kinard WL, Reynolds RP. 2011. Comparative vibration levels perceived among species in a laboratory animal facility. *J Am Assoc Lab Anim Sci*. 2011;50:653-659.
12. Frankovich D. The Basics of Vibration Isolation Using Elastomeric Materials. https://earglobal.com/media/9885/basicsvibrationisolationelastomericmaterials.pdf Accessed December 11, 2017.
13. Griffin MJ. Whole-Body Vibration and Health. In: Griffin MJ, ed. *Handbook of Human Vibration*. San Diego, CA: Elsevier Academic Press; 1996:171-220.
14. Yang G, Zhou J, Zhang L, et al. Research on resonance frequency with mouse liver. *J Biomech*. 2007;22:398-402.
15. Rabey KN, Li Y, Norton JN, Reynolds RP, Schmitt D. Vibrating frequency thresholds in mice and rats: implications for the effects of vibrations on animal health. *Ann Biomed Eng*. 2015;43:1957-1964.
16. MacMillian R. Human Vibration: basic Characteristics. In: Guo J, ed. *Work Health and Safety*, Practitioner. Rev edn. West Perth, Australia: Worksafe; 2013:15-16.
17. Ushakov IB, Soloshenko NV, Koslovskij AP. The examination of resonance frequencies of vibration in rats. *Kosm Biol Aviakosm Med*. 1983;17:65-68.
18. Li Y, Rabey KN, Schmitt D, Norton JN, Reynolds RP. Characteristics of vibration that alter cardiovascular parameters in mice. *J Am Assoc Lab Anim Sci*. 2015;54:372-377.
19. Garner AM, Norton JN, Kinard W, Kissling GE, Reynolds RP. Vibration-induced behavioral changes and observational response threshold in female, C57BL/6 Mice. *J Am Assoc Lab Anim Sci*. In press.
20. Rozema R. Noise and vibration considerations for the animal lab environment. ALNmag.com. https://www.alnmag.com/article/2009/03/noise-vibration-considerations-animal-lab-environment. Published March 31, 2009. Accessed December 11, 2017.
21. Carman R, Jue DA, Glickman GM. Vibration effects on laboratory mice during building construction. *J Acoust Soc Am*. 2008;123:3670.
22. Atanasov NA, Sargent JL, Parmigiani JP, Palme R, Diggs HE. Characterization of train-induced vibration and its effect on fecal corticosterone metabolites in mice. *J Am Assoc Lab Anim Sci*. 2015;54:737-744.
23. Carman RA, Quimby FW, Glickman GM. The effect of vibration on pregnant laboratory mice. *Noise-Con Proc*. 2007;209:1722-1731.
24. Zymantiene J, Zelvyte R, Pampariene I, et al. Effects of long-term construction noise on health of adult female Wistar rats. *Polish J Vet Sci*. 2017;20:155-165.
25. Raff H, Bruder ED, Cullinan WE, Ziegler DR, Cohen EP. Effect of animal facility construction on basal hypothalamic pituitary-adrenal and renin-aldosterone activity in the rat. *Endocrinology*. 2011;152:1218-1221.
26. Blaustein JD. Nearby construction influences the physiology of research animals: beyond stress hormones. *Endocrinology*. 2011;152:1197-1198.
27. Briese V, Fanghanel J, Gasow H. Effect of pure sound and vibration on the embryonic development of the mouse. *Zentralbl Gynakol*. 1984;106:379-388.
28. Rasmussen S, Glickman G, Norinsky R, Quimby FW, Tolwani RJ. Construction noise decreases reproductive efficiency in mice. *J Am Assoc Lab Anim Sci*. 2009;48:363-370.
29. Pritchett KR, Taft RA. Reproductive Biology of The Laboratory Mouse. In: Davisson MT, Quimby FW, Barthold SW, Newcomer CE, Smith AL, Fox JG, eds. *The Mouse in Biomedical Research*. Vol 3. 2nd ed. Burlington, MA: Academic Press; 2007:91-121.
30. Gebresenbet G, Aradom S, Bulitta FS, Hjerpe E. Vibration levels and frequencies on vehicle and animals during transport. *Biosyst Eng*. 2011;110:10-19.
31. Hurst K, Litwak KN. Accelerative forces associated with routine in-house transportation of rodent cages. *J Am Assoc Lab Anim Sci*. 2012;51:544-547.
32. Cavigelli SA, Guhad FA, Ceballos RM, et al. Fecal corticoid metabolites in aged male and female rats after husbandry-related disturbances in the colony room. *J Am Assoc Lab Anim Sci*. 2006;45:17-21.
33. Shehu A, Mohammed A, Magaji RA, Muhammad MS. Exposure to mobile phone electromagnetic field radiation, ringtone and vibration affects anxiety-like behaviour and oxidative stress biomarkers in albino wistar rats. *Metab Brain Dis*. 2016;31:355-362.

34. Kákosy T. Vibration disease. *Baillieres Clin Rheumatol*. 1989;3:25-50.
35. Carlsöö S. The effect of vibration on the skeleton, joints and muscles. A review of the literature. *Appl Ergon*. 1982;13:251-258.
36. Wen-bo L, Yi L, Ming C, Da-qiang S. An introduction to Chinese safety regulations for blasting vibration. *Environ Earth Sci*. 2012;67:1951-1959.
37. Occupational Health and Safety Authority. Work place (minimum health and safety requirements for the protection of workers from risks resulting from exposure to vibration) regulations. Subsidiary Legislation 424.31. 2005. http://www.justiceservices.gov.mt/DownloadDocument.aspx?app=lom&itemid=10735&l=1. Accessed December 11, 2017.
38. Croy I, Smith MG, Waye KP. Effects of train noise and vibration on human heart rate during sleep: an experimental study. *BMJ Open*. 2013;3:e002655.
39. Rosenberger A, Liphardt AM, Bargmann A, et al. EMG and heart rate responses decline within 5 days of daily whole-body vibration training with squatting. *PLoS ONE*. 2014;9:e99060.
40. Edwards RG, McCutcheon EP, Knapp CF. Cardiovascular changes produced by brief whole-body vibration of animals. *J Appl Physiol*. 1972;32:386-390.
41. Minka NS, Ayo JO. Physiological responses of food animals to road transportation stress. *Afr J Biotechnol*. 2009;8:7415-7427.
42. Stephens DB, Bailey KJ, Sharman DF, Ingram DL. An analysis of some behavioural effects of the vibration and noise components of transport in pigs. *Q J Exp Physiol*. 1985;70:211-217.
43. Scott G. Effects of short-term whole body vibration on animals with particular reference to poultry. *Worlds Poult Sci J*. 1994;50:25-38.
44. Thompson WR, Yen SS, Rubin J. Vibration therapy: clinical applications in bone. *Curr Opin Endocrinol, Diabetes Obes*. 2014;21:447-453.
45. Musumeci G. The use of vibration as physical exercise and therapy. *J Funct Morphol Kinesiol*. 2017; 2:1-10.
46. Judex S, Lei X, Han D, Rubin C. Low-magnitude mechanical signals that stimulate bone formation in the ovariectomized rat are dependent on the applied frequency but not on the strain magnitude. *J Biomech*. 2007;40:1333-1339.
47. Rubin C, Turner AS, Muller R, et al. Quantity and quality of trabecular bone in the femur are enhanced by a strongly anabolic, noninvasive mechanical intervention. *J Bone Miner Res*. 2002;17:349-357.
48. Weinheimer-Haus EM, Judex S, Ennis WJ, Koh TJ. Low-intensity vibration improves angiogenesis and wound healing in diabetic mice. *PLoS ONE*. 2014;9:e91355.
49. Kang J, Bushi JA, Ratamess NA, et al. Acute effects of whole-body vibration on energy metabolism during aerobic exercise. *J Sports Med Phys Fitness*. 2016;56:834-842.
50. Rubinacci A, Marenzana M, Cavani F, et al. Ovariectomy sensitizes rat cortical bone to whole-body vibration. *Calcif Tissue Int*. 2008;82:316-326.
51. Liang YQ, Qi MC, Xu J, et al. Low-magnitude high-frequency loading, by whole-body vibration, accelerates early implant osseointegration in ovariectomized rats. *Mol Med Rep*. 2014;10:2835-2842.
52. Gnyubkin V, Guignandon A, Laroche N, Vanden-Bossche A, Malaval L, Vico L. High-acceleration whole body vibration stimulates cortical bone accrual and increases bone mineral content in growing mice. *J Biomech*. 2016;49:1899-1908.
53. Krajnak K, Riley DA, Wu J, et al. Frequency-dependent effects of vibration on physiological systems: experiments with animals and other human surrogates. *Ind Health*. 2012;50:343-353.
54. Cardinale M, Pope MH. The effects of whole body vibration on humans: dangerous or advantageous? *Acta Physiol Hung*. 2003;90:195-206.
55. Occupational Health and Safety Representatives. Effects of vibration: what are the health effects of exposure to vibration? http://www.ohsrep.org.au/hazards/vibration/effects-of-vibration. Updated May 2017. Accessed December 29, 2017.
56. O'Loughlin PF, Morr S, Bogunovic L, Kim AD, Park B, Lane JM. Selection and development of preclinical models in fracture-healing research. *J Bone Joint Surg Am*. 2008;90:79-84.
57. Katz RJ, Roth KA, Carroll BJ. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neurosci Biobehav Rev*. 1981;5:247-251.
58. Schoner J, Heinz A, Endres M, Gertz K, Kronenberg G. Post-traumatic stress disorder and beyond: an overview of rodent stress models. *J Cell Mol Med*. 2017;21:2248-2256.
59. Bondi CO, Rodriguez G, Gould GG, Frazer A, Morilak DA. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. *Neuropsychopharmacology*. 2008;33:320-331.
60. Murrough JW, Iacoviello B, Neumeister A, Charney DS, Iosifescu DV. Cognitive dysfunction in depression: neurocircuitry and new therapeutic strategies. *Neurobiol Learn Mem*. 2011;96:553-563.
61. Lam RW, Kennedy SH, McIntyre RS, Khullar A. Cognitive dysfunction in major depressive disorder: effects on psychosocial functioning and implications for treatment. *Can J Psychiatry*. 2014;59:649-654.
62. Costantine MM, Ferrari F, Chiossi G, et al. Effect of intrauterine fetal programming on response to postnatal shaker stress in endothelial nitric oxide knockout mouse model. *Am J Obstet Gynecol*. 2009;201:301.e1-301.e6.
63. Bernatova I, Key MP, Lucot JB, Morris M. Circadian differences in stress-induced pressor reactivity in mice. *Hypertension*. 2002;40:768-773.
64. Nakagami G, Sanada H, Matsui N, et al. Effect of vibration on skin blood flow in an in vivo microcirculatory model. *Biosci Trends*. 2007;1:161-166.
65. Prisby RD, Lafage-Proust MH, Malaval L, Belli A, Vico L. Effects of whole body vibration on the skeleton and other organ systems in man and animal models: what we know and what we need to know. *Ageing Res Rev*. 2008;7:319-329.
66. Murfee WL, Hammett LA, Evans C, et al. High-frequency, low-magnitude vibrations suppress the number of blood vessels per muscle fiber in mouse soleus muscle. *J Appl Physiol*. 2005;98:2376-2380.
67. Perremans S, Randall JM, Rombouts G, Decuypere E, Geers R. Effect of whole-body vibration in the vertical axis on cortisol and adrenocorticotropic hormone levels in piglets. *J Anim Sci*. 2001;79:975-981.
68. Govindaraju SR, Curry BD, Bain JL, Riley DA. Effects of temperature on vibration-induced damage in nerves and arteries. *Muscle Nerve*. 2006;33:415-423.
69. Curry BD, Govindaraju SR, Bain JL, et al. Evidence for frequency-dependent arterial damage in vibrated rat tails. *Anat Rec A Discov Mol Cell Evol Biol*. 2005;284:511-521.
70. Ariizumi M, Okada A. Effect of whole body vibration on the rat brain content of serotonin and plasma corticosterone. *Eur J Appl Physiol Occup Physiol*. 1983;52:15-19.
71. Monteiro MOB, de Sá-Caputo DDC, Moreira-Marconi E, et al. Effect of a short period whole body vibration with 10 Hz on blood biomarkers in Wistar rats. *Afr J Tradit Complement Altern Med*. 2017;14:11-18.
72. Santos IFC, Rahal SC, Shimono J, Tsunemi M, Takahira R, Teixeira CR. Whole-body vibration exercise on hematology and serum biochemistry in healthy dogs. *Top Companion Anim Med*. 2017;32:86-90.
73. McLain RF, Weinstein JN. Effects of whole body vibration on dorsal root ganglion neurons. Changes in neuronal nuclei. *Spine (Phila Pa 1976)*. 1994;19:1455-1461.
74. Xie L, Jacobson JM, Choi ES, et al. Low level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. *Bone*. 2006;39:1059-1066.

75. Vanleene M, Shefelbine SJ. Therapeutic impact of low amplitude high frequency whole body vibrations on the osteogenesis imperfecta mouse bone. *Bone*. 2013;53:507-514.
76. Garman R, Gaudette G, Donahue LR, Rubin C, Judex S. Low-level accelerations applied in the absence of weight bearing can enhance trabecular bone formation. *J Orthop Res*. 2007;25:732-740.
77. Christiansen BA, Silva MJ. The effect of varying magnitudes of whole-body vibration on several skeletal sites in mice. *Ann Biomed Eng*. 2006;34:1149-1156.
78. Butezloff MM, Zamarioli A, Leoni GB, Sousa-Neto MD, Volpon JB. Whole-body vibration improves fracture healing and bone quality in rats with ovariectomy-induced osteoporosis. *Acta Cir Bras*. 2015;30:727-735.
79. Komrakova M, Sehmisch S, Tezval M, et al. Identification of a vibration regime favorable for bone healing and muscle in estrogen-deficient rats. *Calcif Tissue Int*. 2013;92:509-520.
80. Stuermer EK, Komrakova M, Sehmisch S, et al. Whole body vibration during fracture healing intensifies the effects of estradiol and raloxifene in estrogen-deficient rats. *Bone*. 2014;64:187-194.
81. Minematsu A, Nishii Y, Imagita H, Takeshita D, Sakata S. Whole-body vibration can attenuate the deterioration of bone mass and trabecular bone. microstructure in rats with spinal cord injury. *Spinal Cord*. 2016;54:597-603.
82. Wei FY, Chow SK, Leung KS, et al. Low-magnitude high-frequency vibration enhanced mesenchymal stem cell recruitment in osteoporotic fracture healing through the SDF-1/CXCR4 pathway. *Eur Cell Mater*. 2016;31:341-354.
83. Li M, Wu W, Tan L, et al. Low-magnitude mechanical vibration regulates expression of osteogenic proteins in ovariectomized rats. *Biochem Biophys Res Commun*. 2015;465:344-348.
84. Chung SL, Leung KS, Cheung WH. Low-magnitude high-frequency vibration enhances gene expression related to callus formation, mineralization and remodeling during osteoporotic fracture healing in rats. *J Orthop Res*. 2014;32:1572-1579.
85. Rubin C, Turner AS, Bain S, et al. Anabolism: low mechanical signals strengthen long bones. *Nature*. 2001;412:603-604.
86. Blottner D, Salanova M, Puttmann B, et al. Human skeletal muscle structure and function preserved by vibration muscle exercise following 55 days of bed rest. *Eur J Appl Physiol*. 2006;97:261-271.
87. Bogaerts A, Delecluse C, Claessens AL, Coudyzer W, Boonen S, Verschueren SM. Impact of whole-body vibration training versus fitness training on muscle strength and muscle mass in older men: a 1-year randomized controlled trial. *J Gerontol A Biol Sci Med Sci*. 2007;62:630-635.
88. Elfering A, Burger C, Schade V, Radlinger L. Stochastic resonance whole body vibration increases perceived muscle relaxation but not cardiovascular activation: a randomized controlled trial. *World J Orthop*. 2016;7:758-765.
89. McGee-Lawrence ME, Wenger KH, Misra S, et al. Whole-body vibration mimics the metabolic effects of exercise in male leptin receptor deficient mice. *Endocrinology*. 2017;158:1160-1171.

How to cite this article: Reynolds RP, Li Y, Garner A, Norton JN. Vibration in mice: A review of comparative effects and use in translational research. *Animal Model Exp Med*. 2018;1:116–124. https://doi.org/10.1002/ame2.12024

Journal of the American Association for Laboratory Animal Science
Copyright 2020
by the American Association for Laboratory Animal Science

Vol 59, No 6
November 2020
Pages 665–672

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

Jeremy G Turner,[1,2*]

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

DOI: 10.30802/AALAS-JAALAS-19-000131

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.[11,16,24,29,36,37,40,41,48,49] 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[19] 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).[28] 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.[28] 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,[11,16,24,29,36,37,40,41,48,49] 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

Received: 09 Sept 2019. Revision requested: 15 Oct 2019. Accepted: 03 Mar 2020.
[1]*Department of Psychology, Illinois College, Jacksonville, Illinois; and* [2]*Turner Scientific, Jacksonville, Illinois*
[*]*Corresponding author. Email: jturner@ic.edu, jturner@turnerscientific.com*

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.[25] 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.[27] 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.

1. **P**lan. Facilities should maintain a written, comprehensive noise and vibration measurement, training, communication, and action plan.
2. **A**nnual Assessment. Facilities should conduct an annual comprehensive noise and vibration assessment.
3. **C**hanges. 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. **T**hresholds. 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.

Figure 1. Recommended Practices (PACT) for minimizing negative effects on research animals, ongoing research studies, and relationships with scientists.

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.

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.[48,49] 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.[23] 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.[21,34]

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.[52] The World Health Organization (WHO) confirmed and adopted the EPA's 24-h noise exposure threshold of 70 dB in 1999,[5] and more recently, confirmed that limit by conducting a comprehensive review of the human and animal research data.[53] Both the EPA and WHO have also recognized

Noise		Vibration
• ≥70 dB SPL Chronic Noise Level		•* ≥0.025g RMS Chronic Vibration Level
• *note that lower level signals ≥45 dB SPL might be disruptive to more sensitive models. • Set low and high-end frequency filters to the hearing range of the species being studied.		• *note that lower level signals might be disruptive to more sensitive models and breeding animals, so this level would be recommended for such situations.
• Use a measurement system capable of processing signals in the range of the filter settings for the animal being studied.		• 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).
• Report unweighted measurements in dB SPL (re 20 uPa). Do not use human A-weighted measurements. Report whether sound is measured as instantaneous levels or time-weighted averages.		• Report vibration in acceleration (not displacement or velocity) using either g or m/s/s (1g = 9.81 m/s²). • 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.
• Note that regardless of baseline background noise level, changes of 3 dB (JND) or more can likely be heard by the animal.		• Note that regardless of baseline background vibration level, changes of 10% (JND) or more can likely be felt by the animal.

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.[5,52,53]

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.[3,15,32,33,50] Some evidence further indicates that such low-level, 70 dB noise effects can also be complicated by sex-specific effects.[51]

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.[39,49] 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.[4,10,36,39,42,53] 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.[42] 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.[53] 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.[4,43] In addition, numerous additional negative consequences of noise, including sleep disturbances, cardiovascular stress, and learning and memory impairments can occur.[6-8,10,13,36,46]

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.[44] 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.[12,35] 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[11,29,37] 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.[1,2,31,38] At magnitudes as low as 0.1 to 0.3 g, fetal pigs showed a significant increase in plasma cortisol and adrenocorticotropin hormone levels.[31] Likewise, vibration levels of only approximately 0.025 g have been shown to increase fecal corticosterone metabolites in female (but not male) mice,[2] and to result in overt behavioral responses in female mice indicative of arousal.[11] 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.[2,24,47]

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.[30,45] 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)[20] sets an action level for vibration for an 8-h work day at approximately 0.05 g (0.5 m/s^2); the standard describes vibration in the approximately 0.05 to 0.1 g (0.5 to 1 m/s^2) range as "fairly uncomfortable", and levels over approximately 0.08 g (0.8 m/s^2) as "uncomfortable". While no current standards exist for vibration in animal facilities, the approximately 0.05 g (0.5 m/s^2) 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.[18] 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,[22,54] and for vibration it is approximately a 10% increase from the background vibration.[26] 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.[14] 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[17] 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;[9]). 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[14] and a referenced listing of detailed hearing ranges of different species is maintained at 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^2, 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^2 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[37] and mice appear to be most impacted by frequencies in the 70 to 100 Hz range.[41] 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. Therefore, 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.[11] 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.[41]

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[53] 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[19] 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.

Acknowledgments

I thank Dr Linda Toth for her helpful review and comments, Amanda

Henton for help assembling some papers for review, and to Leanna Pantier and Michael Brunt for their comments on an earlier version of this manuscript. The author declares that he is a co-owner and Chief Scientific Officer at Turner Scientific, a company that provides preclinical hearing research and vivarium noise and vibration monitoring equipment and services.

References

1. **Ariizumi M, Okada A.** 1983. Effect of whole body vibration on the rat brain content of serotonin and plasma corticosterone. Eur J Appl Physiol Occup Physiol **52:**15–19. https://doi.org/10.1007/BF00429019.
2. **Atanasov NA, Sargent JL, Parmigiani JP, Palme R, Diggs HE.** 2015. Characterization of train-induced vibration and its effect on fecal corticosterone metabolites in mice. J Am Assoc Lab Anim Sci **54:**737–744.
3. **Attarha M, Bigelow J, Merzenich M.** 2018. Unintended consequences of white noise therapy for tinnitus— Otolaryngology's cobra effect. JAMA Otolaryngol Head Neck Surg **144:**938–943. https://doi.org/10.1001/jamaoto.2018.1856.
4. **Babisch W.** 2003. Stress hormones in the research in cardiovascular effects of noise. Noise Health **5:**1–11.
5. **Berglund B, Lindvall T, Schwela DH, editors.** [Internet]. 1999. WHO guidelines for community noise. [Cited 11 November 2018]. Available at: http://whqlibdoc.who.int/hq/1999/a68672.pdf.
6. **Chang EF, Merzenick MM.** 2003. Environmental noise retards auditory cortical development. Science **300:**498–502. https://doi.org/10.1126/science.1082163.
7. **Cheng L, Wang SH, Chen QC, Liao XM.** 2011. Moderate noise induced cognition impairment and its underlying mechanisms. Physiol Behav **104:**981–988. https://doi.org/10.1016/j.physbeh.2011.06.018.
8. **Cui B, Wu M, She X.** 2009. Effects of chronic noise exposure on spatial learning and memory of rats in relation to neurotransmitters and NMDAR2B alteration in the hippocampus. J Occup Health **51:**152–158. https://doi.org/10.1539/joh.L8084.
9. **Davis MF.** 2007. Audio and electroacoustics. p 743–778. In: Rossing TD, editor. Handbook of acoustics. New York (NY): Springer. https://doi.org/10.1007/978-0-387-30425-0_18
10. **Gannouni N, Mhamdi A, Tebourbi O, El May M, Sakly M, Rhouma KB.** 2013. Qualitative and quantitative assessment of noise at moderate intensities on extra-auditory system in adult rats. Noise Health **15:**406–411. https://doi.org/10.4103/1463-1741.121236.
11. **Garner AM, Norton JN, Kinard WL, Kissling GE, Reynolds RP.** 2018. Vibration-induced behavioral responses and response thresholds in female C57BL/6 mice. J Am Assoc Lab Anim Sci **57:**447–455. https://doi.org/10.30802/AALAS-JAALAS-17-00092.
12. **Grimsley JMS, Sheth S, Vallabh N, Grimsley CA, Bhattal J, Latsko M, Jasnow A, Wenstrup JJ.** 2016. Contextual modulation of vocal behavior in mouse: Newly identified 12 kHz "Mid-Frequency" vocalization emitted during restraint. Front Behav Neurosci **10:**1–14. https://doi.org/10.3389/fnbeh.2016.00038.
13. **Harding GW, Bohne BA.** 2004. Temporary DPOAE level shifts, ABR threshold shifts and histopathological damage following below-critical-level noise exposures. Hear Res **196:**94–108. https://doi.org/10.1016/j.heares.2004.03.011.
14. **Heffner HE, Heffner RS.** 2007. Hearing ranges of laboratory animals. J Am Assoc Lab Anim Sci **46:**20–22.
15. **Heinrich UR, Brieger J, Silvanova O, Feltens R, Elmemacher A, Schafer D, Mann WJ.** 2006. COX-2 expression in the guinea pig cochlea partly altered by moderate sound exposure. Neurosci Lett **394:**121–126. https://doi.org/10.1016/j.neulet.2005.10.039.
16. **Hogan MC, Norton JN, Reynolds RP.** 2018. Chapter 20 Environmental factors: macroenvironment versus microenvironment. In: Weichbrod RH, Thompson GAH, Norton JN, editors. Management of animal care and use programs in research, education, and testing, 2nd ed. Boca Raton (FL): CRC Press.
17. **Hughes LF.** 2007. The fundamentals of sound and its measurement. J Am Assoc Lab Anim Sci **46:**14–19.
18. **Hurst K, Litwak KN.** 2012. Accelerative forces associated with routine in-house transportation of rodent cages. J Am Assoc Lab Anim Sci **51:**544–547.
19. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press. https://doi.org/10.17226/12910.
20. **International Organization for Standardization (ISO).** [Internet]. 1997. Mechanical vibration and shock—Evaluation of human exposure to whole-body mechanical vibration—Part 1: General requirements. ISO 2631-1. [Cited 30 September 2019]. Available at: https://www.iso.org/standard/7612.html
21. **Ison JR, Allen PD.** 2003. Low-frequency tone pips elicit exaggerated startle reflexes in C57BL/6J mice with hearing loss. J Assoc Res Otolaryngol **4:**495–504. https://doi.org/10.1007/s10162-002-3046-2.
22. **Kobrina A, Toal KL, Dent ML.** 2018. Intensity difference limens in adult CBA/CaJ mice (*Mus musculus*). Behav Processes **148:**46–48. https://doi.org/10.1016/j.beproc.2018.01.009.
23. **Lauer AM, May BJ, Hao ZY, Watson J.** 2009. Analysis of environmental sound levels in modern rodent housing rooms. Lab Anim (NY) **38:**154–160. https://doi.org/10.1038/laban0509-154.
24. **Li Y, Rabey KN, Schmitt D, Norton JN, Reynolds RP.** 2015. Characteristics of vibration that alter cardiovascular parameters in mice. J Am Assoc Lab Anim Sci **54:**372–377.
25. **Logge W, Kingham J, Karl T.** 2014. Do individually ventilated cage systems generate a problem for genetic mouse model research? Genes Brain Behav **13:**713–720. https://doi.org/10.1111/gbb.12149.
26. **Morioka M, Griffin MJ.** 2000. Difference thresholds for intensity perception of whole-body vertical vibration: effect of frequency and magnitude. J Acoust Soc Am **107:**620–624. https://doi.org/10.1121/1.428331.
27. **Murphy TJ, Rienstra AA.** 2012. Hear more: a guide to using ultrasound for leak detection and condition monitoring. Ft Myers (FL): Terence O'Hanlon Publishers. ISBN 978-0-9825-1633-1.
28. **National Institutes of Health Design Requirements Manual.** 2016. Issuance Notice 12/12/2016, Rev. 1.0: 02/13/2018 Accessed Nov 18, 2018: https://www.wbdg.org/FFC/NIH/nih_design_requirements_rev_1.0_2018.pdf
29. **Norton JN, Kinard WL, Reynolds RP.** 2011. Comparative vibration levels perceived among species in a laboratory animal facility. J Am Assoc Lab Anim Sci **50:**653–659.
30. **Obernier JA, Baldwin RL.** 2006. Establishing an appropriate period of acclimatization following transportation of laboratory animals. ILAR J **47:**364–369. https://doi.org/10.1093/ilar.47.4.364.
31. **Perremans S, Randall JM, Rombouts G, Decuypere E, Geers R.** 2001. Effect of whole-body vibration in the vertical axis on cortisol and adrenocorticotropic hormone levels in piglets. J Anim Sci **79:**975–981. https://doi.org/10.2527/2001.794975x.
32. **Pienkowski M, Eggermont JJ.** 2012. Reversible long-term changes in auditory processing in mature auditory cortex in the absence of hearing loss induced by passive moderate-level sound exposure. Ear Hear **33:**305–314. https://doi.org/10.1097/AUD.0b013e318241e880.
33. **Pienkowski M, Munguia R, Eggermont JJ.** 2013. Effects of passive, moderate-level sound exposure on the mature auditory cortex: spectral edges, spectrotemporal density, and real-world noise. Hear Res **296:**121–130. https://doi.org/10.1016/j.heares.2012.11.006.
34. **Popelář J, Díaz Gómez M, Lindovský J, Rybalko N, Burianová J, Oohashi T, Syka J.** 2017. The absence of brain-specific link protein Bral2 in perieneuronal nets hampers auditory temporal resolution and neural adaptation in mice. Physiol Res **66:**867–880. https://doi.org/10.33549/physiolres.933605.
35. **Portfors CV, Perkel DJ.** 2014. The role of ultrasonic vocalizations in mouse communication. Curr Opin Neurobiol **28:**115–120. https://doi.org/10.1016/j.conb.2014.07.002.
36. **Rabat A.** 2007. Extra-auditory effects of noise in laboratory animals: The relationship between noise and sleep. J Am Assoc Lab Anim Sci **46:**35–41.

37. **Rabey KN, Li Y, Norton JN, Reynolds RP, Schmitt D.** 2015. Vibrating frequency thresholds in mice and rats: implications for the effects of vibrations on animal health. Ann Biomed Eng **43:**1957–1964. https://doi.org/10.1007/s10439-014-1226-y.
38. **Raff H, Bruder ED, Cullinan WE, Ziegler DR, Cohen EP.** 2011. Effect of animal facility construction on basal hypothalamic-pituitary-adrenal and renin-aldosterone activity in the rat. Endocrinology **152:**1218–1221. https://doi.org/10.1210/en.2010-1432.
39. **Rasmussen S, Glickman G, Norinsky R, Quimby FW, Tolwani RJ.** 2009. Construction noise decreases reproductive efficiency in mice. J Am Assoc Lab Anim Sci **48:**363–370.
40. **Reynolds RP, Kinard WL, Degraff JJ, Leverage N, Norton JN.** 2010. Noise in a laboratory animal facility from the human and mouse perspectives. J Am Assoc Lab Anim Sci **49:**592–597.
41. **Reynolds RP, Li Y, Garner A, Norton JN.** 2018. Vibration in mice: A review of comparative effects and use in translational research. Animal Model Exp Med **1:**116–124. https://doi.org/10.1002/ame2.12024.
42. **Sales GD, Wilson KJ, Spencer KV, Milligan SR.** 1988. Environmental ultrasound in laboratories and animal houses: A possible cause for concern in the welfare and use of laboratory animals. Lab Anim **22:**369–375. https://doi.org/10.1258/002367788780746188.
43. **Samson J, Sheeladdevi R, Ravindran R, Senthilvelan M.** 2006. Stress responses in rat brain after different durations of noise exposure. Neurosci Res **57:**143–147. https://doi.org/10.1016/j.neures.2006.09.019.
44. **Schwarting RKW, Wöhr M.** 2012. On the relationships between ultrasonic calling and anxiety-related behavior in rats. Braz J Med Biol Res **45:**337–348. https://doi.org/10.1590/S0100-879X2012007500038.
45. **Silva MJ, Dias A, Barreta A, Nogueira PJ, Castelo-Branco NAA, Boavida MG.** 2002. Low frequency noise and whole-body vibration cause increased levels of sister chromatid exchange in splenocytes of exposed mice. Teratog Carcinog Mutagen **22:**195–203. https://doi.org/10.1002/tcm.10012.
46. **Skellett RA, Crist JR, Fallon M, Bobbin RP.** 1996. Chronic low-level noise exposure alters distortion product otoacoustic omissions. Hear Res **98:**68–76. https://doi.org/10.1016/0378-5955(96)00062-7.
47. **Smith MG, Croy I, Ögren M, Waye KP.** 2013. On the influence of freight trains on humans: a laboratory investigation of the impact of nocturnal low frequency vibration and noise on sleep and heart rate. PLoS One **8:**1–9. https://doi.org/10.1371/journal.pone.0055829.
48. **Turner JG, Bauer CA, Rybak LP.** 2007. Noise in animal facilities: why it matters. J Am Assoc Lab Anim Sci **46:**10–13.
49. **Turner JG, Parrish JL, Hughes LF, Toth LA, Caspary DM.** 2005. Hearing in laboratory animals: strain differences and nonauditory effects of noise. Comp Med **55:**12–23.
50. **Turner JG, Willott JF.** 1998. Exposure to an augmented acoustic environment alters auditory function in hearing-impaired DBA/2J mice. Hear Res **118:**101–113. https://doi.org/10.1016/S0378-5955(98)00024-0.
51. **Turner JG, Parrish JL, Zuiderveld L, Darr S, Hughes LF, Caspary DM, Idrezbegovic E, Canlon B.** 2013. Acoustic experience alters the aged auditory system. Ear Hear 34:151–159. https://doi.org/10.1097/AUD.0b013e318269ca5b.
52. **US Environmental Protection Agency (EPA).** [Internet]. 1974. Information on levels of environmental noise requisite to protect public health and welfare with an adequate margin of safety. [Cited 11 November 2018]. Available at: http://www.nonoise.org/library/levels74/levels74.htm
53. **World Health Organization (WHO).** [Internet]. 2018. Environmental noise guidelines for the European Region. [Cited 30 September 2019]. Available at: http://www.euro.who.int/en/publications/abstracts/environmental-noise-guidelines-for-the-european-region-2018. ISBN: 9789289053563.
54. **Whitmer WM, McShefferty D, Akeroyd MA.** 2016. On detectable and meaningful speech-intelligibility benefits. Adv Exp Med Biol **894:**447–455. https://doi.org/10.1007/978-3-319-25474-6_47.